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Moxifloxacin's Limited Efficacy in the Hollow-Fiber Model of *Mycobacterium abscessus* Disease

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Current regimens used to treat pulmonary *Mycobacterium abscessus* disease have limited efficacy. There is an urgent need for new drugs and optimized combinations and doses. We performed hollow-fiber-system studies in which *M. abscessus* was exposed to moxifloxacin lung concentration-time profiles similar to human doses of between 0 and 800 mg/day. The minimum bactericidal concentration and MIC were 8 and 2 mg/liter, respectively, in our *M. abscessus* strain, suggesting bactericidal activity. Measurement of the moxifloxacin concentrations in each hollow-fiber system revealed an elimination rate constant (k_{el}) of 0.11 ± 0.05 h^{-1} (mean ± standard deviation) (half-life of 9.8 h). Inhibitory sigmoid maximal effect (E_{max}) modeling revealed that the highest E_{max} was 3.15 ± 1.84 $\log_{10}$ CFU/ml on day 3, and the exposure mediating 50% of E_{max} (EC_{50}) was a 0- to 24-h area under the concentration time curve (AUC_{0-24})-to-MIC ratio of 41.99 ± 31.78 ($r^2 = 0.99$). The EC_{80} was an AUC_{0-24}/MIC ratio of 102.11. However, no moxifloxacin concentration killed the bacteria to burdens below the starting inoculum. There was regrowth beyond day 3 in all doses, with replacement by a resistant subpopulation that had an MIC of >32 mg/liter by the end of the experiment. A quadratic function best described the relationship between the AUC_{0-24}/MIC ratio and the moxifloxacin-resistant subpopulation. Monte Carlo simulations of 10,000 patients revealed that the 400- to 800-mg/day doses would achieve or exceed the EC_{80} in ≤12.5% of patients. The moxifloxacin susceptibility breakpoint was 0.25 mg/liter, which means that almost all *M. abscessus* clinical strains are moxifloxacin resistant by these criteria. While moxifloxacin's efficacy against *M. abscessus* was poor, formal combination therapy studies with moxifloxacin are still recommended.

Mycobacterium abscessus is a rapidly growing mycobacterium that is notorious because of resistance to most antibiotics (1). Pulmonary disease due to *M. abscessus* infection is chronic and relentless. Current regimens used to treat *M. abscessus* consist of a combination of amikacin, a macrolide, and either cefoxitin or imipenem; however, the regimens fail in most patients (2). Based on static *in vitro* models, amikacin is considered the key antibiotic in the treatment regimens (3–6). We recently demonstrated that the efficacy of amikacin based on concentration-time profiles achievable in human lungs as recapitulated in the hollow-fiber-system model of *M. abscessus* (HFS-*M. abscessus*) was poor, with failure to kill the bacteria below stasis (7). This, as well as clinical experience of high failure rates of amikacin-based regimens, means that there is an urgent need to find new antibiotics and optimize their doses. Here, we approached this by conducting a recommended formal pharmacokinetic/pharmacodynamic (PK/PD) evaluation of alternative antibiotics with potential bactericidal activity (8).

The 8-methoxy fluoroquinolone, moxifloxacin, has been shown to have excellent efficacy against *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium kansasii* (9–12). In addition, continuation regimens currently used to treat pulmonary *M. abscessus* include moxifloxacin in the combination, starting from the second month of therapy. Given these prior successes of moxifloxacin, as well as its current role in the treatment of *M. abscessus*, we tested it against this species. We performed formal moxifloxacin PK/PD work in the hollow-fiber-system model of pulmonary *M. abscessus*. We exposed *M. abscessus* to moxifloxacin concentration-time profiles as encountered in lungs of patients with pneumonia (13). Repetitive day-to-day sampling of hollow-fiber systems is a major advantage in identifying the evolution of bacteria in response to the periodic fluctuation of antibiotic concentrations, vital given *M. abscessus*' propensity to develop acquired drug resistance (8, 14–17).

MATERIALS AND METHODS

Bacteria, antibiotic, and growth conditions. Stock cultures of *M. abscessus* ATCC 19977 (American Type Culture Collection, Manassas, VA), stored at −80°C in Middlebrook 7H9 broth supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC; Remel, Lenexa, KS) and 15% glycerol, were used for all the experiments. One vial was thawed before each assay and incubated for 24 to 48 h at 30°C to achieve logarithmic growth phase. Moxifloxacin hydrochloride was purchased from the Baylor University Medical Center pharmacy. On the day of use, the moxifloxacin was diluted in sterile water to desired concentrations for the assays.

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Antimicrobial susceptibility testing and mutation frequency. The MIC was identified using broth macrodilution in Middlebrook 7H9 broth (here termed "broth"), as well as by use of the Etest (bioMérieux, Durham, NC). In addition to the turbidity test, the CFU per ml were enumerated for each concentration evaluated in the broth macrodilution test. In this test, the MIC was defined as the lowest concentration associated with ≥99% decrease in CFU/ml compared to the growth of the untreated control and the minimum bactericidal concentration (MBC) as the concentration corresponding to >99.9% kill. Mutation frequency was determined for the inoculum by culturing 0.2 ml on Middlebrook 7H10 agar plates (here termed "agar") supplemented with 3 times the moxifloxacin MIC. Cultures were incubated for 5 days.

Exposure-response studies in the hollow-fiber system. The hollow-fiber-system model of pulmonary *M. abscessus* (HFS-*M. abscessus*) has been used previously to perform PK/PD evaluation of amikacin (7). Twenty milliliters of 6 $\log_{10}$ CFU/ml *M. abscessus* in log phase was inoculated into the peripheral compartment of each of seven hollow-fiber cartridges (FiberCell Systems, Frederick, MD). Doses that mimicked the non-protein-bound plasma 0- to 24-h area under the concentration time curve (AUC_{0-24}), peak concentrations, and time to maximum concentration achieved in humans treated with moxifloxacin doses of 0, 25, 50, 100, 200, 400, and 800 mg were administered to the central compartment once daily via computerized syringe pumps (18). These exposures were chosen because they represent the range of clinically tolerated doses of moxifloxacin. Treatment was for 21 days of daily therapy. A plasma-to-lung epithelial lining fluid penetration ratio of 1 was assumed, based on the literature (13, 19, 20). As an example, the standard 400-mg-a-day dose was expected to achieve a peak concentration of 4.2 mg/liter and a half-life of 10 h, translating to an AUC_{0-24}/MIC ratio of 28.3. The actual moxifloxacin concentrations achieved in all the systems were validated by repetitive sampling of 1 ml from the central compartment of each HFS-*M. abscessus* during the first 3 days at 0, 1, 6, 9, 12, 18, 23.5, 25, 30, 33, 36, 42, and 47.5 h postdose. In order to quantify the *M. abscessus* burden, 1 ml of the peripheral compartment culture contents was removed from each system on days 0, 1, 2, 3, 5, 7, 10, 14, and 21. The samples were washed with saline to avoid antibiotic carryover, after which samples were serially diluted and cultured on agar. To quantify the moxifloxacin-resistant *M. abscessus* CFU/ml, the same samples were also inoculated onto agar supplemented with 3 times the moxifloxacin MIC.

Drug assay. The moxifloxacin concentrations in the samples collected from the central compartment of the HFS-*M. abscessus* were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Moxifloxacin and moxifloxacin-13CD3 (internal standard) were purchased from Sigma (St. Louis, MO) and Santa Cruz Biotech (Santa Cruz, CA), respectively. Calibrator, controls, and internal standard were included in each analytical run for quantitation. Stock solutions of moxifloxacin and the internal standard were prepared in 80:20 methanol-water at a concentration of 1 mg/ml and stored at −20°C. A 7-point calibration curve was prepared by diluting moxifloxacin stock solution in drug-free medium (0.1, 0.2, 1, 2, 5, 10, and 20 mg/liter). Quality control samples were prepared by spiking medium with stock standards for two levels of controls. Samples were prepared in 96-well microtiter plates by the addition of 10 μl of calibrator, quality controls, or sample to 190 μl of 0.1% formic acid in water containing 10 mg/liter internal standard, followed by vortexing. Chromatographic separation was achieved on an Acquity UPLC high-strength silica (HSS) T3 analytical column (1.8 μm particle size, 50 by 2.1 mm; Waters) maintained at 30°C at a flow of 0.2 ml/min with a binary gradient and a total run time of 6 min. The observed ion values (*m/z*) of the fragment ions were as follows: for moxifloxacin, *m/z* 402.2→384.2, and for the internal standard, moxifloxacin-13CD3, *m/z* 406.2→388.3. Sample injection and separation were performed by using an Acquity UPLC column interfaced with a Xevo TQ mass spectrometer (Waters). All data were collected using MassLynx version 4.1 with software change note 810 (SCN810). The limit of quantitation for this assay was 0.1 mg/liter. The inter- and intraday variations were 1.5% and 9.4%, respectively.

TABLE 1 Moxifloxacin pharmacokinetic parameter estimates utilized in Monte Carlo simulations

Pharmacokinetic parameter	Observed in patients (entered into subroutine PRIOR)		Simulated for 10,000 patients	
	Parameter estimate	IIV[a] as %CV	Parameter estimate	IIV as %CV
Total clearance (liters/h)	11.3	23.7	11.3	14.58
Intercompartmental clearance (liters/h)	47.7	—[b]	47.7	19.19
Absorption rate constant (h^{-1})	1.09	135	1.09	143
Central vol (liters)	55.6	—	55.6	18.40
Peripheral vol (liters)	59.6	15.3	59.6	14.99

[a] IIV, interindividual variability.
[b] —, fixed in the pharmacokinetic model by Kees et al. (27).

Pharmacokinetics and pharmacodynamics modeling. All drug concentrations from each of the HFS-*M. abscessus* units at all time points were comodeled using ADAPT 5 software (Biomedical Simulations Resource, University of Southern California). The steps used in the pharmacokinetic parameter analysis were as described in detail in prior studies (10, 21). The pharmacokinetic parameter estimates identified were used to calculate the observed AUC_{0-24} values and AUC_{0-24}/MIC ratios. Exposure-response was modeled using the inhibitory sigmoid maximal effect (E_{max}) model, a standard model recommended for examining the effects of antibiotics whether they are static or cidal, for both clinical and laboratory data (22–24). Total bacterial burden was used as the response parameter, while drug exposure was expressed as the AUC_{0-24}/MIC ratio. For moxifloxacin resistance emergence, we used the quadratic model that we identified previously, with drug exposure examined versus the size of the moxifloxacin-resistant subpopulation on each sampling day (8, 25).

Monte Carlo simulations. In order to put our findings into clinical context, we performed a 10,000-patient Monte Carlo simulation to identify two important clinical aspects. First, we wanted to identify the clinical dose best able to achieve or exceed the EC_{80}, which is the exposure mediating 80% of the maximal kill (E_{max}). This exposure is considered optimal since E_{max} is on an asymptote and it is independent of whether a drug is cidal or static. Second, we wanted to identify the susceptibility breakpoint, which we considered the MIC below which >10% of patients achieve the EC_{80} with standard dosing or with the highest dose that can be administered. The steps in the Monte Carlo simulations were as detailed in recommendations elsewhere and in our prior work (8, 26). We examined moxifloxacin doses of 400 mg a day, 600 mg a day, and 800 mg a day. We entered the two-compartment model pharmacokinetic parameter estimates and covariance identified by Kees et al. into subroutine PRIOR of ADAPT 5, as shown in Table 1 (27). We assumed a 1:1 AUC_{0-24} ratio in the lung versus plasma (13). An MIC distribution from South Korea (28) was used, and data on MIC distribution from Nijmegen, the Netherlands, and the Dallas-Fort Worth metroplex, United States, were included for comparison of the impact of the new susceptibility breakpoint.

RESULTS

In this study, the moxifloxacin MIC for the *M. abscessus* laboratory strain was 2 mg/liter. In addition, the concentration associated with >99.9% kill, or MBC, was 8 mg/liter. Thus, the MBC/MIC ratio was 4, which means that moxifloxacin would be considered bactericidal according to the standard convention (29). The frequency of mutation to 3 times the moxifloxacin MIC was $(2.11 \pm 0.16) \times 10^{-5}$ in repeat experiments.

In the HFS-*M. abscessus* dose-effect studies, the moxifloxacin

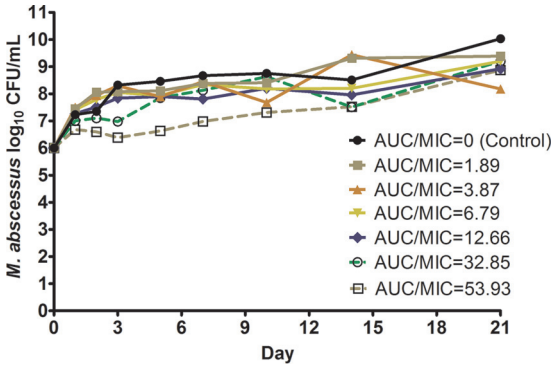

FIG 1 Moxifloxacin exposure-effect against *M. abscessus* in the HFS. None of the moxifloxacin exposures evaluated attained killing below the starting inoculum of 6.0 $\log_{10}$ CFU/ml at any point during the study. Regrowth was observed after day 3 in all systems.

concentrations measured in each HFS-*M. abscessus* unit revealed an elimination rate constant (k_{el}) of 0.11 ± 0.05 h^{-1}, which translates to a half-life of 9.8 h, consistent with the moxifloxacin pharmacokinetics in a recent 241-patient clinical study (18). As an example, the r^2 for the intended drug concentrations, exposures, and half-life of the 400-mg standard dose versus the observed concentrations was 0.98, which means the intended pharmacokinetics were recapitulated well. Figure 1 shows the time-kill curves for each of the moxifloxacin exposures in the HFS-*M. abscessus*. None of the exposures evaluated achieved a considerable killing effect of the *M. abscessus*.

The relationship between the AUC_{0-24}/MIC ratio and the *M. abscessus* burden in the HFS-*M. abscessus* is shown in Fig. 2; the highest E_{max} was encountered on day 3. The day 3 parameters were an E_{max} of 3.15 ± 1.84 $\log_{10}$ CFU/ml, a Hill slope of 1.56 ± 0.63, and an EC_{50} that was an AUC_{0-24}/MIC ratio of 41.99 ± 31.78 ($r^2 = 0.987$). Based on the inhibitory sigmoid E_{max} relationship for day 3, the EC_{80} was calculated as a non-protein-bound AUC_{0-24}/MIC ratio of 102.11. The E_{max} appears high mainly because in nontreated controls, *M. abscessus* grew to large bacterial burdens of about 10 $\log_{10}$ CFU/ml, starting from 6.0 $\log_{10}$ CFU/ml at the start of the experiment. Indeed, the data in Fig. 2 show that even

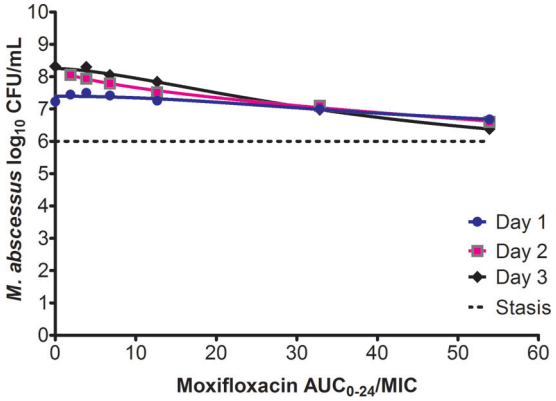

FIG 2 Moxifloxacin exposure versus *M. abscessus* burden. Inhibitory sigmoid E_{max} curves are shown for the first 3 days, prior to replacement of microbial population by drug-resistant subpopulation. The highest E_{max} was encountered on day 3, but not even that produced kill below the stasis level.

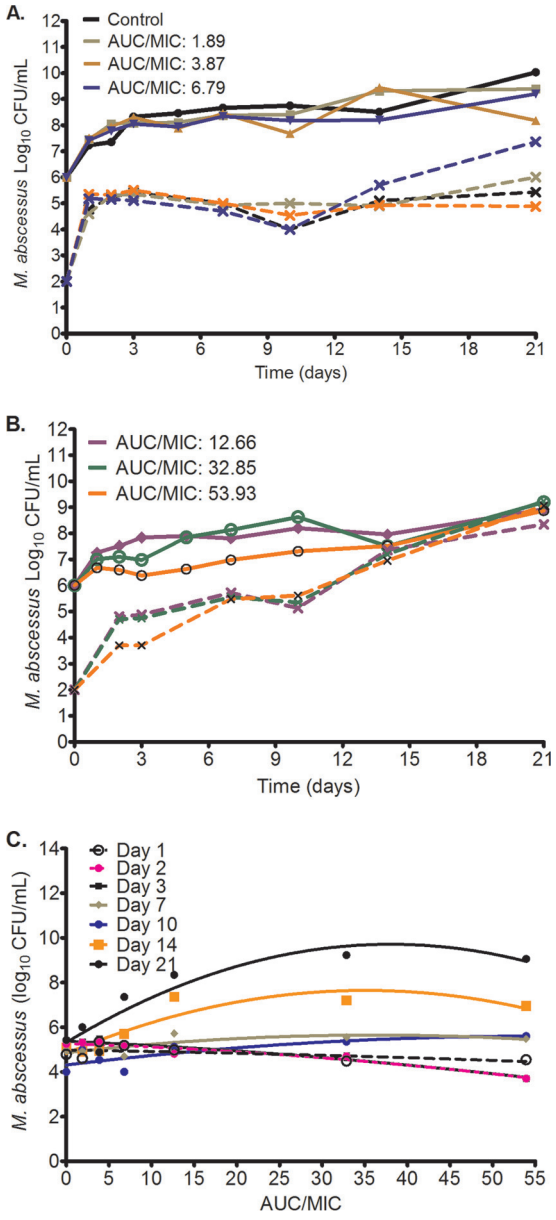

FIG 3 Changes in total and moxifloxacin-resistant subpopulations with time. Total *M. abscessus* population (solid lines) and moxifloxacin-resistant subpopulation (hatched lines) over the course of 21 days of treatment with different moxifloxacin AUC_{0-24}/MIC exposures. (A) Control and three lower exposures. While the drug-resistant subpopulation increased with time, especially beyond day 10, it had not completely replaced the total population in these lower doses, with the highest dose among these lower doses associated with the largest moxifloxacin-resistant subpopulation. (B) At the three highest exposures, the total population had been completely replaced by the moxifloxacin-resistant subpopulation by day 14. (C) The relationship between moxifloxacin AUC/MIC and size of subresistant subpopulation is described by systems of evolving U-shaped curves. At day 1 it was a straight line, then it switched to an inverted U-shaped curve that was more pronounced by day 21.

on day 3, moxifloxacin never reduced the bacterial burden below the starting inoculum (stasis).

The data in Fig. 3 show that acquired drug resistance emerged after 10 days of exposure. These data further show that the moxifloxacin-resistant subpopulation had replaced the total popula-

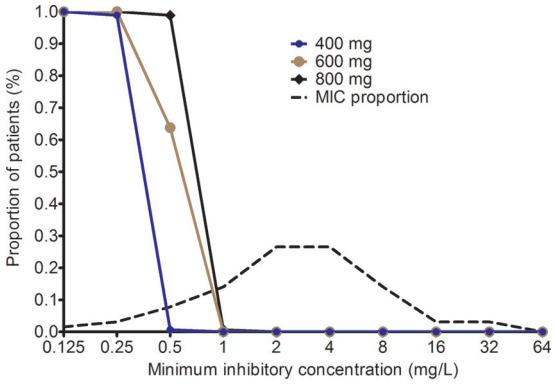

FIG 4 Probability of target attainment of three different moxifloxacin doses in 10,000 patients. Target attainment probability at each MIC fell to 0% after the 0.25-mg/liter MIC for a dose of 400 mg and to just above 60% for 600 mg. The target attainment probability for a dose of 800 mg a day plummeted after the 0.5-mg/liter MIC. Since the proportion of clinical isolates with an MIC of >0.25 mg/liter is large (and 0% of these achieve the EC_{80}), the fraction of all patients who will achieve the EC_{80} is very small.

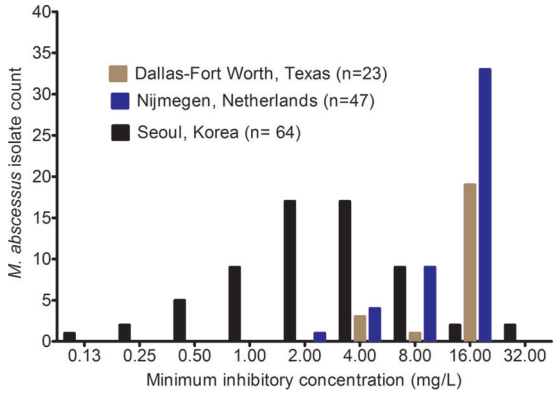

FIG 5 Moxifloxacin MIC distribution in three different practice locations. The MIC distribution from South Korea was used for the Monte Carlo simulations; the other two settings are used as comparators. Notably, the MIC distributions from the Netherlands and from Texas, United States, show most isolates to be resistant to moxifloxacin.

tion in the systems exposed to the 3 highest doses by day 21.The relationship between the size of the resistant subpopulation and the moxifloxacin exposure was described by a system of changing U-shaped curves, in effect a quadratic function. However, even at the highest AUC_{0-24}/MIC ratios tested, moxifloxacin was not able to suppress acquired drug resistance. Indeed, the MICs of cultures from the systems exposed to the 3 highest doses had changed from 2 mg/liter to >32 mg/liter by day 21.

Figure 4 shows the result of Monte Carlo simulations of 10,000 simulated subjects, based on the largest published moxifloxacin MIC distribution of *M. abscessus*, which is from South Korea (28). Overall, the cumulative fraction of response, or proportion of patients that achieved or exceeded EC_{80}, given the MIC distribution, was poor for all doses. For the standard dose of 400 mg daily, only 4.7% of patients would achieve the EC_{80}, for 600 mg a day, 9.7% would, and for 800 mg a day, 12.5% would. If the penetration ratio of moxifloxacin AUC achieved in the lung versus plasma were 2, as suggested by two outlier pharmacokinetic studies (19, 20), the cumulative fraction of response would become 12.5% for 400 mg a day, 21.5% for 600 mg a day, and 26.6% for 800 mg a day. Either way, standard and high doses of moxifloxacin performed poorly.

The data in Fig. 4 also show the target attainment at different moxifloxacin MICs. These data show that at the standard dose and at 600 mg a day, the target attainment to achieve EC_{80} fell to below 0.9 (i.e., 90%) after the MIC of 0.25 mg/liter. This is the PK/PD-derived moxifloxacin susceptibility breakpoint for *M. abscessus*, since above this MIC, most patients will not achieve optimal microbial effect. It should be borne in mind that even this optimal kill would still not kill below the bacterial burden at the start of treatment. If the dose was increased to 800 mg a day, the susceptibility breakpoint only changed by one tube dilution. In contrast, the epidemiologic cutoff point for the South Korean MIC distribution would be 16 mg/liter. In Fig. 5, we show the MIC distributions of *M. abscessus* from our two clinical practice locations in the Netherlands and the Dallas-Fort Worth metroplex in Texas. Given the MIC distributions shown in Fig. 5 and the proposed susceptibility breakpoint of 0.25 mg/liter, then almost all *M. abscessus* isolates in North Texas and the Netherlands as of 2015 were intrinsically resistant to moxifloxacin. Accordingly, the cumulative fraction of responses in these two locales would fall to 0% even for a dose of 800 mg a day.

DISCUSSION

Moxifloxacin and quinolones in general are considered ideal antibiotics given that they are bactericidal, are active against a broad spectrum of bacteria, are available both in oral and parenteral formulations, have a broad distribution in organs and tissues, and can achieve therapeutic concentrations both in the intra- and extracellular environment (21, 30). Indeed, these agents have now found broad use in the treatment of many slow-growing and rapidly growing mycobacteria. The MBC/MIC ratio calculated in this study would seem to indicate that the drug would work well against *M. abscessus*. However, in our formal PK/PD study, we found that moxifloxacin does not reduce the bacterial burden below the starting inoculum even at high exposures and that resistance arises fairly quickly during monotherapy. Therefore, the meaning of standard MBC/MIC assays used to determine bactericidal effect is questionable.

Moxifloxacin has been used for *M. abscessus* pulmonary disease at 400 mg/daily, after the discontinuation of parenteral antibiotics that are administered during the first 1 to 2 months. In that role, it is used as part of combination therapy. However, we show that its performance in the HFS-*M. abscessus* does not inspire continued confidence even for that role. Inhibitory sigmoid E_{max}-based target exposures such as EC_{80} and EC_{90} have been used to identify the susceptibility breakpoints of several bacteria, including mycobacteria (8, 10–12, 31–36). We chose the EC_{80} exposure as the optimal kill exposure that we have found to best translate to the clinic for other mycobacteria, such as *M. tuberculosis* and *M. avium*, and indeed, to other Gram-negative and Gram-positive organisms, even with drugs that do not kill below stasis (31–35). Indeed, indices of microbial kill, such as 3.0 $\log_{10}$ CFU/ml, that are commonly used in work with Gram-positive cocci and Gram-negative bacilli are rarely achieved in work with mycobacteria with a single agent. Nevertheless, an approach similar to ours has been used to identify susceptibility breakpoints, even at exposures that are merely associated with stasis, for several drugs and bacteria (31–36). Work done with *M. tuberculosis* in the hollow-fiber system to identify the EC_{80} in the inhibitory sigmoid E_{max} model for

drugs that kill below stasis, as well as those that do not, in tandem with Monte Carlo simulations, has been shown to predict optimal clinical exposures, doses, and susceptibility breakpoints to within 94% of the value later identified in the clinic, based on FDA and European Medicines Agency (EMA) submissions (8, 37–41). The hollow-fiber work that identified these exposures and breakpoints later confirmed in the clinic was done with single strains of *M. tuberculosis*, with the MIC distribution taken into account only at the stage of Monte Carlo simulations. We report here on moxifloxacin dosing and optimal exposures using the same hollow-fiber system and the same inhibitory sigmoid E_{max} models and EC_{80} exposures in tandem with Monte Carlo simulations, demonstrating limited activity at doses up to 800 mg a day, and we expect the same degree of accuracy as in tuberculosis.

In the treatment of *M. abscessus*, moxifloxacin is used as part of combination therapy. Thus, it could be that it would show efficacy in combination therapy. Formal combination therapy studies with moxifloxacin in the treatment of *M. abscessus* will need to be performed in the HFS-*M. abscessus* in order to explore potential synergy with other agents before definitive recommendations can be made to remove it from current regimens. So far, we have examined high-dose moxifloxacin in combination with tigecycline and ceftaroline and found that it did not add to the effect of these drugs (unpublished data). Thus, the findings for moxifloxacin monotherapy are likely to be borne out for its use in combination therapy.

Acquired moxifloxacin resistance has been described in mycobacteria, particularly for *M. tuberculosis* (11). In the absence of other companion antibiotics, moxifloxacin monotherapy rapidly leads to resistance, as was the case here for *M. abscessus*. Unfortunately, in this study, no moxifloxacin exposure was associated with suppression of resistance. The relationship between acquired moxifloxacin resistance and the moxifloxacin AUC/MIC was best described by a quadratic function, similar to our findings with *M. tuberculosis* (25). However, the underlying mechanism for moxifloxacin resistance in *M. abscessus* deserves further exploration; DNA gyrase is still an interesting bacterial target against which new inhibitors are being developed, with evaluation of their potential for use against *M. abscessus* and other nontuberculous mycobacteria (42).

Finally, according to guidelines, the current breakpoint to determine moxifloxacin resistance to rapidly growing mycobacteria, such as *M. abscessus*, is ≥4 mg/liter in cation-adjusted Mueller-Hinton broth (43), which in a wide MIC distribution (28) will barely allow the use of moxifloxacin in 50% of cases. A 1.0-mg/liter susceptibility breakpoint has also been used by the CLSI (43). In this study, although using a different type of broth (Middlebrook 7H9), we found a PK/PD-derived breakpoint of 0.25 mg/liter. Examination of the MIC distributions in our clinical practices demonstrated that at this proposed breakpoint, most isolates would be *a priori* resistant to moxifloxacin. This comports well with failure of the moxifloxacin-containing regimens in clinical practice. Our findings suggest that the poor outcome observed when treating *M. abscessus* is no more than a reflection of the amount of intrinsically resistant isolates that are circulating and infecting patients. The approach that utilizes the hollow-fiber-system model and the EC_{80} or EC_{90} followed by Monte Carlo simulations has been found to be highly accurate in predicting MICs above which combination therapy fails in clinical studies, even for drugs that do not kill below the stasis line, in the case of tuberculosis and disease caused by other more mundane bacteria, such as Gram-negative rods (25, 44–52). Thus, our findings are likely accurate, but this will require clinical validation in the future. Such confirmation will require multivariable pharmacometric analyses of clinical data, which do not currently exist for moxifloxacin and *M. abscessus* pulmonary disease (53). Indeed, no such data exist, as far as we know, for any of the drugs used in treatment of pulmonary *M. abscessus*, since there is a lack of clinical trials and large prospective clinical cohort studies for this disease. Thus, the PK/PD-derived breakpoint is a proposed breakpoint to be used for clinical decision making until a large-enough data set can better identify further breakpoints.

There are some limitations in this study. We performed our evaluation with one laboratory strain. Although the strain used was the type strain, ideally this work should be extended to clinical isolates with different susceptibility profiles. Indeed, the type strain was not from a patient with pneumonia but from an abscess after trauma. It is not clear whether strains that cause lung disease are intrinsically different from those that cause extrapulmonary disease. Nevertheless, even when single strains have been used in PK/PD studies in the hollow-fiber system with other bacteria, the results have been found to be fairly accurate and to allow generalizations (11, 25, 37–41, 44–52). Moreover, here we only report the effect of moxifloxacin alone, and there is still a need to evaluate its performance as part of combination therapy. However, studies evaluating different moxifloxacin-based combinations to explore the potential of synergistic combinations, as well to explore the intracellular model for pulmonary disease, have been performed and are being prepared for publication.

In summary, we used a recently developed preclinical model for *M. abscessus* disease to evaluate and then identify the efficacy of moxifloxacin, which demonstrated rapid appearance of resistance. Efficacy was poor, and moxifloxacin even at high doses is not expected to work in the clinic. We used the PK/PD studies to identify and propose a new moxifloxacin susceptibility breakpoint of 0.25 mg/liter.

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