

Gatifloxacin pharmacokinetics/pharmacodynamics-based optimal dosing for pulmonary and meningeal multidrug-resistant tuberculosis

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Running title: Gatifloxacin doses for drug-resistant TB

Summary

We identified the optimal doses gatifloxacin of 800 mg and 1200 mg for the treatment of drug-resistant meningeal and pulmonary tuberculosis, respectively. We also identified the gatifloxacin clinical susceptibility breakpoint, and introduce a susceptible dose-dependent category.

ABSTRACT

Background: Gatifloxacin is used for the treatment of multidrug-resistant tuberculosis (MDR-TB). The optimal dose for use in these patients is unknown.

Methods: We performed a 28-day gatifloxacin hollow fiber system model of tuberculosis (HFS-TB) study in order to identify the target exposures associated with optimal kill and resistance suppression. Monte Carlo experiments (MCE) were used to identify the dose that would achieve the target exposure in 10,000 adult patients with MDR-TB. The optimal doses identified were validated using probit analyses of clinical data from two prospective clinical trials of patients with pulmonary and meningeal tuberculosis. Classification and regression-tree (CART) analyses were used to identify the gatifloxacin minimum inhibitory concentration (MIC) below which patients failed or relapsed on combination therapy.

Results: The target exposure associated with optimal microbial kill and resistance suppression in the HFS-TB was a 0-24 hour area under the concentration-time curve-to-MIC of 184. MCE identified an optimal gatifloxacin dose of 800 mg/day for pulmonary and 1200 mg/day for meningeal MDR-TB, and a clinical susceptibility breakpoint $\text{MIC} \leq 0.5$ mg/L. In clinical trials, CART identified that 79% patients failed therapy if MIC was >2 mg/L, but 98% were cured if MIC was ≤ 0.5 mg/L. Probit analysis of clinical data demonstrated $>90\%$ probability of cure in patients if treated with 800 mg/day and 1200 mg/day for pulmonary and meningeal tuberculosis, respectively. Doses ≤ 400 mg/day were suboptimal in MCE and probit analyses.

Conclusions: Gatifloxacin doses of 800 mg/day and 1200 mg/day are recommended for pulmonary and meningeal MDR-TB treatment, respectively.

The emergence of multidrug-resistant tuberculosis (MDR-TB) has been well chronicled, as have the potential reasons [1]. In patients with MDR-TB treated with gatifloxacin-containing regimens, the treatment success rate was found to be 87% in gatifloxacin-

susceptible tuberculosis versus 51% in patients with isolates showing high-level gatifloxacin-resistance [2]. Successful treatment outcomes in extensively drug resistant tuberculosis (XDR-TB), which is MDR-TB with resistance to quinolones and aminoglycosides, are a dismal 28% [3]. Thus, the use of 8-methoxy fluoroquinolones in treating MDR-TB is crucial for therapy success: moxifloxacin and gatifloxacin are part of the World Health Organization (WHO) Group A drugs. While preclinical pharmacokinetics/pharmacodynamics (PK/PD) work has been performed for moxifloxacin, no PK/PD derived target exposure for gatifloxacin has been derived for *Mtb* [4]. Since high dose gatifloxacin could be less arrhythmogenic than high dose moxifloxacin, it is imperative that equivalent dosing be established for high dose gatifloxacin [5]. Moreover, given that quinolone and aminoglycoside acquired drug resistance (ADR) arises in 9-14% of patients after an average of 5 months of combination therapy for MDR-TB, it is crucial to identify a gatifloxacin dose that suppresses the acquired resistance [6, 7]. Here, we performed PK/PD studies for gatifloxacin microbial kill and ADR suppression in the hollow fiber model of tuberculosis (HFS-TB).

The HFS-TB, with repetitive sampling for drug concentrations and bacterial burden, allows the derivation of the relationships between drug exposure, microbial kill rates, and resistance suppression for monotherapy and for combination therapy [4, 8-11]. Delineation of these PK/PD relationships allows for estimation of clinical doses [8, 12-15]. Since drug concentrations achieved in patients with tuberculosis are a major driver of both therapy failure and ADR, the estimation of clinical doses takes into account the pharmacokinetic variability [16-21]. Similarly, the antibiotic minimum inhibitory concentration (MIC) is also an important determinant of patient outcomes, with dramatic fall in patient response once a breakpoint MIC is achieved, and is also taken into account in clinical dose selection [21-26]. Here, we utilized population pharmacokinetics

(and thus variability) of gatifloxacin from 169 patients with tuberculosis, gatifloxacin MICs from 243 *Mtb* isolates, and the PK/PD target derived from the HFS-TB to identify gatifloxacin doses likely to be effective in pulmonary and meningeal tuberculosis, and the MIC breakpoint above which therapy is expected to fail, in Monte Carlo experiments (MCE) [27, 28]. The dose versus outcome relationships and MIC breakpoints derived thereof were then validated using clinical trial data from actual patients. Currently, doses of 200 mg to 1200 mg are given in MDR-TB.

METHODS

Materials, isolates, and reagents

Mtb H37Ra (ATCC # 25177) was utilized for all HFS-TB experiments. Stock *Mtb* H37Ra culture stored at -80°C in Middlebrook 7H9 broth was thawed before each experiment, and grown into logarithmic growth phase (log-phase) in Middlebrook 7H9 broth supplemented with 10% oleic acid, dextrose, and catalase (OADC) at 37°C under 5% CO_2 , under shaking conditions.

Gatifloxacin powder was purchased from Sigma-Aldrich (USA), and moxifloxacin- $^{13}\text{C}_3$ from United States Pharmacopeia (Rockville, MD, USA). Hollow fiber cartridges were purchased from FiberCell (Frederick, Maryland). We utilized the BACTEC MGIT 960 Mycobacterial Growth Tube Indicator System (MGIT) for monitoring growth (Franklin Lakes, New Jersey).

MICs and screening for gatifloxacin intracellular effect

MICs of the laboratory strain were identified using, the standard macrobroth dilution reference method, and the MGIT assay, as described before [29, 30]. The following 11 concentrations were used for MIC determination: 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1,

2, 4, 8, 16 mg/L. We used the 1% proportion method. Next, we examined the microbial kill characteristics of gatifloxacin, in extracellular and intracellular *Mtb*, using the same concentrations, and co-incubation over 7 days. The methods have described in detail in prior studies [29, 30].

Hollow fiber system model of tuberculosis and PK-PD modeling

The mechanistic details of the HFS-TB, have been extensively published [5, 15, 17, 31]. We grew *Mtb* cultures into log-phase as described earlier and inoculated them into seven HFS-TB units. We started treatment with gatifloxacin doses 24 hours after, at a half-life of 11 hours, daily for 28 days. The central compartment was sampled for gatifloxacin concentration measurements at 0, 1, 4, 8, 12, 22 and 23.5 hours post first dose with methods described in the supplement. The peripheral compartment was sampled for total bacterial burden using both time-to-positivity (TTP) assay in the MGIT, and colony forming units (CFU) on Middlebrook 7H10 agar supplemented with 10% OADC [5, 9, 30, 32, 33]. Gatifloxacin-resistant subpopulation was captured by culturing on agar supplemented with gatifloxacin 3 times the MIC. Gatifloxacin-resistant colonies were picked and underwent whole genome sequencing (WGS), with specific mutations confirmed by Sanger sequencing, as described before [30, 33].

PK/PD modeling of gatifloxacin AUC_{0-24}/MIC exposure predictive of microbial kill was performed using the inhibitory sigmoid E_{max} model and that for resistance suppression using the quadratic equation as described in the introduction paper [8, 34].

Dose finding and validation of findings using Monte Carlo experiments

We performed 10,000 patient MCE for gatifloxacin doses using ADAPT 5 software [35]. Gatifloxacin parameter estimates shown in Table 1 were used [28]. For pulmonary tuberculosis, concentrations of gatifloxacin were 1.5-1.8-fold higher in epithelial lining fluid (ELF) and bronchial mucosa compared to serum in prior studies [36, 37]. Work in

granulomas suggests a 5-6 fold higher total concentration, with a caseum unbound proportion of 0.23, which leaves the effective (non-protein bound) concentration about 1.3-fold in granulomas compared to serum, which is similar to the non-protein bound ELF ratios [36, 38]. Thus, the gatifloxacin concentration in tuberculosis cavities was assumed to be 1.3-fold; sensitivity testing was performed for a 1-fold change (i.e., similar concentration to serum). On the other hand, the gatifloxacin AUC₀₋₂₄ penetration ratio into cerebrospinal fluid (CSF) of patients is 0.48, which is virtually identical with entry ratios into rabbit CSF in experimental meningitis [25, 39]. The 0.48 penetration ratio was used in MCE for doses to treat tuberculous meningitis (TBM).

Gatifloxacin doses of 200, 400, 600 and 800 mg/day were examined for target attainment in pulmonary and TBM. We validated the MCE using recommended steps [8], including comparison to the AUC and peak concentrations achieved by doses of 200 mg and 400 mg used for FDA licensing: https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/21061s023,024,21062s026,037lbl.pdf. We calculated the target attainment probability for the AUC₀₋₂₄/MIC for the EC₈₀ and resistance suppression target exposure at each MIC within the 243 isolate distribution from a study of isolates from Spain [27]. This MIC distribution from Spain was based on Middlebrook 7H11 medium (agar), which could have higher MICs by one-tube dilution compared to MGIT. Therefore, in a further sensitivity analysis, to account for differences in methods used to identify MIC, we also utilized a distribution derived in the MGIT by Isaeva et al who examined isolates from patients who had not been exposed to fluoroquinolones [40]. Cumulative fraction of response (CFR) was calculated by summing over this MIC range. The CFR is the proportion of 10,000 patients treated with specified dose who achieved target exposure over the MIC range.

External validation of MCE dose and MIC findings using clinical trials data

Data on the relationship between gatifloxacin dose, MIC, and CSF exposure, and patient outcomes in 15 patients with TBM, has been published by Thwaites and colleagues [25]. The patient level data was used to examine the relationship between dose (mg/kg) and proportion of patients with a specified outcome in probit regression modeling. Outcomes examined were (1) patient survival, (2) death or disability, (3) relapse, and (4) a composite therapeutic success or good outcome defined as not experiencing either death or disability or failure or relapse. This regression was compared to the CFR from the MCEs.

Similarly, we examined clinical outcomes in 161 patients based on 161 single-isolate patient-with confirmed pulmonary MDR-TB who were treated with high dose in Bangladesh [26]. The main outcomes and patient characteristics in this study have been reported before [3, 26]. Patient isolates had gatifloxacin MICs identified based on the Löwenstein–Jensen medium; mutations in the gene encoding the DNA gyrase subunit A (*gyrA*) were also identified. Since patients received doses starting at 200 mg up to 800 mg by weight band, we also performed probit regression for the relationship between cure and gatifloxacin dose in mg/kg. Next, we utilized a machine learning algorithm, classification and regression tree analyses (CART), to identify the MIC above which combination therapy fails, following steps in our prior work [23, 24]. We then performed a probit analysis of dose versus good clinical outcome for patients with MICs above and below this threshold.

RESULTS

Gatifloxacin microbial kill of intracellular and extracellular *Mtb*

The gatifloxacin MIC of the laboratory strain was 0.06 mg/L with the MGIT assay but 0.125 mg/L by the macrobroth dilution method. Since the macrobroth assay is the

reference method, we adopted an MIC of 0.125 mg/L; however for sensitivity analysis for dose findings we compared findings to those using the MGIT-derived MICs (see below). Next, we compared microbial kill of intracellular and extracellular *Mtb* by gatifloxacin, with results shown in **Figure 1**. Gatifloxacin achieved a maximal kill (E_{\max}) and 95% confidence interval (CI) of 6.79 (95% CI: 6.30-7.32) \log_{10} CFU/mL against log-phase growth extracellular *Mtb* in 7 days (**Figure 1A**). The concentration associated with 50% of E_{\max} (EC_{50}) was 0.08 (95% CI: 0.07-0.10) mg/L ($r^2=0.98$). **Figure 1B** shows results of the intracellular *Mtb* study. The E_{\max} was 4.55 (95% CI: 3.94-5.65) and EC_{50} 0.64 (95% CI: 0.50-1.01) mg/L ($r^2=0.95$).

HFS-TB study results

The gatifloxacin-concentration time curves achieved in the HFS-TB are shown in **Figure 2A**. Pharmacokinetic modeling revealed a mean \pm standard deviation elimination rate constant of 0.06 ± 0.01 hr $^{-1}$ and the volume of 0.52 ± 0.05 L achieved. The pharmacokinetic model predicted versus observed concentrations had a slope of 1.00 ± 0.01 ($r^2>0.99$), indicating no bias.

Figure 2B demonstrates the time-kill curves in the HFS-TB based on \log_{10} CFU/mL, and shows that at $AUC_{0-24}/MIC>171.2$, gatifloxacin completely sterilized the HFS-TB on day 10, but rebounded by end of study. The kill rates demonstrated here are similar to those of moxifloxacin in the HFS-TB in our prior studies [4]. **Figure 2C** shows that a gatifloxacin-resistant subpopulation arose in the three lowest gatifloxacin exposures, starting after day 10, and was responsible for the rebound. However, there were exposures above which no drug-resistant subpopulation arose during the 28 days of the study. **Figure 2D** shows the kill curves based on TTP. The TTP assay shows that the *Mtb* population was actually not all killed on day 10, demonstrating the higher sensitivity of TTP compared to CFU/mL assay [9, 32].

PK/PD modeling for microbial kill and ADR suppression

Inhibitory sigmoid E_{\max} modeling of CFU/mL versus exposure is shown in **Figure 3A**. Given the rebound growth in the three lowest doses on day 28, the model achieved moderate fit at the end of the experiment ($r^2=0.89$), with an EC_{80} that was an AUC_{0-24}/MIC of 82. On the other hand, the TTP assay based results demonstrated better model fits (**Figure 3B**), with the lowest Akaike Information Criteria score noted on day 10, associated with an EC_{80} that was an AUC_{0-24}/MIC of 147, while the EC_{80} at the end of the experiment was an AUC_{0-24}/MIC of 184.

The % of gatifloxacin-resistant subpopulation versus exposure were as shown in **Figure 3C**. The figure shows that based on the quadratic function model [8, 34], gatifloxacin AUC_{0-24}/MIC associated with resistance suppression was 171.2. Taken together, the gatifloxacin exposure that would achieve both maximal kill and suppress ADR was an $AUC_{0-24}/MIC \geq 184$.

WGS of gatifloxacin-resistant isolates

WGS of the gatifloxacin-resistant isolates identified several *gyrA* mutations. These were confirmed by Sanger sequencing, with results shown in **Figure 3D**. The gatifloxacin-resistant isolates carried either an A>G Asp94Gly or a G>A Asp94Asn or a G>T Gly88Cys *gyrA* mutation, all in the quinolone resistance-determining region (QRDR) [41].

Monte Carlo experiment dose-finding for pulmonary and meningeal tuberculosis

Target attainment probability (TAP) in 10,000 patients with pulmonary tuberculosis treated with different gatifloxacin doses is shown in **Figure 4A**. As the gatifloxacin MIC increased the TAP fell, and for the dose of 400 mg the precipitous fall was at 0.25 mg/L, but with 800 mg/day this occurred at 0.5 mg/L. When the TAP was summated over the

MIC range of the 243 *Mtb* isolates from Spain, the proportion of patients who achieved target exposures with each dose were as shown in Figure **4B**. The optimal dose was 600 mg/day, which achieved target exposure in 91% compared to 84% for 400 mg. However, on sensitivity analysis assuming a 1:1 penetration ratio, the 600 mg dose fell just short of 90%, and optimal dose was 800 mg a day (92% of patients). At this dose, the gatifloxacin TAP falls just short of 90% at the MIC of 0.5 mg/L, based on the Middlebrook 7H 11 agar dilution test. In a second sensitivity analysis that accounted for method used to identify MICs, we utilized the MGIT-derived MICs of our laboratory isolate (0.0625 mg/L), which increased the PK/PD exposure targets by a factor of two from an $AUC_{0-24}/MIC \geq 184$ to ≥ 368 , together the MGIT-derived MIC distribution of *Mtb* isolates from Russian patients with no prior quinolone treatment. Results are shown in **Figure 4C**. The figures shows that the MCE-derived MGIT susceptibility breakpoint for 800 mg/day and 1200 mg/day would be one-tube dilution lower. However, since the MIC distribution is 1-2 tube dilutions lower in MGIT, the CFR for 800 mg a day would be >99% for both a 1.3 and 1:1 penetration rates, driven by the lower MICs from the MGIT.

Next, we examined the performance of doses in achieving the target $AUC_{0-24}/MIC \geq 184$ in CSF, given the gatifloxacin penetration ratio of 0.48. The summated results are shown in **Figure 4D**. The dose of 400 mg/day was associated with a mean CSF AUC_{0-24}/MIC of 208.6 with a range of 1.9 to 901.0. The cumulative fraction of response for 400 mg was 15% in TBM, and that for 600 mg 48%. **Figure 4D** also shows sensitivity analysis results based on the higher MGIT-MIC based $AUC_{0-24}/MIC \geq 368$ in CSF, and the MGIT-based MIC distribution, in treatment of meningeal tuberculosis. The dose of 1200 mg/day remained as the best for TBM.

Validation of MCE-identified doses with clinical data

Next, we examined clinical outcomes in 161 Bangladesh patients, whose clinical characteristics are shown in **Table 2**. Patients received high dose gatifloxacin plus ethambutol, pyrazinamide, clofazimine, kanamycin, prothionamide, and isoniazid during the first 4 months, followed by gatifloxacin, ethambutol, pyrazinamide, and clofazimine for 5 months. We examined for the probability of outcome by dose in mg/kg, in probit analysis versus favorable outcome (cure without relapse), with results shown in **Figure 5A**. The figure shows relatively flat response between doses of 6-12 mg/kg with favorable outcomes of less than 20%, followed by a steep dose-versus success curve up until dose of 18.18 mg/kg with a probability of success of 73%. The curve is still on an upswing, such that based on the shape of the curve 90% cure would be achieved by a dose of 801.37 mg, given the weights of patients. That dose is virtually the same as that identified by MCE.

The distribution of LJ-based gatifloxacin MIC in the 161 MDR-TB patients is shown in **Figure 5B**; 78% of isolates had wild type genes, while 22% had mutations in *gyrA* and or *gyrB*. We divided the 161 patient data set randomly into two equal halves, and trained a CART model to identify the gatifloxacin MIC above which patients with pulmonary tuberculosis failed the combination therapy or relapsed after cure; and then used the second dataset for validation. The validated CART model shown in **Figure 5C** had a receiver operating characteristic curve (ROC) score of 0.86, a precision of 0.96 and specificity of 0.87. Since the ROC on the learn model was 0.91 ± 0.04 , this CART model will be highly reproducible when applied to independent data. The CART model shown in **Figure 5C** identified two breakpoints: the primary node was MIC<1.0 mg/L, and the secondary node MICs 1-2mg/L. Thus, there is susceptibility breakpoint of <1.0 mg/L, and an intermediate susceptibility zone of 1-2 mg/L. The odds ratio for failure/relapse at an MIC>1 mg/L versus MICs <1 mg/L for the entire data was 21.74 (95% CI: 7.09-66.67), $p<0.001$. Given these findings, we performed a probit analysis of mg/kg dose in patients

infected with *Mtb* that had MIC<1.0 mg/L versus MIC 1.0-2.0 mg/L versus those with MICs \geq 1 mg/L, which revealed results shown in **Figures 5D**. At the dose of 800mg/day, favorable outcomes were observed in 54% of patients with MIC>1.0 mg/L versus 84% with MIC<1.0 mg/L. There were 13 patients with gatifloxacin MIC<1 who were cured but had the weight and gatiflocaxcin dose data missing, otherwise the proportion cured in this group would have been 96%.

Gatifloxacin dose versus cure in patients with meningeal tuberculosis

There were a total of 15 patients with TBM, who were treated with gatifloxacin 400 mg a day for the first 60 days of treatment; full clinical details of these patients have been published elsewhere [25]. In addition patients were also treated with dexamethasone, 5 mg/kg isoniazid, 10 mg/kg rifampin, 30 mg/kg pyrazinamide, and 20 mg/kg streptomycin; and 30 mg/kg/day ethambutol was added to the regimen for 3 months for those previously treated for tuberculosis. The gatifloxacin dose achieved a mean CSF AUC₀₋₂₄/MIC of 148.2 (95% CI: 74.4-222.3); the 95% CI is within values identified in MCE with this dose. Overall favorable outcome (no disability, death, or relapse) at this dose was 7/15 (47%). Probit analysis of dose mg/kg versus outcome is shown in **Figure 6** ($r^2=0.95$); the probability of disability/relapse-free survival at the median dose of 8.33 mg/kg was 20.0-26.7%. The curves mirror those for MCE in TBM patients. However, the confidence intervals in the probits were too wide to accurately calculate dose associated with 90% probability of favorable response.

DISCUSSION

Firstly, we present a comprehensive gatifloxacin PK/PD study. The kill curves were reminiscent of those of moxifloxacin, while the time to ADR was better than for moxifloxacin in the same model in the past [4]. Based on this, we utilized MCE and identified a gatifloxacin dose for patients that could minimize the commonly observed

ADR rates in MDR-TB treatment, while optimizing microbial kill. We identified the dose of 800 mg a day as optimal. This dose has been given before to patients with MDR-TB, and was well tolerated by patients [2, 26]. In the probit analysis, a similar dose was calculated to be associated with at least 90% cure in MDR-TB patients with pulmonary TB. Thus, the gatifloxacin doses of 200-400 mg/day for pulmonary tuberculosis currently utilized by some are suboptimal, that of 600 mg/day is good but not optimal if drug penetration into cavities is not higher than in the blood, while that of 800 mg would be the best especially for replacement of high dose moxifloxacin. An equivalent dose for levofloxacin for the same target has been derived elsewhere [42].

Secondly, the MCE performances of different doses to achieve target exposures for efficacy and suppression of resistance in the subarachnoid space were examined. Thwaites et al have demonstrated that quinolone AUC/MIC versus favorable outcome on patients with TBM is “U” shaped curve, with an optimal zone of CSF AUC₀₋₂₄/MIC of 14-252 for survival outcome, 0-240 for post-treatment disability, and 94-352 for relapse [25]. Interestingly, our target exposure AUC₀₋₂₄/MIC of 184 falls squarely in the middle of this optimal zone of exposure ranges. Probit analyses curves mirrored those of MCE, confirming the dose-response relationship. We found that the dose of 1200 mg would be best able to accomplish the target exposure. This is a high dose, however, and would be only applicable to patients with MDR-TB in whom there are otherwise no other good second drugs that penetrate the CSF and achieve sufficient kill [43]. With drug susceptible TB, other drugs that penetrate CSF would also be able to kill the *Mtb*, and the gatifloxacin optimal dose may be less under those circumstances [44].

Thirdly, we propose a gatifloxacin susceptibility breakpoint of 0.25 mg/L in MGIT assays and 0.5 mg/L with macrobroth dilution assays at the 800mg/day dose. Interestingly, the susceptibility breakpoint of 0.5 mg/L on LJ agar was identified by the agnostic CART

algorithm in patients; in these patients' isolates MICs had been determined using LJ medium. Further, based on the findings from the clinical study, we define an intermediate susceptibility dose-dependent (ISDD) zone of 1-2mg/L on LJ, while based MCE the ISDD zone would be 0.5-1 mg/L in broth, at which 1200 mg of gatifloxacin could lead to microbial kill.

Finally, the gatifloxacin-resistant isolates from the HFS-TB were found to have Asp94Gly or Asp94Asn or Gly88Cys *gyrA* mutation, which are the most commonly encountered in fluoroquinolone-resistant MDR-TB strains [41]. These QRDR mutations confer high-level fluoroquinolone resistance, while Gly88Cys has been associated with intermediate susceptibilities [45, 46]. The ability to repetitively sample the HFS-TB through time in order to document evolution of resistance, and the WGS, allowed us to catch the “antibiotic resistance arrow of time” in process [10, 11]. Mutations arose at specific “resistance amplifying” exposures, but not at high exposures, suggesting that high enough concentrations can shut down, or at least delay, quinolone-resistance.

There are several limitations to our studies. First, more precise PK/PD exposure targets could be identified if different *Mtb* isolates were used in the HFS-TB experiments. To partially mitigate this, however, MCE take into account isolates with a large range of MICs in calculating optimal dose. In addition, we calculated our PK/PD target exposure using two different MICs (by MIC assay), and identified the same optimal doses. Moreover, the clinical validation portion of our study suggests that the findings have clinical meaning. A second limitation is that optimal exposures and doses are regimen specific, you may need lower doses in the case of a regimen with drugs that are synergistic or have high efficacy [21, 32]. In addition, the HFS-TB does not have an immune system, and the contribution of the immune system to therapy success could reduce the requirement for a large dose. Thus, our dose choices should be considered a

worst case scenario. However, the validation study suggests that in the case of gatifloxacin, the doses derived using the HFS-TB, reflect clinical reality.

AUTHOR CONTRIBUTIONS. T.G. and D.D designed the study; T.G., D.D., and P.B performed the hollow fiber studies; D.D wrote the first draft of the manuscript; T.K. performed DNA extraction; T.G and S.S performed the WGS analysis; T.G. performed PK-PD modeling and MCE; W.S. and H.M. performed the gatiflocaïn population pharmacokinetic analyses; J.P. and T.G. performed Probit and CART analysis of clinical data; S.B., P. A., H. M., G.T., A.vD., performed the clinical studies and wrote the paper with special emphasis of clinical details from the clinical studies; M.G and A.vD performed MIC and gyrA sequencing in the 161 clinical isolates; D.D., J.P., S.S., and T.G. wrote the manuscript; all authors edited and contributed to the final version of the manuscript.

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Fig.1 Gatifloxacin effect against extracellular and intracellular *Mtb* at static concentrations

Concentrations was examined in triplicate, and results shown are for mean and SD (error bar).

The dotted line indicates the starting (day 0) bacterial burden. **(A)** After 7 days of co-incubation, gatifloxacin completely eliminated log-phase growth extracellular *Mtb*, with a kill of 5.16 ± 0.03 \log_{10} CFU/mL below stasis. **(B)** Gatifloxacin showed considerable effect against intracellular *Mtb* with a kill of 4.82 ± 0.40 \log_{10} CFU/mL below stasis. However, this was slightly less than that observed against extracellular *Mtb*.

Figure 2. Gatifloxacin pharmacokinetics and time-kill curves in the HFS-TB

A. Pharmacokinetic model predicted gatifloxacin concentrations are shown with the shaded graph lines, while the concentrations observed on direct measurement are shown as the symbols. The concentrations achieved were used to calculate the 24hr area under the concentration-time curve (AUC), and AUC/MICs. **B.** Time-kill curves based on \log_{10} CFU/mL versus the AUC/MIC achieved in each HFS-TB unit reveals a biphasic decline at each exposure. **C.** There was emergence of gatifloxacin-resistant subpopulation on days 14 and 21 in the three lowest exposures, while higher exposures suppressed resistance. In the non-treated controls, the % of subpopulation that was gatifloxacin-resistant did not change and remained close to 0%. **D.** When bacterial burden was expressed as time-to-positivity, the bacterial burden demonstrated the same biphasic pattern, but starting at day 14, and with rebound or regrowth in virtually all systems. None of the HFS-TB units demonstrated complete sterilization with the monotherapy.

Figure 3. Exposure versus effect models for microbial kill and acquired resistance

A. Shown are inhibitory sigmoid E_{\max} curves for gatifloxacin microbial kill expressed as CFU/mL for each day of sampling. The EC_{80} s can be read off the graphs for each day, at the inflection point of each curve. **B.** The same inhibitory sigmoid curves, based on TTP readout, are shown. The model fit was better with this readout (except day 3 in blue) than CFU/mL. **C.** Quadratic function model for acquired drug resistance revealed a model fit associated with an $r^2=0.82$ on day 21 (blue) and $r^2=0.90$ on day 28 (magenta). The AUC/MIC of 171.2 is shown, which was associated with resistance suppression. **D.** Illustration of mutations in *gyrA* of some gatifloxacin-resistant isolates. The mutations cluster at position 7563 of *gyrA* in all resistant isolates but one.

Figure 4. Performance of different gatifloxacin doses in Monte Carlo simulations

A. Shown is the target attainment probability (TAP) for the $AUC_{0-24}/MIC \geq 184$, associated with both maximal kill and resistance suppression in pulmonary cavities. The MIC distribution shown is for 243 isolates from Spain, based on Middlebrook 7H11 medium [27]. At a dose of 400 mg/day the TAP fell below 90% at MICs above 0.25 mg/L, at 800 mg/day above an MIC of 0.25 mg/L, and at dose of 1200 mg/day fell at the MIC of 0.5 mg/L. The clinical susceptibility breakpoint would be one tube dilution higher than each of these MICs. **B.** The cumulative fraction of response (CFR) for the $AUC_{0-24}/MIC \geq 184$ shows the dose of 600 mg/day would be optimal; however in the sensitivity analysis assuming a 1:1 penetration into cavities it was the dose of 800 mg that achieved CFR > 90% (shown in gray). **C.** TAP for $AUC_{0-24}/MIC \geq 368$ based on MGIT MIC of 0.06 mg/L, and the MGIT-derived MIC distribution from Russia. The MGIT-derived susceptibility breakpoint was 0.25 mg/L for 800 mg/day and 0.5 mg/L for 1200 mg/day. However, the MIC distribution led to better CFR for each dose. **D.** The proportion of patients achieving the target exposures for efficacy and suppression of resistance for TB meningitis is shown. The gatifloxacin dose of 1200 mg a day was required to meet target exposures in >90% of patients. Shown in gray is sensitivity analysis that assumed the MGIT-based MICs and the $AUC_{0-24}/MIC \geq 368$; the dose of 1200 mg/day was still the best for the meningitis.

Figure 5 Gatifloxacin MIC and dose thresholds predictive of microbiologic cure in patients.

A. Probability of therapeutic success (cure without relapse) increases as gatifloxacin doses increase ($r^2=0.96$). A dose of 654 mg would achieve success of about 73%, while that of 801.37 mg would achieve success of about 90%. **B.** LJ based MIC distribution in 161 Mtb clinical isolates at the start of therapy. About 30% of the MDR-TB isolates had MICs of 1.0 to 4 mg/L. **C.** A pruned classification and regression in the test-sample tree shows that the highest node which separates patients who succeeded (98%) was an MIC of <1.0 mg/L, with the rest having favorable outcomes in only 48%. The second node was of MICs of 1 and 2 mg/L with a favorable outcome in 71% of patients versus 21% of patients when $MIC > 2.0$ mg/L, which are

exactly the success rates for XDR-TB tuberculosis. Half of the data was used for training the model, which explains why 84 patients shown in figure. Fav denotes favorable outcome, i.e., therapeutic success, while Unfav denotes unfavorable outcomes, i.e., therapeutic failures **D**. There are in fact three different dose response curves on probits ($p < 0.01$), one for patients with LJ-based MICs < 1.0 mg/L, a different one at MICs of 1 and 2 mg/L, and a third flat one at MIC > 2 mg/L, indicating resistance.

Figure 6. Gatifloxacin dose versus probability of favorable outcome

A. Plot of probability of gatifloxacin dose (mg/kg) versus survival that was relapse and disability-free survival in 15 patients treated with 400mg of gatifloxacin for tuberculous meningitis. All neurological disability was taken as a poor outcome, regardless of severity, as was death and relapse. **B.** Plot of probability of survival versus dose in mg/kg. In both cases, confidence intervals for the C_{50} were too wide to be able to calculate optimal dose with precision.

Fig.1 Gatifloxacin effect against extracellular and intracellular *Mtb* at static concentrations

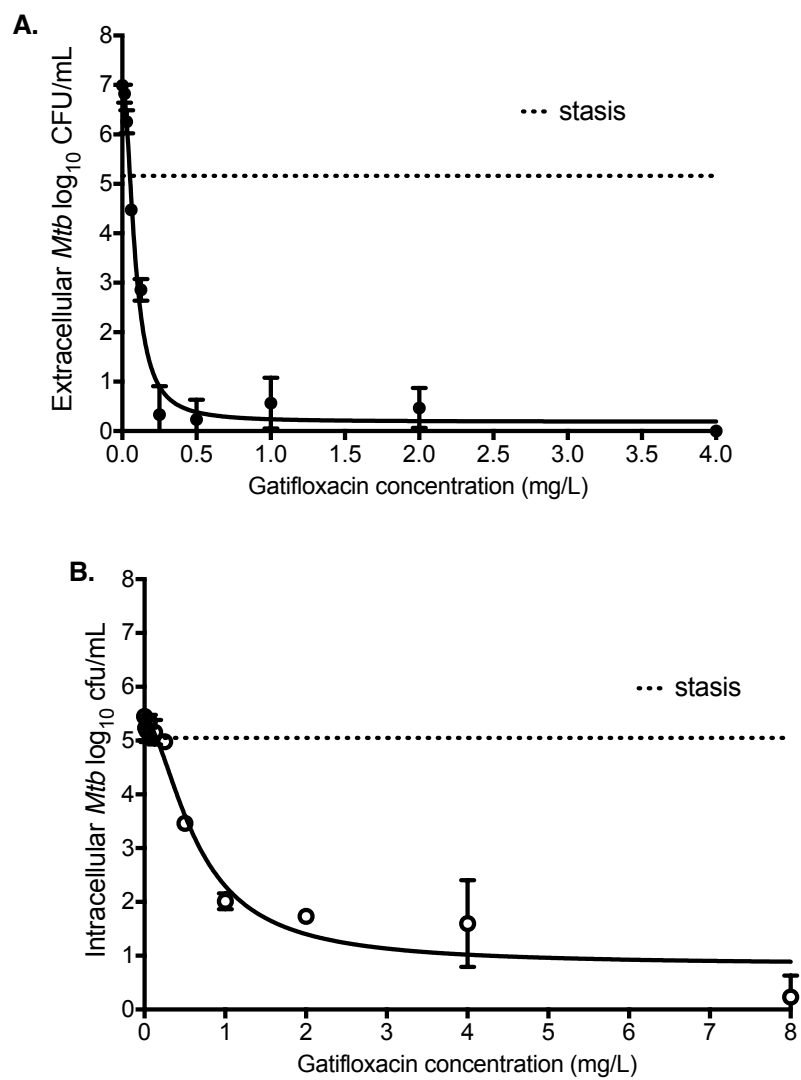


Figure 2. Gatifloxacin pharmacokinetics and time-kill curves in the HFS-TB

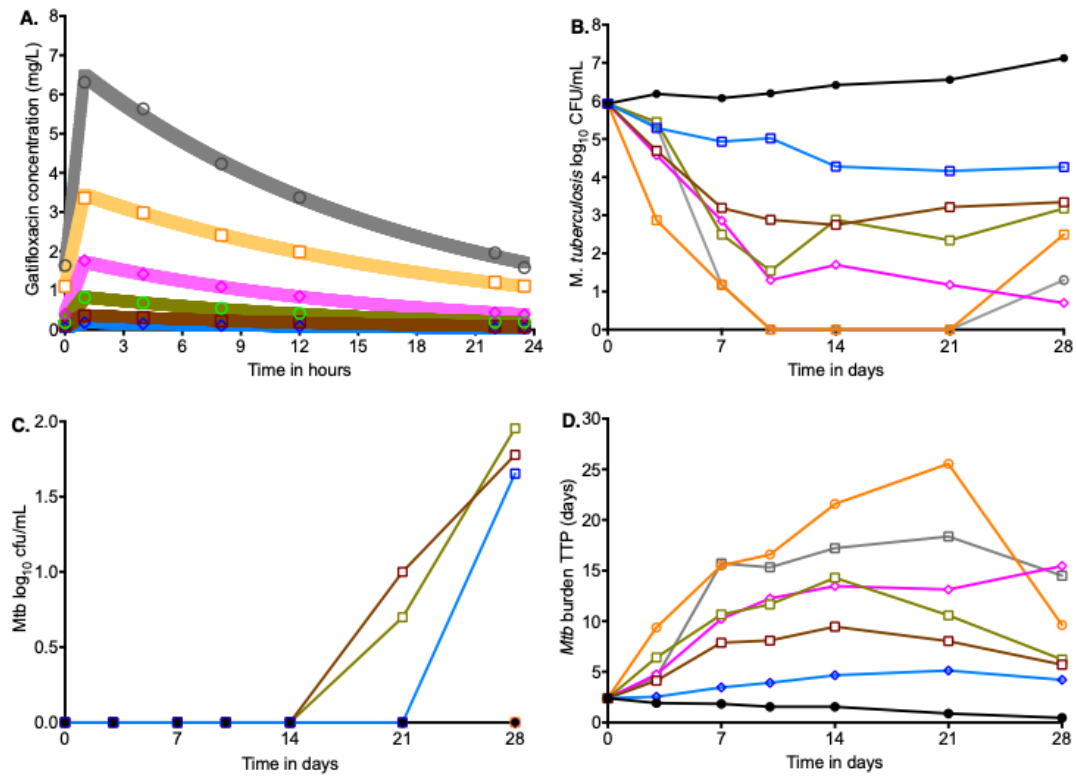


Figure 3. Exposure versus effect models for microbial kill and acquired resistance

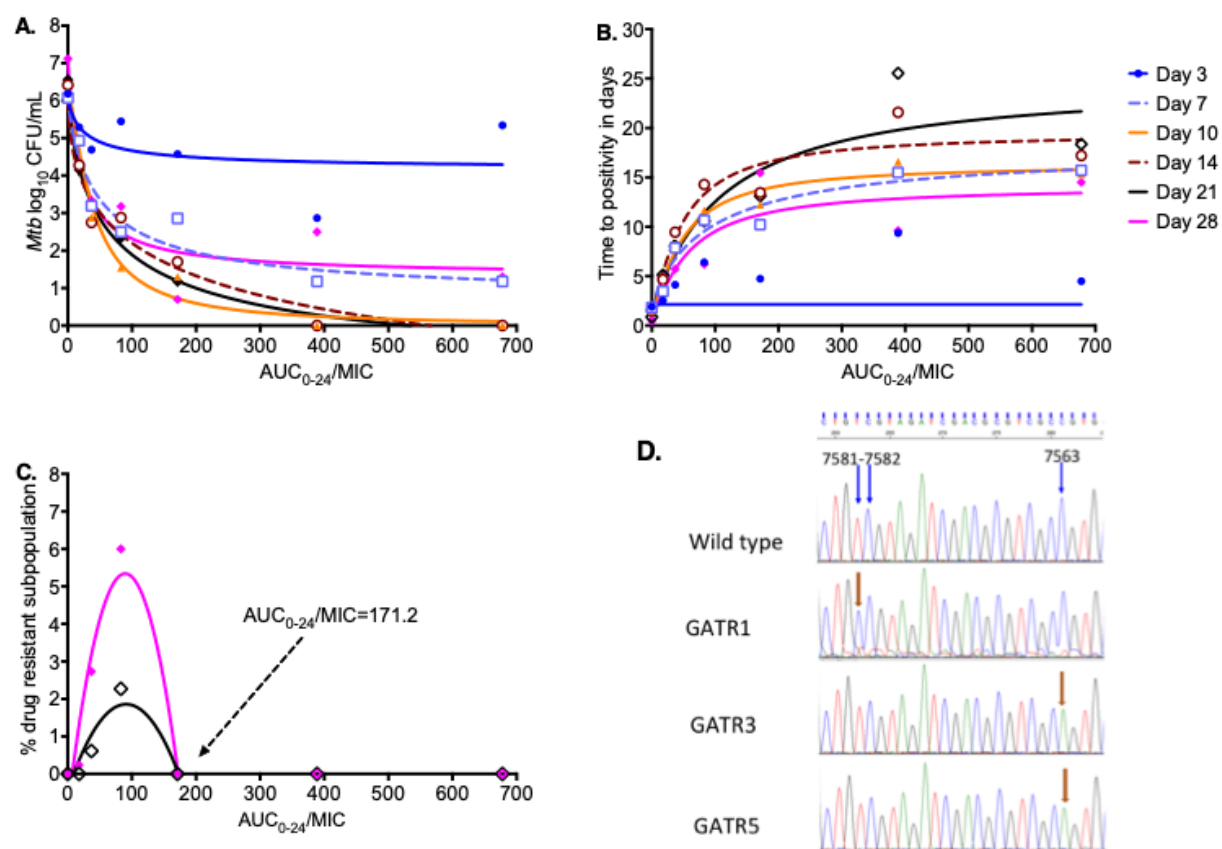


Figure 4. Performance of different gatifloxacin doses in Monte Carlo simulations

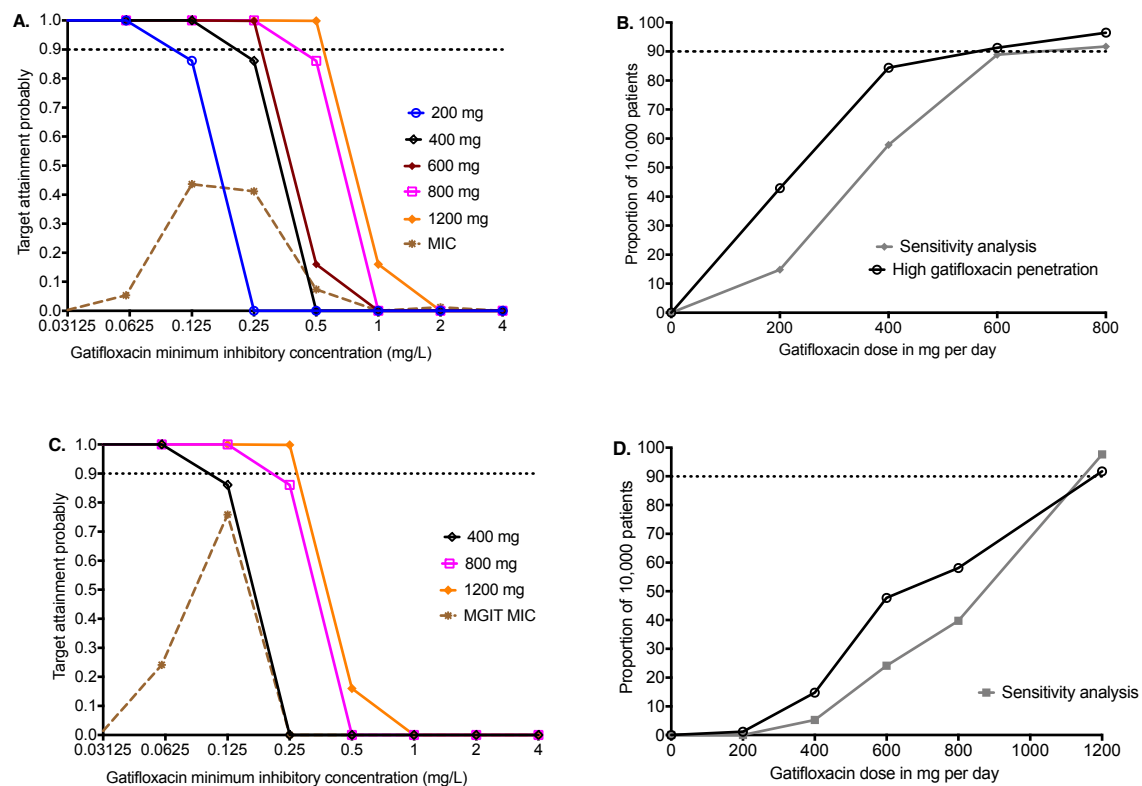


Figure 5 Gatifloxacin MIC and dose thresholds predictive of microbiologic cure in patients

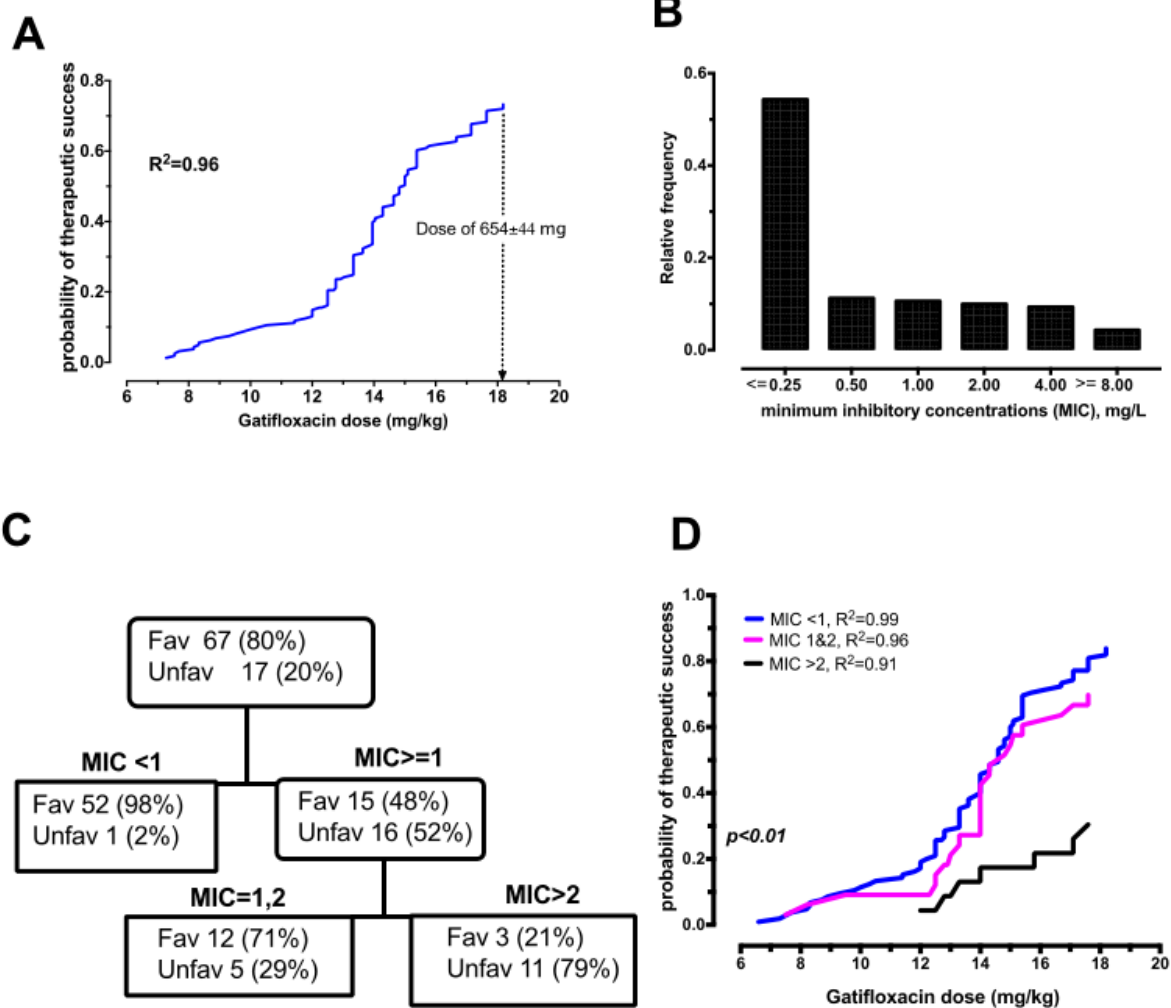


Figure 6. Gatifloxacin dose versus probability of favorable outcome

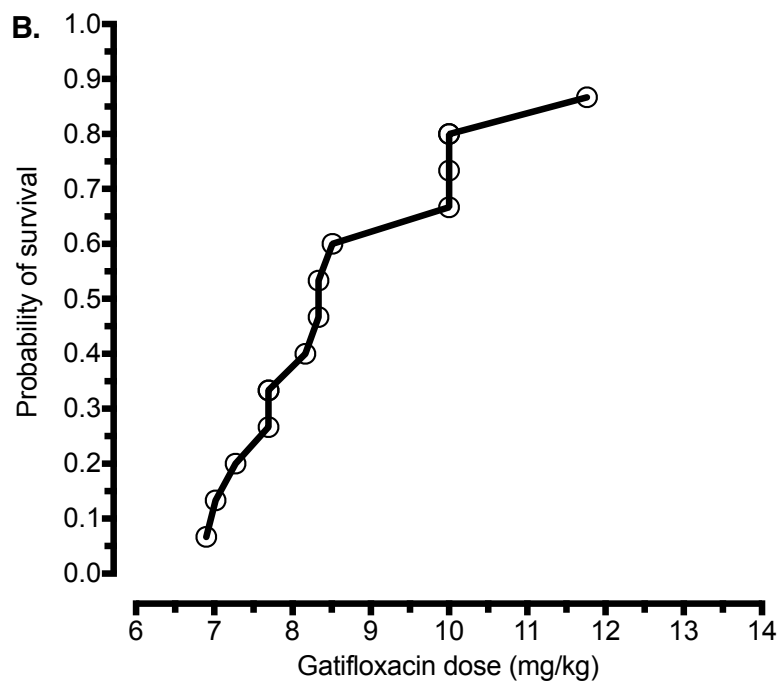
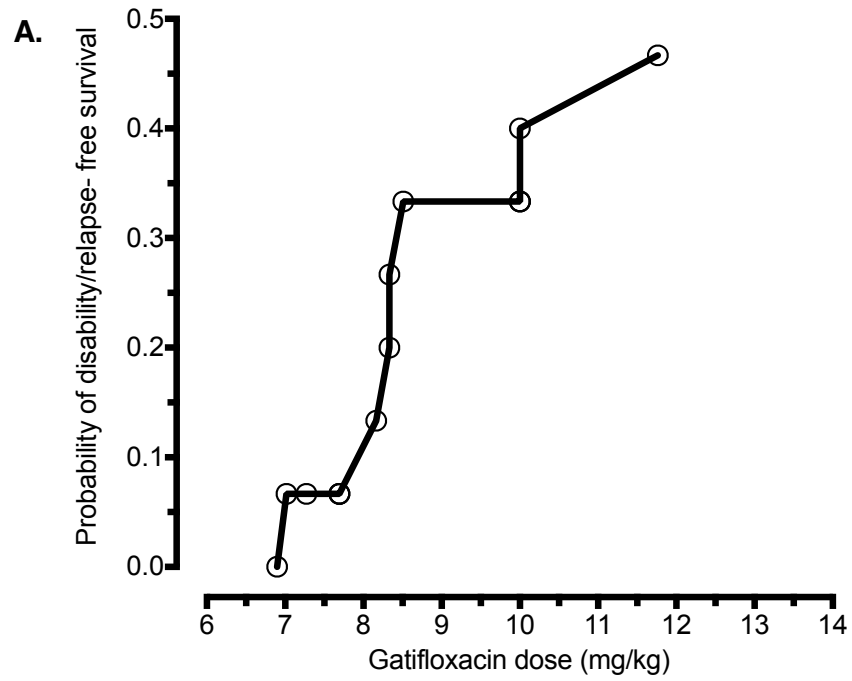


Table 1. Comparison of pharmacokinetic parameters in simulation to patients

	Domain of input	10,000 simulated subjects	FDA submission
Clearance in L/hr (mean±SD)	6.2 ±2.0	6.1±2.0	8.8±1.5 ^b
Volume in L (mean±SD)	141.0±31.0	141.0±8.8	-
Absorption constant (hr ⁻¹)	1.3±0.4	1.3±0.6	-
200 mg oral			
AUC ₀₋₂₄ mg*hr/L	-	20.8±2.8	14.2 ± 0.4 ^a
Peak concentration in mg/L	-	1.3±0.1	2.0 ± 0.4 ^a
400 mg oral			
AUC ₀₋₂₄ mg*hr/L	-	41.6±5.6	51.3 ± 20.4 ^b
Peak concentration in mg/L	-	2.5±0.2	4.2 ± 1.9 ^b

^aHealthy volunteers, single dose: ^bMultiple dose in patients with non-tuberculous infection.

Table 2. Demographic and clinical characteristics of 161 patients with MDR-TB

Clinical or laboratory feature	Estimate
Gender	

Age	
Weight in kg; median (range)	43 (26-65)
Pulmonary tuberculosis; n (%)	161 (100%)
Gatifloxacin dose; median (range)	600 (400-800)
Gatifloxacin dose mg/kg; median (range)	14.0 (6.6-18.2)
Minimum inhibitory concentration in mg/L; median (range)	0.25 (<0.25 to >8)
Gyrase mutations in pretreatment isolates	
Wild type	108 (67%)
GyrA mutations	53 (33%)
Outcomes	
Cure	131 (81%)
Failure	23 (14%)
Relapse	7 (4%)
