Pharmacokinetic-Pharmacodynamic Evaluation of Gepotidacin Against Gram-Positive Organisms Using Data From Murine Infection Models

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Short title: Gepotidacin PK-PD Against Gram-Positive Organisms

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Gepotidacin (formerly GSK2140944) is a novel triazaacenaphthylene bacterial topoisomerase inhibitor with in vitro activity against conventional and biothreat pathogens, including *Staphylococcus aureus* and *Streptococcus pneumoniae*. Using neutropenic murine thigh- and lung-infection models, the pharmacokinetics-pharmacodynamics (PK-PD) of gepotidacin against *S. aureus* and *S. pneumoniae* were characterized. Single dose PK data from uninfected mice (16-128 mg/kg SC) were fit with candidate models. Dose-fractionation studies (one isolate/organism; 2-512 mg/kg/day) and dose-ranging studies (5 isolates/organism; 2-2048 mg/kg/day; MIC ranges: *S. aureus* 0.5–2 mg/L; *S. pneumoniae*: 0.125–1 mg/L) were conducted. *In vivo* post-antibiotic effects (PAE) were also evaluated. Relationships between change from baseline in log$_{10}$ CFU at 24 h and free-drug plasma AUC:MIC ratio, Cmax:MIC ratio, and %T>MIC were evaluated using Hill-type models. Plasma and ELF PK data were best fit by a 4-compartment model with linear distributional clearances, a capacity-limited clearance, and a first-order absorption rate. The ELF penetration ratio in uninfected mice was 0.65. Since the growth of both organisms was poor in the murine lung-infection model, lung efficacy data were not reported. As determined using the murine thigh-infection model, free-drug plasma AUC:MIC ratio was the PK-PD index most closely associated with efficacy ($r^2=0.936$ and 0.897 for *S. aureus* and *S. pneumoniae*, respectively). Median free-drug plasma AUC:MIC ratios of 13.4 and 58.9 for *S. aureus*, and 7.86 and 16.9 for *S. pneumoniae*, were associated with net bacterial stasis and a 1-log$_{10}$ CFU reduction from baseline, respectively. Dose-independent PAEs
of 3.07-12.5h and 5.25-8.46h were demonstrated for *S. aureus* and *S. pneumoniae*, respectively.
Introduction

Infections due to Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, are associated with great morbidity and mortality [1, 2]. *S. aureus*, including methicillin-resistant isolates, one of the most common bacterial pathogens causing both community- and hospital-acquired infections, is often responsible for acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP). *Streptococcus* spp. represent an additional group of Gram-positive pathogens associated with ABSSSI and CABP with *S. pneumoniae* often identified as one of the leading causes of CABP [3]. Quinolones, due to their demonstrated clinical efficacy in numerous infections, have historically been one of the most widely used classes of antimicrobials to treat numerous infection types including ABSSSI and CABP [4]. However, resistance to quinolones, which results from either mutations that alter the drug target enzymes, most often DNA gyrase and topoisomerase IV, or by efflux pumps or decreased uptake, has steadily increased over time [4].

Quinolone resistance in *S. aureus* and *S. pneumoniae* is thought to first occur in the ParC subunit of topoisomerase IV and results in a reduced affinity of topoisomerase IV for the quinolone [5]. The prevalence of quinolone resistance in *S. aureus* isolates, in particular methicillin-resistant *S. aureus* (MRSA), has been reported to be greater than 70% [6]. In contrast to *S. aureus*, the *S. pneumoniae* resistance rate for quinolones is generally considered low in North America [7]. However, due to the emergence of *S. pneumoniae* isolates resistant to beta-lactam and macrolide antibiotics, the quinolones
have been relied upon more extensively for the treatment of CABP and as such, the potential for the emergence of quinolone resistance is greater [7]. To combat the increase in resistance rates, additional antibiotics that are efficacious in the treatment of patients with CABP and ABSSSI and do not demonstrate cross-resistance to existing antibiotics are needed.

Gepotidacin (formerly GSK2140944), a novel triazaacenaphthylene bacterial topoisomerase inhibitor, selectively inhibits bacterial DNA replication through a novel binding mode with the GyrA subunit of bacterial DNA gyrase and the ParC subunit of bacterial topoisomerase IV that is distinguished from the binding mode of other antibacterials [8]. As a result of this novel mode of action, gepotidacin displays in vitro activity against target pathogens carrying resistance determinants for established antibacterials including the fluoroquinolones. The spectrum of in vitro activity of gepotidacin includes Gram-positive pathogens and certain Gram-negative pathogens, including those that give rise to CABP and ABSSSI [9]. Gepotidacin is currently being developed by GlaxoSmithKline Pharmaceuticals for potential use as oral (PO) and intravenous (IV) antibacterial therapy [10, 11, 12, 13].

Preclinical in vivo efficacy data for gepotidacin against S. pneumoniae have been previously described [14]. However, these experiments were not designed to identify the pharmacokinetic-pharmacodynamic (PK-PD) index associated with the efficacy of gepotidacin or to determine the magnitude of the index that is associated with various levels of reduction in bacterial burden. Neutropenic murine thigh- and lung-infection...
models are frequently utilized in drug development to characterize PK-PD relationships for efficacy; PK-PD targets derived based on such models can then be used to support dosing regimen selection. Use of such models would allow for the determination of both the PK-PD index and magnitude associated with the in vivo efficacy of gepotidacin against S. aureus and S. pneumoniae.

Using murine thigh- and/or lung-infection models in which gepotidacin was evaluated against various S. aureus and S. pneumoniae isolates, the objectives of these analyses were the following: 1) to develop a pharmacokinetic (PK) model to describe the disposition of gepotidacin in both the plasma and epithelial lining fluid (ELF) of neutropenic uninfected mice; 2) to identify the free-drug plasma PK-PD index most closely associated with the efficacy of gepotidacin; 3) to determine the magnitude of the free-drug plasma PK-PD index associated with various levels of bacterial reduction from baseline; and 4) to evaluate for the presence of an in vivo post-antibiotic effect.
Methods

Bacterial Isolates

A total of 12 Gram-positive isolates, six S. aureus and six S. pneumoniae, were used in these studies. The MIC values for gepotidacin were determined in triplicate by broth microdilution, using methods outlined by the Clinical and Laboratory Standards Institute [15]. Modal MIC values for each isolate were reported.

Inoculum Preparation

Frozen stocks of S. aureus were inoculated onto agar plates and grown overnight. Colonies were sub-cultured from these agar plates into Mueller Hinton II broth and grown overnight to an absorbance of 0.3 at 580 nm. Frozen stocks of S. pneumoniae were inoculated onto sheep blood agar plates and grown overnight. Colonies were taken directly from the sheep blood agar plates and diluted into Mueller Hinton II broth to an absorbance of 0.3 at 580 nm.

Antimicrobial Test Agent

Analytical grade gepotidacin (GlaxoSmithKline, Collegeville, PA) was utilized for all in vitro and in vivo studies. Using the supplied potency, gepotidacin powder was weighed in a sufficient quantity to achieve the required stock concentration and reconstituted using DMSO, with a final DMSO concentration of 1%. Subsequent concentrations were prepared by dilution using 0.15 M NaCl before use for each of the studies.

Animal Infection Models
Specific-pathogen free female CD-1 mice (Charles River, St Constant, Canada) weighing approximately 19 to 21 grams were used in all experiments. All studies were conducted after review by the Institutional Animal Care and Use Committee at the University of Wisconsin and in accordance with the GSK policy on the Care, Welfare, and Treatment of Laboratory Animals. For the PK, dose-ranging, dose-fractionation, and post-antibiotic effect (PAE) studies, the animals were rendered neutropenic (i.e., having no more than 100 polymorphonuclear leukocytes/mm³) by two intraperitoneal injections of cyclophosphamide consisting of 150 mg/kg 4 days and 100 mg/kg 1 day before infection [16]. For all but the PK studies, the animals were then infected with *S. aureus* or *S. pneumoniae* for evaluation in a murine-thigh or -lung infection model. The murine-thigh infection model, in which mice were inoculated in each posterior thigh via a 0.1 mL intra-muscular injection of bacterial suspension containing approximately 10⁷ CFU of bacteria 2 h prior to the initiation of antimicrobial therapy, was used to conduct dose-fractionation, dose-ranging, and PAE studies. The murine-lung infection model, in which diffuse pneumonia was induced in the mice by intranasal instillation of a 50 µL bacterial inoculum containing approximately 10⁸ CFU of *S. aureus* or *S. pneumoniae*, was used to conduct dose-ranging studies.

**Pharmacokinetic Studies**

PK were undertaken in neutropenic, uninfected mice administered gepotidacin as single subcutaneous (SC) doses of 16, 32, 64, or 128 mg/kg. Terminal plasma samples were collected via cardiac puncture at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose (three mice/time point). Bronchoalveolar lavage (BAL) samples were collected for
determination of gepotidacin ELF concentrations for all dosing cohorts at 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose. The recovered BAL fluid was centrifuged to separate the alveolar macrophages (cell pellet) and the ELF fluid (supernatant).

In order to evaluate the impact of infection on the PK profile of gepotidacin, a second plasma PK study was conducted in both neutropenic infected and uninfected mice administered gepotidacin as a single SC dose of 32 mg/kg and neutropenic infected mice administered gepotidacin 16, 64, and 128 mg/kg.

Plasma and BAL samples for the first PK study were assayed for gepotidacin concentrations on two separate occasions by GlaxoSmithKline using a validated high-performance liquid chromatography tandem mass spectrometry method with a lower limit of quantification of 0.05 mg/L and 0.1 mg/L for the plasma samples and 0.0025 mg/L and 0.005 mg/L for the ELF samples. Plasma samples for the second PK study were assayed for gepotidacin concentrations by GlaxoSmithKline using the same methodology as above with a lower limit of quantification of 0.010 mg/L [Data on file, GlaxoSmithKline Pharmaceuticals]. The assay was reproducible with precision ranging between 0.16 and 2.31% for the plasma samples, and between 2.13 and 7.30% for the BAL samples. The assay also showed good accuracy with gepotidacin concentrations within 97 to 107% of the expected plasma concentrations and within 92 to 105% of the expected BAL concentrations [Data on file, GlaxoSmithKline Pharmaceuticals].

Plasma and ELF urea concentrations were used to correct the ELF drug concentrations using the method described by Rennard et al. [17]. Urea concentrations were determined in triplicate using a commercial colorimetric assay kit (Pointe Scientific, Inc.).
Efficacy as Assessed by Change in Bacterial Density

Two hours after infection for the murine-thigh infection model studies and 4 h after infection for the murine-lung infection model studies, gepotidacin treatment regimens were administered as 0.2 mL SC injections and continued for a 24 h period in all studies. Control animals received sterile, normal saline using the same volume, route, and schedule as the active-treatment regimens. Non-drug treated control animals were sacrificed just prior to antibiotic initiation (0 h) and after 24 h. All animals were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. After sacrifice, mice in the murine-thigh infection models had their thighs removed and individually homogenized in normal saline. Serial dilutions of the thigh homogenates were plated for CFU determination and incubated overnight at 37°C. Counts from each thigh were considered as independent observations. Similarly, for the murine-lung infection model, following euthanasia, the lungs were aseptically excised, homogenized, diluted, plated, and incubated. Viable plate counts were determined as log$_{10}$ CFU per thigh or lung. Efficacy was calculated as the change in the log$_{10}$ CFU obtained for antibiotic-treated mice after 24 h compared with the pre-antibiotic baseline CFU measured for the 0 h control animals.

Dose-Fractionation Studies

Dose-fractionation studies were conducted in neutropenic mice infected in each thigh with \textit{S. aureus} 33591 or \textit{S. pneumoniae} 10813. Groups of two mice each were administered gepotidacin total daily doses of 2, 8, 32, 128, or 512 mg/kg SC divided
evenly every 3, 6, 12, or 24 h. Untreated mice for the growth control assessment were euthanized at 2 and 24 h after inoculation.

Dose-Ranging Studies

Murine-Thigh Dose-Ranging Studies
Dose-ranging studies were conducted in neutropenic mice infected in each thigh with one of five *S. aureus* isolates or one of five *S. pneumoniae* isolates. Groups of two mice each were administered gepotidacin total daily doses of 2, 8, 32, 128, 512, and 2048 mg/kg SC, divided into four equal doses administered every 6 h. Untreated infected mice for growth control assessment were euthanized at 2 and 24 h after inoculation for each of the ten isolates studied.

Murine-Lung Dose-Ranging Studies
Dose-ranging studies were conducted in neutropenic mice infected in the lung via intranasal instillation with one of three *S. aureus* isolates or one of three *S. pneumoniae* isolates. Groups of three mice each were administered gepotidacin total daily doses of 2, 8, 32, 128, 512, and 2048 mg/kg SC divided into four equal 0.2 mL doses administered every 6 h. Untreated infected mice for growth control assessment were euthanized at 4 and 24 h after inoculation for each of the six isolates studied.

Pharmacokinetic Analyses

Pharmacokinetic Model Development
The murine plasma and ELF concentration data from uninfected mice from the first PK study were fit by candidate PK models using maximum likelihood objective function, as implemented in S-ADAPT 1.57 [18]. Weighting of the plasma and ELF concentrations was based on the reciprocal of the estimated observation variance (the error model standard deviation (SD) squared), which was predicted as a function of the fitted concentrations. Given that the study design for PK sampling was destructive in nature (i.e., animals were sacrificed at the evaluated time points), the data were combined and fit using a pooled PK analysis approach.

**Calculation of ELF Penetration Ratio**

Using the PK model described above that was developed using data from uninfected mice from the first PK study, fitted total-drug plasma and ELF concentration-time profiles were examined for each dose that was evaluated (16, 32, 64, and 128 mg/kg/day). Using total-drug plasma and ELF concentration-time profiles from 0 to 24 h on Day 1, area under the concentration-time curve values over 24 h (AUC$_{0-24}$) were calculated for each dose. Gepotidacin murine plasma protein binding was determined by GlaxoSmithKline Pharmaceuticals using plasma from normal, uninfected CD-1 mice and equilibrium dialysis over a range of gepotidacin concentrations (5, 9, 18, and 36 µM) [Data on file, GlaxoSmithKline Pharmaceuticals]. Concentration-dependent protein binding was negligible. Based on these data, the mean estimate for plasma protein binding was 0.237 [Data on file, GlaxoSmithKline Pharmaceuticals]. Using the corresponding mean unbound gepotidacin estimate of 0.763, free-drug plasma AUC$_{0-24}$
values were determined. The ELF penetration ratio was calculated by dividing the ELF
\text{AUC}_{0-24}^\text{ELF} \text{ value by the free-drug plasma } \text{AUC}_{0-24}^\text{free-drug plasma} \text{ value.}

\textit{Evaluation of the Impact of Infection on the Pharmacokinetic Profile of Gepotidacin}

Using NONMEM version 7.1.2, PK models were developed based on data from
uninfected and infected mice evaluated in the second PK study using a first order
conditional estimation with interaction. Total-drug plasma \text{AUC}_{0-24} values based on the
PK data for 32 mg/kg were calculated by integrating the respective PK profiles. The
geometric mean total-drug plasma \text{AUC}_{0-24} \text{ value was computed for both infected and
uninfected mice. The ratio of the uninfected to infected geometric mean } \text{AUC}_{0-24} \text{ and
associated 90\% confidence interval around this ratio was computed.}

\textit{Pharmacokinetic-Pharmacodynamic Data Analyses}

Using the final PK model, plasma and ELF concentration-time profiles for gepotidacin
total doses ranging from 2 to 2048 mg/kg were generated. These concentration-time
profiles and the free-fraction estimate of 0.763 for gepotidacin were used to generate
the PK-PD indices. Free-drug plasma and ELF \text{AUC}_{0-24} \text{ values were calculated for each
dose by numerically integrating the individual fitted functions for the free-drug plasma
and ELF concentration-time profiles, respectively. These were then divided by the
gepotidacin MIC value for each isolate to obtain ratios of free-drug plasma and ELF
\text{AUC}_{0-24} \text{ values to MIC (AUC:MIC ratio). Maximum free-drug plasma and ELF
concentration values (C_{\text{max}}) for each dose were divided by the MIC value of each isolate
to obtain ratios of free-drug plasma and ELF C_{\text{max}} \text{ to MIC (C_{\text{max}}:MIC ratio). The percent
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of time that free-drug plasma and ELF concentrations were above the MIC (%T>MIC) were also calculated for each isolate by numerical integration of the fitted functions.

**Dose-Fractionation Studies**

Relationships between efficacy, as measured by change from baseline in log_{10} CFU, and the gepotidacin free-drug plasma PK-PD indices AUC:MIC ratio, C_{max}:MIC ratio, and %T>MIC, were evaluated to determine the PK-PD index most associated with efficacy using data from the murine-thigh infection model. These relationships were evaluated by fitting Hill-type models to the data using maximum likelihood estimation in S-ADAPT 1.57 [18].

**Dose-Ranging Studies**

**Murine-Thigh Dose-Ranging Studies**

Data from the dose-fractionation and dose-ranging studies carried out using a neutropenic murine-thigh infection model in which mice were infected with *S. aureus* were combined for analysis. The corresponding data for *S. pneumoniae* were also combined for analysis. Relationships between efficacy, as measured by change from baseline in log_{10} CFU, and the PK-PD index most associated with the efficacy of gepotidacin were evaluated. These relationships were evaluated by fitting Hill-type models to the *S. aureus* and *S. pneumoniae* data independently, using a population PK-PD analysis approach and as such, account for inter-isolate variability in parameter estimates. Using the post-hoc parameter estimates from the Hill-type models, free-drug
plasma PK-PD targets associated with net bacterial stasis, and a 1- and 2-log\textsubscript{10} CFU reduction from baseline were identified.

Murine-Lung Dose-Ranging Studies

The relationships between the change from baseline in log\textsubscript{10} CFU and the ELF and free-drug plasma PK-PD index most associated with efficacy of gepotidacin were evaluated using data obtained from a neutropenic murine-lung infection model. These relationships were evaluated by fitting Hill-type models to the \textit{S. aureus} and \textit{S. pneumoniae} data, independently, using a population PK-PD analysis approach in S-ADAPT 1.57 [18]. Using the relationships described by these models, PK-PD targets that were associated with net bacterial stasis and a 1- and 2-log\textsubscript{10} CFU reduction from baseline for \textit{S. aureus} and \textit{S. pneumoniae} were identified.

Post-Antibiotic Effect Study

Post-antibiotic effect studies were conducted using \textit{S. aureus} 33591 and \textit{S. pneumoniae} 10813 in a neutropenic murine-thigh infection model. In this model, the mice were administered gepotidacin single doses of 16, 32, 64, or 128 mg/kg SC. Groups of three mice per dose were euthanized at 2, 4, 6, 12, and 24 h after the start of dosing. The PAE for each dose was calculated using the following formula [19]:

$$\text{PAE} = T - C - M$$

where T is the time required for the bacterial counts of treated mice to increase by 1-log and was calculated using linear interpolation; C is the time required for bacterial counts to increase by 1-log in the control animals; and M is the time for
which free-drug plasma concentrations exceed the MIC value, which was determined using the murine PK model.
Results

Bacterial Isolates and In vitro Susceptibility

The gepotidacin MIC ranges for the isolates evaluated were 0.5 to 2 mg/L for *S. aureus* and 0.125 to 1 mg/L for *S. pneumoniae*. The gepotidacin MIC values for all isolates are shown in Table 1.

Pharmacokinetic Analysis

The plasma and ELF PK of gepotidacin based on the first study in uninfected mice were best described by a four-compartment model with three compartments for plasma, one compartment for ELF, linear distributional clearances, a parallel first-order and capacity-limited clearance, and a first-order absorption rate. The PK data were well fit by the PK model as evidenced by an even scatter of the data around the line of identity and $r^2$ values of 0.852 and 0.850 for the plasma and ELF data, respectively (Supplemental Figure 1 and 2). Total-drug plasma and ELF concentration-time profiles at each of the evaluated gepotidacin doses based on the observed data and fitted profile based on the final PK model are provided in Supplemental Figure 3. Table 2 shows the PK model parameter estimates and standard errors. As evidenced by the rate constants for the distribution of gepotidacin into ($k_{ce}$) and the removal from the ELF ($k_{ec}$), gepotidacin rapidly penetrates and departs the ELF compartment of mice and does not accumulate. The ELF penetration ratio for gepotidacin in non-infected neutropenic mice was 0.65 across all of the doses assessed. Since the $k_{ce}$ and $k_{ec}$ rate constants were determined without variability across doses, the above-described ELF penetration ratio estimate was independent of dose.
Evaluation of the Impact of Infection on the Pharmacokinetic Profile of Gepotidacin

As shown in Supplemental Figure 4 and evidenced by an even scatter of data about the line of identity and $r^2$ values of 0.892 and 0.940 for the uninfected and infected mice, respectively, the PK data from both uninfected and infected mice were well fit by the respective PK models. While the observed total-drug plasma concentrations following administration of gepotidacin 32 mg/kg from uninfected mice were generally lower relative to that for infected mice, the total-drug plasma concentrations from these two groups overlapped (Supplemental Figure 5). The resulting geometric mean of the ratio of the total-drug plasma AUC$_{0-24}$ for uninfected mice to the total-drug plasma AUC$_{0-24}$ for infected mice was 0.86 (90% CI 0.72-1.02). Thus, gepotidacin exposure in infected and uninfected animals after administration of the same dose was not statistically different.

Pharmacokinetic-Pharmacodynamic Analyses

Evaluation of the dose-fractionation data demonstrated relationships between change from baseline in log$_{10}$ CFU and each of the gepotidacin PK-PD indices. Examination of the log$_{10}$ CFU data at 24 h for the once every 24 h (q24h) dosing regimen revealed systematically higher bacterial counts than those for dosing regimens based on the other intervals studied. Due to the short half-life of gepotidacin in mice, the q24h dosing interval resulted in inadequate drug concentrations, and therefore, re-growth within the murine infection model. As such, these data were removed from the analyses. As shown in Figure 1 A and B, which illustrate the relationships between the free-drug plasma AUC:MIC ratio, $C_{\text{max}}$:MIC ratio, and %T>MIC and the change from baseline in log$_{10}$ CFU per thigh for S. aureus and S. pneumoniae, respectively, data from the dose-
fractionation studies were well described using Hill-type models. As evidenced by the $r^2$
values for free-drug plasma AUC:MIC ratio and %T>MIC of 0.936 and 0.936,
respectively, for *S. aureus*, and 0.897 and 0.9, respectively, for *S. pneumoniae*, the data
were well described by both indices. Given that gepotidacin is a DNA replication
inhibitor (as are other agents, including fluoroquinolones) and that free-drug plasma
%T>MIC is more difficult than AUC to estimate with precision, free-drug plasma
AUC:MIC ratio was considered to be the more informative PK-PD index.

Results of dose-ranging studies conducted in the neutropenic murine-thigh and -lung
infection models demonstrated activity for gepotidacin against all of the *S. aureus* and
*S. pneumoniae* isolates evaluated. However, as evidenced by a mean (SD) increase of
0.876 (0.472) and 0.0667 (0.468) $\log_{10}$ CFU relative to baseline for the *S. aureus* and
*S. pneumoniae* growth controls, respectively, in the murine-lung infection model as
compared to 1.64 (0.410) and 2.26 (1.07) $\log_{10}$ CFU in the murine-thigh infection model,
the growth of *S. aureus* and *S. pneumoniae* isolates in the neutropenic murine-lung
infection model was less than that in the murine-thigh infection model. Given less than a
1-$\log_{10}$ CFU increase for either *S. aureus* or *S. pneumoniae* in the murine-lung infection
model, the results of the PK-PD analyses for the murine-lung infection model were not
reported.

**Figure 2** A and B show the relationships between the free-drug plasma AUC:MIC ratio
and change from baseline in $\log_{10}$ CFU for the *S. aureus* and *S. pneumoniae* isolates,
respectively, based on data from the dose-fractionation and dose-ranging studies. The
relationships between free-drug plasma AUC:MIC ratio and change from baseline in
log_{10} CFU for *S. aureus* and *S. pneumoniae* were well described by the Hill-type models, as evidenced by $r^2$ values of 0.925 for both pathogens. The parameter estimates and associated standard errors for these models are provided in Table 3.

Table 1 summarizes the magnitude of free-drug plasma AUC:MIC ratio targets associated with net bacterial stasis, and a 1- and 2-log_{10} CFU reduction from baseline for *S. aureus* and *S. pneumoniae* based on the Hill-type models presented in Table 3.

The median free-drug plasma AUC:MIC ratio target associated with net bacterial stasis for *S. aureus* and *S. pneumoniae* was 13.4 and 7.86, respectively. The median free-drug plasma AUC:MIC ratio target associated with a 1- and 2-log_{10} CFU reduction from baseline for *S. aureus* was 58.9 and 257.2, respectively, while that for *S. pneumoniae* was 16.9 and 69.8, respectively.

**Evaluation of the Presence of a PAE**

Figure 3 A and B show the relationship between log_{10} CFU and time overlaid with the free-drug plasma concentration profiles for gepotidacin doses evaluated and the associated PAE for *S. aureus* and *S. pneumoniae*, respectively. A dose-independent PAE was observed with gepotidacin against *S. aureus* and *S. pneumoniae*. The duration of PAE for *S. aureus* at doses of 16, 32, 64, and 128 mg/kg was 3.07, 10.4, 4.44, and 12.5 h, respectively. The duration of PAE for *S. pneumoniae* at these same doses was 6.68, 7.43, 5.25, and 8.46 h, respectively.
Discussion

The results of the above-described studies undertaken using murine thigh- and lung-infection models allowed for the following insights: 1) characterization of the disposition of gepotidacin in the plasma and ELF of neutropenic mice and, using the PK model developed based on these data, reliable estimation of PK-PD indices across the dose range studied; 2) identification of free-drug plasma AUC:\text{MIC} ratio and \%T>\text{MIC} as the PK-PD indices most closely associated with efficacy; 3) calculation of the magnitude of free-drug plasma AUC:\text{MIC} ratios associated with various levels of reduction in bacterial burden of \textit{S. aureus} and \textit{S. pneumoniae}; and 4) identification of the presence of a dose-independent \textit{in vivo} post-antibiotic effect.

The plasma and ELF PK of gepotidacin in uninfected mice was best described by a four-compartment model with three compartments for plasma, one compartment for ELF, linear distributional clearances, a parallel first-order and capacity-limited clearance, and a first-order absorption rate.. Results of studies conducted in infected and uninfected mice to evaluate the impact of infection of the PK profile of gepotidacin demonstrated that the presence of infection did not have a statistically significant impact on total-drug plasma concentrations. These data provide support for use of exposures for the PK-PD analyses described herein using the PK model developed using data from uninfected mice.

Gepotidacin exposure in uninfected mice, as measured by the AUC$_{0-24}$ value, was less for ELF compared to that for free-drug plasma, as evidenced by a penetration ratio of 0.65. Comparison of this ELF penetration ratio for mice compared to that determined...
using ELF and plasma AUC values over 0 to 12 hours based on PK data from healthy volunteer demonstrated higher ELF penetration, with a ratio of 1.88 [13]. This difference in ELF penetration between mice and humans is an important factor to consider when predicting effective human dosing regimens for pneumonia using data from murine studies.

For both *S. aureus* and *S. pneumoniae*, free-drug plasma AUC:MIC ratio and %T>MIC were the PK-PD indices which best described gepotidacin efficacy in dose fractionation studies. While the mechanism of action of gepotidacin, a DNA replication inhibitor, is similar to that of the fluoroquinolones for which efficacy is best predicted by AUC:MIC ratio, gepotidacin has a binding mode that is different than that of the fluoroquinolones [8, 20]. Given the similar mechanism of action and that free-drug plasma %T>MIC is more difficult to estimate with precision than AUC, preference was given to free-drug plasma AUC:MIC ratio over free-drug plasma %T>MIC. When evaluating the Hill-type models for free-drug plasma AUC:MIC ratio derived based on the dose-fractionation and dose-ranging data, a difference in the efficacy of gepotidacin against *S. aureus* versus *S. pneumoniae* was revealed. As evidenced by the smaller $E_{\text{max}}$ value for *S. aureus* versus that for *S. pneumoniae* (3.54 versus 4.49, respectively), gepotidacin had greater in vivo efficacy against *S. pneumoniae*.

Attaining the PK-PD target associated with net bacterial stasis for *S. aureus*, based upon data from a neutropenic murine-thigh infection model, has been shown to be predictive of high probabilities of successful response in patients with ABSSSI treated with linezolid or tigecycline [21]. The results of these analyses for *S. aureus* and *S. pneumoniae*...
pneumoniae (which can be used as a surrogate for Streptococcus pyogenes; unpublished data, Dr. William A. Craig) demonstrated that median free-drug plasma AUC:MIC ratios of 13.4 and 7.86, respectively, were associated with net bacterial stasis. When these free-drug plasma AUC:MIC ratio targets for the same endpoint were compared with those of other fluoroquinolones (free-drug plasma AUC:MIC ratio of 36 to 81 and 25 to 34 for net bacterial stasis against S. aureus and S. pneumoniae, respectively), it is interesting to note that the targets for gepotidacin were lower [21, 22, 23, 24]. Based upon the above-described free-drug plasma AUC:MIC ratio of 13.4 for S. aureus, a ratio of 14 was selected as the PK-PD target for the evaluation of gepotidacin dosing regimens for patients with ABSSSI. Using this free-drug plasma AUC:MIC ratio target in combination with Phase 1 PK data and Monte Carlo simulation, PK-PD target attainment analyses were conducted [Data on file, GlaxoSmithKline Pharmaceuticals]. The results of these analyses were used to select the gepotidacin dosing regimens for evaluation in a Phase 2 ABSSSI study [25].

The translational value of the PK-PD target associated with a 1-log10 CFU reduction from baseline in a murine infection model was demonstrated in a study by Bulik et al., which evaluated the relationship between the probability of attaining the PK-PD target associated with this endpoint and the probability of obtaining regulatory approval for antibacterial agents for the treatment of pneumonia [26]. The results of this study showed that the probability of regulatory drug approval increased with increasing PK-PD target attainment for the target associated with a 1-log10 CFU reduction from baseline in the murine infection model [26]. The murine-lung infection studies described herein
were limited by the poor growth of S. pneumoniae observed at 24 hours in the no-
treatment control groups relative to those in the thigh-infection model. The growth of the
no-treatment control group at 24 hours can be used as an overall indicator of the fitness
of the pathogen in an infection model. If robust growth of the pathogen is observed in
the no-treatment control group at 24 hours, one can assume robust growth in all groups
of the study over the same 24 hours. Accordingly, poor growth can be assumed in all
groups if poor growth is observed in the no-treatment control group at 24 hours. Drug
efficacy in the murine-infection model is measured as the change in log_{10} CFU from
baseline at 24 hours and does not consider the 24 hour data from the no-treatment
control group. As such, there is the potential to attribute greater efficacy to the drug than
would be warranted given the indicator of poor bacterial growth in the no-treatment
control group at 24 hours. A further impact of the overestimation of drug effect is the
underestimation of the magnitude of PK-PD targets associated with efficacy. For the
above-described reasons, ELF and free-drug plasma AUC:MIC ratio targets for
gegotidacin efficacy based on the murine-lung infection model were not reported. The
impact of poor growth, as demonstrated in the no-treatment control group, on the
magnitude of PK-PD targets for efficacy underscores the importance of ensuring that
this study design criterion is met. Future studies achieving sufficient growth in the no-
treatment control groups with different isolates or which use alternative methods with
the current isolates (e.g., use of higher inoculums or direct introduction of the inoculum
into the trachea of mice) may allow for the PK-PD of getotidacin to be characterized
using the murine-lung infection model.
The PAE of an antibiotic, which can be evaluated using either *in vitro* or *in vivo* methods, can be described as the persistent suppression of the growth of the pathogen following exposure to the antibiotic [27]. While the clinical utility of the results of an *in vitro* PAE study is questionable, the presence of an *in vivo* PAE has been a useful characteristic to determine the length of dosing interval required for various antibiotics, such as aminoglycosides and quinolones [27]. Gepotidacin was found to demonstrate an *in vivo* PAE against both *S. aureus* (range, 3.07 to 12.5 h) and *S. pneumoniae* (range, 5.25 to 8.46 h) that is comparable to that of fluoroquinolones [28]. Since the gepotidacin PAE on both organisms was not found to be dose-dependent, it appears to be a predictable characteristic that can be used to inform decisions regarding clinical dosing interval. In general, the presence of a prolonged *in vivo* PAE allows for wider clinical dosing intervals since bacterial regrowth would be expected to be minimal during this period when plasma and tissue concentrations fall below the MIC value for the infecting pathogen [28].

In summary, using PK data from neutropenic mice and data from a murine-thigh infection model in which mice were infected with *S. aureus* or *S. pneumoniae*, free-drug plasma AUC:MIC ratio was identified as the PK-PD index most associated with the efficacy of gepotidacin. Data from these studies also allowed for characterization of the magnitudes of the free-drug plasma AUC:MIC ratios associated with various levels of reduction in bacterial burden and the characterization of PAE. Such data are useful to support future drug development decisions, including dose selection, for gepotidacin.
References


15. **Clinical and Laboratory Standards Institute.** 2010. CLSI document M100 S20. Wayne, PA.


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Figure 1. Relationships between change from baseline in log_{10} CFU and gepotidacin free-drug plasma AUC:MIC ratio, C_{max}:MIC ratio, and %T>MIC for (A) S. aureus 33591 and (B) S. pneumoniae 10183 excluding the q24h dosing regimens. Symbols represent the change from baseline in log_{10} CFU per thigh for each mouse at 24 h. The horizontal lines represent no net change from baseline.
Figure 2. Relationship between the change from baseline in log_{10} CFU and gepotidacin free-drug plasma AUC:MIC ratio for (A) six *S. aureus* isolates and (B) six *S. pneumoniae* isolates, with fitted functions based on the Hill-type models overlaid on the observed data. The r^2 values for these relationships were both 0.925. The horizontal lines represent no net change from baseline. The observed data for each isolate is represented by a different symbol with a fitted function overlaid.
Figure 3. Relationships between log_{10} CFU and time overlaid with the free-drug plasma concentration profiles for gepotidacin doses evaluated and the associated PAE for (A) *S. aureus* and (B) *S. pneumoniae* in the murine-thigh infection model.
Table 1. Gepotidacin free-drug plasma AUC:MIC ratio targets associated with various levels of bacterial reduction from baseline for *S. aureus* and *S. pneumoniae* derived using the Hill-type model based on data from a neutropenic murine-thigh infection model.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Isolate</th>
<th>Phenotype</th>
<th>MIC (mg/L)</th>
<th>Post-hoc free-drug plasma AUC:MIC ratio estimate (%SEE)</th>
<th>Net bacterial stasis</th>
<th>1-log$_{10}$ CFU reduction</th>
<th>2-log$_{10}$ CFU reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>29213</td>
<td>MSSA</td>
<td>0.5</td>
<td>35.5 (18.4)</td>
<td>103.2 (16.3)</td>
<td>1102 (115.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WCUH29</td>
<td>MRSA</td>
<td>0.5</td>
<td>7.77 (22.2)</td>
<td>56.5 (40.4)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33591</td>
<td>MRSA</td>
<td>1</td>
<td>4.38 (14.3)</td>
<td>13.9 (12.5)</td>
<td>117.2 (50.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B-3</td>
<td>MRSA</td>
<td>1</td>
<td>21.2 (18.3)</td>
<td>61.2 (16.1)</td>
<td>397.1 (53.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25923</td>
<td>MSSA</td>
<td>1</td>
<td>3.97 (21.9)</td>
<td>12.6 (19.9)</td>
<td>103.8 (57.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M709</td>
<td>MRSA</td>
<td>2</td>
<td>19.1 (19.0)</td>
<td>64.7 (16.5)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.4</td>
<td>58.9</td>
<td>257.2</td>
<td></td>
</tr>
</tbody>
</table>

| *S. pneumoniae* | MNO-418 | FQR | 0.125 | 8.30 (22.1) | 18 (22.3) | 54.3 (33.6) |
| | 10813 | PSSP | 0.25 | 35.3 (26.5) | 78.8 (24.8) | 286.1 (53.5) |
| | 503167 | PSSP, FQR | 0.5 | 8.38 (23.2) | 16.6 (22.5) | 38.1 (23.9) |
| | 10127 | PSSP | 0.5 | 7.42 (24) | 17.2 (24.4) | 85.2 (83.5) |
| | 1396 | PISP, macrolide-resistant mef | 0.5 | 1.13 (9.8) | 2.57 (10.4) | 16 (46) |
| | TPS3 | FQR, macrolide-resistant mef | 1 | 4.15 (21.8) | 9.76 (22.24) | 91 (153.7) |
| Median | - | - | - | 7.86 | 16.9 | 69.8 |

a. “–” indicates the level of bacterial reduction was not achieved for the isolate.

MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*; PSSP = penicillin-sensitive *S. pneumoniae*; PISP = penicillin-intermediate *S. pneumoniae*; FQR = fluoroquinolone-resistant.


Table 2. Gepotidacin PK model parameter estimates and %SEE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Plasma and ELF data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>First-order absorption rate</td>
<td>$K_a$ (hr$^{-1}$)</td>
<td>1.44</td>
</tr>
<tr>
<td>Maximum rate of clearance</td>
<td>$V_{max}$ (mg/hr/kg)</td>
<td>3.63</td>
</tr>
<tr>
<td>Apparent volume of the central compartment</td>
<td>$V_c/F$ (L/kg)</td>
<td>0.244</td>
</tr>
<tr>
<td>Apparent distributional clearance 1</td>
<td>$C_l/F$ (L/hr/kg)</td>
<td>1.68</td>
</tr>
<tr>
<td>Apparent volume of the peripheral compartment 1</td>
<td>$V_p/F$ (L/kg)</td>
<td>14.6</td>
</tr>
<tr>
<td>Apparent distributional clearance 2</td>
<td>$C_l/F$ (L/hr/kg)</td>
<td>0.973</td>
</tr>
<tr>
<td>Apparent volume of the peripheral compartment 2</td>
<td>$V_p/F$ (L/kg)</td>
<td>2.88E-03</td>
</tr>
<tr>
<td>Concentration at which there is half-maximal clearance</td>
<td>$K_m$ (mg/L)</td>
<td>4.45E-02</td>
</tr>
<tr>
<td>First-order clearance</td>
<td>$C_l$ (L/hr/kg)</td>
<td>3.12</td>
</tr>
<tr>
<td>Rate constant for distribution into the ELF</td>
<td>$k_{ce}$ (hr$^{-1}$)</td>
<td>7.91</td>
</tr>
<tr>
<td>Rate constant for removal from the ELF</td>
<td>$k_{ec}$ (hr$^{-1}$)</td>
<td>16.0</td>
</tr>
<tr>
<td>Plasma error variance model intercept</td>
<td>$S_dP_{int}$</td>
<td>2.50E-02</td>
</tr>
<tr>
<td>Plasma error variance model slope</td>
<td>$S_dP_{slp}$</td>
<td>0.4277</td>
</tr>
<tr>
<td>ELF error variance model intercept</td>
<td>$S_dE_{int}$</td>
<td>1.75E-02</td>
</tr>
<tr>
<td>ELF error variance model slope</td>
<td>$S_dE_{slp}$</td>
<td>0.121</td>
</tr>
</tbody>
</table>
Table 3. Parameter estimates and associated %SEE for the Hill-type model for S. aureus and S. pneumoniae based on data from a neutropenic murine-thigh infection model\(^a\)

<table>
<thead>
<tr>
<th>Parameter(^b)</th>
<th>Mean Estimate</th>
<th>Inter-isolate variability (%CV) Estimate</th>
<th>%SEE</th>
<th>%SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean %SEE</td>
<td>Estimate %SEE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{con}})</td>
<td>1.56</td>
<td>14.6</td>
<td>13.0</td>
<td>120</td>
</tr>
<tr>
<td>(E_{\text{max}})</td>
<td>3.54</td>
<td>7.58</td>
<td>12.7</td>
<td>81.6</td>
</tr>
<tr>
<td>(H_{\text{ill}})</td>
<td>1.02</td>
<td>1.08</td>
<td>2.53</td>
<td>85.6</td>
</tr>
<tr>
<td>(E_{\text{C50}})</td>
<td>13.9</td>
<td>95.3</td>
<td>40.8</td>
<td>92.4</td>
</tr>
<tr>
<td>(S_{\text{dint}})</td>
<td>0.403</td>
<td>-</td>
<td>5.56</td>
<td>-</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{con}})</td>
<td>2.02</td>
<td>39.7</td>
<td>16.6</td>
<td>61.6</td>
</tr>
<tr>
<td>(E_{\text{max}})</td>
<td>4.49</td>
<td>21.5</td>
<td>9.70</td>
<td>65.1</td>
</tr>
<tr>
<td>(H_{\text{ill}})</td>
<td>1.20</td>
<td>14.6</td>
<td>9.78</td>
<td>62.8</td>
</tr>
<tr>
<td>(E_{\text{C50}})</td>
<td>7.41</td>
<td>134</td>
<td>56.4</td>
<td>60.1</td>
</tr>
<tr>
<td>(S_{\text{dint}})</td>
<td>0.566</td>
<td>-</td>
<td>5.56</td>
<td>-</td>
</tr>
</tbody>
</table>

\(a\). Based on datasets containing 6 S. aureus and 6 S. pneumoniae isolates.

\(b\). \(E_{\text{con}}\)=the change from baseline in log\(_{10}\) CFU at 24 h; \(E_{\text{max}}\)=the maximum change in log\(_{10}\) CFU from \(E_{\text{con}}\); \(E_{\text{C50}}\)=the free-drug plasma AUC:MIC ratio associated with half-maximal effect; \(H_{\text{ill}}\)=the Hill coefficient; and \(S_{\text{dint}}\)=the intercept term for the error variance model.

\(c\). \(E_{\text{C50}}\) was modeled as normally distributed and as such, the arithmetic mean and standard deviation is reported.
Figure 1A:
Change in $\log_{10}$ CFU/Thigh

- Free-drug AUC:MIC ratio
- Free-drug Cmax:MIC ratio
- Free-drug %T > MIC

$r^2 = 0.986$
$r^2 = 0.911$
$r^2 = 0.956$
Figure 1 B

[Graph showing the change in Log_{10} CFU/Thigh as a function of Free-drug AUC:MIC ratio, Free-drug Cmax:MIC ratio, and Free-drug % T > MIC. Each panel includes data points and trend lines with correlation coefficients r² = 0.897, r² = 0.845, and r² = 0.9 respectively.]
Figure 2 A
Figure 2 B
Figure 3 A
Figure 3 B