The Discovery of AMS-I-1274, a High Potent and Orally Active Capsid-assembly Modulator against Hepatitis B Virus

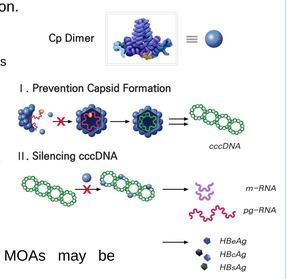
J. KIM, M. LEE, H. SIM, R. KIM, B. PAK, P. PRASIT, N. JEONG

AM Sciences, Seoul, Republic of Korea

INTRODUCTION

- New drugs that inhibit HBV replication are needed to enhance the treatment of chronic hepatitis B resulting in a higher number of durable responses and functional cures.
- Capsid-assembly modulators (CAM) are a novel class of HBV antivirals and they have been suggested to be effective anti-HBV agents in both preclinical and clinical studies.
- CAMs have multiple mechanisms of action.
  - ✓ Inhibition of virus proliferation
    - Inhibit creation of additional hepatitis viruses
    - Prevent cccDNA accumulation
  - ✓ Eradication cytolytic infected cells
    - Decreasing level of HBsAg and HBeAg
  - ✓ Eradication non-cytolytic infected cells
    - Restoration of antiviral cytokine activity
    - Promoting APOBEC activity

Figure 1. CAM mechanism of action



- Potent antiviral activity via multiple MOAs may be important for optimal patient responses.

AIM

- To describe the preclinical characterization of AMS-I-1274, a novel CAM with high potency against viral replication and cccDNA formation

METHOD

- *In vitro* anti-viral activity were determined in HepAD38 cells, HBV-infected HepG2-hNTCP cells and primary human hepatocytes (PHH).
  - ✓ The level of intracellular HBV DNA was measured by quantitative real-time PCR.
  - ✓ The levels of extracellular HBs/eAg were measure by ELISA.
  - ✓ The level of HBV RNA was measured by quantitative real-time RT-PCR.
- The effect on cccDNA levels was determined in HBV-infected PHH by Southern Blot analysis.
- Serum-shift assay was conducted using HepAD38 cells cultured with tetracycline-media supplemented with human serum albumin (HSA).
- Anti-viral activity on HBV genotype (Gt), core-, and NA-resistant variants were evaluated in a HepG2/plasmid DNA transfection system. Potency of anti-viral activity was evaluated with the level of HBV DNA.
- Single-dose pharmacokinetic (PK) studies were conducted in rodent (rat) and nonrodent species (monkey and dog).
- *In vivo* efficacy were determined in uPA/SCID mice with humanized liver (PXB-mice) infected with HBV.
- Human hepatotoxicity was ascertained by *in vitro* model using Hurelhuman™ primary hepatocyte micro liver model.

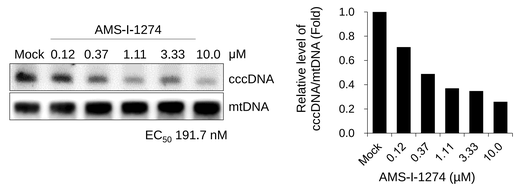
RESULTS

- **AMS-I-1274 potently inhibits HBV replication and cccDNA formation in PHH as well as HepAD38 cell and HepG2-hNTCP cells.**

Table 1. Potency of AMS-I-1274 in HepAD38 cells, HepG2-hNTCP cells and PHH

Cells	Parameter	AMS-I-1274 EC_{50} ± SD (nM)	AMS-I-1274 EC_{90} ± SD (nM)
HepAD38	HBV DNA	6.23 ± 2.92	58.0 ± 26.4
HepG2-NTCP	HBV DNA	6.41 ± 1.94	104 ± 62.0
	HBV RNA	7.67 ± 5.05	273 ± 255
	HBeAg	58.1 ± 17.8	522 ± 160
	HBsAg	28.8 ± 4.88	259 ± 43.9
PHH	HBV DNA	5.55 ± 3.38	49.9 ± 36.1
	HBV RNA	6.27 ± 4.32	56.4 ± 26.3
	HBeAg	13.5 ± 14.4	121 ± 129
	HBsAg	8.38 ± 5.85	73.3 ± 51.7

Figure 2. AMS-I-1274 reduced the cccDNA formation in HepG2-hNTCP



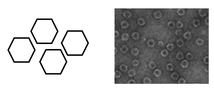
- **Presence of 40% of HSA causes a slight reduction in potency (EC_{50}=15.6 nM, 2.5-fold increase). AMS-I-1274's anti-viral activity was 65-fold more potent compared to Novira 3-778 (EC_{50} = 5,712 nM at 40% HSA).**

Table 2. Serum protein shift value for AMS-I-1274 in HepAD38 cells

HAS (%)	AMS-I-1274 EC_{50} ± SD (nM)	AMS-I-1274 Fold Shift	NVR 3-778 EC_{50} ± SD (nM)	NVR 3-778 Fold Shift
0	6.20 ± 2.9	1.0	425.3 ± 50.7	1.0
2	9.17 ± 4.7	1.4	774.3 ± 52.5	1.8
10	14.1 ± 4.0	2.2	2455 ± 91.4	5.7
40	15.6 ± 9.4	2.5	5712± 1657	13

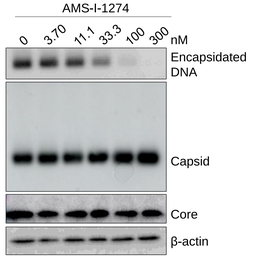
- **AMS-I-1274 induces the formation of empty capsid particles devoid of pgRNA and rcDNA.**

✓ MOA: Class II capsid inhibitor



Empty Capsid

Figure 3. Capsid assembly assay



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- **AMS-I-1274 shows pan-genotypic activity against isolates from Gt A to H.**

Table 3. Pan-genomic activity of AMS-I-1274

Genotype	EC_{50} (nM) AMS-I-1274	EC_{50} (nM) ETV
A2	9.11	3.68
B	10.34	1.63
C	4.92	1.94
D	26.51	2.58
E	124.50	2.59
F2	25.85	2.20
G	13.75	1.98
H	16.92	1.66

- **Most core variants show no (<2 fold) or modest (<10 fold) decrease in potency of AMS-I-1274; except for T33N (80-fold).**

Table 4. Anti-HBV potency of AMS-I-1274 against core variants

Core Variants	AMS-I-1274 EC_{50} (nM)	AMS-I-1274 Fold Shift	NVR 3-778 EC_{50} (nM)	NVR 3-778 Fold Shift	ETV EC_{50} (nM)	ETV Fold Shift	GLS4 EC_{50} (nM)	GLS4 Fold Shift
WT	10.95	1.00	307.10	1.00	3.87	1.00	11.48	1.00
F23Y	90.48	8.26	929.00	3.03	4.20	1.09	35.28	3.07
P25A	27.81	2.54	1055.00	3.44	4.70	1.22	135.10	11.77
D29G	40.69	3.72	706.90	2.30	6.24	1.61	8.09	0.71
T33N	884.50	80.78	>5000	>16.28	3.71	0.96	>1000	>87.11
T109M	55.90	5.11	414.00	1.35	3.03	0.78	20.62	1.80
Y118F	9.30	0.85	449.10	1.46	2.73	0.71	48.78	4.25
V124F	13.60	1.24	530.00	1.73	3.24	0.84	410.00	35.71
T128I	67.34	6.15	761.10	2.48	2.94	0.76	1.26	0.11
I105T	126.40	11.54	1566.00	5.10	2.67	0.69	24.62	2.14
S106T	3.71	0.34	234.00	0.76	7.45	1.93	2.77	0.24

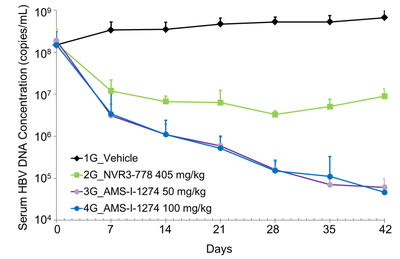
- **Nucleoside reverse transcriptase inhibitors (NrtI) mutants are fully susceptible to AMS-I-1274.**

Table 5. Anti-HBV potency of AMS-I-1274 against NrtI mutants

NrtI mutants	AMS-I-1274 EC_{50} (nM)	AMS-I-1274 Fold Shift	NVR 3-778 EC_{50} (nM)	NVR 3-778 Fold Shift	ETV EC_{50} (nM)	ETV Fold Shift	LAM EC_{50} (nM)	LAM Fold Shift
WT	11.46	1.00	323.20	1.00	83.05	1.00	2.76	1.00
rtM204I	34.45	3.01	358.70	1.11	>100000	>1197.60	-	-
rtL180M+M204V	14.35	1.25	375.50	1.16	>100000	>1197.60	-	-
rtA181T+N236T	42.72	3.73	429.30	1.33	3584.00	43.15	-	-
rtM204I+S202G +M250V	14.91	1.30	522.50	1.62	-	-	>500	>181.16
L180M+M204V +M250V+I169T	29.95	2.61	560.60	1.73	-	-	74.63	27.04
L180M+M204V +T184G+S202I	16.07	1.40	313.20	0.97	-	-	252.30	91.41

- **Oral administration of AMS-I-1274 at 50 mg/kg twice daily in HBV-infected PBX-mice resulted in robust multi-log reduction (-3.49 log10) of serum HBV DNA after 42 days of treatment.**

Figure 4. Pharmacology study in HBV-infected PBX-mice model



- **Liver toxicity evaluation**
  - ✓ *In vitro* cytotoxicity: CC_{50} > 100 μM (HepAD38 Cells)
  - ✓ Liver transporters: No inhibition of BSEP, MRP3, MRP4 at 10 μM
  - ✓ Hurelhuman™ primary hepatocyte micro liver model: Low cholestatic potential (TC_{50}'s ~200 μM); No appreciable change in toxicity ± Bile acids
    - This is >25 times above therapeutic C_{max} in the mouse model
  - ✓ No covalent binding of GSH to proteins in liver microsomes
  - ✓ No ALT/AST increase in PBX mice when chronically dosed for 7 days at 100 mg/kg/day.
  - ✓ In-life portion of 1-month GLP toxicity studies in monkey and mouse are complete without obvious toxicity
  - ✓ No inhibition or induction of CYPs, Not a time-dependent inhibitor of CYP3A4
  - ✓ Low plasma protein binding: %unbound =15% (Human)
  - ✓ No hit (>50%) in CEREB screening at 10 μM
  - ✓ Not a strong inhibitor of hERG: >25 μM, Negative in AMES & MNT

CONCLUSIONS

- AMS-I-1274 is a novel class II capsid-assembly modulator with high potency against both pgRNA and cccDNA formation.
- AMS-I-1274 potently inhibits downstream hepatitis B "e" antigen (HBeAg) in the mouse model.
- AMS-I-1274 has a favorable preclinical profile and excellent antiviral activity.
- AMS-I-1274 is fully active against NrtI-resistant HBV.
- AMS-I-1274 is pangenotypic.
- AMS-I-1274 has low potential for drug-drug interaction.
- AMS-I-1274 has low potential for liver toxicity.
- AMS-I-1274 has favorable oral bioavailability in rodents and non-human primates.
- AMS-I-1274 has favorable overall safety profile.
- Phase 1 studies with AMS-I-1274 are planned for 1Q 2023.

CONTACT INFORMATION

E-mail. njeong@amsciences.co.kr, kimjh@amsciences.co.kr