# Pharmacokinetics/Pharmacodynamics (PK/PD) of Plazomicin (PLZ) against Carbapenem-Resistant Enterobacteriaceae (CRE) in Neutropenic Murine Thigh Infection and Pneumonia Models

**Abstract 100**

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## Background

Plazomicin is an aminoglycoside designed to overcome the vast bulk of aminoglycoside modifying enzymes, the most common aminoglycoside resistance mechanism in Enterobacteriaceae.

Plazomicin is FDA approved for cUTI/pyelonephritis. An additional study in patients with CRE serious bacterial infections has been conducted.

Dose-range studies in a neutropenic murine thigh infection model (TIM) and a neutropenic murine pneumonia model (MPM) of CRE infection and dose fractionation studies in the TIM were performed to identify the PD indices and exposure intensities that optimize the killing of CRE in each infection site.

Pharmacokinetic studies were carried out, including ELF penetration studies, to link mg/kg/day doses to PD exposure indices.

Understanding the PD index and exposure targets allows identifying optimal doses and schedules in man to attain the best clinical results.

- **How does site of infection influence exposure target?**

## Materials & Methods

***Organisms for Study*** Eight *K. pneumoniae*, *E. coli* and Enterobacter strains were evaluated in dose-range studies for plazomicin in the neutropenic TIM. Seven were used for the MPM studies. The strains were selected and supplied by Achaogen, Inc. Some of the isolates were resistant to one or more carbapenem antibiotics and/or to the legacy aminoglycoside gentamicin, tobramycin or amikacin

***In vitro Susceptibility Testing*** The *in vitro* susceptibilities were performed using the broth microdilution method described by CLSI.

***Neutropenic Murine Thigh Infection Model***

Methods for TIM are shown in **Abstract 101**

***Neutropenic Murine Pneumonia Model***

All animal experimentation was approved by the University of Florida Institutional Animal Care and Use Committee.

Female, 16 – 18 g BALB/c mice (Charles River [NCI colony], Frederick, MD) were rendered transiently neutropenic with 150 mg/kg of cyclophosphamide given intraperitoneally (i.p.) 4 days prior to infection plus 100 mg/kg given i.p. 1 day before infection.
The neutropenic mice were anesthetized with ketamine 100 mg/kg and xylazine 6 mg/kg given as a single IP injection. The anesthetized mice were inoculated in each nostril with $10^6$ or $10^7$ CFU of a bacterial isolate. The bacterium was administered in volumes of 15 µL/nostril. The animals were monitored until they recovered from the anesthesia.

Two hours after pathogen challenge, plazomicin and vehicle control were administered by the subcutaneous route at the indicated dosing schedule assigned to each group. At sacrifice, lungs were collected for colony count determination for the efficacy studies. For the PK studies plasma and a bronchoalveolar lavage were collected at designated time points and plazomicin and urea concentrations were measured by LC-MS/MS.

***Mathematical Analysis*** Inhibitory Sigmoid-Emax Analysis: A 4 parameter system ($E_{con}$; $E_{max}$; $E_{50}$; H) was employed to link exposure to response. Fit of the model to the data was accomplished with the Estimation Module of the ADAPT V package of programs. A maximum likelihood estimator was employed. Stasis was defined as the AUC/MIC ratio exposure which was required to keep the bacterial burden at the size measured when therapy was initiated. Other Log Kill targets were also calculated. Population PK analysis was performed for all plasma and ELF determinations with the BigNPAG program. ELF penetration was calculated as the ratio of $AUC_{ELF}/AUC_{Plasma}$

## Objective

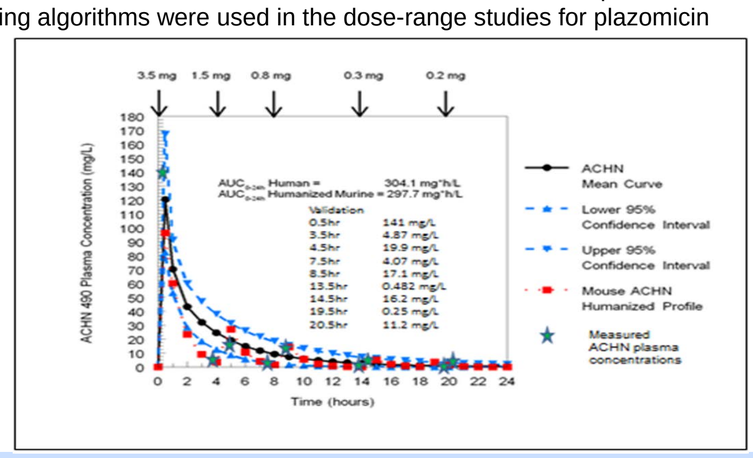
***Identify the dynamically-linked index which drives the efficacy of plazomicin and the dose intensities to achieve stasis as well as 1-Log and 2-Log kill in the TIM and MPM***

## Results

**Table 1 (found in Abstract 101):** shows the activity of plazomicin and comparator antibiotics against 8 clinical isolates of Enterobacteriaceae and an ATCC isolate (AKPN001, ATCC 43816). Plazomicin and amikacin MICs are shown in Table 4.

- **Plazomicin generates potent activity against these isolates**
- **Plazomicin's activity is superior to legacy aminoglycosides**

**Figure 1:** Humanized concentration-time profile in murine plasma for plazomicin at a dose in mice of 225 mg/kg/d. The mean human concentration-time curve and its 95% confidence intervals for plazomicin (formerly ACHN-490) in humans at a dose of 15 mg/kg is shown. A validation study was performed to demonstrate that the humanization did achieve the desired profile. Humanized dosing algorithms were used in the dose-range studies for plazomicin




- **The humanization scheme provided a good approximation of a human plasma concentration-time profile in mouse plasma.**

**Table 2:** Pharmacokinetic parameter values derived from analyzing mean plazomicin concentrations obtained from a neutropenic murine thigh infection model.

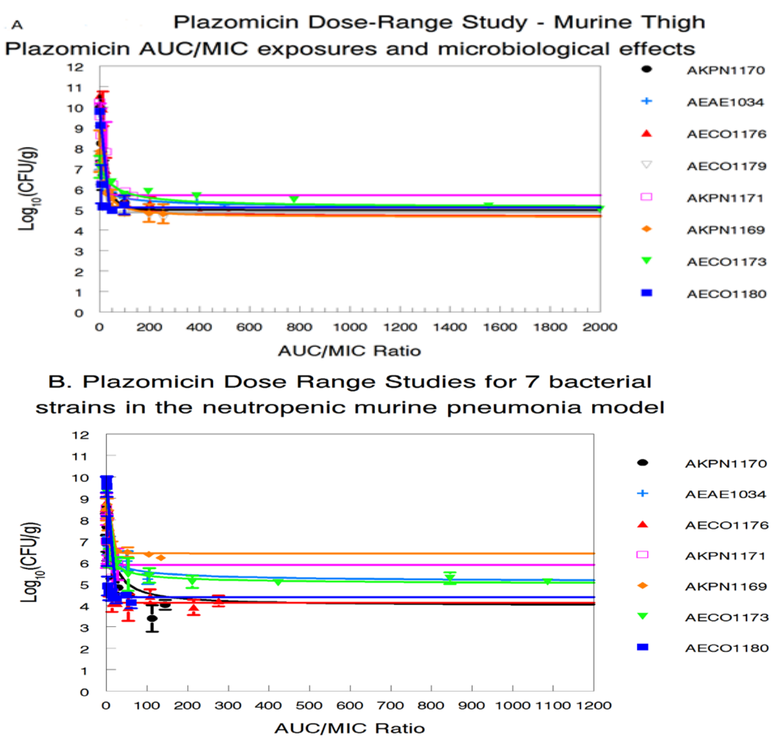
| Parameter | $V_c/F$ | CL/F | $K_{12}$ | $K_{21}$ | $K_a$ |
|---|---|---|---|---|---|
| Units | L | L/h | $h^{-1}$ | $h^{-1}$ | $h^{-1}$ |
| Median | 0.00649 | 0.00647 | 7.235 | 9.642 | 7.393 |
| SD | 0.00286 | 0.00317 | 3.172 | 4.388 | 0.9425 |

**Table 3:** Pharmacokinetic parameter values derived from analyzing mean plazomicin concentrations obtained from a neutropenic murine pneumonia model.

| Parameter | $V_c/F$ | CL/F | $K_{23}$ | $K_{32}$ | $K_{24}$ | $K_{42}$ | $V_{ELF}$ | $K_a$ |
|---|---|---|---|---|---|---|---|---|
| Units | L | L/h | $h^{-1}$ | $h^{-1}$ | $h^{-1}$ | $h^{-1}$ | L | $h^{-1}$ |
| Median | 0.00486 | 0.00882 | 6.017 | 18.01 | 14.34 | 6.74 | 0.00491 | 6.203 |
| SD | 0.00713 | 0.00491 | 1.966 | 2.875 | 2.967 | 0.888 | 0.000498 | 2.510 |

ELF Penetration was 84.6%; If calculated from individual values, penetration was 69.7%.

**Figure 2:** Dose-range study showing the microbiologic effect of different plazomicin doses on bacterial kill for (A) 8 isolates in a murine thigh infection model and (B) 7 isolates in the neutropenic murine pneumonia model. . Plazomicin was given using the algorithm which humanizes its PK profile in mice. The doses were transformed to AUC/MIC ratios.




- **Plazomicin produced good activity against the CRE isolates in both neutropenic thigh and pneumonia models**

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## Results (Cont'd)

**Table 4:** Calculated doses (mg/kg/day) and daily exposures (AUC/MIC ratios) of plazomicin needed to achieve bacterial stasis and 1-log and 2-log (CFU/g) reductions in the bacterial densities of 8 Enterobacteriaceae strains in a neutropenic murine thigh infection model.

| | | | | Plazomicin Dose (mg/kg/day) | | | Plazomicin Exposure (AUC/MIC ratio) | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Plazomicin MIC (µg/mL) | Amikacin MIC (µg/mL) | Challenge Inoculum (CFU/thigh) | Stasis | 1 Log (CFU/g) Reduction | 2 Log (CFU/g) Reduction | Stasis | 1 Log (CFU/g) Reduction | 2 Log (CFU/g) Reduction |
| AECO1176 | 1 | 32 | $10^5$ | 14.4 | 26.5 | ------[a] | 33.0 | 60.7 | ------[a] |
| AECO1179 | 2 | 32 | $10^5$ | 7.0 | 10.0 | ------ | 7.6 | 11.34 | ------[a] |
| AKPN1171 | 4 | 8 | $10^5$ | 27.4 | ------[a] | ------[a] | 15.9 | ------[a] | ------[a] |
| AKPN1169 | 2 | 32 | $10^5$ | 18.0 | 135.0 | ------[a] | 20.2 | 152.1 | ------[a] |
| AEAE1034 | 1 | 4 | $10^5$ | 4.9 | ------[a] | ------[a] | 10.9 | ------[a] | ------[a] |
| AECO1173 | 0.25 | 1 | $10^5$ | 4.9 | 58.5 | ------[a] | 43.5 | 518.3 | ------[a] |
| AECO1180 | 4 | 8 | $10^5$ | 10.1 | 14.4 | ------[a] | 5.7 | 8.1 | ------[a] |
| AKPN1170 | 2 | 32 | $10^5$ | 20.8 | 75.0 | ------[a] | 23.6 | 85.0 | ------[a] |

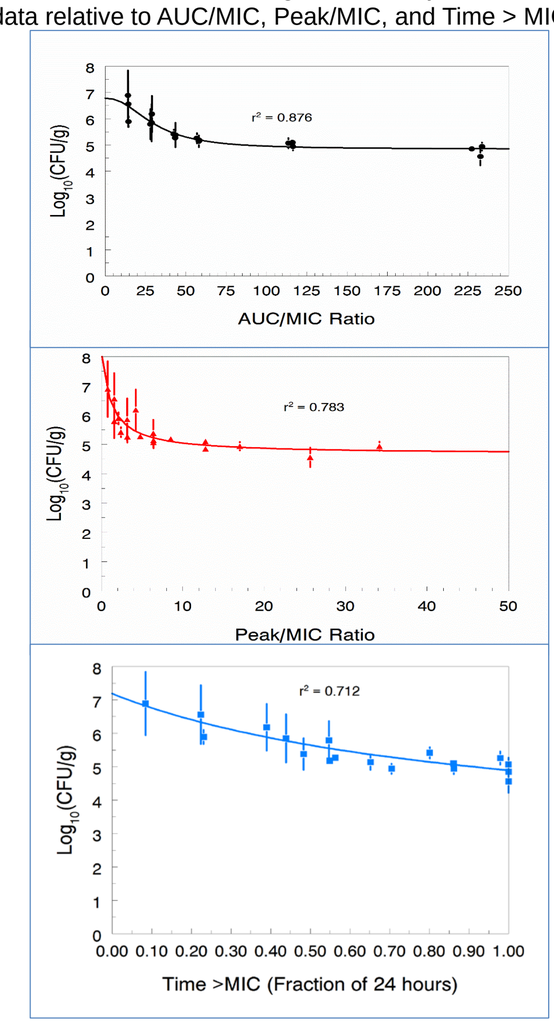
a: ------ = end point not achieved with plazomicin 225 mg/kg/d.

**Table 5:** Calculated doses (mg/kg/day) and AUC/MIC exposures of plazomicin needed to achieve net bacterial stasis and 1-log and 2-log (CFU/g) reductions in the bacterial densities of 7 Enterobacteriaceae strains in a neutropenic murine pneumonia model.

| | | | | Plazomicin Dose (mg/kg/day) | | | Plazomicin Exposure (AUC/MIC ratio) | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Plazomicin MIC (µg/mL) | Amikacin MIC (µg/mL) | Challenge Inoculum (CFU/nare) | Stasis | 1 Log (CFU/g) Reduction | 2 Log (CFU/g) Reduction | Stasis | 1 Log (CFU/g) Reduction | 2 Log (CFU/g) Reduction |
| AECO1176 | 1 | 32 | $10^6$ | 4.6 | 4.9 | 5.3 | 5.6 | 6.0 | 6.5 |
| AKPN1171 | 4 | 8 | $10^6$ | 4.4 | 31.0 | ------[a] | 2.3 | 16.4 | ------[a] |
| AKPN1169 | 2 | 32 | $10^7$ | 4.6 | 10.6 | ------[a] | 2.7 | 6.3 | ------[a] |
| AEAE1034 | 1 | 4 | $10^7$ | 1.0 | 4.9 | 35.1 | 1.2 | 5.6 | 40.3 |
| AECO1173 | 0.25 | 1 | $10^7$ | 0.1 | 0.6 | 4.9 | 0.9 | 2.9 | 23.7 |
| AECO1180 | 4 | 8 | $10^7$ | 5.0 | 6.26 | 8.1 | 1.4 | 1.7 | 2.2 |
| AKPN1170 | 2 | 32 | $10^6$ | 2.44 | 9.65 | 33.7 | 1.6 | 6.2 | 21.6 |

a: the end point was not achieved with plazomicin doses as high as 225 mg/kg/d.

**Figure 3:** Dose-fractionation study for plazomicin in the neutropenic TIM. The figures show the non-linear regression analyses of the quantitative culture data relative to AUC/MIC, Peak/MIC, and Time > MIC exposures.




- **The dose fractionation experiment identified AUC/MIC ratio as the PD-index linked with plazomicin efficacy.**

## Conclusions

- **Exposure-responses were seen in both neutropenic thigh and neutropenic pneumonia models**
- **Stasis and 1-$Log_{10}$(CFU/g) reductions were achieved with plazomicin doses of 4.9 - 27.4 mg/kg/day (stasis) and 10 - 135 mg/kg/day (1-$Log_{10}$(CFU/g)) in the thigh infection model, which equates to AUC/MIC ratio ranges of 5.7 - 43.5 and 8.1 - 518.3, respectively.**
- **Stasis, 1 and 2-$Log_{10}$(CFU/g) reductions were achieved with plazomicin doses of 0.1 – 4.6 mg/kg/day (stasis), 0.6 - 31 mg/kg/day (1-$Log_{10}$(CFU/g)) and 4.9 – 35.1 mg/kg/day (2-$Log_{10}$(CFU/g)) in the murine pneumonia model, which equates to AUC/MIC ratio ranges of 0.9 – 5.6, 1.7 – 16.4 and 2.2 - ≥40, respectively. Two isolates did not achieve 2 Log reductions with plazomicin doses as high as 225 mg/kg/day.**
- **ELF penetration was 70-84%**
- **AUC/MIC ratio was the PD-linked index**
- **Plazomicin was effective in both infection models**
- **These data support an MIC breakpoint of 4 mg/L for a 15 mg/kg/day plazomicin dose in man against CRE (See Abstract 101)**