4794: Characterization of the Toxicity Profile of AT-527 (Bemnifosbuvir), a Novel Guanosine Nucleotide Prodrug with Antiviral Activity for COVID-19 Infection

Poster Board: P858

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Introduction

AT-527 (bemnifosbuvir), the hemisulfate salt of AT-511 (free base), is an orally administered double prodrug of a guanosine nucleotide analog that is converted after multi-step activation to the active 5′-triphosphate (TP) metabolite (AT-9010), that inhibits the replication of COVID-19 viruses through a dual mechanism of action targeting both NiRAN and RdRp domains (Shannon et al. 2022). It has demonstrated potent anti-COVID-19 activity in vitro (EC_{90}=0.5 μM) and exhibited a well-tolerated safety profile in patients. Here we report the general nonclinical toxicity profile of AT-527. The developmental and reproductive toxicity of AT-527 is presented in P#857.

Materials and Methods

AT-527 was supplied by Atea Pharmaceuticals, Inc. and formulated in dimethyl sulfoxide (DMSO) for in vitro assays or in 0.5% (w/v) CMC / 0.5% (w/v) Tween 80 in purified water or in PEG400 (40%, v/v) / Solutol HS15 (10%, v/v) / 100 mM Citrate buffer pH 4.5±0.2 (50%, v/v) for in vivo studies. The concentrations and dose levels of AT-527 are expressed as free base (AT-511)-equivalent.

Sprague Dawley rats (SPF) were obtained from BioLASCO Taiwan Co., Ltd. (Taipei, ROC). Cynomolgus monkeys (*Macaca fascicularis*) were obtained from Suzhou Xishan Zhongke Laboratory Animal Co., Ltd. (Suzhou, China) or Hainan Jingang Biotech Co., Ltd. (Haikou, China). All animal studies were conducted in facilities that were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and Animal Welfare and approved by the local Institutional Animal Care and Use Committee (IACUC). Pivotal studies were conducted in compliance with GLP Regulations.

One-way analysis of variance (ANOVA) and pairwise comparisons (Dunnett's test) were performed. Statistical significance was reported at the probability levels of 0.05 and 0.01.

Results

The nonclinical safety assessment studies designed to characterize the toxicity profile of AT-527 are summarized in Table 1.

Table 1: Summary of Results of Nonclinical Safety Studies to Characterize the Safety Profile of AT-527

Study Type	Summary of Results
Selectivity against Human DNA Polymerases	The effect of AT-9010 (the active triphosphate metabolite of AT-527) on the human DNA polymerases α, β, and γ was assessed in an in vitro polymerase inhibition assay by measuring incorporation of α-^{32}P labeled GTP. In this assay, AT-9010 was inactive against all three human DNA polymerases with an IC_{50} > 100 μM.
Human Mitochondrial RNA Polymerase (POLRMT) as an Off Target for AT-9010	The effect of AT-9010 on POLRMT was investigated in an in vitro human mitochondrial RNA polymerase nucleotide incorporation assay. The results show that AT-9010 is used by POLRMT and is both a substrate and an inhibitor of POLRMT in vitro, however, with an efficiency similar to that of the triphosphate of sofosbuvir and lower than the naturally occurring nucleotide GTP, indicating its low potential for mitochondrial toxicity.
Mitochondrial toxicity	AT-527 did not show mitochondrial toxicity up to the highest tested concentration of 20 μM. In K562 cells, there was no activity on membrane integrity or adenosine triphosphate (ATP) production, and, in PC3 cells, there was no effect on the mitochondrial protein biogenesis pathway.
Cytotoxicity	AT-511 (the free base of AT-527) did not lead to cytotoxicity up to the highest tested concentration of 100 μM in primary human bone marrow progenitor cells and in human iPS cardiomyocytes.
In vitro hERG in HEK293 cells (GLP)	A minimal effect of 14%, 24%, 16% and 29% inhibition at 3, 10, 30 and 100 μM was observed. This small effect might be attributable to the relatively high vehicle concentration of 1% DMSO used in the study.
CNS in rats (as part of 14-day oral tox study) (GLP)	There were no AT-527-related effects on the neurobehavioral function in rats following single dose oral administration at doses up to 1000 mg/kg.
CV and respiratory function in monkeys (GLP)	There were no AT-527-related effects on blood pressure, electrocardiograms, or respiratory function were noted in monkeys following a single oral dose of AT-527 at doses up to 300 mg/kg.
Ames (GLP)	No mutagenicity was detected in a reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli* tester strains up to the highest tested concentrations of 5000 μg/plate.
Chromosome aberration assay in human PBL (GLP)	AT-527 did not cause chromosomal aberrations in cultured human peripheral blood lymphocytes at concentrations up to 500 μg/mL or limited by cytotoxicity.
Micronucleus test in rats	No clastogenicity or aneugenicity was noted in an in vivo bone marrow micronucleus assay in rats administered oral doses of AT-527 at doses up to 2000 mg/kg.
2-week GLP toxicity and TK study with 2-week recovery in rats	A slight increase in liver weights was only observed in females dosed at 300 and 1000 mg/kg/day with no associated histology findings and no correlating liver enzyme clinical pathology findings. The liver weight increase was fully reversible following the 14-day treatment-free period. The NOAEL was 1000 mg/kg/day.
13-week GLP toxicity and TK study with 4-week recovery in rats	Increased liver weights (Table 2) and correlating hepatocellular hypertrophy (Table 3) were observed at 300 and 1000 mg/kg/day. These findings were reversible following a 4-week recovery. There were no associated degenerative or inflammatory findings, and no correlating liver enzyme clinical pathology findings. The NOAEL was 1000 mg/kg/day.
2-week GLP toxicity and TK study with 2-week recovery in cynomolgus monkeys	Body weight loss associated with loose stools, emesis, and lower food consumption was observed and required reduction of the highest dose from 1000 to 650 mg/kg/day starting on Day 8. There were no findings indicative of liver injury or adaptive changes. The NOAEL was 650 mg/kg/day.
13-week GLP toxicity and TK study with 4-week recovery in cynomolgus monkeys	A statistically significant increase in liver to body weight ratio was observed in males dosed at 650 mg/kg/day, which could be confounded by the slightly lower body weight. There was no statistically significant effect on absolute or liver-to-brain weight ratio. The NOAEL was 650 mg/kg/day.

Table 2: Changes in Liver Weights in the 13-Week Rat Study

Interval	Endpoint	Male (mg/kg/day)				Female (mg/kg/day)			
		0	100	300	1000	0	100	300	1000
Day 92	Absolute (g)	12.62	13.79	14.86**	15.49**	7.29	7.93	9.29**	9.73**
	(% of Control)		*(109%)*	*(118%)*	*(123%)*		*(109%)*	*(127%)*	*(133%)*
	Relative to BW	0.024	0.026	0.028**	0.030**	0.025	0.027	0.031**	0.033**
	(% of Control)		*(107%)*	*(115%)*	*(125%)*		*(108%)*	*(122%)*	*(133%)*
	Relative to Brain	5.55	6.29*	6.65**	6.89**	3.57	3.87	4.59**	4.76**
	(% of Control)		*(113%)*	*(120%)*	*(124%)*		*(108%)*	*(128%)*	*(133%)*
Day 120	Absolute (g)	12.81	13.17	13.46	13.12	6.88	6.98	7.53	8.21*
	(% of Control)		*(103%)*	*(105%)*	*(102%)*		*(102%)*	*(109%)*	*(119%)*
	Relative to BW	0.024	0.024	0.025	0.025	0.025	0.025	0.026	0.026
	(% of Control)		*(99%)*	*(105%)*	*(106%)*		*(100%)*	*(105%)*	*(106%)*
	Relative to Brain	5.68	6.03	6.11	5.77	3.37	3.32	3.63	4.02
	(% of Control)		*(106%)*	*(108%)*	*(102%)*		*(99%)*	*(108%)*	*(119%)*

Data are expressed as group mean. N = 10 for Day 92 and N = 5 for Day 120 (Recovery).
ANOVA & Dunnett: * = p ≤ 0.05, ** = p ≤ 0.01 vs. control.

Table 3: Incidence of Hepatocyte Hypertrophy in the 13-Week Rat Study

Dose (mg/kg/day)	0	100	300	1000
Sex (No. Examined)	M/F (10/10)	M/F (10/10)	M/F (10/9)	M/F (9/9)
Minimal	-/-	-/-	-/1	1/-
Mild	-/-	-/-	1/1	5/1
Moderate	-/-	-/-	1/-	2/-

- = absence of the finding, M – male, F = female

Liver weight increases associated with hepatocellular hypertrophy are well known consequences of metabolic activation in rats. Rats are very sensitive to these effects, and, because of the absence of degenerative histologic or liver enzyme changes, these findings are considered non-adverse and reflective of an adaptive response to AT-527, which is of little relevance to humans (Hall et al. 2012, Pandiri et al. 2017). Thus, the NOAEL for both the 2- and 13-week rat toxicology studies was determined to be 1000 mg/kg/day. These changes were not noted in monkeys. With regards to hepatocellular hypertrophy, the cynomolgus monkey is considered a more predictive species to human compared to rodents (Hall et al. 2012).

Conclusions

- AT-527 exhibited low potential for QTc prolongation. The IC_{50} for hERG inhibition was > 100 μM. There was no AT-527-related effect observed in the rat FOB or NHP CV&R study.
- AT-527 and its metabolites were negative in a battery of in vitro and in vivo genetic toxicity assays.
- In repeat dose oral toxicity studies in rats or monkeys up to 13 weeks, no target organ of toxicity was identified. Dose- related reversible liver weight increases were noted in rats with correlating hepatocellular hypertrophy in the rat 13-week study. These changes were considered adaptive. The NOAEL was 1000 mg/kg/day in rats and 650 mg/kg/day in cynomolgus monkeys following up to 13 weeks of dose administration.

Reference

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2. *Pandiri A. R., Kerlin R. L., Mann P. C., et al. Is It Adverse, Nonadverse, Adaptive, or Artifact? Toxicol Pathol 2017;45(1):238-247.*
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