Potency of omadacycline against *Mycobacteroides abscessus* clinical isolates *in vitro* and in a mouse model of pulmonary infection

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The incidence of nontuberculous mycobacterial diseases in the US is rising and has surpassed tuberculosis. Most notable among the nontuberculous mycobacteria is *Mycobacteroides abscessus*, an emerging environmental opportunistic pathogen capable of causing chronic infections. *M. abscessus* disease is difficult to treat and the current treatment recommendations include repurposed antibiotics, several of which are associated with undesirable side effects. In this study, we have evaluated the activity of omadacycline, a new tetracycline derivative, against *M. abscessus* using *in vitro* and *in vivo* approaches. Omadacycline exhibited an MIC$_{90}$ of 0.5 µg/ml against a panel of 32 contemporary *M. abscessus* clinical isolates several of which were resistant to antibiotics that are commonly used for treatment of *M. abscessus* disease. Omadacycline when combined with clarithromycin, azithromycin, cefdinir, rifabutin or linezolid also exhibited synergy against several *M. abscessus* strains and did not exhibit antagonism when combined with an additional nine antibiotics also commonly considered to treat *M. abscessus* disease. Concentration-dependent activity of omadacycline was observed in time-kill assessments. Efficacy of omadacycline was evaluated in a mouse model of lung infection against four *M. abscessus* strains. A dose equivalent to the 300 mg standard oral human dose was used. Compared to the untreated control group, within four weeks of treatment, 1 to 3 log$_{10}$ fewer *M. abscessus* colony forming units were observed in the lungs of mice treated with omadacycline. Treatment outcome was biphasic, with bactericidal activity observed after the first two weeks of treatment against all four *M. abscessus* strains.
**INTRODUCTION**

*Mycobacteroides abscessus* (formerly *Mycobacterium abscessus*) (1) is an environmental non-tuberculous mycobacteria (NTM) and causative pathogen of pulmonary and soft tissue infections, among others. This organism has been described as a “clinical and antibiotic nightmare” (2, 3) as it demonstrates intrinsic resistance to a wide range of antibiotic classes (4, 5). As only a few oral antibiotics show activity against *M. abscessus*, the current treatment regimen, which requires regular and frequent administration of intravenous drugs is a logistical challenge for patients. Acquired resistance to important antibiotic classes, particularly macrolides and aminoglycosides has further limited therapeutic options. Therefore, *M. abscessus* disease treatment is challenging and there are currently no FDA approved treatment agents or regimens for this indication. Current treatment options include repurposed antibiotics and regimens developed on the basis of empirical evidence and consensus from experts (6–10). Cure rates using antibiotics alone are as low as 25-40% (11, 12).

*M. abscessus* is one of the most frequently recovered NTM from patients, comprising ~10% of all NTM pulmonary infections (13). It was the second most common NTM group identified in a large registry of bronchiectasis patients in the US (14). Further, a steady increase in the incidence of *M. abscessus* was observed in UK clinics over 2000-2013 (15). A recent study of a cystic fibrosis patient cohort in the US examined 341 NTM strains and reported that *M. abscessus* comprised the majority of the isolates (16). These results suggest that the incidence of *M. abscessus* is increasing and so too will the need for new treatments that are effective options against this emergent disease. Additionally, many patients with chronic *M. abscessus* disease that are refractory to prescribed treatment are likely to harbor strains resistant to antibiotics that comprise their treatment regimen (17). An ideal new treatment would also be effective against these antibiotic-resistant strains.

Omadacycline is an aminomethylcycline, which is a semisynthetic derivative of the tetracycline class (18). It is a broad-spectrum antibiotic (19) that received FDA approval in 2018 for...
treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia, and is available in intravenous and oral formulations (20). Tigecycline is another tetracycline that is included in the recommendation for treating *M. abscessus* disease and often used in combination with other antibiotics (8, 10). Tigecycline and omadacycline exhibit similar *in vitro* activity against a range of clinical *M. abscessus* isolates (21–23). Additional studies that have demonstrated potent *in vitro* activity of omadacycline against NTMs including *M. abscessus* (24, 25) have generated further interest in this antibiotic for treating *M. abscessus* disease. Some advantages of omadacycline compared to tigecycline include: a) omadacycline is available in both intravenous and oral formulations making administration simpler for patients and allowing for oral therapy, b) omadacycline has an elevated and sustained concentration in epithelial lining fluid, alveolar cells and plasma as well as improved pulmonary PK compared to tigecycline (26), and c) omadacycline may have better tolerability than tigecycline (25). In addition, in a recent preliminary, real-world multi-center study, clinical success has been demonstrated with regimens containing omadacycline to treat *M. abscessus* infections in the majority of patients (27).

In clinical trials for treatment of acute bacterial infections of the skin and skin structures, and community acquired bacterial pneumonia, omadacycline was found to be non-inferior and tolerable compared to standard of care treatments (28–31). In pre-clinical studies, omadacycline demonstrated dose-dependent activity against *Streptococcus pneumoniae* in a murine pneumonia model (32) and *Staphylococcus aureus* in neutropenic murine pneumonia and thigh infection models (33, 34). Based on the demonstrated efficacy and tolerability in treating pneumonia in clinical trials, efficacy in murine pneumonia models and *in vitro* activity against *M. abscessus*, we asked whether omadacycline has efficacy in treating *M. abscessus* lung disease. Here, using a collection of distinct contemporary *M. abscessus* clinical isolates we evaluated the activity of omadacycline *in vitro* and *in vivo* and compared it to standard of care where applicable. Our studies included determination of minimum inhibitory concentration (MIC), time-
kill analysis, in vitro activity in combination with other antibiotics, and efficacy in a mouse model of pulmonary M. abscessus infection (35).

RESULTS

MICs of omadacycline against clinical isolates of M. abscessus: We initiated this study by determining the MICs of omadacycline and other antibiotics that have shown in vitro activity in prior studies, some of which are included in the current treatment recommendations for M. abscessus infection in humans (6–8, 10). These include tigecycline, amikacin, clarithromycin, azithromycin, linezolid, moxifloxacin, rifabutin, vancomycin, teicoplanin, doripenem, imipenem, cefoxitin, cefdinir, ceftazidime and amoxicillin, and those that have shown promise in recent studies such as clofazimine and bedaquiline (17, 36–45). These antibiotics represent a broad spectrum of antibiotic classes including tetracycline, aminoglycoside, macrolide, fluoroquinolone, rifamycin, glycopeptide, β-lactam, phenazine, and diarylquinoline.

Thirty-one independent M. abscessus clinical strains were included. The majority of strains (29 of 31) were isolated from cystic fibrosis and bronchiectasis patients (17) and two strains were from gastrointestinal infections (46). In addition, American Type Culture Collection (ATCC) 19977, a strain that has been historically designated as the reference M. abscessus strain and is widely used by laboratories studying this organism (47), was also included. Of the 32 M. abscessus strains, 17 belonged to subspecies abscessus and 10 were massiliense. Subspecies determination for the remaining 5 strains has not been completed. Subspeciation was undertaken using whole genome sequencing as described (48). For MIC determination, in addition to cation-adjusted Mueller Hinton broth (CAMHB) that is recommended by the Clinical and Laboratory Standards Institute (CLSI) (49), we also used Middlebrook 7H9 broth to assess if there were any distinct differences in MIC values obtained with the different media types.
The MICs of each drug were determined in duplicate against each strain in CAMHB (Table 1) and Middlebrook 7H9 broth (Supplementary Table 1); if two different MIC values were obtained, an average MIC value was calculated (e.g., an independent MIC of 0.5 and 1 µg/ml were averaged to 0.75 µg/ml). The omadacycline MIC values in CAMHB ranged from 0.06 to 1 µg/ml with MIC$_{50}$ and MIC$_{90}$ values of 0.25 µg/ml and 0.5 µg/ml against the 32 strains tested, respectively. The median omadacycline MIC value was 0.25 µg/ml for both subspecies abscessus and massiliense. In Middlebrook 7H9 broth, omadacycline MIC values ranged from 0.25 µg/ml to 2 µg/ml with MIC$_{50}$ and MIC$_{90}$ values of 0.5 µg/mL and 1.0 µg/ml, respectively. The median omadacycline MIC value was 0.5 and 0.75 µg/ml for abscessus and massiliense, respectively.

The MICs of certain antibiotics against M. abscessus differed between the two broths. MIC values of omadacycline, clarithromycin, azithromycin, cefoxitin, cefdinir, linezolid, clofazimine and rifabutin consistently trended lower for a majority of M. abscessus strains in CAMHB broth compared to MIC values in Middlebrook 7H9 broth. For example, of the 32 isolates tested, 78% (25 of 32) had omadacycline MIC values ≥2-fold higher in Middlebrook 7H9 broth compared with CAMHB. While a 2-fold variation is typically within the standard acceptable variability of an MIC assay, this trend should be reported. Further, 31% (10 of 32) of isolates tested indeed had omadacycline MIC values ≥4-fold higher in Middlebrook 7H9 broth compared with CAMHB, which is considered meaningful. Other antibiotics were even more strikingly different, for example, 100% (32 of 32) of azithromycin MIC values were ≥2-fold higher in Middlebrook 7H9 broth compared with CAMHB and of these, 75% (24 of 32) were ≥4-fold higher, and 66% (21 of 32) were ≥8-fold higher. On the other hand, the MIC values of imipenem consistently trended higher in CAMHB broth compared to Middlebrook 7H9 broth, where MIC values were ≥2-fold for 68% (19 of 32) of the M. abscessus strains (of note, only 9%, 3 of 32, differed by ≥4-fold).

These data highlight the need for the utilization of consistent and appropriate media for
susceptibility testing and therefore, CAMHB was utilized for other in vitro testing in this study in accordance with CLSI guidelines.

Omadacycline is active in vitro against M. abscessus clinical isolates that are resistant to currently used antibiotics: Several strains included in this study were resistant to a range of antibiotics based on CLSI breakpoints (highlighted in Table 1). The collection of M. abscessus strains included in our study (n=32) were randomly selected without prior knowledge of MIC values to omadacycline and the MIC results showed that 100% were inhibited by ≤1 µg/mL of omadacycline when tested according to CLSI methodology, confirming its potent in vitro activity. The MIC of omadacycline was consistently within one dilution of its median MIC (0.25 µg/mL in CAMHB) against strains that displayed a high level of resistance to antibiotics that are frequently used to treat M. abscessus disease such as amikacin, clarithromycin, azithromycin, cefoxitin and imipenem. The MIC of omadacycline was also within one dilution of its median MIC against strains that were resistant to antibiotics less commonly used to treat M. abscessus disease, such as moxifloxacin, doripenem and linezolid.

Omadacycline in combination with clarithromycin, azithromycin, cefdinir, linezolid or rifabutin exhibits synergy in vitro against a subset of clinical M. abscessus isolates: Using a checkerboard assay (50), we assessed if omadacycline acts with synergy, indifference or antagonism when combined with antibiotics currently used to treat M. abscessus disease. For this assessment, omadacycline in combination with 14 different antibiotics from a wide range of classes were tested against 10 M. abscessus clinical isolates and the reference strain ATCC 19977 (Figure 1). The most stringent interpretation of combined activity of the antibiotics (51) was used. Omadacycline in combination with clarithromycin exhibited synergy against 6 of the 11 (55%) strains tested as indicated by the Fractional Inhibitory Concentration Index (FICI) ≤0.5. Omadacycline also exhibited synergy in combination with azithromycin, cefdinir or linezolid against four of the tested strains (36%) as well as with rifabutin against three strains (27%). Omadacycline in combination with amikacin, imipenem, doripenem, ceftazidime or amoxicillin
did not exhibit synergy against any of the 11 *M. abscessus* strains. We did not observe antagonistic activity of omadacycline when combined with any of the antibiotics tested. Strain-specific synergy trends were not observed (Figure 1). We also determined that when omadacycline was combined with clarithromycin, azithromycin, cefdinir or linezolid, the combination often reduced the MIC values of these antibiotics against *M. abscessus* strain from intermediate/resistant to susceptible (Supplementary Table 3), according to CLSI interpretive criteria (Supplementary Table 2). For instance, the MIC of clarithromycin when assessed alone against isolate M9501 was 4.0 µg/ml (Table 1) and would be considered intermediate according to CLSI guidelines (Supplementary Table 2). In combination with omadacycline, the MIC of clarithromycin was 0.5 µg/ml (Supplementary Table 3) as this is the lowest concentration at which growth of M9501 could not be observed irrespective of omadacycline concentration (Supplementary Table 4).

**Time-kill activity of omadacycline against *M. abscessus***: MIC and FICI assays only provide one time-point on the inhibitory activity of an antibiotic on bacterial growth. To generate insight into the activity of omadacycline over time, we undertook time-kill assessments against ATCC 19977 and four randomly selected *M. abscessus* clinical isolates. Each strain was exposed to a single dose of omadacycline at 0.5x, 1x, 2x, 4x, 8x and 16x the MIC specific to each strain. As a control, each strain was also assessed in the absence of antibiotic. Omadacycline exhibited concentration-dependent activity, as demonstrated during the first 24 hours of exposure, where omadacycline at 1X MIC and increasing fold concentrations above the MIC reduced CFU levels of all isolates (Figure 2). Time-dependent activity was also observed against all isolates where further reductions in CFU occurred until 3 days when the isolates were exposed to 8x MIC or higher concentrations of omadacycline. However, at lower concentrations, *M. abscessus* strains were able to recover after three days of exposure and exhibited sustained growth. CFU levels at 3 and 7 days post-exposure correlated inversely with the concentration of omadacycline.
In addition to omadacycline alone, we also undertook time-kill analysis of omadacycline in combination with clarithromycin, azithromycin, cefdinir, linezolid and rifabutin, as they exhibited synergy against some *M. abscessus* strains in checkerboard assays. Amikacin was also included as it is one of the most frequently utilized antibiotics to treat *M. abscessus* disease. For antibiotic pairs, 0.25x, 0.5x and 1x the respective MICs of omadacycline and each antibiotic were evaluated in combination against the same five *M. abscessus* isolates as above. Variable bactericidal activity among different clinical isolates for the same antibiotic combination was observed. For instance, when omadacycline and rifabutin were combined (*Figure 3*), bactericidal activity was observed at 1x MIC of each antibiotic against the reference strain ATCC 19977 and M9510 for up to three days, but growth of these isolates recovered thereafter. For M9529 and M9530, this same antibiotic combination at 1x MIC was bactericidal throughout and resulted in no detectable CFU at 7 days post exposure. Based on reduction in CFU, of the six antibiotic pairs evaluated, the combination of omadacycline and rifabutin exhibited the most potent time-kill activity profile against the greatest number of isolates. The combinations of omadacycline and clarithromycin, azithromycin, cefdinir, linezolid or amikacin produced reduction in the CFU of most isolates during the first 24 hours of exposure (*Supplementary Figure 1-5*). Beyond 24 hours, each drug produced variable change in the CFU of the five isolates. As the objective of this assessment was to compare activities of combinations of omadacycline and a companion antibiotic at different concentrations over a defined duration against *M. abscessus* isolates, single antibiotic comparators were not included. Therefore, the *M. abscessus* CFU changes reflect the net activities of two-drug combinations as specified in the assessments.

**Pharmacokinetics of omadacycline in C3HeB/FeJ and BALB/c mice:** To identify the omadacycline dose that best represents the human equivalent exposure or area under the curve (AUC\(_{0-24h}\)) of the human 300 mg oral dose (20), we determined pharmacokinetic (PK) parameters of omadacycline in C3HeB/FeJ and BALB/c mice. The human steady state AUC\(_{0-24h}\)
for the 300 mg dose is 11.156 hr*µg/mL for total drug and 8.92 hr*µg/mL for free drug based on
20% plasma protein binding in humans (NUZYRA® prescribing information). The C3HeB/FeJ
mouse strain PK parameters were determined because this mouse strain was utilized to
evaluate omadacycline efficacy in an *M. abscessus* pulmonary infection model (35). PK
parameters were also determined in the BALB/c mouse strain as this strain has also been
considered for studying *M. abscessus* infection (46, 52). Additionally, the BALB/c strain is used
in *in vivo* efficacy studies against related non-tuberculous mycobacteria such as *M. avium* (53),
*M. ulcerans* (54) and also *M. tuberculosis* (55, 56). Further, BALB/c mouse plasma is
commercially available to facilitate determination of the free and bound fraction of
omadacycline. Therefore, the BALB/c dataset is not only important for potential future work but
also aids in determination of free AUC$_{0-24h}$ for C3HeB/FeJ mice, in the absence of commercially
available C3HeB/FeJ mouse plasma.

Omadacycline pharmacokinetic (PK) parameters were determined in C3HeB/FeJ mice by taking
plasma samples at 0.5, 1, 2, 3, 6, 12, and 24 hours after subcutaneous injection of
omadacycline at 7.5, 15, or 30 mg/kg. Omadacycline PK parameters were also determined in
BALB/c mice by taking plasma samples at 0.25, 0.5, 1, 2, 6, 12, and 24 hours after
intraperitoneal injection, of omadacycline at 2.5, 7.5, 15, or 30 mg/kg. Plasma concentrations of
omadacycline versus time were plotted (Figure 4A&B) to determine PK parameters for
C3HeB/FeJ mice (Table 2A) and for BALB/c mice (Table 2B). For C3HeB/FeJ mice, both AUC$_{0-24h}$
and C$_{max}$ were dose linear within the dose range of 7.5 to 30 mg/kg (Figure 4C&D). The
AUC$_{0-24h}$ is numerically dose proportional (slope=0.99) and the C$_{max}$ is numerically less than
dose proportional (slope=0.91, less than 1.0). For BALB/c mice, both AUC$_{0-24h}$ and C$_{max}$ were
also dose linear (Figure 4C&D). The AUC$_{0-24h}$ is numerically dose proportional (slope=1.05) and
the C$_{max}$ is numerically less than dose proportional (slope=0.89, less than 1.0). These data show
that omadacycline AUC$_{0-24h}$ and C$_{max}$ while similar in these two mouse strains at the doses
tested are not identical, with differences in PK parameters attributable to the different routes of
administration. Specifically, intraperitoneal administration typically yields higher $C_{\text{max}}$ values than does subcutaneous injection (57).

Free $AUC_{0-24\text{h}}$ values were determined using an average mouse plasma protein binding of 33.9% as determined in BALB/c mice via equilibrium dialysis (data not shown). Linear regression analysis of the dose versus the C3HeB/FeJ mouse free $AUC_{0-24\text{h}}$ was performed and determined that an omadacycline subcutaneous dose of 15 mg/kg would result in a mouse free $AUC_{0-24\text{h}}$ of 9.2 hr*µg/ml, which best represents the human free $AUC_{0-24\text{h}}$ of 8.92 hr*µg/ml. Of note, omadacycline lacks oral bioavailability in rodents (personal communication; Paratek Pharmaceuticals) and thus extrapolation of the AUC obtained via subcutaneous administration in the mouse to the AUC obtained via oral administration in the human was required.

Omadacycline concentrations in plasma from infected and uninfected C3HeB/FeJ mice were also measured. Plasma samples from infected and uninfected mice were compared and showed no significant difference in omadacycline concentrations indicating that *M. abscessus* infection had no effect on omadacycline plasma levels in mice (data not shown).

**Efficacy of omadacycline against *M. abscessus* pulmonary infection in mice:** Efficacy of omadacycline was evaluated against four independent *M. abscessus* strains in a mouse model of pulmonary *M. abscessus* infection (35). These included ATCC 19977, and recent pulmonary clinical isolates M9501, M9529 and M9530. The four strains have a range of MIC values for antibiotics most frequently used to treat *M. abscessus* infections such as amikacin (16 to >256 µg/ml), clarithromycin (≤0.06 to 3 µg/ml) and imipenem (16 to 256 µg/ml) and were thus chosen to represent different phenotypes that may be encountered in the clinic (Table 1). For example, the clinical strain M9501 is susceptible to most antibiotics, M9529 is resistant to both amikacin and imipenem, and M9530 is resistant to imipenem. All three clinical strains and ATCC 19977 are resistant to linezolid and moxifloxacin. Omadacycline MIC values ranged from 0.25 to 1 µg/ml against the four strains tested.
In the negative control group, mice that received 1x PBS treatment at the same frequency as the test group, *M. abscessus* lung burden increased through the course of infection. In the positive control group, which received imipenem treatment, *M. abscessus* lung burden for all four strains decreased gradually over the course of the study. Omadacycline produced a biphasic effect on lung *M. abscessus* burden (Figure 5). At the final time-point of four weeks of treatment, the lungs of mice harbored $>3 \log_{10}$ fewer CFUs of ATCC 19977 compared to mice in the untreated group. Similarly, omadacycline reduced lung burdens of M9501, M9529 and M9530 by an average of $2.02 \log_{10}$, $1.02 \log_{10}$ and $2.84 \log_{10}$, respectively, at the conclusion of four weeks of treatment. During the first two weeks of treatment, the relative growth rate of each *M. abscessus* isolate was lower when compared to the growth rate in untreated mice. After two weeks, omadacycline reduced *M. abscessus* lung burden and thereby exhibited bactericidal activity. This trend was observed against all strains except M9529. In M9529 infected mice, omadacycline produced a low-grade reduction in lung burden that was maintained throughout the four-week treatment.

**MICs of omadacycline against *M. abscessus* remained unchanged after four weeks of exposure in mice:** To assess if prolonged exposure to omadacycline as a monotherapy in this model alters its MIC against *M. abscessus*, the MICs of ATCC 19977 and M9501 recovered from lung homogenates of mice after four weeks of omadacycline treatment were determined along with the parent strains. Two colonies from each mouse, five mice per infection group, were randomly selected from 7H11 agar plates inoculated with lung homogenates. Overall, the MICs of omadacycline against the recovered isolates were identical or similar to the MIC value obtained against the parent strains (Supplementary Table 5). Among the 10 colonies derived from ATCC 19977 infected mice, the MIC of omadacycline remained unchanged for eight isolates (MIC $\leq 0.5 \, \mu g/ml$). For two isolates the MIC was 0.75 $\mu g/ml$, which is within one dilution. The MIC of omadacycline against all 10 colonies derived from M9501 infected mice were identical to the MIC of omadacycline against the parent M9501 strain.
DISCUSSION

New antibiotics are needed for the treatment of *M. abscessus* disease as demonstrated by the intrinsic resistance of this species to most antibiotics (2–4), the treatment duration of at least 12 months with multi-drug regimens containing poorly tolerated agents, and cure rates as low as 25-40% (11, 12). There have been no antibiotics evaluated in randomized clinical trials for the treatment of *M. abscessus* disease and thus there are no antibiotics approved by the FDA for this indication; current treatment recommendations are based on limited observational studies and consensus from experts. As contemporary *M. abscessus* isolates exhibit resistance to an increasing number of antibiotics (3, 17), there is emerging interest in repurposing existing antibiotics and developing new ones. Tigecycline, a tetracycline class of antibiotic, is among the antibiotics included in the current recommendations and has been valuable in treating this disease (6–8, 10). A study evaluated the utility of tigecycline as part of a multidrug regimen in a cohort of 52 patients, the majority of whom had *M. abscessus* infection. While the addition of tigecycline produced improvement in 60% of the patients, adverse effects including nausea and vomiting were reported in >90% of the cases (58). In the past few years, *in vitro* studies of omadacycline have reported low MICs against *M. abscessus* and therefore offer promising potential against this bacteria (21–23, 25). Additionally, recent real-world case reports and case series have reported promising clinical outcomes with regimens containing omadacycline in treating *M. abscessus* lung disease (27, 59–61). These observations have warranted evaluation in a pre-clinical model that permits efficacy determination of omadacycline alone against multiple *M. abscessus* isolates in a controlled laboratory setting. This critical knowledge would provide insight and aid in clinical trial design to evaluate omadacycline against *M. abscessus* disease in patients. The study thus described herein includes *in vitro* assessment and pre-clinical efficacy evaluations in a mouse model of pulmonary *M. abscessus* disease to fill this knowledge gap.
The clinical isolates of *M. abscessus* included in this study trace their origin to pulmonary infections in various structural lung diseases such as bronchiectasis, cystic fibrosis and chronic obstructive pulmonary disease and were obtained within the past 15 years (17). In addition, two isolates from a gastrointestinal infection (46) were evaluated in MIC determination studies. We included isolates from subspecies *abscessus* and *massiliense*, which represent the most frequently isolated subspecies in the clinic. As the omadacycline MIC$_{90}$ of 0.5 µg/ml observed in our study is similar to the MIC$_{90}$ values of omadacycline observed against other Gram-positive species (e.g., *S. aureus*), it is possible that the omadacycline dose currently approved for treatment of community acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) may also prove favorable for the treatment of *M. abscessus* infections. Indeed, the FDA-approved 300 mg oral dose is currently being evaluated in a Phase 2, double-blind, randomized, parallel-group, placebo-controlled study to evaluate the efficacy, safety and tolerability of oral omadacycline in adults with NTM pulmonary disease caused by *M. abscessus* complex (NCT04922554). Importantly, we demonstrated that mice treated with 15 mg/kg, a dose that best represents the human equivalent exposure of the 300 mg oral dose, is efficacious in a murine model of pulmonary infection due to *M. abscessus* (20). Reduction in the CFUs of ATCC 19977, M9501 and M9530 in the lungs of mice occurred after two weeks of treatment with omadacycline. During the first two weeks, lung burden of these isolates increased although the CFUs were consistently lower in mice that received omadacycline compared to control group that received PBS. Omadacycline is a bacteriostatic agent, which may explain in part the delayed kill that was observed. In addition, changes in pharmacokinetics of omadacycline during the course of infection or alterations in the microenvironment where *M. abscessus* exist during the infection may also explain the biphasic activity of omadacycline. Additional mechanisms including changes in metabolism of *M. abscessus* or the host that affect omadacycline activity cannot be ruled out. Determining the basis for the delayed bactericidal activity of omadacycline observed against these isolates will require further study.
Additionally, continuous exposure to omadacycline for four weeks in mice did not alter the MICs of omadacycline against ATCC 19977 and M9501. A potential limitation of this assessment is that MIC of omadacycline was determined for only 10 colonies originating from ATCC 19977 and M9501. Although these colonies were randomly picked and therefore can be expected to represent the *M. abscessus* population in mice following continuous exposure to omadacycline for four weeks, it would require determination of MIC of omadacycline against all surviving colonies to definitively ascertain if any changes to the MIC occurred. Since we did not observe any alterations in MICs against these two strains, we considered it incremental and potentially unnecessary to repeat this assessment in colonies isolated from mice infected with M9529 and M9530.

In the time-kill assay, concentration- and time-dependent activity of omadacycline was observed consistently against all five *M. abscessus* isolates tested. The reduction in *M. abscessus* CFU at 24-hours post-exposure and subsequent increase in CFU after 24 hours at concentrations up to 2x MIC of omadacycline indicates a likely reduction in effective concentration of active omadacycline and hence suggests that continued exposure to the active antibiotic is vital in realizing its anti-*M. abscessus* activity. This hypothesis is supported by a previous report that demonstrated concentration-dependent killing of *M. abscessus* by omadacycline over 7 days with the re-supplementation of 20% omadacycline to cultures daily (24). Another report noted the preference of utilizing oxyrase in *in vitro* studies to stabilize omadacycline against potential degradation over time (62). A low inoculum of *M. abscessus* was used in the time-kill assays to minimize introduction of any pre-existing spontaneous resistant mutants whose outgrowth would confound the interpretation of CFU levels.

Omadacycline and 17 additional standard of care antibiotics were tested in two biological replicate MIC assays against the 32 *M. abscessus* strains each using two different media types, CAMHB (*Table 1*) and Middlebrook 7H9 broth (*Supplementary Table 1*). A surprising and clinically relevant observation was that the MIC values obtained for omadacycline, and several
other antibiotics trended lower in CAMHB when compared with Middlebrook 7H9 broth but that the MIC values of imipenem trended higher in CAMHB when compared with Middlebrook 7H9 broth. These results suggest that the selection of an appropriate media is critical for accurate susceptibility testing and MIC reporting of *M. abscessus* and that CLSI guidelines should be followed when available. In particular, we recommend that CLSI susceptibility testing guidelines are followed for evaluation of omadacycline MICs against NTM.

Additionally, the MIC of imipenem against M9529 is 256 µg/ml in both CAMHB and Middlebrook 7H9 broth and is considered resistant according to CLSI breakpoint guidelines (49) (Supplementary Table 2) but imipenem was efficacious against this strain in mice (Figure 5). These observations bring into question whether *in vitro* MICs of imipenem against some *M. abscessus* strains in either of these two broths can be informative in making clinical decisions on considering imipenem in treatment regimens. Compared to ATCC 19977, M9501 and M9530, growth of M9529 in the lungs of mice was attenuated through the four-week period (Figure 5C). *M. abscessus* isolates exhibit significant heterogeneity in their growth *in vivo* and more than 3 log_{10} CFU difference in the lungs of C3HeB/FeJ mice between the fastest and slowest growing isolates have been described (35).

Treatment regimens for *M. abscessus* pulmonary disease require the use of multiple antimicrobials in combination (6–8, 10) and therefore it is critical to confirm that there is absence of antagonistic activity when antimicrobials are combined. No antagonism was observed when omadacycline was combined with any of the antibiotics assessed against any strains in this study. Instead, omadacycline exhibited synergy in combination with several other antibiotics. For example, omadacycline in combination with clarithromycin exhibited synergy against the largest number (54.5%; 6 of 11) of *M. abscessus* isolates. Because of the small sample size (n=11), and inclusion of strains that are resistant to several antibiotics, the actual proportion of clinical isolates against which the combination of omadacycline and clarithromycin may exhibit synergy may be slightly different. Additionally, although omadacycline in combination with
rifabutin exhibited synergy against fewer strains than in combination with clarithromycin, the 
FICI range of omadacycline+rifabutin was the narrowest at 0.68 ± 0.20 (mean ± standard 
deviation). The FICI values for omadacycline+clarithromycin showed a slightly wider range with 
mean ± standard deviation of 0.55 ± 0.30. An important finding was that antibiotics with which 
omadacycline exhibited synergy including clarithromycin, azithromycin, cefdinir, linezolid and 
rifabutin are available in oral formulation. Therefore, there is potential, pending further 
evaluation in animal models of efficacy and clinical evaluation in humans, that a fully oral 
regimen containing omadacycline may be feasible. This type of regimen would reduce the 
logistical challenges associated with administering prolonged intravenous therapy in an 
outpatient setting, as is often required for treatment of *M. abscessus* lung disease.

The most recently published clinical practice guidelines for the treatment of NTM pulmonary 
disease were published in 2020 but failed to incorporate new antibiotics into these guidelines 
(10). A subsequent publication in July 2021 by two guideline authors provides updated 
treatment recommendations for *M. abscessus* pulmonary infections (63). The authors 
recommend oral omadacycline as a preferred drug but note that while there is ‘impressive *in 
vitro* activity’ and reports of ‘anecdotal clinical successes’ it is not yet clear if treatment with 
omadacycline will contribute to better outcomes compared to the current regimens (63). In 
addition, the FDA granted omadacycline orphan drug designation in August of 2021 for the 
treatment of infections caused by NTM, and this designation includes NTM pulmonary disease 
caused by *M. abscessus* complex, the focus of the ongoing Phase 2 clinical trial 
(NCT04922554). In total, the data presented in our study provides additional support to this new 
treatment recommendation as well as the continued study of omadacycline in patients with 
pulmonary disease due to *M. abscessus*.
MATERIALS AND METHODS

 Ethics. Animal procedures used in the studies described herein were performed in adherence to the Johns Hopkins University Animal Care and Use Committee and to the national guidelines.

 Bacterial strains and in vitro growth conditions. *M. abscessus* reference strain ATCC 19977 (47) was purchased from ATCC (Manassas, VA) and authenticated by sequencing its genome (48). Strains M9563 and M9535, isolated from gastrointestinal infections, were kind gifts from Dr. Thomas Byrd, University of New Mexico School of Medicine and correspond to strains 390R and 390V, respectively, as previously described (46, 64). Remainder of the strains were obtained from Dr. Nicole Parrish at the Johns Hopkins University Clinical Microbiology Laboratory who isolated the strains from cystic fibrosis and bronchiectasis patients between 2006-2018 (17). All strains were grown in either cation adjusted Mueller Hinton Broth (CAMHB) (Sigma-Aldrich, 90922) or Middlebrook 7H9 broth (Difco, 271310) as specified. Middlebrook 7H9 broth was supplemented with 0.5% glycerol, 10% albumin-dextrose-catalase enrichment. *M. abscessus* cultures were grown in an orbital shaker, 220 RPM @ 37 °C. Omadacycline was obtained from Paratek Pharmaceuticals Inc. All antibiotics were purchased from Sigma-Aldrich Limited. Mouse lung homogenates were cultured on Middlebrook 7H11 selective agar (Difco, 283810) supplemented with 10% albumin-dextrose-catalase enrichment, 50 µg/ml carbenicillin (Research Product International, C46000) and 50 µg/ml cycloheximide (Sigma-Aldrich, C7698) as described (65).

 Determination of MICs. The standard broth microdilution method (66, 67) with conditions specified in the CLSI guidelines specific for *M. abscessus* (49) was used to determine the MIC of each antibiotic against 32 different *M. abscessus* strains (Table 1). Sterile deionized water was used to dissolve powdered drug stocks and if insoluble in water they were dissolved in dimethyl sulfoxide (DMSO) at a high concentration and diluted in water. CAMHB broth was used as specified in the CLSI guidelines (49) and separately Middlebrook 7H9 broth was also used.
Two-fold serial dilutions of each antibiotic were prepared in each broth and final antibiotic concentrations ranging from 256 µg/ml to 0.06 µg/ml in 200 µl final volume in each well in a 96-well culture plate was generated. Using an exponentially growing culture of each strain, 10^5 CFU of *M. abscessus* was inoculated into each well. As positive and negative controls, two wells containing 10^5 CFU of *M. abscessus* without drug and two wells containing broth alone were included in each plate and incubated at 30 °C for 72 h in accordance with CLSI guidelines. A Sensititre Manual Viewbox was used to determine growth or lack thereof of *M. abscessus* and the lowest concentration at which *M. abscessus* growth was not observed was recorded as the MIC of the antibiotic. Each MIC assay was performed in duplicate, and the final MIC reported in Table 1 was an average of the biological replicates of the assays.

**Checkerboard Titration Assay.** This assay, a modification of the standard broth microdilution assay was performed as described (50, 68). Briefly, in sterile U-bottom 96-well plates with 300 µl well capacity, stock solutions of two antibiotics were added to CAMHB broth each starting at 2x MIC and serially diluted up to 1/64x MIC such that all possible 2-fold dilution combinations from 2x to 1/64x MIC of each antibiotic were included. Using an exponentially growing culture of each strain, 10^5 CFU of *M. abscessus* was inoculated into each well and positive and negative controls were included as described above. In accordance with CLSI guidelines, plates were incubated for 72 h at 30 °C and evaluated for *M. abscessus* growth using a Sensititre Manual Viewbox. The fractional inhibitory concentration index (FICI) was calculated as described (50).

As per the stringent interpretation recommended, an FICI of ≤0.5 was interpreted as synergy, an FICI of >0.5-4 as indifference, and an FICI of >4 as antagonism (51). The reference strain ATCC 19977 and 10 clinical isolates, M9501, M9502, M9507, M9510, M9513, M9522, M9524, M9526, M9529 and M9530 were included. Omadacycline in combination with 14 antibiotics were assayed. A total of 165 separate assays testing 14 antibiotic combinations against 11 strains were performed. For those combinations that produced a FICI of ≤0.5, a biological repeat of the assay was performed to verify synergy.
**Time-Kill Assay.** Activities of omadacycline alone and in combination with clarithromycin, azithromycin, cefdinir, linezolid and rifabutin against the reference strain ATCC 19977 and four randomly selected clinical isolates M9510, M9526, M9529, and M9530 were determined. Each *M. abscessus* isolate was grown in CAMHB broth to exponential phase and a suspension at an optical density $A_{600nm} = 0.01$ was prepared by diluting the culture in fresh broth. 50 ml culture tubes containing omadacycline at 0.5x, 1x, 2x, 4x, 8x and 16x MIC specific to each strain in 4.8 ml CAMHB broth were prepared and inoculated with 200 µl of the *M. abscessus* suspension, or $\sim 10^5$ CFU. A positive control for growth of *M. abscessus* without omadacycline was included in each assessment. The samples were incubated in an orbital shaker, 220 RPM @ 37 °C. At 0, 1, 3 and 7 days, 100 µl aliquot was obtained from each sample, appropriate ten-fold serial dilutions were prepared in CAMHB broth, inoculated onto CAMHB agar and CFU were enumerated after 3 days of incubation at 37 °C. Similarly, to determine time-kill activities of omadacycline in combination with another antibiotic, the five *M. abscessus* isolates were exposed to omadacycline and clarithromycin, azithromycin, cefdinir, linezolid or rifabutin at 1x, 0.5x and 0.25x MIC of each antibiotic specific to each strain, grown and CFU determined. For each sample, CFU counts were converted to CFU/ml and mean ± standard deviation data vs. time were plotted.

**Determination of omadacycline PK parameters in C3HeB/FeJ mice and in BALB/c mice.** Uninfected C3HeB/FeJ mice, 6-8 weeks old, female, were injected subcutaneously with 7.5, 15, or 30 mg/kg omadacycline solution in 1xPBS. The blood samples were taken via terminal cardiac puncture at 0.5-, 1-, 2-, 3-, 6-, 12-, and 24-hours post-injection, and at each time-point a single blood sample was collected from a single mouse. Five mice per dose and time-point were utilized. Uninfected BALB/c mice, 6-8 weeks old, female, were injected intraperitoneally with 2.5, 7.5, 15, or 30 mg/kg omadacycline and blood samples were taken via terminal cardiac puncture at 0.25-, 0.5-, 1-, 2-, 6-, 12-, and 24-hours post-injection. Blood was transferred to lithium
heparin vials and centrifuged at 1,500 xg for 10 minutes to separate the plasma. Plasma was stored at -80 °C until omadacycline concentration analysis.

The mean (±SD) plasma concentration versus scheduled time points for uninfected BALB/c mice are shown in Figure 4A&4B. These mean concentration profile data were used to estimate the PK parameters of omadacycline in plasma by standard noncompartmental methods using a WinNonlin (Phoenix) validated SAS program for all dose groups. For the calculation of PK parameters, Below the Limit of Quantification (BLQ) values before the first quantifiable concentration were treated as zero. BLQ values after the first quantifiable concentration were retested and confirmed and were included in the analyses. Missing concentrations were treated as missing.

To determine omadacycline concentrations in M. abscessus infected C3HeB/FeJ mice, mice that had been infected were treated with 15 mg/kg omadacycline as described and blood samples were taken via cardiac puncture from 5 mice at 24 hours post-treatment at 0-, 1-, 2-, and 4-weeks post-infection. Blood was transferred to lithium heparin vials and centrifuged at 1,500 xg for 10 minutes to separate the plasma. Plasma was stored at -80°C until omadacycline concentration analysis.

**Determination of omadacycline concentrations.** Omadacycline plasma concentrations were determined by Institute for Clinical Pharmacodynamics (ICPD; Schenectady, NY) using a qualified liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Samples were subjected to protein precipitation and chromatographically separated on an ACE 3 C18 100 × 3 mm, 3 μm particle size HPLC column (ACE, Advanced Chromatography Technologies Ltd) using a Sciex Exion LC AC system. Analyte molecules were detected using a Sciex 5500 mass spectrometer scanning in positive ion mode. Omadacycline tosylate drug substance (omadacycline) was used to prepare the stock standard and working standard omadacycline.
solutions. D9-Omadacycline was used as the internal standard for the analysis of omadacycline.

The peak areas of omadacycline and its internal standard, d9-omadacycline, were acquired using Analyst 1.6.3 software (Sciex, Framingham, MA). The calibration curves were obtained by fitting the peak area ratios of omadacycline/d9-omadacycline and the standard concentrations to a linear 1/x^2 regression model using Analyst 1.6.3 software. The equations of the calibration curves were then used to interpolate the concentrations of omadacycline in the samples using their peak area ratios. The peak areas and peak area ratios used for the calculations were rounded to three precision points.

Mice, infection and efficacy studies. C3HeB/FeJ mice, female, 5-6 weeks old, were procured from Jackson Laboratories (Bar Harbor, Maine). As described in the protocol for a mouse model of pulmonary *M. abscessus* infection (35), mice were treated daily with 5 mg/kg/day dexamethasone beginning one week prior to infection with *M. abscessus* and continued throughout the duration of the study. *M. abscessus* strains ATCC 19977, M9501, M9529 and M9530 were used to infect mice. Infection with each strain was performed separately; 110 mice were infected with each strain. In a Glas-Col inhalation exposure system, all 110 mice were infected concurrently with aerosol generated from 10 ml of exponentially growing *M. abscessus* culture diluted to an A_{600nm} of 0.1 in sterile 1x PBS, pH 7.4 according to the manufacturer’s instructions (Glas-Col, Terre Haute, Indiana). The infection cycle included preheat for 15 min, aerosol nebulization for 30 min, cloud decay for 30 min followed by surface decontamination for 15 min. This study was conducted in two phases. The first phase included evaluation of omadacycline efficacy in mice infected with ATCC 19977 and M9501. At the conclusion of this phase, two *M. abscessus* colonies were randomly selected from 7H11 agar plates containing lung homogenates of each mouse following completion of 4 weeks of daily omadacycline treatment. The MIC of omadacycline was determined against these 20 colonies to assess if daily exposure to omadacycline for four weeks altered its MIC against these strains. In the second phase, efficacy of omadacycline was evaluated in mice infected with M9529 and M9530.
An identical protocol was used in both phases, but the studies were separated in time. Five mice per infecting strain group were sacrificed at 24 h post-infection (designated week -1), lungs were homogenized, inoculated onto Middlebrook 7H11 selective plates described above, incubated at 37 °C for 5 days and CFU recorded to determine the initial *M. abscessus* burden in the lungs of mice. Similarly, five mice were sacrificed at one-week post-infection (week 0) and CFU enumerated. CFU counts from each mouse lung were converted into CFU per lung comprising the average of three consecutive steps of a 10-fold dilution series of a given lung sample. Mean CFU ± standard deviation of lung *M. abscessus* burden in five mice per group per time-point was plotted determine the growth of each isolate under conditions tested.

**Antibiotic regimens.** At one-week post infection (week 0), mice infected with each strain were further divided into three groups of 15 mice per group. All antibiotics and control treatments were administered via subcutaneous injection in the dorsal flank of hind limbs. Mice in the negative control group were treated once daily with 200 µl sterile 1x PBS. Mice in the positive control group were administered imipenem and mice in the test group were administered omadacycline. Powdered omadacycline and imipenem were resuspended in sterile 1x PBS, pH 7.4 (Quality Biologicals) for administration of 15 mg/kg/q24 and 200 mg/kg/q12 of omadacycline and imipenem, respectively, using a 200 µl bolus. An average body weight of 25 grams per mouse was considered based on our past experience with this mouse strain, age and sex (35). Therefore, omadacycline and imipenem were prepared at concentrations of 1.875 mg/ml and 25 mg/ml for administration. Omadacycline dissolved completely in 1x PBS, pH 7.4 and produced a solution, so a single batch of omadacycline was produced for the experiment and aliquots for each day of administration were stored at -20 °C and thawed at the time of administration. Imipenem dissolved incompletely in PBS and produced a fine suspension. Imipenem powder for each administration was weighed, stored at -20 °C and freshly resuspended in 1x PBS, pH 7.4 moments prior to administration. All treatments were administered via sub-cutaneous injection into the dorsal flank using syringe with 27-gauge needle. Daily is defined as seven days a week.
At one week (week +1), two weeks (week +2) and four weeks (week +4) from the time of antibiotic treatment initiation, five mice per treatment arm were sacrificed and *M. abscessus* lung burden was determined as described above.

**Data analysis.** CFU data from *in vitro* and *in vivo* studies were analyzed to determine mean ± standard deviation for each time-point in each experimental group and graphed using GraphPad Prism 8.4.3.
ACKNOWLEDGEMENT

This study was supported by Paratek Pharmaceuticals.
Figure 1: Combined activity of omadacycline and another antibiotic against 11 M. abscessus isolates. Fractional Inhibitory Concentration Index (FICI) of ≤0.5 was interpreted as synergy, >0.5-4 as indifference, and >4 as antagonism as per the most stringent recommendation (51). The red line at FICI of 0.5 demarcates synergy (at or below the line) from indifference (above the line). Omadacycline was synergistic with the antibiotic indicated for strains at or below the red line. Each ‘x’ mark denotes a single M. abscessus strain.
Figure 2: Time-kill activity of omadacycline against five *M. abscessus* isolates. *M. abscessus* strain (A) ATCC 19977, and clinical isolates (B) M9510, (C) M9526, (D) M9529, and (E) M9530 were exposed to omadacycline at 0.5x, 1x, 2x, 4x, 8x and 16x MIC specific to each strain and no drug in CAMHB broth and surviving colonies were recovered on CAMHB agar in duplicate at 1, 3 and 7 days and enumerated (mean±SD).
Figure 3: Time-kill activity of omadacycline and rifabutin against five *M. abscessus* isolates. *M. abscessus* strains (A) ATCC 19977, and clinical isolates (B) M9510, (C) M9526, (D) M9529, and (E) M9530 were exposed to a combination of omadacycline and rifabutin at 0.25x, 0.5x and 1x MIC of each antibiotic specific to each strain and no drug in CAMHB broth and surviving colonies were recovered on CAMHB agar in duplicate at 1, 3 and 7 days and enumerated (mean±SD). Additional time-kill curves against five other synergistic pairs are included in Supplementary Figures 1-5.
Figure 4. Omadacycline PK parameters in C3HeB/FeJ and BALB/c mice. Mean (±SD) omadacycline plasma concentration versus scheduled time points in A) C3HeB/FeJ mice and B) BALB/c mice. Dose linearity of (C) AUC\textsubscript{0-24} and (D) C\textsubscript{max} in BALB/c and C3HeB/FeJ mice.
Figure 5: *M. abscessus* burden in the lungs of mice. C3HeB/FeJ mice were used. All mice were immunosuppressed with dexamethasone. Lung *M. abscessus* burden assessments at weeks -1, 0, +1, +2, and +4 (n=5 per group per timepoint; represented in mean±SD) are shown. Week -1 represents the day after mice were infected with *M. abscessus* and week 0 represents the day of antibiotic treatment initiation (denoted with vertical dotted line). Data in panel (A) corresponds to mice infected with reference strain ATCC 19977, and clinical isolates (B) M9501, (C) M9529, and (D) M9530. PBS; Phosphate buffered saline control; IMI; imipenem; OMC; omadacycline. PBS and OMC (15 mg/kg) were administered once daily, and IMI (200 mg/kg) was administered twice daily. All agents were administered by subcutaneous injection into the dorsal flank.
| Subspecies      | Isolate ID | DMC     | TGC      | AMK     | CLR     | AZM     | IMI     | DOR     | FOX     | CAZ     | AMK     | VAN     | TEC     | L2D     | CFZ     | MOX     | RFB     | BDQ     |
|-----------------|------------|---------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| abscessus       | M9563      | 0.125   | 0.25     | ≤0.06   | 0.188   | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | ≤0.06   |
| massiliense     | M9532      | 0.25    | 0.125    | >256    | 2       | 64      | 64      | 32      | 32      | 32      | 32      | 32      | 32      | 32      | 0.125   | 0.125   | 0.125   | 0.125   | 0.125   | >256    |

Table 1: MIC (µg/mL) of omadacycline and select antibiotics against 31 M. abscessus clinical isolates and reference strain ATCC 19977 in CAMHB broth.

MIC values are average of two biological replicates. For agents with published CLSI breakpoints, resistant MIC values are denoted in red and intermediate MIC values are denoted in blue (49) (Supplementary Table 2). OMC, omadacycline; TGC, tigecycline; AMK, amikacin; CLR, clarithromycin; AZM, azithromycin; IMI, imipenem; DOR, doripenem; FOX, cefoxitin; CDR, cefdinir; CAZ, ceftazidime; AMX, amoxicillin; VAN, vancomycin; TEC, teicoplanin; L2D, linezolid; CFZ, cefozopran; MOX, moxifloxacin; RFB, rifabutin, and BDQ, bedaquiline. ‘nd’ denotes M. abscessus isolates whose subspecies has not been determined.
Table 2A. Omadacycline PK parameters in C3HeB/FeJ mice dosed via subcutaneous injection.

<table>
<thead>
<tr>
<th>Omadacycline Dose (mg/kg)</th>
<th>Total AUC&lt;sub&gt;0-24h&lt;/sub&gt; (hr*µg/ml)</th>
<th>Free AUC&lt;sub&gt;0-24h&lt;/sub&gt; (hr*µg/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
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<td>8.07</td>
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<td>31.81</td>
<td>17.87</td>
<td>3.00</td>
<td>6.81</td>
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</table>

<sup>a</sup>Free AUC<sub>0-24h</sub> was determined based on 33.9% plasma protein binding in BALB/c mice.

AUC<sub>0-24h</sub> = Area under the plasma concentration versus time curve (AUC) from time 0 to 24 hrs after dosing, calculated using the linear trapezoidal linear interpolation method. C<sub>max</sub>=maximum observed plasma concentration. T<sub>1/2</sub>=Plasma terminal elimination half-life, calculated as T<sub>1/2</sub>=ln(2)/λ<sub>z</sub>; where λ<sub>z</sub> = Terminal elimination rate constant calculated by linear regression of the terminal portion of the natural log-plasma concentration versus time curve.

Table 2B. Omadacycline PK parameters in BALB/c mice dosed via intraperitoneal injection.

<table>
<thead>
<tr>
<th>Omadacycline Dose (mg/kg)</th>
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<th>Free AUC&lt;sub&gt;0-24h&lt;/sub&gt; (hr*µg/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
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</table>

<sup>a</sup>Free AUC<sub>0-24h</sub> was determined based on 33.9% plasma protein binding in BALB/c mice.

AUC<sub>0-24h</sub> = Area under the plasma concentration versus time curve (AUC) from time 0 to 24 hrs after dosing, calculated using the linear trapezoidal linear interpolation method. C<sub>max</sub>=maximum observed plasma concentration. T<sub>1/2</sub>=Plasma terminal elimination half-life, calculated as T<sub>1/2</sub>=ln(2)/λ<sub>z</sub>; where λ<sub>z</sub> = Terminal elimination rate constant calculated by linear regression of the terminal portion of the natural log-plasma concentration versus time curve.
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