Intrapulmonary Pharmacokinetics of Relebactam, a Novel β-lactamase Inhibitor, Dosed in Combination with Imipenem/Cilastatin in Healthy Subjects

Running Title: Relebactam with Imipenem/Cilastatin, Intrapulmonary PK

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Abstract

This Phase I study assessed the intrapulmonary pharmacokinetic profiles of relebactam (MK-7655), a novel β-lactamase inhibitor, and imipenem. Sixteen healthy subjects received 250 mg relebactam with 500 mg imipenem/cilastatin, given IV every 6 hours for 5 doses, and were randomized to bronchoscopy/broncho-alveolar lavage at 0.5, 1, 1.5, or 3 hours after the last dose (4 subjects per time point). Both drugs penetrated the epithelial lining fluid (ELF) to a similar degree, with profiles similar in shape to the corresponding plasma profiles and apparent terminal half-lives in plasma and ELF, respectively, of 1.2 and 1.3 hours for relebactam, and 1.0 hour in both compartments for imipenem. The relative exposure (AUC\textsubscript{0-inf}) in ELF:plasma was 54% for relebactam and 55% for imipenem, after adjusting for protein binding. ELF penetration for relebactam was further analyzed by fitting the data to a two-compartment pharmacokinetic model to capture behavior in the plasma, with a partitioning coefficient capturing behavior in the lung compartment. In this model, the time-invariant partition coefficient for relebactam was found to be 52% based on free drug levels. These results support further clinical evaluation of relebactam with imipenem/cilastatin for the treatment of bacterial pneumonia.
Introduction

Relebactam is a dual Class A and Class C β-lactamase inhibitor that can restore the *in vitro* activity of imipenem against many carbapenem-nonsusceptible isolates of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and Enterobacter spp. (1-4). The pharmacokinetic parameter best correlated with relebactam efficacy is the AUC, with exposures (AUC₀⁻²⁴hr) of ~100 µM*hr required in a thigh infection model (5) and 150 µM*hr in a pulmonary infection model (6). The increased exposures required in the pulmonary infection model are likely partially due to the penetration of relebactam into the murine lung, which is approximately 34% based on the ratio of total drug levels in the lung versus plasma (data on file). Relebactam doses of 125 mg or higher exceed the single-dose AUC₀⁻∞ target of 37.5 µM*hr, which is based on four-times daily dosing and derived from the target of 150 µM*hr established in the pulmonary infection model (6). The pharmacokinetic half-life of relebactam is compatible with four-times daily dosing with imipenem/cilastatin (7-9), and co-administration of relebactam with imipenem/cilastatin has been generally well tolerated in Phase 2 clinical studies (10, 11).

Imipenem is an ideal partner for beta-lactamase inhibitors in Pseudomonad from a resistance perspective. Imipenem is a potent carbapenem antibiotic that is relatively stable to the AmpC class C cephalosporinase of *P. aeruginosa*, requiring concomitant loss of the entry porin OprD along with hyperproduction of AmpC before resistance is achieved (12, 13). Unlike the beta-methyl carbapenems, imipenem is not subject to efflux by any of the resistance-nodulation-cell division (RND)-type efflux pumps of Pseudomonas including mexAB/OprM, mexCD/OprJ, mexEF/OprN, and mexXY/OprM(14, 15). Therefore, inhibition of the chromosomal enzyme by a β-lactamase inhibitor will restore susceptibility to many multidrug resistant isolates of *P. aeruginosa*, including those with over-expression of efflux pumps, but will not restore susceptibility in isolates where a β-lactamase not inhibited by relebactam is present, such as class B metallo-β-lactamases(16).
The penetration of antibiotics into tissues and fluids at the specific site of infection is a potentially valuable indicator for predicting a clinical response (17). For bacterial pneumonia, the distal bronchial lumen and alveolar surface are considered the sites of bacterial invasion (18, 19). Antibiotic concentrations in epithelial lining fluid (ELF) remain the most critical parameter for activity against extracellular pathogens, including most gram-negative bacteria. The recovery of ELF by fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) is a safe, well-tolerated procedure that has become widely used and accepted to study pulmonary drug penetration (20).

In this study, we assessed the pharmacokinetic profiles of relebactam and imipenem in the pulmonary ELF and alveolar cells (AC) obtained from BAL specimens. Relebactam lung penetration was further analyzed by fitting the data to a two-compartment pharmacokinetic model to capture behavior in the plasma, with a partitioning coefficient capturing behavior in the lung compartment.

**Materials and Methods**

This open-label, randomized, parallel-group study (MK-7655 Protocol 007) was conducted from 24-Apr-2012 through 25-Jun-2012 at a single site (Pulmonary Associates PA, Phoenix, AZ) and in conformance with principles of Good Clinical Practice, as well as all applicable statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research. The protocol was approved by Quorum Review IRB, and all subjects gave written informed consent before any study procedures were performed. The primary objective was to determine the relationship between the intrapulmonary pharmacokinetics of relebactam and those of imipenem after four-times daily intravenous administration of relebactam with imipenem/cilastatin in healthy subjects. The primary study hypothesis was that the relebactam concentration in ELF would be >25% of the imipenem concentration in ELF at the T_{max} of imipenem (0.5 hr).
Eligible subjects were healthy men and women 18 to 45 years of age with a body mass index \( \leq 32 \text{ kg/m}^2 \), creatinine clearance \( \geq 80 \text{ mL/min} \), no clinically significant disease, and no history of significant multiple or severe allergies, including allergies to beta-lactam antibiotics and lidocaine or other local anesthetics. Subjects received 5 doses of study drug (relebactam 250 mg in combination with imipenem/cilastatin 500 mg) by intravenous infusion over 30 minutes, one dose every 6 hours, and subsequently underwent bronchoscopy and BAL at either 0.5, 1.0, 1.5, or 3.0 hours after the last dose of study drug. Dosing and administration of imipenem/cilastatin was carried out consistent with the recommended labeled posology.

For each subject, the timing of bronchoscopy/BAL was determined by random assignment to Panel A, B, C, or D (4 subjects per panel). An optional fifth panel (Panel E) was included to allow for the collection of information at a different dose or time point (such as at the end of the dosing interval at 6 hours) contingent upon the analysis of the intrapulmonary and plasma PK data from Panels A-D. Based on the data obtained in Panels A-D, it was decided not to conduct Panel E as the data from Panels A-D were deemed adequate by visual inspection to characterize the intrapulmonary profiles of imipenem and relebactam.

BAL specimens were obtained during bronchoscopy for the determination of relebactam and imipenem concentrations in the ELF and AC. Four aliquots of normal saline (50 mL each) were sequentially instilled and aspirated after each instillation. The first aspirate was discarded, and the remainder were pooled and measured. Aliquots were obtained for cell count/differential, and the remainder was centrifuged. The liquid aspirate and cell pellet were separated and stored at \(-70^\circ \text{C}\). The supernatant was sent for urea and drug level measurements. Blood samples were collected prior to the first and fifth dose of study drug for the determination of relebactam and imipenem plasma concentrations. Blood samples were also collected during the bronchoscopy/BAL procedure for the determination of relebactam, imipenem, and urea concentrations.
The safety and tolerability of relebactam was monitored by clinical assessment of adverse events, measurement of vital signs, physical examination, 12-lead electrocardiogram (ECG), and standard laboratory safety tests (hematology, chemistry, and urinalysis). Renal function (serum/urine creatinine, serum urea, urine protein, and urine albumin) and hepatic function (serum bilirubin, ALT, AST) were carefully monitored during the study. The safety of bronchoscopy and BAL was monitored by clinical assessment including continuous cardiac monitoring and repeated measurements of vital signs, according to the standard operating procedures at the study site.

**Analytical and Pharmacokinetic Methods**

Relebactam and imipenem levels in plasma, pulmonary ELF, and AC were measured simultaneously via acetonitrile protein precipitation and were chromatographed using hydrophilic interaction (HILIC) chromatography and detected via tandem mass spectrometry (LC-MS/MS). The system consisted of a Waters Acquity UPLC (Waters Corp., Milford, MA), and an API 4000 or 5000 triple quadrupole tandem mass spectrometer (Sciex, Framingham, MA) equipped with a turbo-ion spray interface and operated in the positive ionization mode. The Multiple Reaction Monitoring (MRM) transitions monitored were m/z 349→269 for relebactam; m/z 300→98 for imipenem; m/z 354→274 and m/z 307→98 for their respective internal standards. The chromatographic separation of the analytes was achieved using a Waters Atlantis HILIC (50 x 2.1mm x 3 μm) column kept at 35°C and mobile phase consisting of 5 mM ammonium acetate (pH 4.5) in 80/20 acetonitrile/water. The flow rates and run times for the plasma, ELF and AC assays were 0.45 mL/min, 3.0 min and 0.4 mL/min, 4.0 min, respectively.

ELF volumes recovered by BAL were determined by using urea as an endogenous marker to provide a dilution ratio by measurement of urea concentrations in the BAL and serum (21). Concentrations in AC were determined by estimation of intracellular volume of macrophages (i.e., 2.42 mL per 10⁶ cells), based on cell count/differential. The mean value for
each time point was used to conduct the non-compartmental analysis using Phoenix® 6.3 (Pharsight Corporation, Mountain View, CA) and to calculate PK parameters based on the mean profile in each matrix.

Population PK analysis was conducted using NONMEM version 7.3 (ICON plc., Dublin, Ireland). The first order conditional estimation with interaction (FOCEI) method was applied for parameter estimation. Previous population PK analysis showed that the plasma concentration-time profile of relebactam can be sufficiently described by a two-compartment model with linear PK (10). To elucidate the relationship between plasma and ELF concentrations, penetration into the ELF was explored using three approaches, each using a naïve-pooled data approach:

1. a three-compartment model with bidirectional mass transfer between the ELF compartment and the central volume, (2) a time-invariant partition coefficient driven by the predicted unbound plasma concentration, and (3) an effect compartment with an input rate constant driven by the concentration difference between the central volume and effect compartment.

The equations comprising the partition coefficient model are as follows:

\[
\frac{dA_1}{dt} = \left(\frac{Q_2}{V_1}\right)A_2 - \left(\frac{Q_2}{V_1}\right)A_1 - \left(\frac{C_L}{V_1}\right)A_1 \quad (\text{Eq.1})
\]

\[
\frac{dA_2}{dt} = \left(\frac{Q_2}{V_2}\right)A_1 - \left(\frac{Q_2}{V_2}\right)A_2 \quad (\text{Eq.2})
\]

\[
C_{\text{ELF}} = k_{\text{ELF}} \cdot f_{u,\text{REL}} \cdot \frac{A_1}{V_1} \quad (\text{Eq.3})
\]

Where:

\(A_1\): amount of drug in central compartment
\(A_2\): amount of drug in peripheral compartment
\(CL\): plasma clearance
\(Q_2\): inter-compartmental clearance
\(V_1\): central volume
\(V_2\): peripheral volume
\(C_{\text{ELF}}\): relebactam concentration in the ELF
\(f_{u,\text{ELF}}\): free fraction of relebactam in plasma (~80%)
\(k_{\text{ELF}}\): partition coefficient into ELF space
Free fraction in the ELF was assumed to be 100%. Different residual error models (additive, proportional, and combined) were tested for both plasma and ELF concentration of relebactam. Model development, including the selection of the structural and residual error model, was based upon success of minimization, numerical comparison of the objective function value, precision of parameter estimates, and the generation of standard model diagnostic plots. The PK of imipenem was not modeled, as the lung penetration data from this study was previously described using a population PK approach (22).

**Statistical Analysis**

All data were analyzed according to the treatment actually received. Safety and tolerability were assessed in the All Subjects as Treated (AST) population, defined as all subjects who received at least one dose of study drug. Pharmacokinetic parameters were analyzed in the Per-Protocol (PP) population, defined as subjects who complied sufficiently with the protocol to ensure that the data would likely exhibit the effects of treatment, according to the underlying scientific model.

The concentrations of relebactam and imipenem in pulmonary ELF were log-transformed and analyzed using a linear mixed-effects model containing compound (relebactam and imipenem), time (30 min, 1 hr, 1.5 hr, and 3 hr after the last dose), and compound by time interaction as fixed effects, and subject as a random effect. Point estimates and 90% confidence intervals (CI) were calculated for the geometric mean ratio (GMR) of concentration (relebactam/imipenem) at the time to maximum concentration ($T_{\text{max}}$) of imipenem based on the mean concentration time profile. The log-trapezoidal rule was used to compute the $\text{AUC}_{0-3\text{hr}}$ of relebactam and imipenem in the ELF, AC, and plasma for the mean concentration-time profile.
Results

Seventeen subjects (14 male, 3 female; age range 24-42 years) entered the study. Sixteen subjects completed the study; one subject discontinued early due to an adverse event (see below).

Following 5 consecutive doses of relebactam 250 mg in combination with imipenem/cilastatin 500 mg given every 6 hours, relebactam levels in ELF and AC were consistently lower than relebactam levels in plasma (Figure 1). Penetration of relebactam into the extracellular space was approximately one-third to one-half of the corresponding plasma level, with GMRs for ELF/plasma concentrations ranging from 0.32 to 0.51 across time points (Table 1). The relative exposure (AUC$_{0\text{-}\text{inf}}$) of relebactam in ELF vs plasma was 54% based on the mean profiles (Table 2), after adjustment for protein binding (relebactam is 80% unbound in plasma; free fraction of 100% assumed for the ELF). The $T_{\text{max}}$ and terminal half-life values for relebactam in ELF were similar to those in plasma, with $T_{\text{max}}$ occurring at 0.5 hr in both matrices, and terminal half-lives of 1.2 hr in plasma and 1.3 hr in ELF (Table 2), indicating a lack of any system hysteresis.

Penetration of relebactam into the intracellular space was lower than in ELF, with GMRs for AC/plasma concentrations ranging from 0.14 to 0.51 across time points (Table 1), and a relative exposure (AUC$_{0\text{-}\text{inf}}$) in AC vs plasma of 36% based on the mean profiles after adjustment for protein binding. $T_{\text{max}}$ and terminal half-life for relebactam in AC were slightly different than in plasma, representing slower intracellular penetration and clearance compared to plasma, with $T_{\text{max}}$ occurring at 1.0 hr, and a terminal half-life of 2.3 hr in AC.

As shown in Figure 2, imipenem levels in ELF were consistently lower than imipenem levels in plasma. Because the large majority of imipenem AC concentrations were below the limit of quantitation, pharmacokinetic parameters for imipenem in AC are not reported.

Penetration of imipenem into the extracellular space was approximately one-third to one-half of
the corresponding plasma level, with GMRs for ELF/plasma concentrations ranging from 0.32 to 0.55 across time points (Table 1), and a relative exposure (AUC_{0-inf}) in ELF vs plasma of 55% based on the mean profiles (Table 2), after adjustment for protein binding (imipenem is 80% unbound in plasma; free fraction of 100% assumed for the ELF). T_{max} and terminal half-life for imipenem in ELF were similar to those in plasma, with T_{max} occurring at 0.5 hr and a terminal half-life of 1.0 hr in both matrices (Table 2), again indicating a lack of any observable system hysteresis.

Mean relebactam-to-imipenem ratios in plasma and ELF are shown in Figure 3. The primary hypothesis that the relebactam concentration in ELF would be >25% of the imipenem concentration in ELF at the T_{max} of imipenem (0.5 hr) was confirmed, as the point estimate (90% confidence interval) of the GMR for relebactam versus imipenem in ELF was 47% (45%, 49%) (Table 3).

The population PK analysis of relebactam indicated that among the three approaches explored, the ELF data was best fit by a time-invariant partition coefficient driven by the predicted unbound plasma concentration. Model parameters are shown in Table 4, with corresponding model diagnostics in Figure 4. As shown in Table 4, the partition coefficient estimated by the model (55%) was consistent with the estimated penetration using the AUC-ratio method described above (54%). Plasma and lung PK of relebactam was sufficiently described by the two compartment plasma model with a time-invariant partitioning describing penetration into the lung. The model predicts that the equilibration of relebactam between the plasma and ELF is rapidly established with negligible delay, with substantial penetration into the ELF.

Five subjects reported a total of 9 adverse events during the study; all were non-serious. Five events were deemed to be drug related: mild fatigue in one subject, mild increased creatinine in another subject, and mild diarrhea with moderate nausea and vomiting in a third
subject. The subject with nausea, vomiting, and diarrhea was discontinued from the study during administration of the second dose of study drug. All adverse events resolved, and most events (7 of 9) lasted less than 12 hours.

Discussion

In this study, the penetration of both relebactam and imipenem into the pulmonary extracellular space was similar, with relative exposures in ELF vs plasma of 54% for relebactam and 55% for imipenem, after adjustment for protein binding based on the ratio of AUC values between the respective compartments. These values were confirmed using population PK approaches, where penetration into the ELF was similarly projected to be 55% for both relebactam and imipenem (22). \( T_{\text{max}} \) for both compounds was 0.5 hr in both matrices, and the terminal half-lives were also similar (1.2 hr in plasma and 1.3 hr in ELF for relebactam; 1.0 hr in both matrices for imipenem), indicating rapid establishment of equilibrium for both relebactam and imipenem between plasma and ELF and a lack of any significant delay or system hysteresis. These observations also lend support to the sampling scheme chosen for this study and the decision not to collect additional later time points at the end of the dosing interval, as the parallel elimination phases in plasma and ELF observed for both imipenem and relebactam indicated a robust characterization of the clearance in both compartments. Plasma PK parameters observed in this study for both relebactam and imipenem were consistent with those previously reported in healthy subjects (7, 8).

Penetration of relebactam into the intracellular space resulted in relative exposure of relebactam in AC vs plasma of 36% after adjustment for protein binding, and relebactam was cleared more slowly from AC than from plasma (half-lives of 2.3 vs 1.2 hr). Imipenem levels in AC were undetectable in the majority of patients, consistent with the general observation that \( \beta \)-lactams do not penetrate into intracellular compartments as well as macrolides and fluoroquinolones (18). This finding is not clinically meaningful, since the efficacy of
imipenem/cilastatin for the treatment of pneumonia has been well established in several large, multcenter clinical trials (23).

Although imipenem penetration into the respiratory tract has not been studied previously, extensive clinical experience in the treatment of pneumonia suggests that the exposures achieved in ELF are sufficient for clinical efficacy, even if lower than those observed in plasma.

As detailed above, results from preclinical in vivo infection models indicate the PK parameter best correlated with relebactam efficacy is the AUC, with exposures (AUC_{0-24hr}) of ~100 µM*hr required in the thigh infection model and 150 µM*hr in the pulmonary infection model. The increased exposures required in the pulmonary infection model are likely partially due to the penetration of relebactam into the murine lung, which is approximately 34% based on the ratio of total drug levels in the plasma versus lung (data on file). Because the ELF/plasma concentration ratio for relebactam is slightly lower in mice than in humans, plasma concentrations represent a good surrogate for lung exposure. Thus, the plasma PK target from the mouse lung infection model allows for a direct assessment of the appropriateness of dosing for lung infections, using the corresponding human plasma PK data. The plasma PK derived from Phase 2 studies in patients has been previously analyzed and reported, indicating robust target attainment at the relebactam dose of 250 mg four times daily (10).

Prior studies of the intrapulmonary penetration of carbapenem-class β-lactam antibiotics are limited and have not included imipenem. Other carbapenems have shown penetration ratios that bracket the ELF/plasma ratios observed for imipenem in this study. For example, the ELF/plasma ratio for meropenem ranged from 0.32 to 0.53 after multiple-dose administration of 1 g q8h x 4 doses) in healthy adults (24) and was estimated at 30% based on population modeling in patients with ventilator-associated pneumonia (25). For ertapenem, ratios of ELF concentrations to total plasma concentrations ranged from 0.21 to 0.64 (median 0.32) in a multiple-dose study of adult patients with ventilator-associated pneumonia (26).
Prior studies of the intrapulmonary penetration of β-lactam/BLI combination therapies are also limited. For orally administered amoxicillin/clavulanate, concentrations of both drugs in ELF were less than 20% of those observed in plasma (27). In critically-ill patients receiving multiple doses of piperacillin/tazobactam, mean concentrations in ELF were approximately 57% and 91% of total plasma concentrations, respectively (28). In healthy adults, ceftazidime/avibactam displayed similar plasma and ELF curves, with AUC ELF/plasma ratios of 31%-32% for ceftazidime and 32%-35% for avibactam (29). Ceftolozane/tazobactam have also demonstrated similar ELF and plasma curves, with ELF:plasma AUC ratios of 0.48 for ceftolozane and 0.44 for tazobactam in healthy adults (30). In a recent study of meropenem/RPX7009 in healthy adults, mean penetration ratios based on AUC were 0.63 for meropenem and 0.53 for RPX7009 (31).

The accuracy of antibiotic measurements in ELF and AC can be influenced by several methodological issues. In particular, prolonged dwelling time of fluid during BAL (>1 min) can cause additional urea to diffuse into ELF and overestimate ELF volume. Contamination of BAL with blood can also lead to overestimation of ELF volume and inaccurate drug concentration estimates. Since antibiotic concentrations may differ in fluids and cells, prompt separation of cells from fluid is necessary to avoid lysis of cells that may change concentrations in fluid. To minimize the effect of these factors, this study was conducted by experienced personnel using established bronchoscopy and BAL procedures and included detailed procedures for collection, handling, and storage of BAL samples with careful and prompt separation of ELF and AC. In addition, concentrations at several time-points spanning the dose interval were studied, providing enough data to generate a pulmonary PK profile and to support a PK/PD hypothesis. The primary limitation of this study (as opposed to general limitations of ELF studies) is that it was conducted in healthy volunteers, and there is limited information available regarding the correlation of pulmonary drug penetration in healthy volunteers to that in critically ill patients.
Though limited, current data indicates that lung penetration in healthy volunteers appears to be directionally and often quantitatively similar to penetration ratios observed in patients (32).

Furthermore, conduct of such a study in healthy subjects is common practice (33), due to feasibility considerations as well as the semi-quantitative interpretability of study results (22).

Further, while we observe parallel elimination slopes between the observed plasma and ELF data over the range of observed data, additional sampling through the full dosing interval of 6 hours would provide an even more complete picture of the dynamic lung penetration of relebactam.

In summary, this study in healthy subjects demonstrates that relebactam and imipenem achieve similar relative exposures in pulmonary ELF compared to plasma, and that relebactam and imipenem clearances in pulmonary ELF mirror those seen in plasma. Imipenem was not detected in AC, providing further confirmation that activity of imipenem in the pulmonary extracellular compartment (ELF) may be most relevant to its efficacy in treating pneumonia.

These data suggest that a dose adjustment for either relebactam or imipenem is likely not necessary for the effective treatment of bacterial pneumonia. Relebactam is sufficiently well tolerated to continue with further clinical investigation, and these results further support the investigation of relebactam used in combination with imipenem/cilastatin in a Phase 3 trial for the treatment of bacterial pneumonia (NCT02493764).
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Conflict of Interest Disclosure

M. Rizk, E. Rhee, P. Jumes, T. Zhao, E. Mangin, S. Bi, C. Chavez-Eng, Z. Zhang, and J. Butterton are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and may own stock and/or stock options in the company. M. Gotfried received grant support from Merck & Co., Inc., Kenilworth, NJ, USA for conducting the study.

Author Contributions

MLR and EGR wrote the manuscript. MLR, EGR, PAJ, and JRB designed the research. MHG performed the research. MLR, TZ, EM, SB, CMC-E, and ZZ analyzed the data. All authors reviewed the manuscript and approved the final version for submission.
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Figure Legends

**Figure 1**: Arithmetic Mean (±SD) Concentration Profiles for Relebactam in Plasma, ELF and AC after Multiple-Dose Administration of Relebactam 250 mg with Imipenem/cilastatin 500 mg in Healthy Subjects (N=4/Time Point, Top=Linear Scale, Bottom=Semi-log)

**Figure 2**: Arithmetic Mean (±SD) Concentration Profiles for Imipenem in Plasma and ELF after Multiple-Dose Administration of Relebactam 250 mg with Imipenem/cilastatin 500 mg in Healthy Subjects (N=4/Time Point, Top=Linear Scale, Bottom=Semi-log)

**Figure 3**: Arithmetic Mean Ratios (±SD) of Relebactam to Imipenem Concentrations in Plasma and ELF after Multiple-Dose Administration of Relebactam 250 mg with Imipenem/cilastatin 500 mg in Healthy Subjects (N=4/Time Point)

**Figure 4**: Relebactam population PK model diagnostics (DV: observed value; PRED: model-predicted value; CWRES: conditionally weighted residual) for plasma (black circles) and ELF (red squares) data. REL=relebactam.
| Time (hr) | N  | Relebactam | Imipenem |
|----------|----|------------|----------|
|          |    | ELF / Plasma GMR (90% CI) | AC / Plasma GMR (90% CI) | ELF / Plasma GMR (90% CI) |
| 0.5      | 4  | 0.32 (0.23, 0.43) | 0.14 (0.10, 0.19) | 0.32 (0.25, 0.43) |
| 1.0      | 4  | 0.35 (0.26, 0.47) | 0.25 (0.18, 0.33) | 0.36 (0.27, 0.48) |
| 1.5      | 4  | 0.51 (0.38, 0.69) | 0.38 (0.26, 0.56) | 0.55 (0.42, 0.73) |
| 3.0      | 4  | 0.46 (0.34, 0.62) | 0.51 (0.36, 0.70) | 0.50 (0.38, 0.67) |

† Ratio of least-square means from linear mixed effect model performed on the natural log-transformed values with location, time (4 levels), and location by time interaction as fixed effects, and subject as random effect. CI, confidence interval.
Table 2. Pharmacokinetic Parameters for Relebactam and Imipenem after Multiple-Dose Administration of Relebactam 250 mg with Imipenem/cilastatin 500 mg in Healthy Subjects (N=4/Time Point, non-compartmental analysis conducted on mean profile)

| Analyte   | Matrix       | AUC₀⁻_INF (µM*hr)ᵃ | AUC₀⁻₃hr (µM*hr)ᵇ | C_max (µM)ᵃ | T_max (hr)ᵃ | t₁/₂ (hr)ᵃ | AUC₀⁻_INF ELF/Plasma Ratioᶜ | Adjusted AUC₀⁻_INF ELF/Plasma Ratioᵈ |
|-----------|--------------|---------------------|--------------------|--------------|--------------|------------|-------------------------------|--------------------------------------|
| Relebactam| Plasma       | 81.2                | 64.7               | 47.9         | 0.50         | 1.24       | 43.0                          | 53.7                                 |
|           | ELF          | 34.9                | 26.7               | 15.3         | 0.50         | 1.29       |                               |                                      |
|           | Alveolar Cells (AC) | 23.6              | 12.8               | 7.81         | 1.00         | 2.25       |                               |                                      |
| Imipenem  | Plasma       | 130                 | 114                | 99.6         | 0.50         | 0.95       | 44.2                          | 55.2                                 |
|           | ELF          | 57.4                | 48.4               | 32.6         | 0.50         | 1.03       |                               |                                      |
|           | Alveolar Cells (AC) |  --               |  --                |  --          |  --          |  --        |                               |                                      |

ᵃConcentration values were averaged across 4 subjects at each timepoint and all timepoints were combined into a single dataset for NCA calculation.
ᵇInsufficient data
ᶜCalculated as 100* ELF AUC₀-inf / plasma AUC₀-inf
ᵈCalculated as 100* ELF AUC₀-inf / plasma AUC₀-inf / 0.8 (80% fraction unbound for both relebactam and imipenem).
Table 3. Relebactam and Imipenem Concentrations (µM) in ELF after Multiple-Dose Administration of Relebactam 250 mg with Imipenem/cilastatin 500 mg in Healthy Subjects

| Time | Relebactam in ELF (µM) | Imipenem in ELF (µM) | Relebactam / Imipenem |
|------|------------------------|----------------------|-----------------------|
|      | GM †                   | 95 % CI              | GM †                  | 95 % CI              | GMR ‡                  | 90 % CI              |
| 0.5 hr | 14.93 (9.89, 22.53)    | 32.09 (21.26, 48.44) | 0.47 (0.45, 0.49)     |
| 1 hr   | 10.93 (7.24, 16.50)    | 20.27 (13.43, 30.59) | 0.54 (0.52, 0.56)     |
| 1.5 hr  | 9.49 (6.29, 14.32)     | 16.47 (10.92, 24.87) | 0.58 (0.55, 0.60)     |
| 3 hr   | 4.27 (2.83, 6.45)      | 5.99 (3.97, 9.04)    | 0.71 (0.68, 0.74)     |

† Back-transformed least-squares mean from linear mixed effects model performed on the natural log-transformed values with fixed effect for compound (MK and IPM), time (4 levels) and compound by time interaction and subjects as random effect.
‡ GMR = Geometric least-squares mean ratio.
CI = Confidence Interval.
N=4 at each time point.
### Table 4. Relebactam population PK model parameter estimates

| Parameter                        | Unit     | Estimate | RSE%  |
|----------------------------------|----------|----------|-------|
| **Structural model**             |          |          |       |
| Clearance                        | $\theta_{CL}$ | L/h      | 9.17  | 5.04  |
| Volume, central                  | $\theta_{Vc}$ | L       | 15.3  | 15.9  |
| Volume, peripheral               | $\theta_{Vp}$ | L       | 10.6  | 384.9 |
| Inter-compartmental Clearance    | $\theta_{Q2}$ | L/h     | 2.64  | 107.2 |
| **Residual error**               |          |          |       |
| Additive                         | $\sigma_{addi}$ | mg/L    | 0.01 (fixed) | |
| Proportional                     | $\sigma_{prop}$ | CV%    | 28.0  | 49.5  |
| **ELF penetration**              |          |          |       |
| Penetration coefficient, ELF     | $\theta_{kELF}$ | Ratio   | 0.553 | 9.06  |
| Proportional residual error      | $\sigma_{prop,kELF}$ | CV%   | 39.2  | 44.2  |

RSE: relative standard error
CV: coefficient of variation
Figure 1:

- Alveolar Cells (AC)
- Epithelial Lining Fluid (ELF)
- Plasma
Figure 2:

[Graph showing the mean imipenem concentration over time for Epithelial Lining Fluid (ELF) and Plasma.]

- Epithelial Lining Fluid (ELF)
- Plasma
