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Preclinical Profile of Potent Second Generation CpAMs Capable of Inhibiting the Generation of HBsAg, HBeAg, pgRNA and cccDNA in HBV Infected Cells

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AASLD THE LIVER MEETING® 2017 WASHINGTON, DC OCTOBER 20-24

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Background

Clinical cure remains elusive in chronic HBV (CHB) patients, despite prolonged treatment periods with current available therapies[1]. Core Protein Allosteric Modulators (CpAMs) are a new class of direct acting antivirals that target Core protein and inhibit multiple steps in the viral lifecycle, including the establishment of cccDNA[2,3,4]. Here we characterize potent 2nd generation CpAMs derived from novel and distinct chemical scaffolds that exhibit enhanced potency in inhibiting cccDNA establishment and favorable drug properties.

Materials and Methods

Inhibitors: ASMB CpAMs ABI-H0731, ABI-H2158 and ABI-Nx were discovered and prepared at ASMB, while SBA-R01[5] (believed to be NVR 3-778 or close analog) was synthesized at a CRO by published procedures. ETV (Nuc) was purchased from ACME Bioscience.

HBV DNA Replication Assays: HepAD38 and HepG2 cells producing HBV Genotype A and C viruses were induced and treated with compounds for 4 to 7 days. Intracellular viral DNA were quantified by Taqman quantitative PCR (qPCR) using primers and a probe specific to the HBV core gene.

HBV Transient Transfection Assays: Huh7 cells were transfected with plasmids expressing HBV in 96-well plates and treated with compounds for 8 days. Supernatants were harvest and digested with DNase and quantified by qPCR.

Aberrant Capsid EM Assay: CpAMs were incubated at 10 µM with HBV Core protein (Cp149) with 300 mM NaCl for 5 hr at 37°C, then examined by transmission electron microscopy[6].

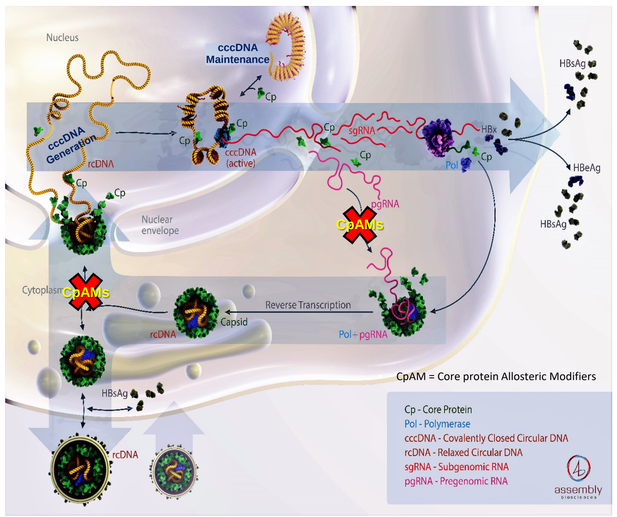
HBV Infection Assays: HepG2-NTCP or Primary Human Hepatocytes (PHH; Lonza) were infected with HBV from HepAD38 at an MOI of 50-250 and treated with inhibitors at the indicated time points and concentrations. Supernatants were harvested and measured for HBV antigen expression (ECL). HBV DNA/pgRNA levels were quantified using bDNA probes (Affymetrix) specific to the relevant HBV sequences[7].

Molecular Profiling of CpAMs: HepAD38 cells were treated with inhibitors and induced simultaneously. Cells were retreated 3 days later and harvested 6 days post induction. HBV capsid, and capsid associated core DNA were detected by Enzyme Immunoassay (EIA), and Southern Blot, respectively, as previously described[8].

cccDNA Southern Blots: HBV infected NTCP-HepG2 or PHH cells were treated with inhibitors. Four days post infection, cells were harvested and extrachromosomal DNA extracted by a Hirt-modified method[9]. Non-supercoiled DNA was removed via T5 exonuclease (NEB) digestion. High-fidelity EcoRI endonuclease (NEB) was then used to linearize supercoiled HBV cccDNA.

EC_{50} Calculations: EC_{50} values were calculated using GraphPad Prism software.

CpAMs Disrupt Multiple Steps of HBV Lifecycle

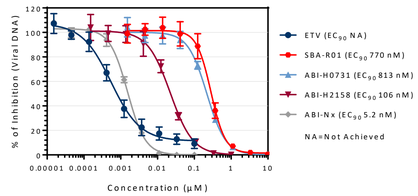


- CpAMs inhibit encapsidation of Pol-pgRNA, preventing subsequent conversion of pgRNA to rcDNA, trafficking to nucleus and conversion to cccDNA

Antiviral Potency and Spectrum of CpAMs

CpAMs Completely Block HBV Viral Replication

Antiviral Activity in HepAD38 (HBV genotype D)



CpAMs Activity vs. Major HBV Genotypes

HBV Virus	Reduction of Viral DNA - EC_{50} (nM)				
	SBA-R01	ABI-H0731	ABI-H2158	ABI-Nx	ETV
GT-A	142	39	6.6	ND	5.4
GT-B	148	67	11	0.6	2.9
GT-C	107	31	7.1	ND	2.4

Effect of Serum Protein on CpAM Activity

Human Serum	Reduction of Viral DNA - EC_{50} (nM)			
	SBA-R01	ABI-H0731	ABI-H2158	ETV
2%	486	253	23	0.5
40%	6,289	986	140	0.6
Fold Shift	13	3.9	6.2	1.2

CpAMs Active Against Nuc^R Virus

HBV Virus	Reduction of Viral DNA - EC_{50} (nM)				
	SBA-R01	ABI-H0731	ABI-H2158	ABI-Nx	ETV
Wt	281	216	22	1.5	0.6
Nuc^R	569	267	19	ND	7.1
Fold	2.0	1.2	0.9	ND	12

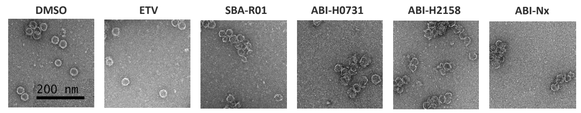
CpAMs Inactive Against Other Viruses

Viruses	Reduction of Viral DNA - EC_{50} (nM)			
	SBA-R01	ABI-H0731	ABI-H2158	ETV
Flu A	>10,000	>10,000	>10,000	>10,000
HSV-1	>10,000	>10,000	>10,000	>10,000
HRV-16	>10,000	>10,000	>10,000	>10,000

- Second generation ASMB CpAMs exhibit enhanced potency in reducing viral DNA levels
- CpAMs display pangenotypic activity, active against major genotypes (A, B, C and D)
- ASMB CpAMs exhibit modest loss of potency in the presence of 40% human serum
- CpAMs retain activity against Nuc resistant (Nuc^R) virus (genotype D, L180M + M204V)
- CpAMs are selective and specific inhibitors of HBV, with no significant activity against Flu A, HRV-16, HSV-1 at 10 µM, and no evidence of cytotoxicity (CC_{50} >10 µM) vs. Huh7, HepG2, Hela, HEK293, MOLT-4, NCI-H226 and PBMC (data not shown)
- Combining CpAMs with ETV results in additive to synergistic outcomes when analyzed using both the MacSynergy II and CompuSyn approaches over a range of concentrations and inhibitor ratios (data not shown)

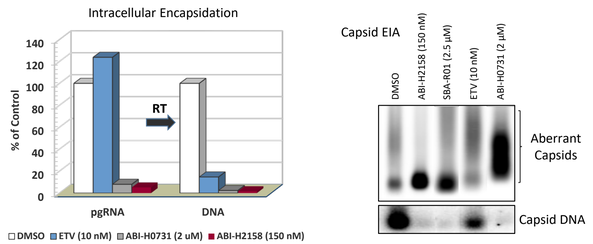
CpAM Mechanism of Action Studies

CpAMs Induce Formation of Aberrant Capsids (EM)



- In contrast to ETV, incubation of CpAMs with HBV Core protein (Cp149) accelerated capsid assembly and induced formation of aberrant, cracked capsids

CpAMs Block pgRNA Encapsidation and Induce Formation of Empty Capsids



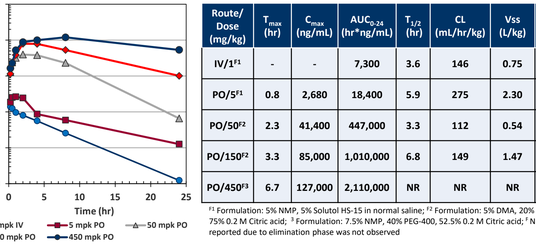
- Induced HepAD38 cells were treated at 10x EC_{50} levels
- CpAMs prevent encapsidation of pgRNA and subsequent Pol synthesis of rcDNA in nucleocapsids
- ETV inhibits conversion of pgRNA to rcDNA directly in nucleocapsids, stabilizing and increasing the levels of encapsidated pgRNA observed
- CpAMs perturb capsid assembly at 100x EC_{50} levels and cause conformationally-induced migration changes on EIA gels
- CpAMs induce empty capsids by Capsid DNA Southern blot (lower panel)
- ETV fails to completely inhibit HBV replication

CpAMs Impair cccDNA Formation in Infected HepG2-NTCP and PHH Cells

Inhibition in HBV Infected HepG2-NTCP Cells

Parameter	HepG2-NTCP EC_{50} (nM)				
	ETV	SBA-R01	ABI-H0731	ABI-H2158	ABI-Nx
Viral DNA	1	487	207	29	1
HBeAg	>100	9,171	5,619	365	34
HBsAg	>100	9,087	6,251	409	47
pgRNA	>100	>10,000	3,465	227	1

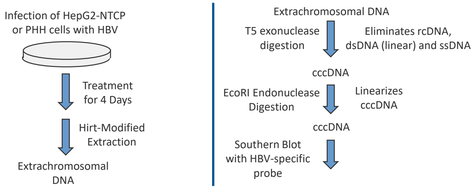
Inhibition in HBV Infected PHH Cells



Parameter	PHH EC_{50} (nM)				
	ETV	SBA-R01	ABI-H0731	ABI-H2158	ABI-Nx
Viral DNA	< 0.025	1,218	306	49	11
HBeAg	Incomplete	3,563	3,597	242	60
HBsAg	Incomplete	3,309	3,518	231	50
pgRNA	Incomplete	3,077	3,526	193	72

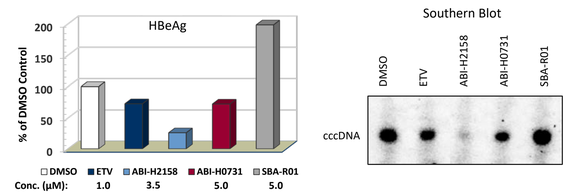
- ASMB CpAMs inhibit viral replication in HBV infected HepG2-NTCP and PHH cells, with potency similar to HepAD38 assays, indicating good stability in human hepatocytes
- Importantly, next generation CpAMs exhibit enhanced potency in reducing HBeAg, HBsAg and pgRNA levels (surrogate markers of cccDNA formation)
- ETV showed incomplete inhibition of antigen production in PHH when tested up to 10 µM

HBV cccDNA Detection Using a Southern Blot Assay



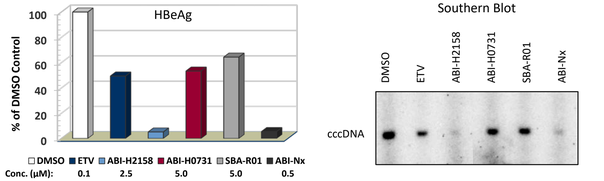
- HepG2-NTCP or PHH cells infected with HBV virus for 3 hr and treated with compounds
- cccDNA was extracted by modified Hirt method – digestion with T5 and EcoRI nucleases

HBV Infected HepG2-NTCP Cells



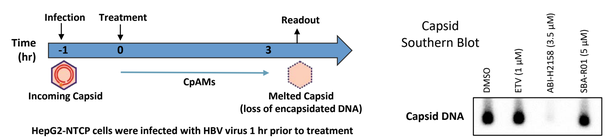
- ABI-H2158 exhibited potent activity in blocking cccDNA establishment
- ETV modestly inhibits cccDNA establishment at concentrations >1,000-fold its EC_{50}
- SBA-R01 increased HBeAg expression and enhanced cccDNA formation at 5 µM

HBV Infected PHHs



- CpAMs reduced cccDNA establishment in PHH cells
- ASMB 2nd generation CpAMs exhibit enhanced potency in reducing cccDNA establishment
- ETV also reduced cccDNA levels in PHH at high concentrations (>10,000x EC_{50})

ABI-H2158 Disassembles Incoming Capsids



- ABI-H2158 exhibited significant ability to prematurely "melt" incoming capsids and cause a loss of viral DNA
- Result correlates with its inhibition of cccDNA establishment

ABI-H2158 PK Profile

ABI-H2158 Single-Dose Pharmacokinetic Results in Rats

Route/ Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24} (hr*ng/mL)	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vss (L/kg)	%F
IV/1[F1]	-	-	7,300	3.6	146	0.75	-
PO/5[F1]	0.8	2,680	18,400	5.9	275	2.30	50
PO/50[F2]	2.3	41,400	447,000	3.3	112	0.54	~100
PO/150[F2]	3.3	85,000	1,010,000	6.8	149	1.47	92
PO/450[F3]	6.7	127,000	2,110,000	NR	NR	NR	64

[F1] Formulation: 5% NMP, 5% Solutol HS-15 in normal saline; [F2] Formulation: 5% DMA, 20% PEG-400, 75% 0.2 M Citric acid; [F3] Formulation: 7.5% NMP, 40% PEG-400, 52.5% 0.2 M Citric acid; [F] NR = not reported due to elimination phase was not observed

- High bioavailability (50-100%) following oral administration to rats
- Plasma exposures were less than dose proportional, but yielded high C_{max} (up to 127 µg/mL or ~260 µM) and AUC_{0-24hr} (up to 2,110 hr*µg/mL or ~4,400 µM*hr) values, as well as a $T_{1/2}$ suggestive of potential QD dosing in humans
- Second generation CpAMs exhibit good stability in liver microsomes (data not shown)
- No significant CYP inhibition observed (IC_{50}s > 10 µM)
- Possesses favorable pharmaceutic properties

Summary

- ASMB has discovered and optimized multiple chemically-distinct CpAM scaffolds
- Second generation CpAMs exhibit enhanced potency in inhibiting both viral replication and cccDNA, while retaining the favorable drugable properties of first generation CpAM ABI-H0731
- ABI-H2158 is currently completing IND-enabling studies for subsequent progression into Phase 1 clinical studies
- An additional clinical candidate from the ABI-Nx class of CpAMs is expected to be nominated in the coming months

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