

Pharmacokinetic-Pharmacodynamic Analysis of Solithromycin (CEM-101) Against *Streptococcus pneumoniae* Using Data from a Murine-Lung Infection Model

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Abstract

Background: CEM-101 is a broad spectrum fluoroketolide with *in vitro* activity against typical and atypical pathogens associated with community-acquired bacterial pneumonia (CABP). Using a murine-lung infection model, epithelial lining fluid (ELF) and plasma pharmacokinetic-pharmacodynamic (PK-PD) measures most closely associated with CEM-101 efficacy against *Streptococcus pneumoniae* and targets based on PK-PD relationships for such indices were identified.

Methods: CEM-101 PK data were obtained from healthy mice administered single CEM-101 doses ranging from 0.625 to 40 mg/kg. Plasma and epithelial lining fluid (ELF) were collected over 24 h (3 mice/time point) and assayed for CEM-101. Urea in plasma and ELF was used to correct ELF concentrations. Neutropenic mice infected with 10⁸ CFU of 1 of 5 *S. pneumoniae* isolates via inhalation were administered daily CEM-101 doses (0.156 to 160 mg/kg) via oral gavage. Dose-fractionation was performed for 1 isolate; CEM-101 was administered to the other 4 isolates as a Q6h or Q12h regimen. PK and PK-PD were evaluated using S-ADAPT 1.56.

Results: A 3-compartment model with a parallel first-order and capacity-limited clearance and a capacity-limited first pass effect with fitted lag-times best described the plasma and ELF data ($r^2 = 0.98$ and 0.83 for observed vs fitted concentrations, respectively). ELF to total- and free-drug (*f*) plasma (based on protein binding of 91.8% in mice) AUC₀₋₂₄ ratios were 0.22 and 2.7, respectively. ELF and *f* plasma AUC₀₋₂₄:MIC ratios were most predictive of efficacy ($r^2 = 0.85$ for ELF and *f* plasma). ELF and *f* plasma AUC₀₋₂₄:MIC ratios (%SE) associated with net bacterial stasis and a 1- and 2-log₁₀ CFU reduction from baseline were 1.26 (51) and 1.65 (38), 15.1 (29) and 6.31 (23), and 59.8 (16) and 12.8 (14), respectively.

Conclusions: AUC₀₋₂₄:MIC ratio was the PK-PD index most predictive of efficacy for CEM-101. PK-PD targets based on these relationships will inform dose selection for future clinical studies.

Introduction

- Solithromycin (CEM-101) is a broad spectrum fluoroketolide antibiotic, a subclass of the macrolide family, with activity against typical and atypical bacterial respiratory organisms.
- CEM-101 is bactericidal against *Streptococcus pneumoniae* including macrolide-resistant isolates, suggesting its potential efficacy for the treatment of patients with community-acquired bacterial pneumonia (CABP).
- Using a neutropenic murine-lung infection model, the objectives of this study were to identify both the pharmacokinetic-pharmacodynamic (PK-PD) measures in plasma and epithelial lining fluid (ELF) of the lung most closely associated with efficacy, and the magnitude of such measures necessary for the efficacy of CEM-101 against *S. pneumoniae*.

Materials and Methods

Bacterial Isolates and Study Drug

- Five *S. pneumoniae* isolates, ATCC 10813, CDC 1329, CDC 1326, CDC 1020, and CDC 673, with MIC values of 0.06, 0.125, 0.06, 0.06, and 0.03 µg/mL, respectively, were evaluated in this study.
- CEM-101 was supplied by Cempra Pharmaceuticals, Inc. Chapel Hill, NC.

Murine Pharmacokinetic Studies

- Healthy CD-1 mice were administered a single 0.625, 2.5, 10, or 40 mg/kg dose of CEM-101 via oral gavage.
- Plasma samples were collected via cardiac puncture at 1, 2, 6, 9, 12, and 24 h after CEM-101 dose administration (3 mice/time point); ELF samples were also collected in the same mice by bronchoalveolar lavage at the same time points.
- The plasma and ELF samples were assayed for CEM-101 using high performance LC/MS/MS with a lower limit of quantification of 10 ng/mL or 0.1 ng/mL for plasma and ELF and a %CV of < 7.67% and < 13.0%, respectively.

Materials and Methods

- Plasma and ELF urea concentrations were also measured and used to correct the measured ELF CEM-101 concentrations using the method described by Rennard, *et. al.* [1].
- Plasma and ELF data from the mice were pooled by dosing cohort and fit by candidate pharmacokinetic (PK) models using Monte Carlo parametric expectation maximization (MC-PEM) as implemented in S-ADAPT 1.56.

Murine Pharmacokinetic-Pharmacodynamic Lung-Infection Model

- Female CD-1 mice were rendered neutropenic (< 100 neutrophils/mm³) via 2 intra-peritoneal injections of cyclophosphamide 4 days (150 mg/kg) and 1 day (100 mg/kg) prior to bacterial infection.
- S. pneumoniae*, in log-phase growth, were used to induce diffuse pneumonia in the neutropenic mice by intranasal instillation of 50 µL of a 10⁸ colony forming units (CFU)/mL inoculum of bacteria.
- Two hours after infection, mice infected with ATCC 10813 were administered total CEM-101 doses of 0.625, 2.5, 10, 40, or 160 mg/kg/day via oral gavage as a Q3h, Q6h, Q12h or Q24h regimen over a 24 h period.
- Mice infected with CDC 1329 and CDC 1396 received CEM-101 doses of 0.156, 0.625, 2.5, 10, or 40 mg/kg/day via oral gavage as a Q12h regimen and mice infected with CDC 1020 and CDC 673 received the same doses as a Q6h regimen.
- Cohorts of two mice per isolate, administered each of the evaluated regimens, were euthanized immediately prior to and at 24 h following the administration of the first dose of CEM-101. Untreated mice served as negative controls.
- Lungs were harvested, homogenized, and the homogenate was then serially diluted and plated on agar. The bacterial burden was determined in log₁₀ CFU/lung.
- Using a point estimate of 91.8% for the protein binding of CEM-101 in mice, and the PK model derived from healthy mice, the free-drug area under the concentration-time curve from time 0 to 24 h (AUC₀₋₂₄), the maximum concentration achieved (C_{max}) and the percent time above the minimum inhibitory concentration (% T > MIC) were determined by simulation of the doses administered to the infected mice.

- The relationships between the ELF or free-drug plasma AUC₀₋₂₄ to MIC (AUC₀₋₂₄:MIC) ratio, the C_{max} to MIC (C_{max}:MIC) ratio and % T > MIC and change in log₁₀ CFU were evaluated for *S. pneumoniae* ATCC 10813 using Hill-type functions implemented in S-ADAPT 1.56.

- Relationships between change in log₁₀ CFU from baseline at 24 h and both ELF or free-drug plasma AUC₀₋₂₄:MIC ratios were also evaluated using data pooled from mice infected with one of the five isolates.

- The log₁₀ CFU data were weighted using the inverse of the estimated measurement variance.

- The residual variability was described using an additive residual error model. Log₁₀ CFU values observed to be less than the lower limit of detection (2 log₁₀ CFU) were noted as such and were fit using the Beal M3 method for censored data [2].

- Using the final parameter estimates from the PK-PD models, the ELF and free-drug plasma PK-PD targets associated with net bacterial stasis and a 1- and 2-log₁₀ CFU net reduction from baseline were identified.

Results

Pharmacokinetic Studies

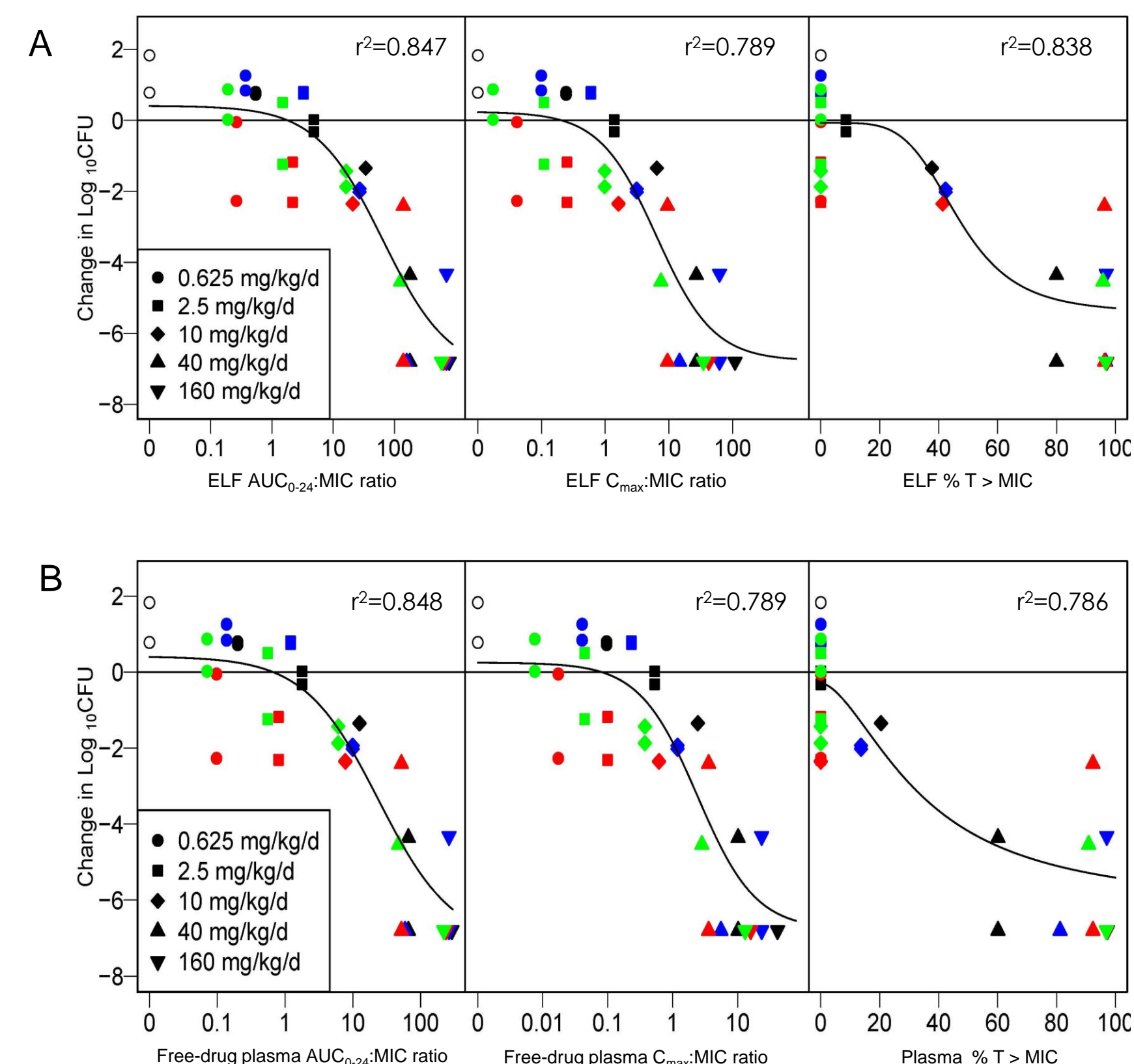
- The murine PK of CEM-101 was best described by a three-compartment model (central, peripheral, and lung compartment) with a parallel first-order and capacity-limited elimination and a first-order absorption process with a fitted lag-time and a capacity-limited first-pass effect.
- The model provided excellent fits to the data, as evidenced by the r^2 of 0.977 and 0.831 for observed vs. individual fitted plasma and ELF concentrations, respectively.

Results

Murine Pharmacokinetic-Pharmacodynamic Lung-Infection Model

- The CEM-101 ELF to total- and free-drug plasma AUC₀₋₂₄ ratios were determined to be 0.22 and 2.7, respectively
- The relationships between the ELF or free-drug plasma AUC₀₋₂₄:MIC ratio, C_{max}:MIC ratio and % T > MIC and change in log₁₀ CFU were well described for *S. pneumoniae* ATCC 10813 by the PK-PD models constructed.
- The fitted relationships between the ELF (A) and free-drug plasma (B) PK-PD indices and change in log₁₀ CFU from baseline at the 24 h time point are shown in **Figure 1**.

Figure 1. Relationship between the ELF (A) and free-drug plasma (B) PK-PD indices and the change in log₁₀ CFU from baseline at 24 h for *S. pneumoniae* ATCC 10813. The black, blue, red, and green shapes represent the Q24h, Q12h, Q6h, and Q3h regimens, respectively



- Because the drug-free control for mice infected with CDC 1020 and CDC 673 had a lower magnitude of bacterial burden in the lungs at the end of 24 h compared to baseline, data for these isolates were considered unreliable and were excluded from the PK-PD analysis.

- The relationships between the ELF or free-drug plasma AUC₀₋₂₄:MIC ratio and change in log₁₀ CFU from baseline at 24 h for *S. pneumoniae*, using data pooled from mice infected with *S. pneumoniae* ATCC 10813, CDC 1396, and CDC 1329, are shown in **Figure 2**.

- Parameter estimates and the associated percent standard errors (%SE) for the PK-PD models describing the relationship between the ELF or free-drug plasma AUC₀₋₂₄:MIC ratio and change in log₁₀ CFU from baseline for the 24 h time point for *S. pneumoniae* are provided in **Table 1**.

- ELF AUC₀₋₂₄:MIC ratios (%SE) associated with net bacterial stasis and a 1- and 2-log₁₀ CFU reduction from baseline were 1.26 (51), 15.1 (29), and 59.8 (16), respectively.

Results

- Free-drug plasma AUC₀₋₂₄:MIC ratios (%SE) associated with net bacterial stasis and a 1- and 2-log₁₀ CFU reduction from baseline were 1.65 (38), 6.31 (23), and 12.8 (14) respectively.

Figure 2. Relationship between the ELF (A) and free-drug plasma (B) AUC₀₋₂₄:MIC and the change in log₁₀ CFU from baseline at 24 h for the *S. pneumoniae* isolates. Each symbol represents the change in log₁₀ CFU from baseline for each mouse at 24 h. The horizontal line represents the bacterial burden at the start of therapy.

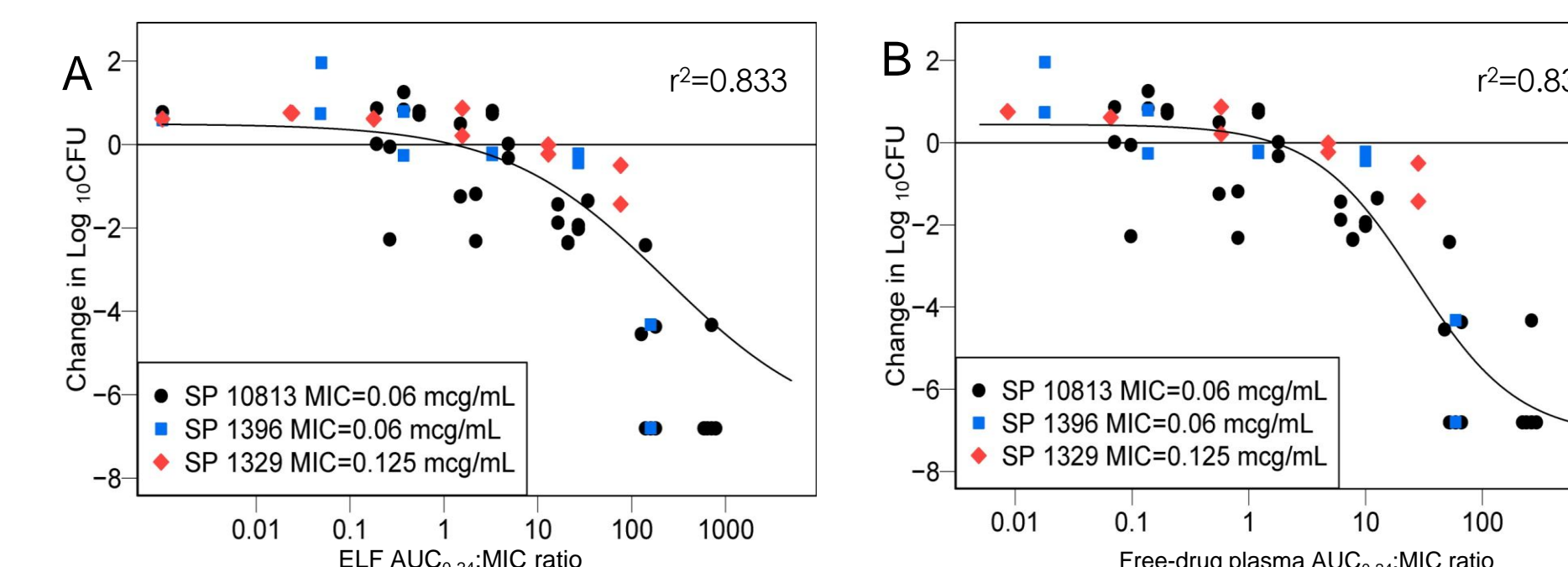


Table 1. Parameter estimates and associated percent standard errors (%SE) for PK-PD models describing the relationship between the ELF or free-drug plasma AUC₀₋₂₄:MIC and change in log₁₀ CFU from baseline for the 24 h time point for *S. pneumoniae*^a

Parameter	Parameter estimates (%SE)	
	ELF	Free-drug plasma
Econ	0.444 (0.337)	0.496 (0.324)
Emax	7.59 (0.669)	7.45 (0.638)
Hill	0.991 (6.97)	0.507 (5.72)
EC ₅₀	27.2 (12.3)	232 (9.58)
r ²	0.834	0.833

a. Based on data pooled from mice infected with *S. pneumoniae* ATCC 10813, CDC 1329, and CDC 1396. Note: Econ is the change in log₁₀ CFU after 24 h from baseline when no drug is administered; Emax is the maximum change in log₁₀ CFU from baseline after 24 h relative to Econ; EC₅₀ is the AUC₀₋₂₄:MIC at which there is half-maximal effect; Hill is the Hill coefficient.

Conclusions

- The PK of CEM-101 was best described by a three-compartment model (central, peripheral, and lung compartment) with a parallel first-order and capacity-limited elimination and a first-order absorption process with a fitted lag-time and a capacity-limited first-pass effect.
- AUC₀₋₂₄:MIC ratio was the PK-PD measure best predictive of CEM-101 efficacy against *S. pneumoniae*, regardless of whether drug exposure was measured in the ELF or plasma.
- PK-PD targets for AUC₀₋₂₄:MIC ratio based on the relationships described herein will inform dose selection for future clinical studies in patients with CABP.

References

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- Beal, S. L. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinetic Pharmacodyn. 2001; 28:481-504.