#549 Pharmacokinetics and Metabolism of [^{14}C]-Bemnifosbuvir in Healthy Male Participants

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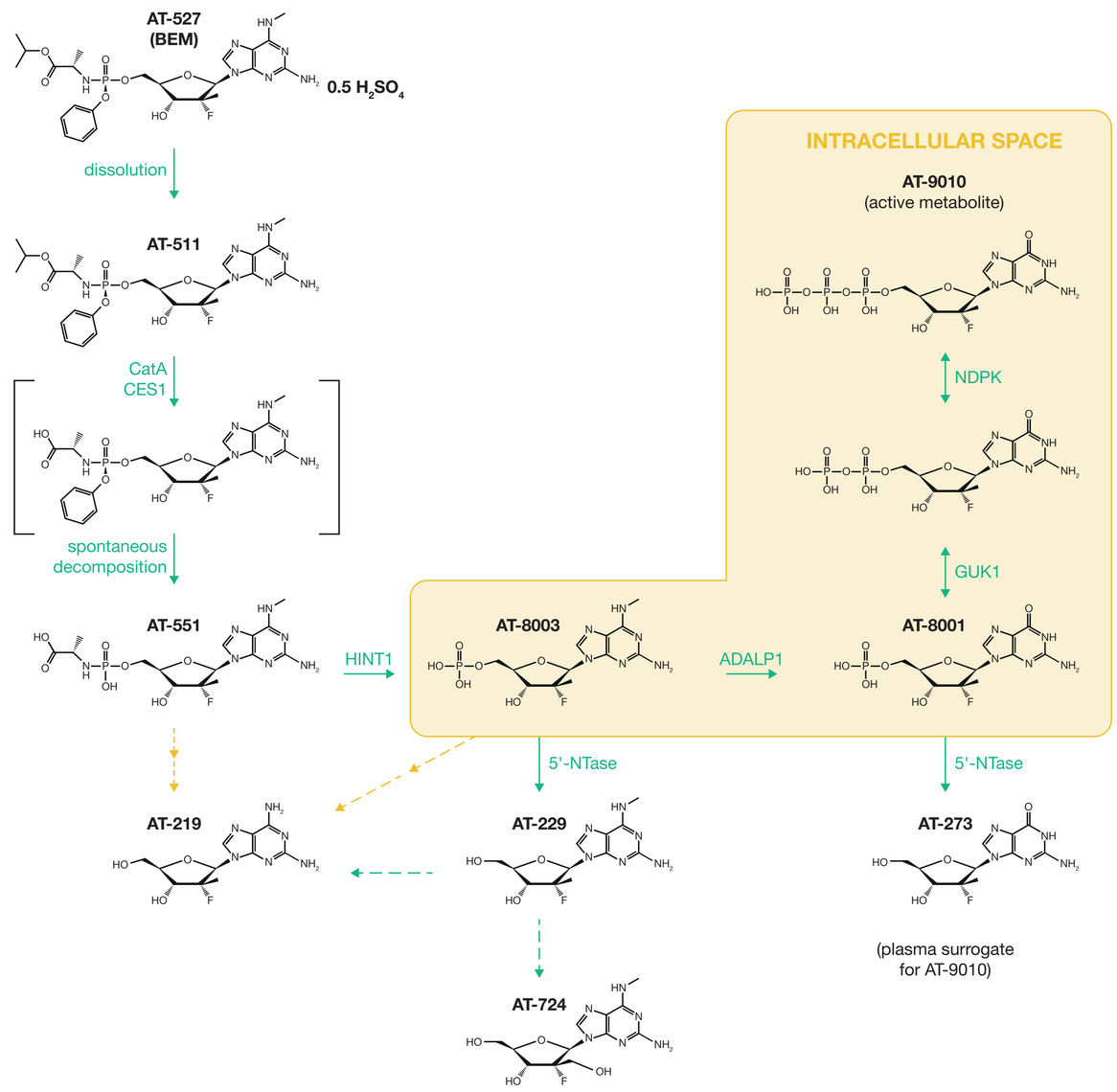
BACKGROUND

- Bemnifosbuvir (BEM, AT-527) is a guanosine nucleotide oral prodrug candidate[1,2] that is being evaluated in the SUNRISE-3 Phase 3 trial for COVID-19 and is in Phase 2 development in combination with ruzasvir for chronic hepatitis C virus (HCV) infection[3,4]
- BEM is rapidly absorbed after oral administration in humans[5] and sequentially metabolized to AT-9010, its active triphosphate (TP)[1,2] **(Figure 1)**
- BEM hemisulfate readily dissolves and releases AT-511, which undergoes multistep metabolic activation **(Figure 1)**:[1,2]
  - AT-511 is hydrolyzed by CatA/CES1, followed by spontaneous decomposition of the phenoxy group to the L-alanyl intermediate AT-551[1,2]
  - AT-551 is further hydrolyzed by HINT1 to AT-8003, the monophosphate (MP) nucleotide with the N^6-methyl modification[1,2]
  - AT-8003 is converted by ADALP1 to AT-8001, the MP nucleotide with the natural guanosine base[1,2,6]
  - AT-8001 is further phosphorylated to AT-9010, the pharmacologically active TP metabolite, which potently inhibits the replication of coronaviruses and flaviviruses, including SARS-CoV-2 and HCV[1,2,7]
- AT-8003, AT-8001, and AT-9010 are formed intracellularly and are not measurable in plasma[2]
- AT-229 and AT-273 are major circulating nucleoside metabolites generated by dephosphorylation.[1,2] Plasma AT-273 is considered a surrogate for AT-9010; AT-219 and AT-724 are minor circulating nucleoside metabolites[1,2,6]
- A Phase 1, open-label, single-dose, mass balance study was conducted to characterize the absorption, metabolism, and elimination profiles of BEM in healthy male participants[8]

METHODS

- Six healthy male participants aged 19–55 years were enrolled and received a single oral dose of BEM 550 mg containing 100 µCi [^{14}C]-BEM on Day 1
- Subjects were discharged from the Clinical Research Unit on Day 8, following blood draw and/or study procedures, if at least one discharge criterion was met:
  - ≥90% of the total dose of radioactivity administered had been recovered in urine and feces
  - There was ≤1% of the total administered radioactivity in each of two consecutive 24-hour intervals where both urine and fecal samples were provided
- Serial whole blood, plasma, urine, and fecal samples were collected pre dose and for up to 216 hours (9 days) post dose
- Concentrations of BEM and major circulating metabolites were quantitated by radioactivity counting and/or by validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) methodologies; samples were subjected to further metabolic identification
- Safety assessments included adverse events, standard clinical laboratory tests, vital sign measurements, electrocardiograms (ECGs), and physical examinations

Figure 1. Metabolic and activation pathway of BEM[1,2,4]



5′-NTase, 5′-nucleotidase; ADALP1, adenosine deaminase-like protein 1; CatA, cathepsin A; CES1, carboxylesterase 1; GUK1, guanylate kinase 1; HINT1, histidine nucleotide triad 1; NDPK, nucleoside diphosphate kinase.

RESULTS

Demographics

- Subjects were mostly White (50%) with a mean age of 37.8 years and a mean body mass index (BMI) of 26.9 kg/m^2 **(Table 1)**

Safety/tolerability

- A single dose of BEM 550 mg was well tolerated
- A total of two treatment-emergent adverse events (TEAEs) were reported by one participant (17%)
  - Both TEAEs (dyspepsia and neck pain) were of mild severity and considered unrelated to the study drug
- There were no clinically significant vital sign, ECG, laboratory test or physical examination findings

Pharmacokinetic evaluation

- Recovery of radioactivity was near complete, with about 64% and 25% recovered from urine and feces after 9 days, respectively, totaling close to 90% of the administered dose. Fraction absorbed was estimated to be approximately 65% **(Figure 2)**
- Total blood and plasma radioactivity over time curves virtually overlapped, with blood-to-plasma ratios of approximately 1, indicative of no red blood cell (RBC) accumulation **(Figure 3)**
- Plasma, urine, and fecal samples were subject to metabolite identification, confirming formation of the L-alanyl intermediate AT-551, the two nucleoside metabolites AT-229 and AT-273, as well as two minor nucleoside metabolites AT-219 and AT-724
  - In plasma, AT-229 was the most abundant metabolite (41.4% of total radioactivity), followed by AT-511 (16.7%) and AT-273 (15.5%)
  - In urine and feces, AT-229 was the most abundant metabolite (26.4% and 10.4% of total radioactivity, respectively), followed by AT-273 (19.3% and 7.8%, respectively)
- Upon oral administration, BEM was rapidly absorbed and extensively metabolized to AT-551 (peak plasma concentrations attained approximately 45 mins post dose), whereas AT-273 exhibited a sustained plasma profile with a mean half-life of 19.7 hours **(Table 2)**
  - Similar to metabolite identification data using radioactivity, AT-229 was the most abundant metabolite in plasma based on AUC using LC/MS-MS
- Both AT-229 and AT-273 were predominantly eliminated via the kidneys, with estimated renal clearance exceeding glomerular filtration rate

Table 1. Demographic characteristics

Characteristic	Total population [N=6]
Mean age, years (SD, min–max)	37.8 (8.4, 24–48)
Race, n (%)	
Black or African American	2 (33)
Multiple	1 (17)
White	3 (50)
Mean weight, kg (SD, min–max)	81.9 (8.1, 69.9–92.9)
Mean BMI, kg/m^2 (SD, min–max)	26.9 (1.4, 25.0–28.9)
Mean GFR, mL/min/1.73 m^2 (SD, min–max)	100.2 (32.8, 75–163)

BMI, body mass index; GFR, glomerular filtration rate; max, maximum; min, minimum; SD, standard deviation.

Table 2. Plasma and urine pharmacokinetics of BEM and metabolites following a single oral dose of 550 mg BEM containing 100 µCi [^{14}C]-BEM

Analyte	Plasma						Urine	
	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\infty}$ (ng/mL×h)	CL/F (L/h)	Vz/F (L)	$t_{1/2}$ (h)	Cumulative dose recovered (%)	CLr (mL/min)
AT-511	5333 ± 1263.3	0.4 [0.2–0.5]	3446 ± 1238.4	188.5 ± 99.8	260.4 ± 91.5	1.0 ± 0.3	2.4 ± 1.3	63.4 ± 11.6
AT-551	989.2 ± 356.0	0.8 [0.5–0.8]	2429 ± 723.9	NA	NA	2.0 ± 0.3	1.1 ± 0.5	37.3 ± 9.4
AT-229	790.3 ± 223.5	1.3 [1.0–3.0]	5842 ± 1881.8	NA	NA	14.7 ± 8.6	22.7 ± 5.9	200.0 ± 45.0
AT-273	206.2 ± 47.0	4.0 [3.0–6.0]	3099 ± 469.8	NA	NA	19.7 ± 7.9	19.1 ± 3.6	304.2 ± 86.0

Data are presented as mean ± SD. T_{max} is presented as median [minimum–maximum]. N=6 throughout.
$AUC_{0-\infty}$, area under the curve from time zero extrapolated to infinity; CL/F, apparent total plasma clearance; CLr, renal clearance; C_{max}, maximum concentration; NA, not applicable; SD, standard deviation; $t_{1/2}$, terminal elimination half-life; T_{max}, time to reach C_{max}; Vz/F, apparent volume of distribution during the terminal elimination phase.

Figure 2. Mean cumulative percentage of dose recovered as total radioactivity in urine, feces, and urine + feces vs time following a single oral dose of 550 mg BEM containing 100 µCi [^{14}C]-BEM

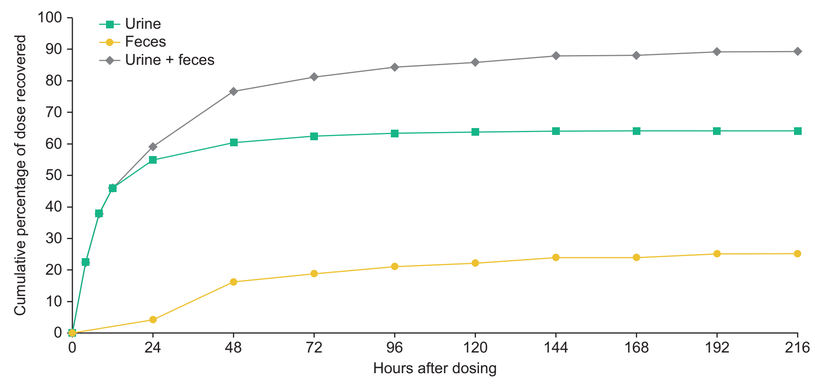
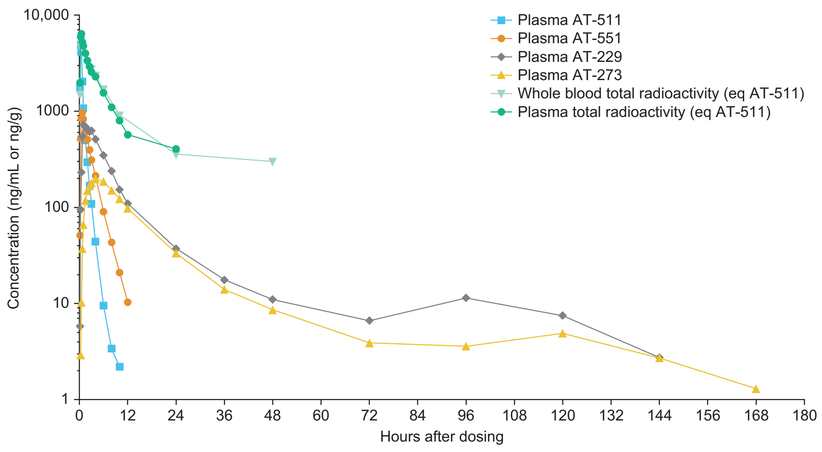


Figure 3. Mean whole blood and/or plasma concentrations vs time profiles of AT-511 and metabolites following a single dose of 550 mg BEM containing 100 µCi [^{14}C]-BEM



Plasma AT-511, AT-551, AT-229, AT-273, and plasma total radioactivity are expressed in ng/mL; whole blood total radioactivity is displayed in ng/g. For the purposes of this figure, 1 g whole blood was considered equivalent to 1 mL whole blood.
Eq, equivalent

CONCLUSIONS

- BEM was well absorbed (fraction absorbed ~65%), with the administered dose nearly completely recovered in urine and feces
- BEM/metabolites did not accumulate in RBCs, with similar plasma and whole blood exposure
- BEM was extensively metabolized to AT-551, which underwent additional metabolism/activation, entering general circulation mostly as nucleoside metabolites AT-229 and AT-273
- Plasma AT-273, considered a surrogate of BEM intracellular phosphates, exhibited a long half-life, supporting once- and twice-daily dosing

References

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Disclosures

Xiao-Jian Zhou, Maureen Montrond, Keith Pietropaolo, Bruce Belanger, Arantxa Horga, and Janet Hammond are employees of and may own stock in Atea Pharmaceuticals, Boston, MA, USA.

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