# *In Vivo* Pharmacodynamic Evaluation of Omadacycline (PTK 0796) against *Streptococcus pneumoniae* in the Murine Pneumonia Model

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**ABSTRACT** Omadacycline is a novel aminomethylcycline antibiotic in clinical development for community-acquired bacterial pneumonia (CABP). We used a neutropenic murine pneumonia infection model to characterize the *in vivo* pharmacodynamic activity of omadacycline against *Streptococcus pneumoniae*. Four strains with various phenotypic resistances to other antimicrobials, including tetracyclines, were utilized. Drug concentration measurements were performed in the plasma and epithelial lining fluid (ELF) after administration of 0.5, 2, 8, and 32 mg/kg. Pharmacokinetic parameters were calculated using a noncompartmental model and were linear over the dose range. Penetration into ELF ranged from 72 to 102%. Omadacycline demonstrated net cidal activity in relation to the initial burden against all four strains. The pharmacokinetic/pharmacodynamic index AUC/MIC correlated well with efficacy ($R^2 = 0.74$). The plasma 24-h static dose AUC/MIC values were 16 to 20 (24-h ELF AUC/MIC of 14 to 18). A 1-$\log_{10}$ kill was achieved at 24-h plasma AUC/MIC values of 6.1 to 180 (24-h ELF AUC/MIC values 6.0 to 200). A 2-$\log_{10}$ kill was achieved at 24-h plasma AUC/MIC values of 19 to 56 (24-h ELF AUC/MIC of 17 to 47). The targets identified in this study in combination with *in vitro* potency and favorable human pharmacokinetics make omadacycline an attractive candidate for further development and study in patients with CABP.

**KEYWORDS** omadacycline, pharmacodynamics, pneumonia

Lower respiratory tract infections are the second leading cause of morbidity and mortality worldwide (1). Despite vaccination efforts, *Streptococcus pneumoniae* continues to be the most common pathogen of community-acquired bacterial pneumonia (CABP) irrespective of age and geographical location (2–4). Morbidity and mortality remain unacceptably high in part due to increasing drug resistance and limited effective antimicrobial options. Indeed, macrolide resistance in *S. pneumoniae* has soared in recent years, rendering macrolides ineffective for many cases of *S. pneumoniae* CABP (5, 6). In addition, fluoroquinolones have recently undergone increased scrutiny regarding the potential risks of side effects or adverse effects during use (7, 8). Therefore, novel antibiotics effective against *S. pneumoniae* are urgently needed.

Omadacycline is a novel aminomethylcycline antibiotic in clinical development for CABP and skin and skin structure infections (9). The goal of our experiments was to characterize the *in vivo* pharmacokinetic/pharmacodynamic (PK/PD) properties of omadacycline. Specifically, we sought to determine (i) the serum and epithelial lining fluid (ELF) pharmacokinetics of omadacycline in the murine model and (ii) the magnitude of

**TABLE 1** Study organisms and omadacycline susceptibility results[a]

| | MIC (mg/liter) | | | |
|---|---|---|---|---|
| S. pneumoniae strain[b] | Omadacycline | Tigecycline | Minocycline | Erythromycin |
| 1293* | 0.0625 | 0.06 | 4 | >8 (Erm) |
| ATCC 10813† | 0.0625 | 0.12 | 0.25 | 0.015 |
| 140† | 0.125 | NA[c] | NA | NA |
| ATCC 49619* | 0.03125 | 0.06 | 0.06 | 0.06 |

[a]Where known, the antimicrobial susceptibility results for other relevant antimicrobial agents are also listed.
[b]*, Penicillin resistant; †, penicillin susceptible.
[c]NA, not available.

the PK/PD parameter AUC/MIC required for efficacy against *S. pneumoniae* in the murine neutropenic pneumonia model.

## RESULTS

*In vitro* **susceptibility studies.** The MICs of omadacycline for the selected strains are listed in Table 1. Also shown are MICs to other relevant antimicrobial agents (when known). The four organisms varied in MIC to omadacycline by only 4-fold despite phenotypic variation in susceptibility to other antimicrobials such as minocycline and tigecycline.

**Pharmacokinetics.** The time course of plasma concentrations of omadacycline in mice after subcutaneous doses of 0.5, 2, 8, and 32 mg/kg are shown in Fig. 1. Over the dose range, the pharmacokinetics were relatively linear (AUC $R^2$ = 1.00, $C_{max}$ $R^2$ = 0.97). Peak levels ranged from 0.11 to 2.19 mg/liter. $AUC_{0-\infty}$ values ranged from 0.58 to 23.12 mg·h/liter. The elimination half-life ranged from 2.8 to 6.3 h.

ELF concentrations were determined from BAL fluid concentrations utilizing the urea correction methodology described in methods above. Pharmacokinetic analysis of the data is presented in Fig. 2. Over the dose range once again the pharmacokinetics were linear (AUC $R^2$ = 0.99, $C_{max}$ $R^2$ = 0.99). Peak levels ranged from 0.10 to 3.65 mg/liter. $AUC_{0-\infty}$ values ranged from 0.47 to 16.76 mg·h/liter. The elimination half-life ranged from 2.4 to 3.9 h. The penetration of omadacycline into ELF relative to plasma drug concentrations was evaluated for each dose. Over the dose range, the percent penetrations based on AUC exposure were 80% at 0.5 mg/kg, 94% at 2 mg/kg, 102% at 8 mg/kg, and 72% at 32 mg/kg.
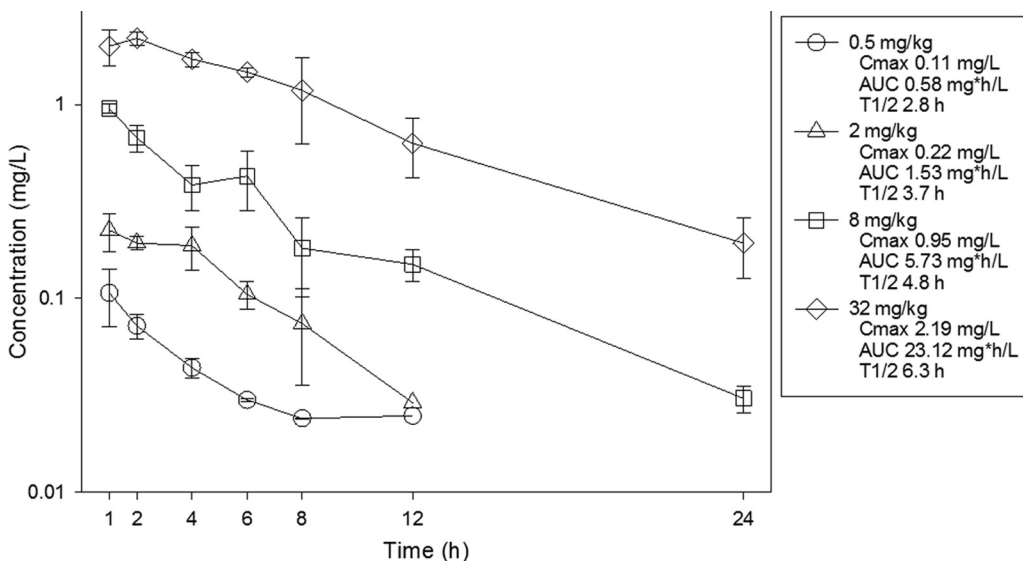


**FIG 1** Plasma concentrations of omadacycline in mice following single subcutaneous doses. Samples were obtained at seven time points over 24 h. Each symbol represents the mean and standard deviation from three mice. $C_{max}$, peak concentration; $t_{1/2}$, beta elimination half-life. The AUC is from 0 to infinity.
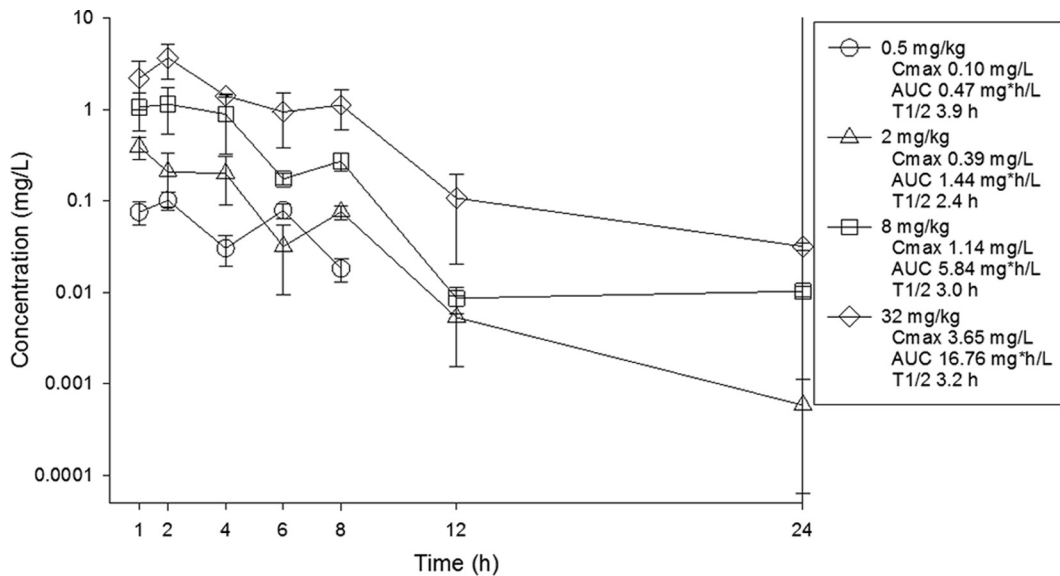
**FIG 2** ELF concentrations of omadacycline in mice following single subcutaneous doses. Samples were obtained at seven time points over 24 h. ELF concentrations were determined using urea concentration correction methods. Each symbol represents the mean and standard deviation from three mice. $C_{max}$, peak concentration; $t_{1/2}$, beta elimination half-life. The AUC is from 0 to infinity.

**Relationship between PK/PD parameter AUC/MIC and efficacy.** At the start of therapy mice had $10^{6.3 \pm 0.3}$ $\log_{10}$ CFU/lungs, and this increased to $10^{7.8 \pm 0.6}$ $\log_{10}$ CFU/lungs. The growth in untreated controls for each strain are shown in Table 2. The *in vivo* dose-response curves for all four strains are shown in Fig. 3. Omadacycline was quite potent over the dose range studied. Bactericidal activity was noted at all doses for two strains (140 and ATCC 49619), and a 1-log kill was achieved for all four strains over the dose range. A $\geq$3-log kill was achieved with three of four strains. The relationship between the $\log_{10}$ CFU in lungs and the 24-h plasma AUC/MIC ratio are illustrated in Fig. 4. The relationship between plasma 24-h AUC/MIC and treatment effect was relatively robust, with an $R^2$ of 0.74. Also shown in the figure is the maximum effect ($E_{max}$), 50% maximal effect point ($ED_{50}$), and the slope ($N$) of the best fit line based on the sigmoid (Hill) $E_{max}$ model. Results for the same PK/PD analysis for 24-h AUC/MIC are shown in Fig. 5 using ELF pharmacokinetic data.

**AUC/MIC magnitude associated with stasis and kill endpoints.** The doses necessary to produce a bacteriostatic effect and a 1-log and 2-log kill are shown in Table 2. The corresponding 24-h AUC/MIC for these doses are also presented utilizing both plasma and ELF pharmacokinetic data. The static doses were 0.92 mg/kg/24 h and 1.28 mg/kg/24 h against the two strains of *S. pneumoniae* for which this endpoint was achieved. The corresponding plasma 24 h AUC/MIC values were 16 and 20. The corresponding ELF 24-h AUC/MIC values were 14 and 18. A 1-$\log_{10}$ kill was achieved for all *S. pneumoniae* strains with 24-h dose range of 0.45 to 18.2 mg/kg. The corresponding plasma and ELF AUC/MIC values were 6.1 to 180 and 6.0 to 200, respectively. The

**TABLE 2** 24-h static and 1-log and 2-log kill doses and associated AUC/MIC values for each strain in the murine pneumonia model

| *S. pneumoniae* strain | 24-h growth in untreated control animals ($\log_{10}$ CFU/lungs) | MIC (mg/liter) | Stasis | | | 1-$\log_{10}$ kill | | | 2-$\log_{10}$ kill | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 24-h total dose (mg/kg) | Plasma AUC/MIC | ELF AUC/MIC | 24-h total dose (mg/kg) | Plasma AUC/MIC | ELF AUC/MIC | 24-h total dose (mg/kg) | Plasma AUC/MIC | ELF AUC/MIC |
| 1293 | 2.34 | 0.06 | 1.28 | 19.83 | 17.80 | 18.24 | 179.98 | 200.64 | NA[a] | | |
| 10813 | 1.64 | 0.06 | 0.92 | 15.79 | 14.18 | 1.26 | 19.66 | 17.61 | 1.81 | 25.05 | 23.19 |
| 140 | 1.13 | 0.125 | NC[b] | | | 0.71 | 6.06 | 6.00 | 3.06 | 18.65 | 17.26 |
| 49619 | 0.85 | 0.03 | NC | | | 0.45 | 15.21 | 13.31 | 2.12 | 56.20 | 47.27 |

[a]NA, endpoint not achieved.
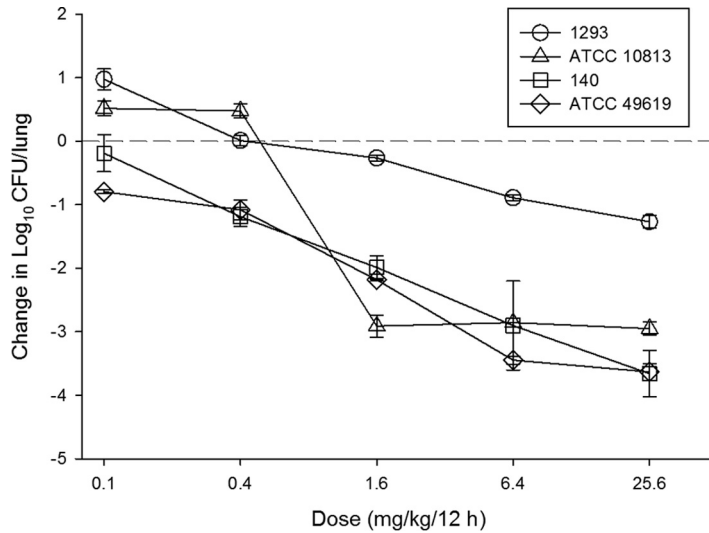[b]NC, not calculated.

**FIG 3** *In vivo* dose-response curves for omadacycline against select *S. pneumoniae* strains using a neutropenic murine pneumonia model. Each symbol represents the mean and standard deviation from three mice. Five total drug dose levels were administered by the subcutaneous route every 12 h. The burden of organisms was measured at the start and end of therapy. The study period was 24 h. The horizontal dashed-line at 0 represents the burden of organisms in the lungs of mice at the start of therapy. Data points below the line represent cidal activity and points above the line represent net growth.

relatively larger range for 1 log kill targets was driven by a single isolate (strain 1293) in which the target increased to a much larger degree when comparing stasis and 1-log kill AUC/MIC targets. The reason for this is not completely clear, but it could be due to inherent variability in the model and/or strain variability. Future studies utilizing more isolates would test whether this is an uncommon outlier. A 2-$\log_{10}$ kill was achieved for three strains with a 24-h dose range of 1.8 to 3.1 mg/kg. The corresponding plasma and
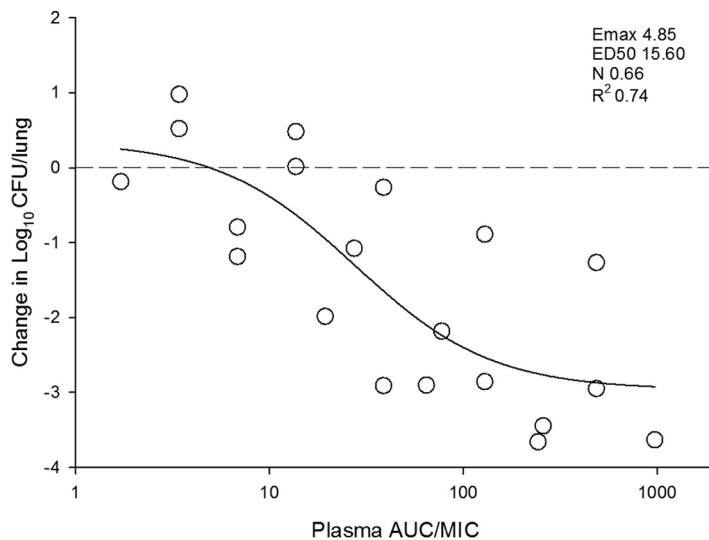


**FIG 4** *In vivo* dose effect of omadacycline against select *S. pneumoniae* strains using a neutropenic murine pneumonia model. Each symbol represents the mean result from three mice. Five total drug dose levels were fractionated into an every-12-h regimen. The omadacycline exposure is expressed as the plasma 24 h AUC/MIC. The burden of organisms was measured at the start and end of therapy. The study period was 24 h. The horizontal dashed line at 0 represents the burden of organisms in the lungs of mice at the start of therapy. Data points below the line represent cidal activity and points above the line represent net growth. The $R^2$ represents the coefficient of determination. The line drawn through the data points is the best-fit line based upon the sigmoid $E_{max}$ formula.
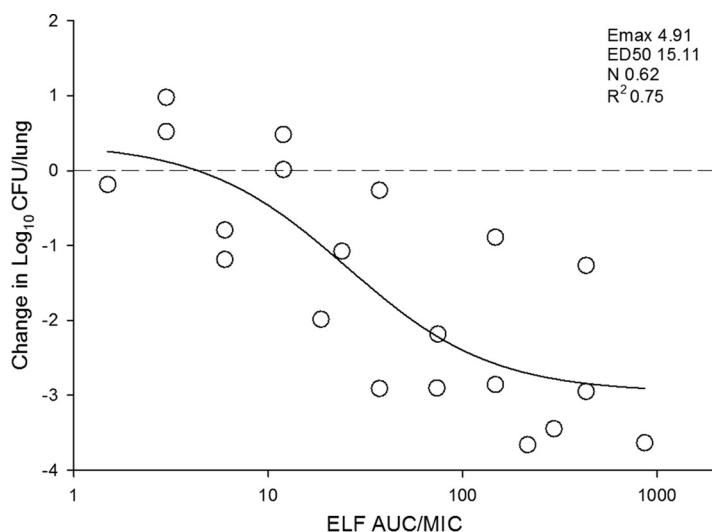
**FIG 5** *In vivo* dose effect of omadacycline against select *S. pneumoniae* strains using a neutropenic murine pneumonia model. Each symbol represents the mean result from three mice. Five total drug dose levels were fractionated into an every-12-h regimen. The omadacycline exposure is expressed as the ELF 24-h AUC/MIC. The burden of organisms was measured at the start and end of therapy. The study period was 24 h. The horizontal dashed line at 0 represents the burden of organisms in the lungs of mice at the start of therapy. Data points below the line represent cidal activity, and points above the line represent net growth. The $R^2$ represents the coefficient of determination. The line drawn through the data points is the best-fit line based upon the sigmoid $E_{max}$ formula.

ELF 24-h AUC/MIC values were 19 to 56 and 17 to 47, respectively. The AUC/MIC values are relatively similar given the relative penetration of drug into ELF ranged from 72 to 102% over the dose range.

## DISCUSSION

The tetracycline class of antibiotics has been around for more than 70 years; however, there has been growing antibacterial resistance, especially in recent years. Advances in synthetic chemistry has created renewed interest in derivatives of tetracycline that maintain antimicrobial activity despite acquired tetracycline resistance mechanisms such as efflux pumps and ribosomal protection. This has been successfully applied in the generation of glycylcyclines, fluorocyclines, and aminomethylcyclines, such as omadacycline which was the focus of these studies. Omadacycline is a novel, first-in-class aminomethylcycline antibiotic in development for the treatment of CABP and skin and skin structure infection. Omadacycline is a broad-spectrum agent with potency against a variety of pathogens, including gram-positives (including organisms such as methicillin-resistant *Staphylococcus aureus*, which is resistant to beta-lactams, and *S. pneumoniae*, which is resistant to macrolides), gram-negatives, anaerobes, and atypical pathogens (9–11). Importantly, omadacycline maintains excellent activity against organisms that have acquired resistance to older tetracyclines (tetracycline, minocycline, and doxycycline) (12, 13).

Pharmacodynamic assessment of antimicrobial efficacy is a critical step in drug development to determine the optimal dosing strategy for clinical studies as well as set preliminary susceptibility breakpoints. We present here the results of a pharmacodynamic assessment of omadacycline activity in a preclinical animal model of *S. pneumoniae* pneumonia. Omadacycline demonstrated *in vitro* and *in vivo* potency against a select group of *S. pneumoniae* strains, including those resistant to other antibiotics such as beta-lactams, macrolides, and earlier generations of the tetracycline class. *In vivo* we observed potent bactericidal activity for all four strains with a ≥3-log$_{10}$ kill in three of four strains tested. We also examined the drug exposures associated with efficacy in terms of plasma pharmacokinetic and ELF pharmacokinetic exposures. Previous unpublished data had suggested favorable pharmacokinetics in lung penetration, and we

affirmed these preliminary results. Almost 100% of the drug in plasma penetrated into the ELF compartment based on drug concentration measurements in both compartments. This translated into similar PK/PD targets when one compared plasma to ELF AUC/MIC targets for stasis or bactericidal endpoints. In addition, the exposure response curves were quite steep, such that small increases in drug exposure led to increasing cidal activity.

There is a paucity of pharmacodynamic studies for the tetracycline class, including new synthetic congeners, to provide a comparison to the current studies. Previous studies have demonstrated tetracyclines exhibit time dependent activity with prolonged postantibiotic effects (14–16). Therefore, the predictive PK/PD index has often been found to be AUC/MIC. This has been demonstrated in dose fractionation studies with two other synthetic tetracycline derivatives: tigecycline (16) and eravacycline (unpublished data). Pharmacodynamic studies evaluating the PK/PD target exposures in *in vivo* animal model studies are even more sparse. Utilizing four *S. pneumoniae* strains in a murine neutropenic thigh model, Christianson et al. demonstrated that a 24-h free-drug AUC/MIC target of 24 for doxycycline was associated with net stasis and that a value of 120 was associated with a 2-$\log_{10}$ kill (17). Tigecycline PK/PD studies have also demonstrated the importance of AUC/MIC as the PK/PD driver of efficacy with net stasis targets that have ranged from 2 to 5 for a variety of pathogens (18–20). The AUC/MIC exposures associated with efficacy in the current preclinical model for omadacycline are similar to other tetracycline class antibiotic PK/PD studies. Importantly, in the case of tigecycline, clinical PK/PD analyses in patients with skin and skin structure infection and community-acquired pneumonia have confirmed the relevance of the PK/PD targets identified in preclinical models on treatment outcome (21, 22).

Human pharmacokinetic studies have evaluated the plasma pharmacokinetics of omadacycline after 100-mg intravenous and 300-mg oral tablet administration (11). The $AUC_{0-\infty}$ is nearly identical at 10.0 and 10.3 mg·h/liter, respectively. In 2014, surveillance antimicrobial susceptibility results for omadacycline against *S. pneumoniae* (>1,800 strains) demonstrated an $MIC_{90}$ of 0.06 mg/liter (range, 0.015 to 0.12 mg/liter) (11). Likewise, in 2015 the *in vitro* activity of omadacycline was examined against tetracycline-resistant strains and demonstrated an $MIC_{90}$ of 0.25 mg/liter (range, 0.015 to 0.25 mg/liter) (11). Utilizing the median AUC/MIC drug exposure targets identified in this study for stasis and kill endpoints, the AUC exposures in humans using the dosing regimens described above would be expected to produce efficacy against most *S. pneumoniae* strains, including those with tetracycline resistance. Given the favorable PK, including low protein binding and penetration into ELF, the *in vitro* potency that included tetracycline-resistant *S. pneumoniae* strains, and the *in vivo* efficacy observed in our animal model study, omadacycline is a promising novel agent for community-acquired pneumonia due to *S. pneumoniae*. These studies should prove beneficial in optimizing clinical dosing regimen design for CABP and setting preliminary susceptibility breakpoints.

## MATERIALS AND METHODS

**Organisms, media, and antibiotic.** Four *S. pneumoniae* strains were used in the studies and are listed in Table 1. Unless specified by American Type Culture Collection (ATCC) labeling, the strains are clinical isolates from invasive infections. Strains were chosen that varied in phenotypic resistance patterns to a number of relevant antimicrobials, including penicillin, minocycline, tigecycline, and erythromycin. All organisms were grown, subcultured, and quantified using sheep blood agar (Remel, Milwaukee, WI). The drug compounds used for *in vitro* and *in vivo* studies were supplied by Paratek Pharmaceuticals (Boston, MA).

***In vitro* susceptibility studies.** The MICs of each compound for the various strains were determined using Clinical and Laboratory Standards Institute microdilution methods (23). All MIC assays were performed in duplicate on three separate occasions. The median MIC of replicate assays is reported and utilized in PK/PD analyses.

**Murine model.** Animals were maintained in accordance with American Association for Accreditation of Laboratory Animal Care criteria (24). All animal studies were approved by the Animal Research Committees of the William S. Middleton Memorial VA Hospital and the University of Wisconsin. Six-week-old, specific-pathogen-free, female ICR/Swiss mice weighing 24 to 27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, IN). Mice were rendered neutropenic (neutrophils < 100/mm³) by

injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) intraperitoneally 4 days (150 mg/kg) and 1 day (100 mg/kg) before lung infection. *S. pneumoniae* strains were grown overnight on sheep blood agar. A sterile loop was then used to transfer organism to sterile saline and absorbance adjusted to 0.3 at 580 nm using a Spectronic 88 spectrophotometer (Bausch and Lomb, Rochester, NY). After a 1:10 dilution, bacterial counts of the inoculum ranged from $10^{8.4-9.1}$ CFU/ml. Lung infections with each of the strains were produced by the administration of 50 $\mu$l of inoculum into the nares of isoflurane-anesthetized mice. Mice were then held upright to allow for aspiration into the lungs. Therapy with omadacycline was initiated 2 h after induction of infection. No treatment controls and zero-hour controls were included in all experiments. After 24 h, organism burden was quantified by CFU counts from whole-organ homogenates.

**Drug pharmacokinetics.** Single dose plasma pharmacokinetics of omadacycline were performed in mice. Dose levels of 0.5, 2, 8, and 32 mg/kg were administered subcutaneously. Groups of three mice were sampled for drug concentration determination at 1, 2, 4, 6, 8, 12, and 24 h. Both plasma and bronchoalveolar lavage (BAL) fluid was obtained for pharmacokinetic analysis. Plasma was obtained from each animal by centrifugation of anticoagulated blood obtained by cardiac puncture. BAL fluid was obtained by instillation of 1 ml of sterile saline into the lungs of each animal, followed by immediate removal. The BAL fluid was centrifuged to remove blood and cellular debris, and the supernatant was collected. Plasma and BAL supernatant was stored at $-70°C$. All drug concentrations were determined by liquid chromatography-tandem mass spectrometry methods by the sponsor. ELF concentrations were calculated from BAL fluid concentrations by urea correction methodology (25) according to the following formula: $[drug]_{ELF} = [drug]_{BAL} \times ([urea]_{plasma}/[urea]_{BAL})$.

Pharmacokinetic parameters (mean $\pm$ the standard deviation), including the elimination half-life ($t_{1/2}$), $AUC_{0-\infty}$, and $C_{max}$, were calculated using a noncompartmental model using mean concentration values from each group of mice. The half-life was determined by linear least-squares regression. The AUC was calculated from the mean concentrations using the trapezoidal rule. Pharmacokinetic estimates for dose levels that were not directly measured were calculated using linear interpolation for dose levels between those with measured kinetics and linear extrapolation for dose levels above or below the highest and lowest dose levels with kinetic measurements. Protein binding of omadacycline is very low, and therefore total drug concentrations were utilized in all PK/PD calculations (26).

**Relationship between PK/PD parameter AUC/MIC and efficacy.** AUC/MIC was chosen as the pharmacodynamic parameter for omadacycline based on previous studies demonstrating this PK/PD index to be predictive of treatment efficacy for the tetracycline class (14–16). *In vivo* treatment studies were performed in the murine pneumonia model for each strain. Groups of three mice per dosing regimen and control group were utilized. Dose-response studies consisted of 4-fold increasing doses (range, 0.1 to 25.6 mg/kg/12 h) administered subcutaneously. The dose-response effect was determined as described above by measurement of CFU in lung homogenates. The correlation between efficacy and the PK/PD parameter AUC/MIC was determined by nonlinear least-squares multivariate regression (SigmaPlot version 12.3; Systat Software, San Jose, CA). The mathematical model used was derived from the Hill equation $E = (E_{max} \times AUC/MIC^N)/(ED_{50}^N - AUC/MIC^N)$, where $E$ is the effector, in this case, the log change in CFU per lung between treated mice and untreated controls after the 24-h period of study, $E_{max}$ is the maximum effect, $D$ is the 24-h total AUC/MIC, $ED_{50}$ is the AUC/MIC required to achieve 50% of the $E_{max}$, and $N$ is the slope of the dose-effect curve. The values for the indices $E_{max}$, $ED_{50}$, and $N$ were calculated using nonlinear least-squares regression. The coefficient of determination ($R^2$) was used to estimate the variance that might be due to regression with the PK/PD parameter AUC/MIC.

**AUC/MIC magnitude associated with stasis and kill endpoints.** Using the Sigmoid $E_{max}$ model described above, the dose required to produce net static effect (static dose) and the 1- and 2-$\log_{10}$ kill compared to the start of therapy were calculated for each drug-organism combination. The plasma and ELF pharmacokinetic results were then used to estimate the AUC/MIC exposure associated with each of the endpoints for each organism. The associated 24-h total drug AUC/MIC targets were calculated.

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