Discovery and preclinical profile of a first-in-class potent hepatitis E virus inhibitor AT-587

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BACKGROUND

Hepatitis E virus (HEV) is one of the most common causes, yet least diagnosed etiologies, of acute viral hepatitis worldwide[1]. Globally, HEV is responsible for an estimated 19.5 million cases of acute hepatitis E annually. While most infections are self-limiting, HEV can lead to fulminant hepatitis during pregnancy, and an aggressive hepatitis in immunocompromised and transplant patients[2]. In those at-risk patients, HEV infection can become chronic and progress to cirrhosis, neurological sequelae, hepatic failure and death, which underscores the need for effective treatment of the infection. Currently, there are no HEV-specific antivirals, and therapeutic options for HEV infection are limited to off-label use of ribavirin (RBV), sofosbuvir (SOF) and pegylated-interferon-α. We report here on a unique nucleotide prodrug, AT-587, that targets HEV RNA polymerase and exhibits a promising antiviral profile as a first-in-class inhibitor for the treatment of life-threatening HEV infection.

METHODS

AT-587 was chosen after screening for anti-HEV activity utilizing a luciferase-based HEV subgenomic replicon and infection system[3]. EC_{50} values for AT-587 and known HEV inhibitors sofosbuvir (SOF) and RBV, were determined against several strains of HEV, genotype 1 & 3.

- AT-587 was incubated with primary human hepatocytes at 37ºC for 24 h. Cells were extracted and the formation of the active metabolite AT-9068 measured by LC-MS/MS.
- The activities of human DNA polymerases α, β and γ were tested in a fluorescence-based assay after the addition of the active triphosphate AT-9068.
- Cytotoxicity of AT-587 was evaluated in Huh-7 replicon cells, human iPS cardiomyocytes and human bone marrow CD34+ cells.
- Genotoxicity of AT-587 was evaluated using Ames and chromosomal aberration assays, and cardiotoxicity was tested with an hERG assay.
- The selectivity of AT-587 was established against other RNA and DNA viruses.
- An in vivo efficacy study was conducted in a gerbil HEV infection model[4]. Animals were inoculated with an HEV-3 isolate at 4×10^6 genome copies per gerbil and randomly divided into four groups (n=6 per group). On Day 5 post-inoculation, they were administered 0, 75, 150 or 250 mg/kg AT-587 daily for 10 days by oral gavage. The control group was administered an equal volume of vehicle only. Fecal samples were collected from the animals on Day 14, 21 and 28 post inoculation, after which the gerbils were euthanized, and liver and intestinal tissues collected to determine viral load. Histological analyses were also conducted on the liver samples using the Ishak scoring system for chronic hepatitis[5].

RESULTS

Antiviral specificity of AT-587 (EC_{50} values, nM)

Virus	AT-587	SOF
HCV GT-1	7	81
DENV-2	194	>10,000
JEV	215	6,228
POWV	298	>10,000
WNV	81	3,108
YFV	54	1,908
ZIKV	102	>10,000
CHIKV	259	>10,000
Rubella	92	12,425
Norovirus	4,868	>10,000
VEEV	3,754	>10,000
HCoV OC43	5,553	>10,000

ND= not determined; DENV, dengue; HCV, hepatitis C; JEV, Japanese encephalitis; POWV, Powassan; WNV, West Nile; YFV, yellow fever; ZIKV, Zika virus; CHIKV, chikungunya; VEEV, Venezuelan equine encephalitis

- AT-587 was active against all flaviviruses (HCV, DENV, JEV, POWV, WNV, YFV, ZIKV) tested using reporter luciferases/GFP in replicon (HCV) or infected Huh-7 cells.
- AT-587 was also active against rubella and CHIKV, but only weakly active against VEEV, HCoV OC43, and human norovirus.
- The prodrug was not active against MERS, SARS-CoV-2, NiV, RSV, hMPV, CCHFV, LASV, RVFV, VSV, HIV, EBOV and Marburg virus, or any of the DNA viruses tested (HSV-1 and HBV).

AT-587 plasma metabolite $AUC_{0\text{-}24h}$ (ng*h/mL)

Species	AT-2485
Monkey	4,375
Rat	2,121
Mouse	2,261

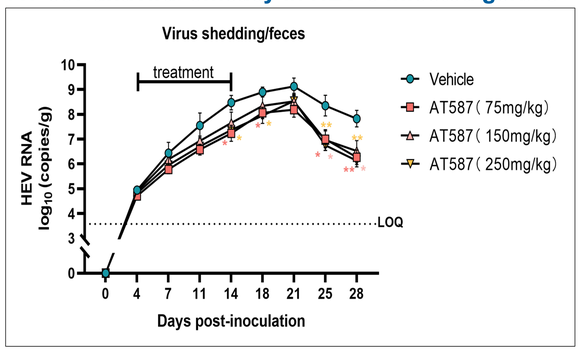
- High plasma concentrations of AT-2485, the surrogate of the active triphosphate metabolite, were measured in monkeys, rats and mice after an oral dose of AT-587 (100 mg/kg).
- AT-587 also formed high levels of the active triphosphate (AT-9068, $AUC_{0\text{-}24h}$= 3920 pmol*h/10^6 cells) in human hepatocytes.

Inhibition of HEV replication by nucleotide analogs

Compound	HEV-3 p6 WT	HEV-3 p6 G1634R (RBV RAS)	HEV-3 p6 A1343V (SOF RAS)	HEV-3 83.2.27	HEV-3a LB rabbit	HEV-1 Sar55
AT-587	80 ± 29	84 ± 2	276 ± 5	142 ± 2	231 ± 53	221 ± 86
SOF	5,129 ± 985	3,925 ± 1,078	13,145 ± 2,293	8,181 ± 1,671	7071 ± 1,465	12,102 ± 1,529
RBV	12,551 ± 256	12,793 ± 945	10,640 ± 1,042	19,111 ± 335	24,441 ± 2,269	29,219 ± 5,586
Fitness (%)	100	144.2	94.7	115	0.6	0.5

EC_{50} values (nM) showed AT-587 was significantly more potent than known HEV inhibitors SOF and RBV in several HEV-3 strains, including Huh-7 Kernow C1 p6/Gluc replicon cells (HEV-3 p6). AT-587 also retained high potency against RBV (G1634R) and SOF (A1343V) clinical resistance strains. Similar efficacy against HEV-1, Sar55 strain, is also presented.

AT-587 antiviral efficacy in HEV-3 infected gerbils



On day 14, 21 and 28 post inoculation, the fecal samples from all three treated groups had significantly lower HEV RNA levels than the vehicle group (control).
In addition, the viral loads in the liver and intestinal samples were significantly lower in the treated groups compared to control (data not shown). However, the Ishak scores were not different between the groups. Overall, these results demonstrated that AT-587 inhibited HEV-3 replication *in vivo*.

AT-587 showed no toxicity in *in vitro* studies

- Active metabolite (AT-9068) did not inhibit human DNA polymerases α, β or γ (IC_{50} >100 µM)
- Not cytotoxic in replicon cells and human iPS cardiomyocytes (CC_{50} >100 µM)
- No effect on viability of bone marrow cells (IC_{50} >30 µM)
- Negative for phototoxicity and negative for genotoxicity in Ames and micronucleus assays
- No hERG inhibition at clinically relevant concentrations
- CTA enabling 2-week *in vivo* toxicology studies have been completed, and planning for Phase I studies is underway

CONCLUSIONS

- AT-587 was 30-150-fold more potent *in vitro* against HEV than known inhibitors SOF and RBV.
- AT-587 formed high levels of AT-2485, the surrogate plasma marker of the active intracellular metabolite (AT-9068), in several species and high levels of the active metabolite in human hepatocytes, the tissue of interest.
- AT-587 showed no toxicity in *in vitro* studies.
- The prodrug was also active against flaviviruses, rubella and chikungunya (EC_{50} range 7 – 300 nM).
- AT-587 inhibited HEV-3 replication in infected gerbils, as measured by fecal HEV RNA post treatment.
- AT-587 is a potential first-in-class oral nucleoside analog being developed for the treatment of life-threatening HEV infection, particularly in the immunocompromised and transplant populations.

References

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