AT-527, an Oral Purine Nucleotide Prodrug Exhibiting Potent *In Vitro* Antiviral Activity Against Human Coronaviruses, Including SARS-CoV-2

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Introduction

Coronaviruses (CoVs) are the causative pathogens of several human diseases, including seasonal respiratory infections (HCoV-229E and HCoV-OC43), Middle East respiratory syndrome (MERS-CoV), severe acute respiratory syndrome (SARS-CoV) and the novel CoV recently identified as the virus responsible for the current COVID-19 pandemic, SARS-CoV-2. AT-527, currently in Phase 2 clinical trials, has demonstrated potent activity and a well-tolerated safety profile in HCV-infected subjects. Here we report the *in vitro* activity of AT-511, the free base form of AT-527, against SARS-CoV-2 and other CoVs. We also show that after dosing with the prodrug, AT-9010, the active triphosphate (TP) of AT-527, is formed in human airway epithelial (HAE) cells *in vitro*, and non-human primate (NHP) lung *in vivo*. Prediction of human tissue levels based on these data suggest that lung intracellular levels of AT-9010 will exceed the *in vitro* EC_{90} of AT-511 against SARS-CoV-2 throughout BID oral therapy at 550 mg AT-527.

Results

In Vitro Potency and Cytotoxicity of AT-511 and Other Oral Drugs Against Several Coronaviruses

Virus (genus)	Cell line	Compound	Cytopathic Effect Assay EC_{50} (μM)	Cytopathic Effect Assay CC_{50} (μM)[a]	Virus Yield Reduction Assay EC_{90} (μM)	Selectivity Index (CC_{50}/EC_{90})
HCoV-229E (alpha)	BHK-21	AT-511 sofosbuvir	1.8 ± 0.3 (2) >100	>100 >100	ND	>55[b] 0
	Huh-7	AT-511 chloroquine hydroxychloroquine	1.7 ± 0.1 (2) 7.8 6.0	>86 28 16	1.2 ± 0.1 (2) <0.050 <0.037	>72 3.6[b] 2.6[b]
HCoV-OC43 (beta)	Huh-7	AT-511	ND[c]	>86	0.5 / <0.03	>170 / >3100
	RD	AT-511	2.8	>86	2.2	>38
MERS-CoV (beta)	Huh-7	AT-511	26 ± 15	>86	37 ± 27	>2.3
SARS-CoV (beta)	Huh-7	AT-511	ND[c]	>86	0.3	>250
SARS-CoV-2 (beta)	HAE	AT-511 N^4-hydroxycytidine	ND[c]	>86[a] >19[d]	0.5 ± 0.1 (3) 3.9	>160 >4.9

The activity of AT-511 and other antiviral compounds was measured in cells infected with different coronaviruses to determine the effective concentration required to achieve 50% inhibition (EC_{50}) of the virus-induced cytopathic effect (CPE), the concentration to reduce virus yield by 1 log_{10} (EC_{90}) and the cytotoxic concentration of the drug to cause death to 50% of viable cells without virus (CC_{50}). Differentiated normal human airway epithelial (HAE) cells (EpiAirway™ AIR-100 or AIR-112) were prepared by MatTek Corporation (Ashland, MA) from a single donor. Values represent results from single or multiple [mean ±SD (n)] experiments.

[a]Highest concentration tested

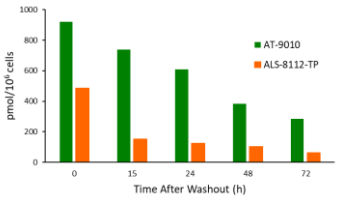
[b]CC_{50}/EC_{50}

[c]Not determined because there was no cytopathic effect with this virus in these cells

[d]Cytotoxicity assessed by visual inspection of cell monolayers

- **AT-511 exhibited a mean EC_{90} value of 0.5 μM against SAR-CoV-2 and similar potencies against HCoV-229E, HCoV-OC43 and SARS-CoV, with no cytotoxicity up to a concentration of 100 μM.**
- **N^4-hydroxycytidine, shown to inhibit SARS-CoV-2 replication (1), had an EC_{90} of 3.9 μM in the same HAE cell model.**

Triphosphate Concentrations in Primary Human Bronchial Epithelial (HBE) Cells Incubated with 10 μM AT-511 or ALS-8112



HBE cells from single donors (PromoCell GmbH) were incubated with 10 μM AT-511 or ALS-8112 for an 8 h incubation, followed by a 72-h washout. At various times post washout, media was removed, cells rinsed, collected and extracted in ice-cold 60% MeOH at -20°C with internal standards, and analyzed for concentrations of AT-9010 and ALS-8112 TP by LC-MS/MS. Untreated cells were collected as negative controls. Cell viability and density, measured after staining with acridine orange and propidium iodide, were determined before the addition of drug and at the end of incubation.

- **Substantial levels (~700 μM) of AT-9010, the active triphosphate of AT-511, were measured in HBE cells after an 8-h incubation.**
- **Two-fold more AT-9010 was formed from AT-511 compared to ALS-8112, a drug clinically effective against respiratory syncytial virus with an *in vitro* EC_{90} value of 1.3-2.7 μM in RSV-infected human airway epithelial (HAE) cells (2).**
- **72 h after drug washout, AT-9010 levels were almost 5-fold more than ALS-8112 TP levels due to the longer half-life of AT-9010 (39 h) as compared to the TP of ALS-8112 (6-8 h).**

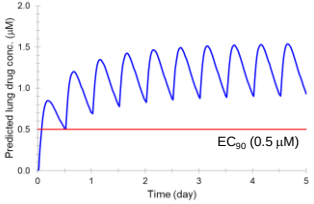
Actual (NHP) and predicted (human) tissue levels of AT-9010

Species	Intracellular AT-9010 concentrations at 12 h post dose (μM)		
	Liver	Kidney	Lung
NHP	0.09	0.13	0.14
Human	0.62	0.91	0.98

Male non-naïve cynomolgus monkeys, ≥ 2 years old and 2 kg BW, were dosed by oral gavage twice a day (BID) with AT-527 to achieve steady state levels: 60 mg/kg loading dose followed by 30 mg/kg every 12 h for 3 days. This regimen approximated allometrically to a human regimen of 1100 mg LD + 550 mg BID. Tissue samples were collected 12-h post last dose, homogenized on ice in a 70% MeOH solution with internal standards, and centrifuged. Aliquots of the supernatants were analyzed for AT-9010 concentrations by LC-MS/MS.

- **Predictions of human tissue levels were based on an observed ratio of 7:1 for AT-9010 concentrations in human versus NHP primary hepatocytes incubated with 10 μM AT-511 over 24 h (3).**

Predicted human lung AT-9010 concentrations for a clinical regimen with 550 mg BID X 5 days of AT-527 in patients with COVID-19



The kinetics of human lung AT-9010 levels were simulated for a 550 mg BID dose regimen for 5 days. Since plasma AT-273 serves as a surrogate marker of intracellular AT-9010, the simulation was performed using published plasma AT-273 data from subjects given daily 550 mg doses of AT-527 (4) and multiplied by 1.6 based on the observed NHP lung-to-liver AT-9010 concentration ratio (see Table) and assuming its applicability to humans. With BID dosing, the predicted AT-9010 steady-state lung peak and trough levels were 1.55 and 0.94 μM.

- **Simulated AT-9010 levels consistently exceeded the *in vitro* EC_{90} of AT-511 for inhibiting replication of SARS-CoV-2 in HAE cells.**
- **The predicted trough level of lung AT-9010 based on simulation was approximately 0.9 μM, which is in close agreement with the predicted trough of 0.98 μM (see Table) based on *in vivo* tissue distribution data in NHP.**

Conclusions

- **AT-511, the free base of AT-527, has potent antiviral activity against several CoVs, including SARS-CoV-2 (EC_{90} = 0.5 μM)**
- **Substantial formation of the active TP metabolite of AT-527 in HBE cells is consistent with the potency of the drug against SARS-CoV-2 replication in the HAE tissue model.**
- **Given that these cell types are actual targets of infection in COVID-19 patients, the potency of AT-527, combined with its favorable safety profile previously established in HCV-infected subjects, suggests that AT-527 may be highly efficacious in treating COVID-19.**

References

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