Omadacycline as a promising new agent for the treatment of infections with *Mycobacterium abscessus*

Hannelore I. Bax¹,²*, Corné P. de Vogel², Johan W. Mouton² and Jurriaan E. M. de Steenwinkel²

¹Department of Internal Medicine, Division of Infectious Diseases, Erasmus University Medical Centre, Rotterdam, The Netherlands; ²Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Centre, Rotterdam, The Netherlands

*Corresponding author. Tel: +31 10 7033510; Fax: +31 10 7038875; E-mail: h.bax@erasmusmc.nl

Received 15 February 2019; returned 5 April 2019; revised 21 May 2019; accepted 27 May 2019

**Background:** Despite intensive treatment regimens, the outcome of *Mycobacterium abscessus* infections is extremely poor and thus novel treatment regimens are needed. Although tigecycline seems to be one of the best options currently available, its long-term use is hampered by severe toxic side effects as well as the need for intravenous administration and the relatively high concentrations required for efficacy.

**Objectives:** To assess the *in vitro* activity of omadacycline against *M. abscessus* and compare it with the activity of tigecycline.

**Methods:** The concentration- and time-dependent killing capacities of omadacycline and tigecycline against *M. abscessus* subspecies *abscessus* were determined using a time–kill kinetics assay. Time–kill curves as well as concentration–effect curves were generated.

**Results:** Time–kill curves showed strong concentration-dependent antimicrobial activity for both omadacycline and tigecycline. Omadacycline showed inhibition of mycobacterial growth at 4 mg/L and mycobacterial killing at concentrations ≥16 mg/L. Tigecycline showed mycobacterial killing at concentrations ≥4 mg/L, achieving elimination at concentrations ≥16 mg/L. The concentration–effect curves after 7 days of exposure showed stasis, 1 log mycobacterial killing and 2 log mycobacterial killing at 3.3, 4.0 and 4.8 mg/L for omadacycline and 2.2, 2.7 and 3.4 mg/L for tigecycline, respectively.

**Conclusions:** The results of this *in vitro* study on omadacycline activity, together with its favourable (pharmacokinetic) properties, suggest that omadacycline is a potential new agent for the treatment of *M. abscessus* infections.

**Introduction**

*Mycobacterium abscessus* belongs to the heterogeneous group of non-tuberculous mycobacteria (NTM) and can cause severe infections in patients with underlying structural lung diseases such as cystic fibrosis (CF). The incidence of NTM infections in CF patients is rising and *M. abscessus* is one of the most frequently isolated species.¹ This is important as pulmonary infections with *M. abscessus* in this patient population have been shown to be responsible for the most rapid lung function decline compared with other pathogens.² In addition, in most medical centres, *M. abscessus* infection is a relative contraindication for lung transplantation. Therefore, appropriate treatment of *M. abscessus* infections in CF patients is crucial.

Among the different NTM species, *M. abscessus* is notorious because of its intrinsic resistance to multiple antibiotics. This is especially true for *M. abscessus* subspecies *abscessus*, characterized by the presence of a functional erythromycin ribosomal methylase (*erm*) gene, conferring inducible resistance to macrolides, which are considered cornerstone agents in treatment.³ As a consequence, *M. abscessus* infections are extremely difficult to treat, requiring a combination of different intravenous and oral antimycobacterial drugs for a prolonged period of time. The drug regimens are usually poorly tolerated and, despite intensive treatment, outcomes are disappointing.⁴ Although good activity and efficacy of tigecycline have been demonstrated both in preclinical models⁵ and in clinical practice,⁶ its long-term use is hampered by severe gastrointestinal side effects, also impeding any further dose increments. Therefore, finding a novel alternative to tigecycline would be a major step towards improving treatment outcome.

Omadacycline is a novel aminomethylcycline antimicrobial agent and a member of the tetracycline class of drugs. Its mechanism of action is similar to that of other tetracyclines, i.e. binding...
to the bacterial ribosome, resulting in inhibition of protein synthesis. Omadacycline has good activity against Gram-positive and a variety of Gram-negative microorganisms. It was recently approved by the FDA for the treatment of skin and skin structure infections and community-acquired pneumonia, showing good efficacy in Phase 3 clinical trials. Because of its structural resemblance to tigecycline, omadacycline might also have good activity against M. abscessus isolates.

The purpose of this study was to explore the potential use of omadacycline for the treatment of M. abscessus infections. In this context, the in vitro activity of omadacycline against M. abscessus was assessed and compared with the activity of tigecycline.

**Methods**

**Bacterial strain and culture**
The M. abscessus subsp. abscessus CIP 104536 (Collection of Institute Pasteur, Paris, France, kindly provided by Dr J. van Ingen, Radboud University Nijmegen Medical Centre) was cultured in CAMHB (Becton, Dickinson and Company (BD), Sparks, MD, USA) supplemented with 10% OADC (BD) and 0.5% glycerol (Scharlau Chemie SA, Sentmenat, Spain) under shaking conditions at 96 rpm at 37°C. Vials with M. abscessus suspensions were stored at −80°C.

**Drug susceptibility testing**
MICs (duplicates) were determined according to CLSI guidelines using broth microdilution in CAMHB at 35°C. Plates were examined after 3–5 days depending on the growth of the control.

**Antimicrobial drugs**
Omadacycline was kindly provided by Paratek Pharmaceuticals (Boston, MA, USA). Tigecycline was purchased from Pfizer (New York, NY, USA).

**Time–kill kinetics assay**
The concentration–time–dependent killing capacities of omadacycline and tigecycline were determined as previously described. Briefly, M. abscessus cultures (log phase) were exposed to antimicrobial drugs at 4-fold increasing concentrations for 7 days at 37°C under shaking conditions at 96 rpm. In the absence of drugs, the mycobacterial population showed an average increase from 3.6×10^9 cfu/mL to 3.5×10^9 cfu/mL within 7 days of incubation. The drug concentrations ranged from 0.063 to 256 mg/L for both compounds. The tested concentrations were based on the MIC values of the individual drugs ranging from 1/64× to 64× MIC comprising a broad range for studying in vitro drug activity. At days 1, 3 and 7 of drug exposure, samples were collected, centrifuged at 14000g to avoid drug carry-over, serially diluted (10-fold, 10^9–10^0) and subcultured onto solid medium. Plates were incubated for 5 days at 35°C with 5% CO₂ to determine cfu counts. The lower limit of detection (LLD) was 5 cfu/mL (log 0.7). All experiments were performed in duplicate. Time–kill curves as well as concentration–effect curves were generated.

**Selection of drug-resistant M. abscessus**
In order to assess the selection of drug-resistant mutants after 7 days of drug exposure, subcultures were also performed on solid medium containing omadacycline or tigecycline. The drug concentrations in the subculture plates were 4× the MIC concentrations, i.e. 16 mg/L for both tigecycline and omadacycline.

**Stability of antimicrobial drugs**
Antimicrobial activity over time was assessed using the standard large-plate agar diffusion assay as previously described in detail. In short, a Staphylococcus aureus strain susceptible to omadacycline and a Micrococcus luteus strain susceptible to tigecycline were plated onto solid diagnostic sensitivity test (DST) agar (Oxoid, Hampshire, UK). A 2-fold increasing standard concentration series was prepared. The standard concentration series and two test concentrations of omadacycline and tigecycline were added onto the DST medium and on days 1, 3 and 7 the inhibition zones were determined. Comparing the inhibition zones of the standard concentration series with the zones of the test concentrations enabled determination of the omadacycline and tigecycline concentrations over time, representing antibiotic stability. A 20% decline in omadacycline concentration was observed within the first 24 h and it was previously shown that the tigecycline concentration declined by 80% daily. To compensate, 20% of the omadacycline concentration and 80% of the tigecycline concentration were added daily in the time–kill kinetics assay.

**Results**
The MICs of omadacycline and tigecycline for this M. abscessus strain were 4 mg/L for both drugs.

In this static in vitro assay, omadacycline and tigecycline both showed concentration-dependent antimicrobial activity. Omadacycline showed inhibition of mycobacterial growth at 4 mg/L and mycobacterial killing at concentrations ≥16 mg/L, but no elimination was achieved. Tigecycline showed mycobacterial killing at concentrations ≥4 mg/L, achieving elimination at concentrations ≥16 mg/L at day 3–7.

No selection of drug resistance above the spontaneous mutation frequency was observed at any of the omadacycline or tigecycline concentrations tested except for 1.5% and 0.6% at omadacycline 4 mg/L and tigecycline 4 mg/L, respectively.

Concentration–effect relationships after 7 days of exposure are shown in Figure 2. The concentration–effect curves showed stasis, 1 log mycobacterial killing and 2 log mycobacterial killing at 3.3, 4.0 and 4.8 mg/L for omadacycline and 2.2, 2.7 and 3.4 mg/L for tigecycline, respectively.

**Discussion**
This in vitro study showed that omadacycline has good activity against M. abscessus subsp. abscessus, which is one of the most difficult-to-treat species among the NTM. Although the in vitro activity of tigecycline was found to be slightly higher, the clinical relevance of this finding is questionable given the favourable pharmacokinetic properties of omadacycline: tigecycline exhibits high protein binding and the free active fraction is therefore relatively low compared with omadacycline. The AUC24 of omadacycline has been shown to be ~3-fold higher compared with tigecycline in both epithelial lining fluid, alveolar cells and plasma.

In our study, omadacycline and tigecycline both showed clear concentration-dependent antimicrobial activity. This is in line with the observation in a recent pharmacokinetic/pharmacodynamic (PK/PD) study on tigecycline activity against M. abscessus. In that hollow-fibre infection study, doubling the currently used clinical dose was needed to achieve a reasonable PTA. However, it should be mentioned that in the time–kill kinetics assay static drug
concentrations were used and therefore important PK/PD information on omadacycline is still lacking. Further PK/PD studies are needed to gain insight into the dose–response relationship for omadacycline and the main PK/PD parameter driving omadacycline activity, as well as to confirm the findings of our study.

Recently, two other in vitro studies reported on omadacycline activity against different strains within the *M. abscessus* complex also showing similar omadacycline and tigecycline MICs.\(^{15,16}\) While the average MIC\(_{90}\)S of both omadacycline and tigecycline in those studies were 2 mg/L, the MIC distribution of both drugs ranged from 0.5 to 4 mg/L\(^{15}\) and from 0.06 to 8 mg/L\(^{16}\), respectively, indicating that MICs of antibacterial agents differ between different *M. abscessus* subspecies as well as between different strains within one subspecies. Therefore, the use of different *M. abscessus* strains should be considered in further preclinical studies evaluating omadacycline activity and efficacy.

Since *M. abscessus* infections require prolonged treatment, omadacycline may provide major advantages over tigecycline since it is available as an oral formulation and is given once daily only. In addition, a recent pharmacokinetic study in healthy volunteers reported only 2.4% nausea in the omadacycline group versus 47.6% in the tigecycline group and vomiting in 0% versus 14.3%, respectively.

Figure 1. Concentration- and time-dependent bactericidal activity of (a) omadacycline (OMC) and (b) tigecycline (TGC) against *M. abscessus* subsp. *abscessus*. Mycobacterial cultures were exposed to OMC or TGC for 7 days at 37°C under shaking conditions. On days 1, 3 and 7, samples were collected, centrifuged and subcultured onto antibiotic-free and OMC- or TGC-containing solid medium and incubated for 5 days at 37°C with 5% CO\(_2\) to determine cfu. Experiments were performed in duplicate. Results shown are from one representative experiment.
which may be of importance during prolonged treatment.\textsuperscript{14} The Phase 3 clinical trials reported higher percentages of gastrointestinal treatment-emergent adverse events (TEAEs), but events were similar between omadacycline and comparator drugs in two out of three trials.\textsuperscript{8,9} In the one trial using a higher oral omadacycline dose during the first 2 days, more gastrointestinal TEAEs were observed for omadacycline. Across the three trials, the gastrointestinal TEAEs led to treatment discontinuation in only 0.4\% of omadacycline-treated patients.\textsuperscript{17} Whether omadacycline tolerability is sustained when used for a prolonged period of time, e.g. for the treatment of \textit{M. abscessus} infections, remains to be determined.

In conclusion, the results of this study on omadacycline activity, together with its favourable (pharmacokinetic) properties, suggest that omadacycline is a potential new agent for the treatment of \textit{M. abscessus} infections.

Acknowledgements
We thank W. Kloezen for assistance in generating the concentration–effect curves.

Funding
This work was supported by internal funding.

Transparency declarations
None to declare.

References
1. Adjemian J, Olivier KN, Prevots DR. Pulmonary mycobacterial sputum positivity in patients with cystic fibrosis in the United States, 2010–2014. Ann Am Thorac Soc 2018; \textbf{15}: 817–26.
2. Qvist T, Taylor-Robinson D, Waldmann E et al. Comparing the harmful effects of nontuberculous mycobacteria and Gram negative bacteria on lung function in patients with cystic fibrosis. J Cyst Fibros 2016; \textbf{15}: 380–5.
3. Guo Q, Chu H, Ye M et al. The clarithromycin susceptibility genotype affects the treatment outcome of patients with \textit{Mycobacterium abscessus} lung disease. Antimicrob Agents Chemother 2018; \textbf{62}: e02360-17.
4. Pasipanodya JG, Ogbonna D, Ferro BE et al. Systematic review and meta-analyses of the effect of chemotherapy on pulmonary \textit{Mycobacterium abscessus} outcomes and disease recurrence. Antimicrob Agents Chemother 2017; \textbf{61}: e01206-17.
5. Ferro BE, Srivastava S, Deshpande D et al. Tigecycline is highly efficacious against \textit{Mycobacterium abscessus} pulmonary disease. Antimicrob Agents Chemother 2016; \textbf{60}: 2895–900.
6. Wallace RJ Jr, Dukart G, Brown-Elliott BA et al. Clinical experience in 52 patients with tigecycline-containing regimens for salvage treatment of \textit{Mycobacterium abscessus} and \textit{Mycobacterium chelonae} infections. J Antimicrob Chemother 2014; \textbf{69}: 1945–53.
7. Pfaller MA, Huband MD, Shortridge D et al. Surveillance of omadacycline activity tested against clinical isolates from the United States and Europe as part of the 2016 SENTRY Antimicrobial Surveillance Program. Antimicrob Agents Chemother 2018; \textbf{62}: e02327-17.
8. O’Riordan W, Green S, Overcash JS et al. Omadacycline for acute bacterial skin and skin-structure infections. \textit{NEJM} 2019; \textbf{380}: 528–38.
9. Stets R, Popescu M, Gongon JR et al. Omadacycline for community-acquired bacterial pneumonia. \textit{NEJM} 2019; \textbf{380}: 517–27.
10. Clinical and Laboratory Standards Institute. \textit{Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes—Second Edition: M24}. CLSI, Wayne, PA, USA, 2011.
11. de Steenwinkel JE, de Knegt GJ, ten Kate MT et al. Time-kill kinetics of anti-tuberculosis drugs and emergence of resistance, in relation to metabolic activity of \textit{Mycobacterium tuberculosis}. \textit{J Antimicrob Chemother} 2010; \textbf{65}: 2582–9.
12. Box HJ, Bakker-Woudenberg JA, ten Kate MT et al. Tigecycline potentiates clarithromycin activity against \textit{Mycobacterium avium} in vitro. Antimicrob Agents Chemother 2016; \textbf{60}: 2577–9.
13. Bennett J, Brodie JL, Benner EJ et al. Simplified, accurate method for antibiotic assay of clinical specimens. \textit{Appl Microbiol} 1966; \textbf{14}: 170–7.
14. Gottfried MH, Henn K, Garrity-Ryan L et al. Comparison of omadacycline and tigecycline pharmacokinetics in the plasma, epithelial lining fluid, and alveolar cells of healthy adult subjects. Antimicrob Agents Chemother 2017; \textbf{61}: e01135–17.
15. Kaushik A, Ammerman NC, Martins O et al. In vitro activity of new tetra-cycline analogs omadacycline and eravacycline against drug-resistant clinical isolates of \textit{Mycobacterium abscessus}. Antimicrob Agents Chemother 2019; \textbf{63}: e00670–19.
16. Shoen C, Benaroch D, Sklaney M et al. In vitro activities of omadacycline against rapidly growing mycobacteria. Antimicrob Agents Chemother 2019; \textbf{63}: e02522-18.
17. Cornely OA, Garrity-Ryan L, Das A et al. Omadacycline gastrointestinal effects—an integrated safety analysis from the phase-3 ABSSSI and CABP studies. In: Abstracts of the Twenty-eighth European Congress of Clinical Microbiology and Infectious Diseases, Madrid, Spain, 2018. Abstract P0271. ESCMID, Basel, Switzerland.