In Vivo Evaluation of Antibiotic Activity Against Mycobacterium abscessus

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Background. The prognosis of Mycobacterium abscessus infections is poor due to the lack of effective drug treatment. The objective of this study was to set up an animal model suitable to test antibiotic activity against M. abscessus.

Methods. The following mouse strains were evaluated: Swiss, BALB/c, C57BL/6, nude, beige, A/J, and GKO. Antibiotic activity was tested for clarithromycin, amikacin, cefoxitin, tigecycline, and bedaquiline (TMC207). Finally, we evaluated the 3-drug combination clarithromycin, cefoxitin, and amikacin.

Results. Nude and GKO mice fulfilled criteria for the model but only nude mice offered sufficient availability for large therapeutic experiments. Among the 3 drugs usually combined for treatment of M. abscessus infection, cefoxitin was the most active because it improved survival and reduced bacillary loads in spleen whereas clarithromycin and amikacin prevented death but had little impact on bacillary loads. The triple-drug combination was not more active than cefoxitin alone. Tigecycline displayed bactericidal activity whereas bedaquiline was almost inactive.

Conclusions. Nude mice are an adequate model for in vivo chemotherapy studies. Among tested drugs, cefoxitin and tigecycline showed promising in vivo activity against M. abscessus. The best drug combination remains to be determined.

Keywords. Mycobacterium abscessus; clarithromycin; amikacin; cefoxitin; tigecycline; bedaquiline; TMC207; cystic fibrosis; murine model; nude mouse.
The study of antibiotic activity in experimental models has been very useful for designing the treatment of tuberculosis [18], leprosy, and some nontuberculous mycobacterial infections, such as Mycobacterium avium and Buruli ulcer [19–21]. Few studies have been carried out for rapidly growing mycobacteria infections, because there is no validated experimental model to test treatment regimens.

We up an experimental model for evaluating antibiotic activity against M. abscessus infection, focusing on disseminated infections, which are more difficult to cure. Because M. abscessus is less virulent than Mycobacterium tuberculosis, the method used for the tuberculosis model was adapted to obtain a model with high bacterial loads in organs persisting for a long time, essential for the study of both the elimination of bacteria and the selection of resistant mutants under treatment. We used this model to study the in vivo activity of the 3 drugs usually used in the treatment of M. abscessus infection, amikacin, cefoxitin and clarithromycin alone and combined. We also evaluated the activity of 2 new antibiotics, tigecycline and bedaquiline (TMC207).

MATERIALS AND METHODS

Mycobacterial Strain

For all the experiments we used the reference strain M. abscessus ATCC 19977, purchased from the Institute Pasteur Collection (CIP 104536T).

Development of Mouse Model

We followed the experimental guidelines provided by the medical faculty of Université Pierre et Marie Curie. Several mice strains were tested for the model of M. abscessus infection: BALB/c, C57BL/6j, nude (athymic mice with depletion of T cells), GKO mice (gamma interferon knot-out), A/J mice (deficient macrophagic functions), and beige mice (deficient in natural killers). BALB/c, Swiss, C57BL/6j, and nude mice were purchased from Janvier Breeding Center, A/J and beige mice from the Jackson Laboratory, and GKO mice from Centre National de la Recherche Scientifique. All mice were 4–6 weeks old, and all were female, except GKO mice owing to low availability.

Each mouse was inoculated intravenously in the tail vein with 0.5 mL of a bacterial suspension containing 10^6–10^8 colony-forming units (CFU) freshly prepared from a 4-day culture on trypticase soy agar (TSA). Seven experiments were conducted successively, because it was not possible to handle all the mice in parallel in a single experiment. The first 5 experiments were allocated to evaluate the development of M. abscessus infection in the different mouse strains. All included BALB/c mice as control. The 2 others were conducted to confirm results obtained with the nude strain which appeared as the more promising. The numbers of mice used in each experiment are presented in Supplementary Table 1. Mice were euthanized on days 1, 7, 14, 21, and 28 after inoculation and, when possible, on days 45, 60, and/or 75. The CFU counts were determined in the spleen, liver, lungs, and kidneys from 2 mice for experiments 1–5 and from 5 mice for experiments 6 and 7.

Experimental Chemotherapy Trials

In vitro antibiotic susceptibility was assessed before inoculation and after treatment experiments in mice by determining the minimum inhibitory concentration (MIC) in liquid medium. The microdilution method with standardized microtiter plates (Sensititre; Trek Diagnostic System) was used for clarithromycin, amikacin, cefoxitin, and tigecycline. The results were assessed after 3–5 days of incubation, as recommended by the Clinical and Laboratory Standards Institute [21], but for clarithromycin results were assessed after 14 days of extended incubation to detect inducible resistance [19]. For bedaquiline, a macrodilution method in brain-heart infusion broth was used with 3-day incubation. Minimum bactericidal concentration (MBC) was measured after 14 days of incubation for bedaquiline. For that purpose, liquid cultures not showing any macroscopic growth were numerated onto TSA as well as the initial inoculum before incubation. The MBC was defined as the lowest drug concentration that killed ≥99.99% of the initial population.

Three trials were conducted in nude mice. Trial 8 evaluated the activity of clarithromycin, cefoxitin, and amikacin alone and combined. Trial 9 evaluated the activity of tigecycline. Trial 10 evaluated the activity of bedaquiline. Drug dosing was chosen to mimic human pharmacokinetics at the usual dosing [22–26]. The details of experimental scheme are presented in Supplementary Table 2. Clarithromycin, cefoxitin, amikacin, and tigecycline were purchased in forms for human use (Zeclar [Abbott], Mefoxin [Merck Sharp & Dohme], Amiklin [Bristol-Myers Squibb], and Tygacil [Pfizer], respectively); bedaquiline was generously provided by Johnson and Johnson laboratories.

Assessment of Results

Mortality, macroscopic lesions in organs, and CFU counts in organs were used as parameters for assessing the in vivo multiplication of the bacilli and the severity of infection. Kidney lesions were scored according to the number of abscesses: 0 indicated no abscess; 1, 1–10 abscesses; 2, 11–20 abscesses; and 3, >20 abscesses.

The CFU counts were determined in the spleen and lungs in all experiments and also in the kidneys and liver in the development of the model. The organs were aseptically removed and homogenized without decontamination. Suspensions were made up to 2 mL for each organ. At least 4 serial 10-fold dilutions of the suspension were plated onto TSA (0.05 mL each) for enumeration of colonies. Because no antibiotic was added to TSA medium, each colony that was not recognized as a
mycobacteria colony was checked with Ziehl-Neelsen staining. For assessment of bedaquiline results, to avoid carryover [27], Lowenstein-Jensen medium was used in place of TSA. The results of the culture were recorded after incubation at 30°C for 7 days. The organs of animals that died the day before sacrifice were cultivated (that is, if the animals were not found in late decomposition).

**Statistical Analysis**

Statistical analysis was performed using the Fisher test for survival and the Mann–Whitney test for mean bacterial loads and kidney lesions.

**RESULTS**

**Mouse Model Development**

The results of the systematic evaluation of *M. abscessus* infection of each strain of mice (experiments 1–5) during the first month of infection are presented in Table 1. A few animals died among Swiss (0%), C57BL/6 (0%), BALB/c (3%), beige (0%), and nude mice (4%). Mortality rates were higher among GKO (10%) and AJ (17%) mice.

No gross lesions were observed in the liver or lungs of mice. However, enlargement of spleen and kidney gross lesions (white spots) were observed in all mice, with no clear differences between mouse strains. Based on this observation, a score was established that was subsequently used in the chemotherapy experiments (see Materials and Methods).

Among BALB/c, Swiss and C57BL/6 mice, CFU counts decreased regularly in the spleen, liver and lungs from day 0 to day 45 after infection. In the kidneys, CFU counts increased initially in BALB/c and Swiss mice, but there was no increase in C57BL/6 mice.

The CFU counts also decreased in the spleen, liver, and lungs of nude mice from day 1 to day 14, but to a lower extent than in the 3 mouse strains listed above. After day 14, the bacterial load

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**Table 1. Mortality, Gross Lesions and Mean Colony-Forming Unit (CFU) Counts During the First Month After Inoculation of Different Mouse Strains With *Mycobacterium abscessus***

<table>
<thead>
<tr>
<th>Outcome</th>
<th>BALB/c Mice(^b) (Experiments 1–5)</th>
<th>Nude Mice(^b) (Experiment 2)</th>
<th>GKO Mice(^b) (Experiment 3)</th>
<th>A/J Mice(^b) (Experiment 4)</th>
<th>Beige Mice(^b) (Experiment 5)</th>
<th>Swiss Mice(^b) (Experiment 2)</th>
<th>C57BL/6 Mice(^c) (Experiments 3 and 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality on day 30</td>
<td>2/60</td>
<td>0/14</td>
<td>1/10</td>
<td>2/12</td>
<td>0/12</td>
<td>0/13</td>
<td>0/27</td>
</tr>
<tr>
<td>Gross lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Spleen, liver, lungs, tail</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>CFU counts, (\log_{10}) \n</td>
<td>Spleen</td>
<td>\n</td>
<td>Day 0</td>
<td>(7.0 \pm 0.3)</td>
<td>(7.4)</td>
<td>(7.1)</td>
<td>(6.7)</td>
</tr>
<tr>
<td>Day 28</td>
<td>(4.2 \pm 0.3)</td>
<td>(6.6)</td>
<td>(5.6^{d})</td>
<td>(5.4^{o})</td>
<td>(3.3)</td>
<td>(4.5)</td>
<td>(3.1 \pm 0.1)</td>
</tr>
<tr>
<td>(\text{Change}^{f})</td>
<td>(-2.8)</td>
<td>(-1.1)</td>
<td>(-1.5)</td>
<td>(-1.3^{o})</td>
<td>(-3.2)</td>
<td>(-2.7)</td>
<td>(-3.7)</td>
</tr>
<tr>
<td>Liver</td>
<td>\n</td>
<td>Day 0</td>
<td>(7.7 \pm 0.3)</td>
<td>(7.9)</td>
<td>(7.8)</td>
<td>(7.8)</td>
<td>(7.3)</td>
</tr>
<tr>
<td>Day 28</td>
<td>(4.2 \pm 0.6)</td>
<td>(6.1)</td>
<td>(7.4^{d})</td>
<td>(5.1^{o})</td>
<td>(3.4)</td>
<td>(3.9)</td>
<td>(3.9 \pm 0.3)</td>
</tr>
<tr>
<td>(\text{Change}^{f})</td>
<td>(-3.4)</td>
<td>(-1.8)</td>
<td>(-0.4)</td>
<td>(-2.7^{o})</td>
<td>(-3.8)</td>
<td>(-3.9)</td>
<td>(-3.5)</td>
</tr>
<tr>
<td>Lungs</td>
<td>\n</td>
<td>Day 0</td>
<td>(5.6 \pm 0.3)</td>
<td>(6.0)</td>
<td>(5.1)</td>
<td>(5.6)</td>
<td>(5.4)</td>
</tr>
<tr>
<td>Day 28</td>
<td>(3.3 \pm 0.4)</td>
<td>(5.0)</td>
<td>(3.5^{d})</td>
<td>(3.1^{d})</td>
<td>(3.0)</td>
<td>(3.2)</td>
<td>(3.0 \pm 0.6)</td>
</tr>
<tr>
<td>(\text{Change}^{f})</td>
<td>(-2.3)</td>
<td>(-1.0)</td>
<td>(-1.6)</td>
<td>(-2.5)</td>
<td>(-2.4)</td>
<td>(-2.6)</td>
<td>(-2.3)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>\n</td>
<td>Day 0</td>
<td>(4.8 \pm 0.6)</td>
<td>(5.0)</td>
<td>(5.0)</td>
<td>(4.7)</td>
<td>(4.3)</td>
</tr>
<tr>
<td>Day 28</td>
<td>(6.2 \pm 0.4)</td>
<td>(7.3)</td>
<td>(10.1^{d})</td>
<td>(7.4)</td>
<td>(6.1)</td>
<td>(5.3)</td>
<td>(4.1 \pm 0.7)</td>
</tr>
<tr>
<td>(\text{Change}^{f})</td>
<td>(+1.4)</td>
<td>(+2.3)</td>
<td>(+5.2)</td>
<td>(+2.7)</td>
<td>(+1.8)</td>
<td>(+0.6)</td>
<td>(+0.2)</td>
</tr>
</tbody>
</table>

\(^{a}\) The inoculum was \(\log_{10} 8.2, 8.4, 8.2, 8.0, \) and 7.4 for experiments 1, 2, 3, 4, and 5, respectively. The strain was ATCC 19977.

\(^{b}\) Mean results of experiments 1–5.

\(^{c}\) Mean results of experiments 3 and 5.

\(^{d}\) Only 1 mouse.

\(^{o}\) Because the culture for day 30 was contaminated, the results for day 21 are reported.

\(^{f}\) Change from day 0 to day 28.
reached a plateau at 6 log_{10} in spleen and liver and at 4–5 log_{10} in lungs until day 75, with no evidence of a decline trend. In contrast, CFU increased in kidneys regularly from day 1 to day 28. The increase was about 2 times higher than among BALB/c mice. The bacterial load reached a plateau at 7 log_{10} at days 28–75.

The CFU counts decreased from day 1 to day 21 in the spleen and liver of GKO mice and, as in nude mice, reached at day 28 a plateau at 5 log_{10} in the spleen and at 6–7 log_{10} in the liver. In the lungs, CFU counts decreased from day 1 to day 14 (by approximately 1 log_{10}). In kidneys, as for nude mice, CFU increased regularly from day 1 to day 28. The increase was about 3 times higher in GKO than in BALB/c mice. The limited availability of GKO mice at the time of the study made impossible to assess CFU counts after day 28. The CFU counts decreased regularly in the spleen, liver, and lungs of A/J and beige mice from day 1 to day 28. The decrease was approximately the same as for BALB/c mice (2–4 log_{10}). The bacterial load was <6 log_{10} at day 28. The CFU counts increased markedly in kidneys from day 1 to day 14, but this increase was followed by a decrease (starting at day 14 for A/J and at day 28 for beige mice).

**Confirmation of the Results Obtained With the Nude Mouse Model**

Results of experiments 6 and 7 were compared with those of experiment 2. No deaths occurred, and lesions were observed only in the kidneys. The evolution of CFU counts was similar in the 4 organs, ending in a plateau in the spleen, liver (approximately 6 log_{10}), and lungs (approximately 5 log_{10}) and reaching 7–8 log_{10} in the kidneys. The variations in the results from one experiment to another were small for the spleen, liver, and lungs (standard deviation, <0.85 log_{10}). Variations were more important for the kidneys, especially at days 28 and 45, with the standard deviations reaching 1.5 and 2.4 log_{10} on days 28 and 45, respectively (Figure 1).

**In Vivo Efficacy of the Recommended Antibiotic Therapy (Clarithromycin, Amikacin, and Cefoxitin)**

Mice were inoculated with 6.3 log_{10} CFU. Pretreatment MICs were 32 µg/mL for cefoxitin and 16 µg/mL for amikacin. For clarithromycin, the MIC increased from 1 µg/mL after 5 days of incubation to >16 µg/mL after 14 days.

In the control group, 8 of 21 mice (38%) died. Among the treated animals, a single mouse died in the group receiving the triple-drug combination and none in the groups receiving monotherapy. Compared with untreated animals, the difference was statistically significant for the monotherapy groups (0/10; \( P = .03 \)) but not for those receiving the triple-drug combination (1/10; \( P = .2 \)), although the absolute difference between the 2 groups was only 1 mouse (Supplementary Table 3). The kidney lesions were less statistically important in all treated groups than in the untreated control group after 2 months, but after 3 months the difference remained significant only for cefoxitin and the triple-drug combination.

The bacterial load remained roughly stable in the spleen and lungs of control mice between day 0 and month 2 (Figure 2). This load decreased at month 3 but was assessed in the 4 surviving mice. In spleen, compared with untreated mice, all treated groups showed a significant decrease in CFU counts at month 2, whereas at month 3 the difference remained significant only for cefoxitin and the triple-drug combination.

![Figure 1. Colony-forming unit (CFU) counts in the organs of nude mice infected with *Mycobacterium abscessus*; the counts represent mean values for experiments 2, 6, and 7.](image-url)
In the lungs, none of the bacillary loads in the treated groups differed significantly from those in the control group. The differences between the untreated control group and the 4 treatment groups can be ranked as follows: cefoxitin $\geq$ triple-drug combination $>$ clarithromycin = amikacin (Supplementary Table 3). There was no modification of antibiotic MICs after treatment. In particular, the strain displayed the same clarithromycin-inducible resistance.

Activity of the New Antibiotics Tigecycline and Bedaquiline

The MIC of tigecycline for the pretreatment isolate was 0.5 µg/mL. Mice were inoculated with 6.3 log$_{10}$ CFU. In the control group, 6 of 11 mice (55%) died. All the euthanized mice (4 at month 1) and all the dead mice except 1 (1 mouse died at day 56) had numerous gross kidney lesions and the bacterial load remained stable from day 1 to month 2 in their spleen and lungs. Tigecycline prevented death (mortality rate, 0/12; $P = .05$ vs control) as well as kidney lesions ($P = .01$ at month 1 and $P = .1$ at month 2 vs untreated control mice; Supplementary Table 3). Compared with untreated mice, CFU counts were significantly smaller in tigecycline-treated mice in the spleen at months 1 and 2 ($P = .02$ and .004) and in lungs at month 2 ($P = .008$) whereas the difference was almost significant in lungs at month 1 ($P = .07$; Figure 3). There was no increase in the tigecycline MIC for the posttreatment isolate.

The MIC of bedaquiline for the pretreatment isolate was 0.5 µg/mL in agar and 0.06 µg/mL in brain-heart infusion broth. The MBC was $>2$ µg/mL. Mice were inoculated with 6 log$_{10}$ CFU. In the control group, 2 of 10 mice (20%) died, on day 55. All the mice (sacrificed and dead) had numerous gross kidney lesions. The bacterial load remained stable from day 1 to month 2 in spleen but decreased by about 1.5 log$_{10}$ in lungs. Bedaquiline did not prevent death (2/10; 20%) or kidney lesions and did not modify the decrease of bacillary load except in the spleen at month 1 ($P = .02$) (Supplementary Table 3 and Figure 4). There was no increase in the bedaquiline MIC for the posttreatment isolate.

DISCUSSION

The results of the experiments comparing the evolution of $M. abscessus$ infection in 7 strains of mice showed that nude and GKO mice are the most suitable for establishing a model of $M. abscessus$ infection for chemotherapy studies, that is, allowing high, stable, and reproducible bacterial loads in organs. Similar
results have already been published for GKO mice [28]. However, only nude mice fulfilled all the desirable criteria, because GKO mice are expensive and have limited availability, and experimental chemotherapy requires high numbers of animals. Moreover, nude mice are largely used for other mycobacterial infections [29–31] and have been recently chosen as a model for *Mycobacterium xenopi* infection [32]. However, because CFU counts initially decrease in nude mice before reaching a plateau, antibiotic activity should be evaluated by comparing treated and untreated mice at simultaneous time points. The reproducibility of the model, as assessed by standard deviations of mean CFU counts observed in 3 independent experiments was good for liver and lungs but not for kidneys. Although the CFU count increase in kidneys seems interesting, the high variability in the CFU counts, probably due to the presence of abscesses, prevented us from choosing bacterial count in this organ for assessing drug treatment efficacy. We did, however, use the gross lesions seen on kidneys to establish a score allowing a rough estimate of drug treatment efficacy (see Material and Methods).

The second objective of this work was to evaluate the in vivo activity of antibiotics against *M. abscessus*. In the 3 therapeutic trials (experiments 8, 9, and 10), lung and spleen CFU and kidney lesions of untreated control mice evolved as in the 3 nude mice model experiments (experiments 2, 6, and 7). Compared with model experiments, mortality in untreated control mice was equivalent in therapeutic trials 8 and 10 (2/10 vs 2/61 after 2 months; *P* = .09) but was higher in therapeutic trial 9 (3/11 vs 1/54 [P = .02] during the first month and 3/6 vs 1/17 [P = .03] during the second). We do not think that this difference limits the interpretation of the results, because tigecycline clearly prevented death. However, this high difference in mortality rates between treated and control groups limits the significance of CFU count comparison, especially at late time points. Conversely, the mortality observed in bedaquiline-treated mice shows differences compared with control animals, because this drug was not able to prevent death.

The reduction in bacterial loads after clarithromycin treatment was limited and comparable to that obtained with bacteriostatic antitubercular drugs, such as ethambutol. For the *M. abscessus* complex, as for *M. avium* [33], the MBC of clarithromycin is high, even for strains with low MICs (data not shown). The reference strain used in our study, displays in vitro inducible clarithromycin resistance, as illustrated by the increased MIC after 14 days of incubation [34]. Human and mouse data, recently published, have demonstrated that the lack of activity of clarithromycin against *M. abscessus* correlates with a functional *erm* gene [14, 35]. However, it should be emphasized that clarithromycin had a favorable impact on mortality and consequently should not be considered as completely inactive in vivo. These results obtained in nude mice are reminiscent of the retrospective analysis by Jeon et al [12] who found that a multidrug regimen including clarithromycin had favorable effect on symptoms in 75% of *M. abscessus* infection cases, on high-resolution computed tomography lesions in 42% of cases but resulted in bacteriological sputum conversion in only 25%.

Amikacin showed little activity in the nude mouse model, a result that is consistent with in vitro data showing high MBC contrasting with low MIC [36]. Recent clinical data showed that the combination of clarithromycin plus moxifloxacin was more active than that of clarithromycin plus amikacin [37]. Taken together with its long-term toxicity, these results do not support the long-term use of amikacin against *M. abscessus* infections.

Cefoxitin was the most active drug among those tested in trial 8. It has been shown recently that the peptidoglycan of *M. abscessus* contains predominantly 3-3 cross-links generated by L,D-transpeptidases [38]. Thus, it is not surprising that cefoxitin showed activity in vivo, even that cephalosporins have in vitro activity against L-D transpeptidases [39]. Because the dosing used for mice in our trial is equipotent to that used in humans [22], we think that cefoxitin should be further evaluated for the treatment of *M. abscessus* infections in humans. Imipenem, a β-lactam that has been shown to be a potent inhibitor of L,D-transpeptidases, may also have in vivo activity and may display synergistic activity with other drugs [39–41].

Figure 4. Lung (A) and spleen (B) colony-forming unit (CFU) counts in nude mice treated with bedaquiline (25 mg/kg/d; trial 10).
The triple-drug combination cefoxitin, amikacin, and clarithromycin was not more active than cefoxitin alone and may even be antagonistic, as suggested by CFU counts after 3 months. Similar antagonism has been shown recently between macrolides and moxifloxacin [42]. These results do not support the use of this combination with the aim to increase antibacterial activity. However, because the main goal of drug combinations in mycobacterial infections is to prevent drug resistance, it seems reasonable to use at least a 2-drug combination. Including >2 drugs could increase toxicity without increasing efficacy.

The in vitro activity of tigecycline against M. abscessus is already established [43], but our current results are the first showing the in vivo activity of this drug. Tigecycline could also be useful in drug combinations because it displays synergistic activity with clarithromycin and amikacin [44]. However, there are concerns about its safety of tigecycline, which may limit its use against M. abscessus infections that require long-term therapy [45].

Bedaquiline did not show any activity in the nude mouse model; it was the only tested drug that did not prevent death. This lack of activity is probably explained by high MBC, as already described for M. avium [46].

A caveat must be emphasized in interpreting the results of these studies. We did not select drug-resistant mutants during monotherapy and thus could not assess the capacity of combined therapy to prevent drug resistance. We believe that this is due to insufficient initial bacillary load (5.5–6 log_{10}). However, it must be recalled that all the published work on experimental chemotherapy for M. abscessus showed initial bacillary loads <7 log_{10} [35, 42].

Apart from the drugs we tested, some other seem promising and may improve the treatment of M. abscessus infections. Among these, moxifloxacin has been shown to be active in a murine model and also in clinical setting [37, 42]. Clofazimine has been shown to act synergistically in vitro with many of the drugs used against M. abscessus [47, 48]. These 2 drugs, given orally and having little toxicity, could be interesting choices for the long-term treatment required for M. abscessus infections. However, because we and others have described antagonism between antibiotics in vivo, these drug combinations should be tested in animal models before clinical use.

In conclusion, we showed that nude mice can be used as a model for in vivo evaluation of antibiotic activity against M. abscessus. Cefoxitin and tigecycline show promising in vivo activity and should be further evaluated.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Potential conflict of interest.** E. C., V. J., C. T., and N. V. have conducted research experiments on bedaquiline (TMC207) activity against M. tuberculosis that have been supported by Janssen Laboratory. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.


