Plasma and tissue disposition of AT-527 and its active triphosphate metabolite in monkeys: implications for dose selection for patients with COVID-19

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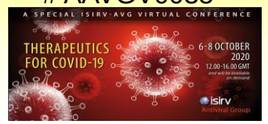


Background

AT-527 is a novel guanosine nucleotide prodrug with potent *in vitro* antiviral activity against HCV and human coronaviruses including SARS-CoV-2 (EC_{90}=0.5 μM), the virus responsible for COVID-19. The safety and efficacy of AT-527 demonstrated in clinical trial HCV subjects prompted clinical evaluation of the drug candidate in subjects with COVID-19.

Methods

Tissue distribution PK in non-human primates (NHP)

- **Animals**: Male non-naïve cynomolgus monkeys, ≥2 yrs old and 2 kg BW (Hainan Jingang Laboratory Animal Co., Ltd., Hainan, China), were group housed during the 5-day acclimation period or individually housed during the study, provided ad libitum access to RO water, fed twice daily.
- **Treatment**: AT-527 was dosed in a suspension via oral gavage to 12 monkeys according to the following BID regimen: 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.
- **Blood and tissue sampling**
  - Blood samples (~0.5 mL) were collected from 3 animals prior to and post the 5th dose, 0.5, 1, 2, 4, 6, 8 and 12 h, and at sacrifice (2 h after the last dose). Blood samples were collected from the rest of the animals, in groups of three, at 12, 24 and 48 h after the last dose, before they were sacrificed for tissues. Plasma was then obtained and prepared for LC/MS/MS analysis.
  - After the terminal blood collection, animals were anesthetized and duplicate samples (~1 g) of lung, liver and kidney tissue were collected from each animal at the same organ location, snap frozen in liquid nitrogen, and stored at -60°C. Portions of the frozen tissues were then homogenized and extracted for LC-MS/MS analysis.

***In vitro* formation of AT-9010 in human and monkey hepatocytes**

- Plated cryopreserved hepatocytes from humans (mixed gender, pool of 10 donors) and male cynomolgus monkey (Sekisui XenoTech, Kansas City, KS) were incubated with 10 μM AT-511. At predetermined time points (0, 2, 4, 8 and 24 h post the start of incubation), hepatocytes were harvested, rinsed and extracted for AT-9010 prior to LC-MS/MS analysis (1).

LC-MS/MS analysis

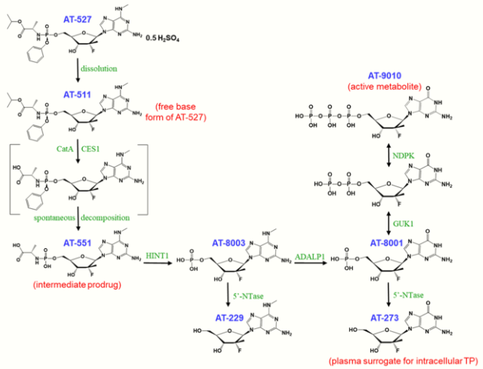
- Plasma concentrations of AT-511 and its L-alanyl metabolite AT-551, nucleoside metabolite AT-273, and tissue levels of the triphosphate active metabolite AT-9010 were analyzed using validated LC-MS/MS methodologies. The lower limits of quantitation were 6 ng/mL for AT-511, AT-551 and AT-273 in plasma and 12 ng/g for AT-9010 in tissue samples, respectively.

Data analysis.

- Plasma concentrations of AT-511, AT-551 and AT-273 were subjected to non-compartmental PK analysis using WinNonlin software (version 6.3 or above, Pharsight, Mountain View, CA).
- Clinical regimen simulation was performed based on steady-state plasma PK data obtained from HCV-infected patients treated with 553 mg/d AT-527 for 7 days (2), using MonolixSuite 2019 (Lixosoft, Antony, France).

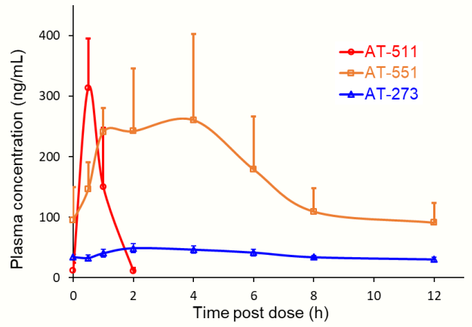
Results

Fig 1. Putative pathway for metabolism of AT-527 to its active triphosphate, AT-9010



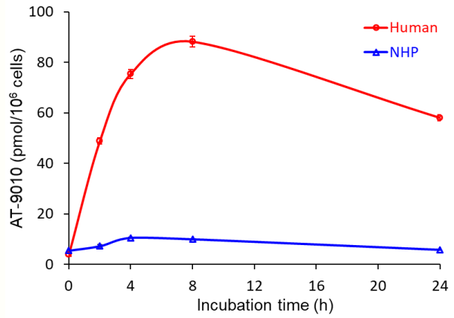
- The parent prodrug (AT-511) is converted to the L-alanyl intermediate AT-551 which further undergoes multistep activation to the intracellular triphosphate active metabolite AT-9010, the predominant phosphate.
- AT-273 can only be formed via dephosphorylation of its intracellular phosphates, and therefore plasma AT-273 serves as the surrogate marker for intracellular AT-9010.

Fig 2. Plasma profile of AT-511 and metabolites in NHP after oral dose of AT-527



- After oral administration, plasma AT-511 was rapidly converted to AT-551 with gradual and sustained AT-273.
- At 12 h post the last dose (steady-state trough level), AT-273 plasma concentration was 0.13 ± 0.04 μM

Fig 3. Formation of the active triphosphate metabolite AT-9010 in human and monkey hepatocytes



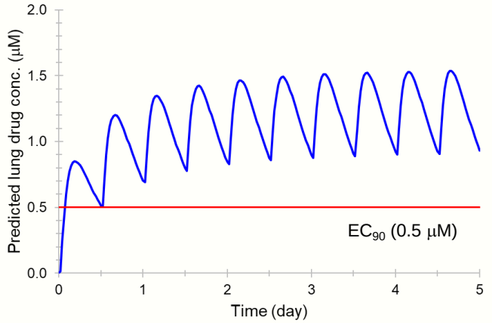
- **Primary human hepatocytes had 7-fold more AT-9010 than NHP liver cells**

Table 1. Actual (NHP) and predicted (human) tissue levels of AT-9010

Species	Intracellular AT-9010 conc. at 12 h post dose (μM)		
	Liver	Kidney	Lung
NHP	0.089	0.13	0.14
Human	0.62	0.91	0.98

- At 12 h post the last dose in NHP, AT-9010 concentrations in lung and kidney were similar and higher than liver.
- The half-life ($t_{1/2}$) of AT-9010 in lung (9.4 h) and kidney (8.0 h), approximately 2-fold longer than liver (4.3 h),
- Prediction of human tissue levels based on an observed ratio of 7 in human versus NHP concentrations of AT-9010 (Fig 3) as assessed by *in vitro* formation in primary hepatocytes suggest that lung intracellular levels of AT-9010 at trough (12 h) will exceed 0.5 μM, the *in vitro* EC_{90} of AT-511 against SARS-CoV-2 replication in human airway epithelial (HAE) cell cultures.
- Predicted AT-9010 levels in human kidney and liver also exceed the *in vitro* EC_{90} of the drug.

Fig 4. Predicted human lung drug concentrations for a clinical regimen with 550 mg BID X 5 days of AT-527 in patients with COVID-19



- Simulations were performed for various regimens including QD and BID without or with a loading dose.
- Results indicate that a simple 550 mg BID regimen can rapidly achieve and consistently maintain throughout therapy lung levels of the active triphosphate metabolite AT-9010 exceeding the *in vitro* EC_{90} of AT-511 in 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 (Fig 4), which is in close agreement with the predicted trough of 0.98 μM (Table 1) based on *in vivo* tissue distribution data in NHP.

Conclusions

- The predicted human trough levels of the active triphosphate metabolite AT-9010 for AT-527 550 mg BID, based on NHP tissue distribution data, exceed the 0.5 μM threshold not only in the lung, the primary organ targeted by SARS-CoV-2, but also kidney and liver.
- Simulation results also supported the selection of a simple 550 mg BID regimen with predicted lung AT-9010 levels closely matching NHP results.
- AT-527 550 mg BID is being evaluated in subjects with moderate COVID-19 in a phase 2 clinical trial (NCT04396106).

References

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Acknowledgements

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