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Pharmacokinetics-Pharmacodynamics of ANT3310, in Combination with Meropenem, Against Carbapenem-Resistant *Acinetobacter baumannii* Using a Neutropenic Murine-Pneumonia Model

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INTRODUCTION

- ANT3310 is a broad-spectrum beta-lactamase inhibitor, which when combined with meropenem, restores activity of the beta-lactam against carbapenem-resistant *Acinetobacter baumannii* (CRAB).
- Previously-completed *in vitro* pharmacokinetics-pharmacodynamics (PK-PD) studies identified ANT3310 free-drug area under the concentration-time curve over 24 hours (AUC) as the exposure measure most predictive of ANT3310 activity [1].
- The studies herein evaluate the magnitude of ANT3310 AUC, in combination with meropenem, associated with restoring meropenem activity against CRAB isolates using a neutropenic murine-pneumonia model.

METHODS

Antimicrobial Agent and Challenge Isolate

- A panel of eight *A. baumannii* isolates (seven CRAB and one meropenem-sensitive *A. baumannii*) were selected based upon their known resistance mechanisms, and meropenem MIC values to be evaluated within the murine model [**Table 1**].
- The challenge isolates were provided by the Centers for Disease Control (CDC) and prevention antimicrobial resistant (AR) bank or were provided by the Sponsor.
- ANT3310 (lot# PRPL2021-019) was provided by the Sponsor (Antabio SAS, Labège, France) and meropenem was purchased from Henry Schein Medical (Melville, NY).

Susceptibility Studies

- The susceptibility of the challenge isolate panel to meropenem was determined alone and in combination with ANT3310 at a fixed concentration of 8 mg/L for each isolate in the challenge panel using broth micro-dilution methodologies recommended by the Clinical and Laboratory Standards Institute (CLSI) [2, 3].

Neutropenic Murine Lung Infection Model

- All animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals [4] and all animal procedures described herein were completed following ICPD protocols approved by an Institutional Animal Care and Use Committee.
- Neutropenia was induced in female CD-1 mice using two intraperitoneal cyclophosphamide injections (150 mg/kg on day -4 and 100 mg/kg on day -1) and mice were infected via intra-nasal inoculation with 25 µL of a bacterial suspension to achieve a final bacterial burden of 10^7 CFU/lung of each challenge isolate.

METHODS

Meropenem and ANT3310 Single-Dose Pharmacokinetic Studies

- Single-dose pharmacokinetic studies were completed in neutropenic mice infected with a meropenem-sensitive *A. baumannii* isolate (AR Bank # 0280).
- Two hours post inoculation, meropenem:ANT3310 dosing regimens of 3.75:0.06, 15:6.0, 30:60, and 75:200 mg/kg were administered via subcutaneous (SC) injection.
- Three mice from each dosing group were sacrificed via CO_2 inhalation at time points of 0.083, 0.25, 0.5, 1, 2, 4, or 6 hours post treatment initiation, at which point blood and epithelial lining fluid (ELF) samples were collected via cardiac puncture and bronchoalveolar lavage (BAL) and stored at -80°C until assayed via qualified liquid-chromatography tandem mass-spectrometry method (LC-MS/MS).
- Plasma and BAL urea concentrations were determined using the Bioassay Systems QuantiChrom Urea Assay, and corrected concentrations of meropenem and ANT3310 in ELF samples were calculated for each mouse using the urea dilution correction factor formula:

$$[Drug]_{ELF} = \frac{[Urea]_{Plasma}}{[Urea]_{BAL}} \times [Drug]_{BAL}$$

Meropenem/ANT3310 Population PK models

- The plasma and ELF PK of meropenem and ANT3310 in CD-1 mice was described using separate population PK models developed using the data obtained from the single-dose PK studies, in R statistical software version 4.0.4 using the nlmixr2 package, version 2.0.9 [5, 6].
- The population mean PK parameters from the ANT3310 population PK model and a murine protein binding percentage of 0% for ANT3310 [7], and 10% for meropenem [8] were used to estimate free-drug plasma AUC values.

Meropenem/ANT3310 Dose-Ranging Studies

- A series of 24-hour dose ranging studies were completed with the goal of identifying the ANT3310 exposure required to restore the activity of a meropenem regimen against the panel of seven CRAB isolates identified in **Table 1**.
- Two hours post inoculation, groups of five mice (n=5), were sacrificed at the initiation of treatment (0h) and 24 hours post treatment initiation for a meropenem 80 mg/kg q4h dose, alone and in combination with ANT3310 doses (0.6 to 100 mg/kg) given every 4 hours, compared to a no treatment control.
- Lung tissues were removed from the sacrificed animals, pooled and homogenized in sterile normal saline, and then serially diluted onto Mueller-Hinton agar plates for enumeration of bacterial burden per gram of lung tissue.

Pharmacokinetic-Pharmacodynamic Analyses

- Using data from the dose-ranging studies and population PK analysis, the relationship between change in log_{10} CFU/g from baseline at 24 hours and free-drug plasma and total-drug ELF AUC were evaluated using Hill-type models.
- ANT3310 free-drug plasma and total-drug ELF AUC targets associated with achieving net bacterial stasis and 1- and 2-log_{10} CFU/g reductions from baseline at 24 hours were determined.

RESULTS

Susceptibility Studies

- The meropenem MIC values determined with and without the presence of 8 mg/L of ANT3310, and known resistance mechanisms for the panel of CRAB isolates evaluated in the neutropenic murine lung infection model are displayed in **Table 1.**
  - Meropenem MIC values for the *A. baumannii* isolates used in the neutropenic murine lung infection model ranged from 32 to 64 mg/L and were attenuated to values of 0.125 to 8 mg/L, when determined in the presence of ANT3310.

Pharmacokinetic-Pharmacodynamic Analyses of ANT3310 Dose-Ranging Studies for Efficacy

- The Hill-type models describing the relationship between the change in bacterial burden in lung homogenate and the ANT3310 free-drug plasma AUC are presented in **Figure 1**, while the corresponding models based on total-drug ELF AUC were evaluated separately (data not shown).
  - The ANT3310 free-drug plasma and total-drug ELF PK-PD targets associated with efficacy for each CRAB isolate are summarized in **Table 2**.
- The median free-drug plasma AUC targets associated with net bacterial stasis and a 1-log_{10} CFU/g reduction were 42.9 and 90.5 mg•h/L, respectively.
- These data were consistent with free-drug AUC targets previously determined to be associated with a 1-log_{10} CFU/mL reduction from baseline in combination with meropenem based on the above-described *in vitro* PK-PD data [1].

Table 1. Modal broth microdilution MIC values (mg/L) determined for the internal control and challenge isolates evaluated in the neutropenic murine lung infection model

A. baumannii isolate	Known resistance mechanism	Broth microdilution MIC (mg/L)	
		Meropenem + ANT3310 at 8 mg/L	Meropenem
AR Bank # 0294	TEM-1B, OXA-23, OXA-65	2	64
AR Bank # 0078	ADC-25, SHV-5, OXA-71	1	64
AR Bank # 0280[a]	ADC-30, OXA-66, TEM-1D	ND	2
ARC 3659	AmpC, OXA-23, PSE-2 (OXA-10)/OXA-69	0.25	32
1729049	GES-20, OXA-23, TEM-OSBL	4	64
1860491	OXA-23	8	64
1729377	OXA-23, TEM-OSBL	4	64
1633238	OXA-23	2	64
NCTC 13438[b]	KPC-3	0.125	64

ND = Not determined.
a. Isolate used in the single-dose pharmacokinetic studies only.
b. Quality control isolate for susceptibility studies only.

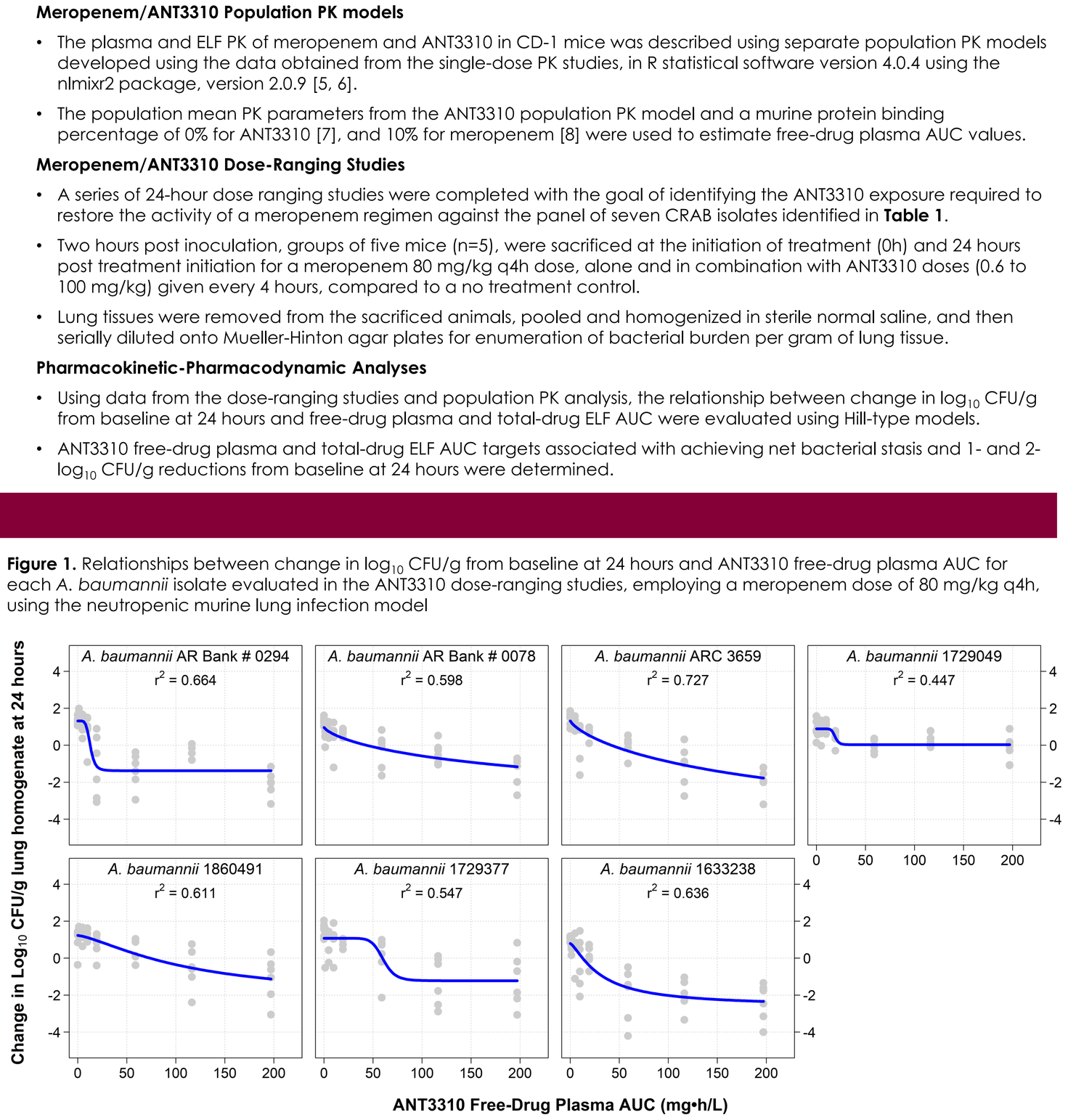

Figure 1. Relationships between change in log_{10} CFU/g from baseline at 24 hours and ANT3310 free-drug plasma AUC for each *A. baumannii* isolate evaluated in the ANT3310 dose-ranging studies, employing a meropenem dose of 80 mg/kg q4h, using the neutropenic murine lung infection model

Table 2. ANT3310 free-drug plasma and total-drug ELF AUC targets associated with net bacterial stasis, and 1- and 2-log_{10} CFU/g reductions from baseline, when evaluated in combination with a meropenem 80 mg/kg q4h dose, using data from the neutropenic murine lung infection model dose-ranging studies

CRAB isolate	Free-drug plasma AUC target (mg•h/L)			Total-drug ELF AUC target (mg•h/L)		
	Net bacterial stasis	1-log_{10} CFU/g reduction	2-log_{10} CFU/g reduction	Net bacterial stasis	1-log_{10} CFU/g reduction	2-log_{10} CFU/g reduction
AR Bank # 0294	12.6	17.6	NA	9.48	13.5	NA
AR Bank # 0078	42.3	166	376	31.8	124	280
ARC 3659	43.5	115	215	33.0	86.2	160
1729049	NA	NA	NA	NA	NA	NA
1860491	73.7	175	829	55.6	131	529
1729377	59.2	65.9	NA	44.2	49.3	NA
1633238	12.6	33.5	97.4	9.51	25.3	73.4
Median	42.9	90.5	296	32.4	67.8	220

NA = AUC target could not be estimated.

CONCLUSIONS

- These data provide a PK-PD basis for evaluating candidate ANT3310 dosing regimens, used in combination with meropenem, for the treatment of infections caused by CRAB isolates.
- The median ANT3310 free-drug plasma AUC targets associated with net bacterial stasis, and 1- and 2-log_{10} CFU/g reductions from baseline, in combination with meropenem, were 42.9, 90.5, and 296 mg•h/L, respectively for *A. baumannii*.

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