Levofloxacin pharmacokinetics-pharmacodynamics, dosing, and susceptibility breakpoints, and artificial intelligence in the treatment of multidrug-resistant tuberculosis

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ABSTRACT

Introduction. Levofloxacin is increasingly being used for the treatment of multidrug-resistant tuberculosis (MDR-TB); however the optimal dose is unknown.

Methods. We used the hollow fiber system model of tuberculosis (HFS-TB) to identify target (0-24 area under the concentration-time curve (AUC_{0-24})-to minimum inhibitory concentration (MIC) ratios associated with maximal microbial kill and suppression of acquired drug resistance (ADR) of *Mycobacterium tuberculosis (Mtb)*. Levofloxacin-resistant isolates underwent whole genome sequencing. Monte Carlo experiments (MCE) of 10,000 patients were used to identify dose best able to achieve the HFS-TB-derived target exposures in cavitary tuberculosis and tuberculous meningitis (TBM). Next we used an ensemble of artificial intelligence (AI) algorithms to identify most important predictors of sputum conversion, ADR, and death in Tanzanian patients with pulmonary MDR-TB treated with levofloxacin-containing regimen. We also performed probit regression to identify optimal levofloxacin doses for TBM in data from Vietnamese patients.

Results. The AUC_{0-24}/MIC associated with maximal *Mtb* kill was 146 and ADR suppression was 360 in the HFS-TB. The most common *gyrA* mutations in resistant *Mtb* were Asp94Gly, Asp94Asn, and Asp94Tyr. The minimum doses achieving target exposures in MCE were 1,500 mg/day. AI algorithms identified an AUC_{0-24}/MIC ratio of 160 as predictive of microbiologic cure, followed by levofloxacin 2-hour peak concentration, and body weight. Probit regression identified an optimal dose of 25 mg/kg as associated with >90% favorable response in adults with pulmonary tuberculosis.

Conclusion. The levofloxacin dose of 1500 mg/day was adequate for replacement of high dose moxifloxacin in treatment of MDR-TB.

The treatment of multidrug-resistant tuberculosis (MDR-TB) relies on latter generation quinolones, considered group A drugs by the World Health Organization (WHO). Moxifloxacin has been most commonly used, and doses of 800 mg a day have been proposed to minimize acquired drug resistance (ADR) [1, 2]. However, at this higher dose, moxifloxacin could be associated with higher rates of QT segment prolongation, which could progress to polymorphic ventricular tachycardia, an important concern especially when co-administered with other anti-tuberculous agents associated with this same adverse event [3]. Levofloxacin is felt to be safer in this regard, and thus, it is important to determine if levofloxacin can replace high dose moxifloxacin, and if so, to identify optimal dose for MDR-TB.

While pharmacokinetics-pharmacodynamics (PK/PD) studies of moxifloxacin, and the earlier generation ofloxacin and ciprofloxacin, have been performed, levofloxacin as of yet has not been examined [1, 4, 5]. Clinical studies have identified the population pharmacokinetic parameters of levofloxacin in patients with tuberculosis, and the penetration of levofloxacin into tuberculosis lesions [6-8]. Since moxifloxacin binds the *Mtb* gyrase better than levofloxacin, in theory it could be a better antituberculosis agent [9]. However, moxifloxacin had lower early bactericidal activity in patients than levofloxacin [10]. On the other hand, murine studies demonstrated that standard dose moxifloxacin was superior to high dose (human equivalent 1,000 mg) levofloxacin when used in combination with ethionamide, amikacin, and pyrazinamide after 4 and 5 months of therapy [10, 11]. Thus, it is as yet unclear if levofloxacin could be a sufficient replacement for moxifloxacin, and if so, what the dose equivalent to moxifloxacin 800 mg a day would be. Here, we performed a PK/PD study in the HFS-TB, and utilized the results and population pharmacokinetic parameters in Monte Carlo experiments (MCE) to evaluate levofloxacin doses for patients with tuberculosis, and identify the MIC above

which levofloxacin ceases to effectively kill *Mycobacterium tuberculosis* (Mtb). Next, we utilized artificial intelligence (AI) methods to identify the magnitude of the PK/PD index associated with clinical outcomes in Tanzanian patients who were treated for pulmonary MDR-TB [12]. We also compared the MCE results dose-versus-outcome in Vietnamese patients treated for tuberculous meningitis (TBM) [13].

METHODS

Materials, organisms, and reagents

Mtb H37Ra (ATCC # 25177) was utilized for all HFS-TB experiments as described elsewhere [14]. Levofloxacin was purchased from Baylor University Medical Center Pharmacy (Dallas, USA), and from SIGMA (St. Louis, USA). Moxifloxacin-13Cd3 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Hollow fiber cartridges were purchased from FiberCell (Frederick, Maryland). We utilized the BACTEC MGIT 960 Mycobacterial Growth Indicator Tube (MGIT) System to determine time-to-positivity (TTP) as a measure of bacterial growth (Franklin Lakes, New Jersey).

MICs and screening for extracellular and intracellular effect

MICs were identified using the standard macrobroth dilution reference method, the MGIT assay, and Epsilometer test (E test) on Middlebrook 7H10 agar, as described previously [15, 16]. The concentrations tested in the MGIT assay were: 0, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1 and 2 mg/L. The macrobroth dilution assay tested the same concentrations but in addition 4, 8 and 16 mg/L. Next, we examined the microbial kill of levofloxacin co-incubated for 7 days with extracellular and intracellular *Mtb* in test tubes and 24-well plates, as described before [15, 16].

Hollow fiber system model of tuberculosis (HFS-TB)

HFS-TB technical specifications, and *Mtb* growth conditions for log-phase growth *Mtb* for levaquin were as described in the introduction paper and elsewhere [1, 14,17-19]. *Mtb* was inoculated into seven HFS-TB units, and treatment with levofloxacin initiated at doses to achieve 0-24 area under the concentration-time curve (AUC_{0-24}) of 0, 1.0, 1.25, 2.0, 10.5, 21, and 42 mg*h/L. Since the levofloxacin half-life in epithelial lining fluid and alveolar macrophages is 8.1-14.3 hours [20], we mimicked the midway half-life of 11hrs. Treatment was delivered daily for 28 days through computerized syringe pumps. We sampled the central compartment at 0, 1, 4, 8, 12, 22 and 23.5 hours after the last dose, to validate that the intended concentration-time profile were achieved. Levofloxacin concentration assay is described in detail in supplementary methods. We sampled the peripheral compartment on days 0, 3, 7, 10, 14, 21 and 28 for TTP and colony forming units (cfu) counts on Middlebrook 7H10 agar supplemented with 10% OADC [16, 21]. The levofloxacin-resistant cfus were captured by culturing on agar supplemented with 8 times the levofloxacin MIC. Levofloxacin-resistant colonies were picked for whole genome sequencing (WGS) and mutations confirmed by Sanger sequencing, as described before [16, 22].

Levofloxacin concentrations from the central compartment of each HFS-TB unit were modeled in ADAPT 5 [23]. The pharmacokinetic model-derived AUC_{0-24}/MIC of levofloxacin achieved in each HFS-TB was modeled for microbial kill and resistance using the inhibitory sigmoid E_{max} model and quadratic function, as described in accompanying background paper [24].The software, types of statistical and pharmacometric analyses, and AI algorithms, are described in the supplemental section.

Monte Carlo experiments using HFS-TB data

The EC_{80} and the AUC_{0-24}/MIC associated with ADR suppression, whichever was the higher value, was defined as the target exposure [25]. The pharmacokinetic parameter estimates and variability, shown in **Table 1**, entered in subroutine PRIOR of ADAPT 5, were based on prior work by others [6, 26]. Kempker et al identified a median tuberculosis cavitary versus serum levofloxacin ratio of 1.33 (range, 0.63 to 2.36) based on microdialysis method, which is remarkably similar to the median 1.4-fold entry into epithelium lining fluid [7, 27]. Therefore, in pulmonary tuberculosis simulations, we used cavitary penetration ratio of 1.3. Pea et al have demonstrated that the cerebrospinal fluid (CSF)-to-serum AUC ratio of 0.71, which we utilized in our simulations [28]. The following levofloxacin doses were examined in each 10,000 subject MCE for pulmonary disease and TBM: 750, 1000, 1250, and 1500 mg. Target attainment probability (TAP) was calculated at each MIC ranging from 0.0625 mg/L to 16 mg/L, based on the MIC distribution of 243 *Mtb* isolates by Rodriguez [29]. Since fluoroquinolone MIC susceptibility breakpoint and distribution in *Mtb* isolates is dependent on the media used, and the MGIT is commonly used for MIC distribution we performed a sensitivity analysis using the MGIT-derived MIC distribution in 30 wild type isolates reported by Kambli et al [30]. Overall cumulative fraction of response (CFR) was then calculated for each dose weighted over this MIC distribution..

Analyses of clinical data using machine-learning (ML) and probit regression

We employed a three-pronged ML approach, which are described in full in the online supplemental methods and elsewhere, to identify levofloxacin PK/PD parameters predictive of outcomes in MDR-TB patients. We examined sputum conversion, ADR and death outcomes of 41 patients in Tanzania with MDR-TB treated with 750 mg levofloxacin, in combination with kanamycin, cycloserine, ethionamide and pyrazinamide, who had levofloxacin concentrations measured 2 hours after dose 2 and 4

weeks after starting levofloxacin [12]. First, we performed compartmental pharmacokinetics modeling, as previously described and compared those with prior studies that used intensive sampling (**Table 1**) [31, 32]. Next, we used random forests and classification and regression tree (CART) analyses, described in detail in the past, to identify and rank most important predictors of sputum conversion [32, 33]. Next, separate probit regression models were used to examine probability of favorable outcomes and levofloxacin dose in patients with pulmonary MDR-TB using data from Tanzania and in TBM from Vietnam [12, 13].

RESULTS

Activity of static levofloxacin concentrations in test tubes and 24 well plates

The levofloxacin MICs for Mtb H37Ra were 0.0625 and 0.125 mg/L in broth macrodilution on two separate occasions, 0.125 mg/L in the MGIT on two separate occasions, and 0.16mg/L on E-test. We adopted an MIC of 0.125 mg/L. The inhibitory sigmoid E_{max} relationship between static concentration and extracellular log-phase growth *Mtb* burden is shown in **Figure 1A**: the EC_{50} was 0.25 (95% confidence interval [CI]: 0.22-0.28) times MIC and E_{max} was 6.62 (95% CI: 6.09-7.16) log_{10} CFU/mL. **Figure 1B** shows results for intracellular *Mtb*: the EC_{50} was 0.44 (95% CI: 0.43-0.51) times MIC and E_{max} was 4.71 (95% CI: 4.38-5.08) log_{10} CFU/mL.

Exposure-response in the HFS-TB

The pharmacokinetic model predicted versus observed concentrations in HFS-TB had a slope of 0.995±0.003 [r^2>0.999] (**Figure 2A),** indicating minimal bias. The concentration-time profiles achieved are shown in **Figure 2B.**The levofloxacin clearance was 0.040±0.002 L/hr, indicating a 5% (minimal) technical variation between the HFS-TB

pharmacokinetic units; the half-life was 10.84 hours, which is 98.55% accurate of the intended 11 hours. The time-kill curves, based on CFU/mL readout, are shown in **Figure 2C**, which demonstrates that the effect of the exposures are clustered into two: those with an $AUC_{0\text{-}24}$/MIC<20 with effect similar to the non-treated controls, and the higher exposures which demonstrated considerable microbial kill. **Figure 2D** shows the same pattern when TTP was used as a measure of bacterial burden: the lower the TTP the higher the bacterial burden. Inhibitory sigmoid E_{max} modeling of $AUC_{0\text{-}24}$/MIC versus $\log_{10}$ CFU/mL for each day of therapy is shown in **Figure 2E**. Supplemental **Table 1** demonstrates a remarkably consistent EC_{50} and Hill slope on all sampling days. The TTP-based inhibitory sigmoid E_{max} fits are shown in **Figure 2F** and supplemental **Table 1**; the EC_{50} estimates for the TTP were generally higher than CFU/mL readout. We calculated the EC_{80} averaged across all sampling days, which was an $AUC_{0\text{-}24}$/MIC of 146.40 (95% CI: 112.1-180.8).

Evolution of resistance in the HFS-TB

The evolution of levofloxacin-resistant *Mtb* subpopulation is shown in **Figure 3A**; ADR arose on day 21. The relationship between $AUC_{0\text{-}24}$/MIC and the levofloxacin-resistant subpopulation, is shown in **Figure 3B**. The figure shows that the EC_{80} $AUC_{0\text{-}24}$/MIC of 146 also amplified the proportion of levofloxacin-resistant *Mtb* to near maximal, similar to what was noted with moxifloxacin and ciprofloxacin [1, 5]. Suppression of ADR was only encountered at an $AUC_{0\text{-}24}$/MIC of 360. Levofloxacin-resistant isolates from the HFS-TB underwent WGS that identified the following *gyrA* mutations: Asp94Gly, Asp94Asn, and Asp94Tyr. The results were confirmed by Sanger sequencing, shown in **Figure 3C**. Indeed, results show that all mutations were in quinolone resistance determining region [34].

MCE for dose selection for pulmonary and meningeal tuberculosis

With regards to the EC_{80} target exposure, the TAP of different doses in pulmonary tuberculosis is shown in **Figure 4A**. When the TAP was averaged over the entire MIC range, the proportion of patients with pulmonary tuberculosis who achieved target exposures with each dose were as shown in **Figure 4B**. However, the EC_{80} target maximally amplifies ADR. Therefore we performed MCE using the target exposure associated with ADR suppression, with results shown in **Figures 4C-D**, which revealed an optimal dose of 1,500 mg a day, based on CFR. At that dose, the proposed susceptibility breakpoint was 0.5 mg/L. Next, we performed a sensitivity analysis utilizing MGIT-derived levofloxacin MIC distribution from Kambli et al: 83.33% *Mtb* had an MIC $\leq$0.38 mg/L and 16.77% an MIC of 0.75 mg/L. The 1,500 mg/day TAP for resistance suppression was 99.29% at MIC of 0.38 mg/L and 15.67% at MIC of 0.75 mg/L; thus the proposed susceptibility breakpoint would remain the same at 0.5 mg/L even in MGIT assays.

Next, we examined the performance of doses in achieving the EC_{80} target in the CSF, with results shown in Figure **4E**. The CFR for each dose is shown in **Figure 4F**. A dose of 1,250 mg/day is required to achieve the EC_{80} in >90% of patients with TBM. For completeness, we also examined the higher target for suppression of ADR, with results shown in **Figure 4G-H** show that even at a dose of 3,000 mg/day (i.e., 4 times current standard dose) only 78% of patients would achieve that target. However, since TBM is a paucibacillary disease with low risks of ADR, the EC_{80} target will likely be clinically sufficient.

Relationship between exposure, concentration, dose and outcome in patients

Clinical details of the 41 Tanzanian patients with pulmonary MDR-TB have been published [12]. MIC were performed for ofloxacin in 31 of the 41 patients, but not levofloxacin, using Sensititre, with distribution shown in **Figure 5A**. The mean ±standard deviation pharmacokinetic model-derived levofloxacin AUC_{0-24} was 83.02±65.00 mg*h/L in the Tanzanian patients, which is virtually the same as those identified with more intensive pharmacokinetic sampling in **Table 1** elsewhere. The following unfavorable outcomes were ascertained: sputum conversion status unknown 2 (5%), defaulted 6 (15%), death 6 (15%) and development of ADR 1 (2%). There was no significant difference in the distribution of the median levofloxacin peak concentration, AUC_{0-24} or AUC_{0-24}/MIC by treatment outcome based on the Kruskal-Wallis test (**Figure 5B**). **Figure 5C** shows the ranking of important variables from two random forest models: (i) comparing microbiologic cure versus failure and (ii) favorable versus unfavorable outcomes. For microbiologic cure versus failure levofloxacin AUC_{0-24}/MIC ratio was the most important predictor at 100% followed by levofloxacin 2-hour peak concentration at 86% and body mass index (BMI) at 85%. For favorable versus unfavorable outcomes in the entire dataset, peak concentration was the primary node (100%), followed by weight at 93%, then BMI at 85% and then AUC_{0-24}/MIC ratio at 81%. **Figure 5D** shows representative CART tree, which revealed that the AUC_{0-24}/MIC threshold predictive of microbiologic failure was 160. However, the MIC used was for ofloxacin, which is often 1-tube dilution higher than levofloxacin, so that the threshold value would translate to a putative AUC_{0-24}/MIC of 320.

The levofloxacin serum AUC distribution in Tanzanian patients with pulmonary MDR-TB is shown in **Figure 6A**. We utilized a probit regression models of levofloxacin dose (mg/kg) versus outcome in the same patients, with results shown in **Figure 6B.**

Interestingly, the maximum probability of favorable outcomes was 60% in pulmonary disease, which is suboptimal. The dose on that flat portion of the probit curve was about 25 mg/kg or a total 1,250mg, given the weight of patients in Tanzania.

Next, we analyzed data from Vietnamese patients with TBM on treatment with a levofloxacin-based regimen. The levofloxacin serum and CSF AUCs in these patients were as shown in **Figure 6C**. The levofloxacin penetration ratio into CSF observed was 0.71, which is close to the 0.75 used in our MCE. A probit analysis revealed the relationship between dose and probability of disability free survival shown in **Figure 6D.** The disability-free survival rates were also poor; however they were in line with the current outcomes even with first line antituberculosis therapy.

DISCUSSION

Firstly, Aubry et al have shown that the ability of a quinolone to inhibit *Mtb* gyrase DNA supercoiling activity by 50% (IC_{50}) was 3 mg/L for gatifloxacin, 4.5 mg/L by moxifloxacin, and 5 mg/L by levofloxacin; the concentration producing 50% of the maximum DNA cleavage (CC_{50}) was 4 mg/L for gatifloxacin, 4 mg/L for moxifloxacin, but 12 mg/L for levofloxacin [35]. In **Table 2** we compare levofloxacin PK/PD parameters, MCE-derived dose, to those of moxifloxacin and gatifloxacin, derived from our separate but similarly designed studies [1, 14]. Based on speed of kill, and time to ADR, the ranking would be gatifloxacin > levofloxacin > moxifloxacin. ADR arose faster with moxifloxacin than levofloxacin, while gatifloxacin suppressed ADR the best. Indeed, the gatifloxacin-resistant subpopulation was only 5% of total on day 28 in the systems that amplified resistance the most, as opposed to 100% for both moxifloxacin and levofloxacin (**Table 2**). Thus, based on PK/PD considerations levofloxacin can indeed replace moxifloxacin in pulmonary tuberculosis, while gatifloxacin could be the best drug to start off with.

However, gatifloxacin increased the rate of dysglycaemia 4.3-fold compared to other antibiotics, while levofloxacin increased it 1.5-fold, and moxifloxacin did not, in elderly patients [36]. Dysglycaemia has been encountered in 2-9% of MDR-TB patients of gatifloxacin, and the incidence of this adverse event may be higher at the advocated doses of 800 mg [14, 37].

Second, we identified the levofloxacin dose that gives equivalent effect to moxifloxacin 800 mg/day, using MCE, AI-based analyses, and probit models of clinical data. The equivalent dose should be able to minimize ADR, which is encountered for quinolones and aminoglycosides in 9-14% of patients on treatment for MDR-TB at standard doses [38, 39]. We have utilized amplicon-based next generation and confirmatory Sanger sequencing in 158 MDR-TB isolates from four countries and identified that 6.3% of 1811 loci examined exhibited at least some mutant population (≥1% of reads) in at least one isolate, with the largest being gyrA, thus subpopulations of fluoroquinolones resistance are encountered commonly in MDR-TB [40]. Mutations were within the same resistance determining codon (gyrA 94) we identified in the HFS-TB with suboptimal levofloxacin exposures, consistent with our antibiotic resistance arrow of time model [41, 42]. Since resistance to quinolones reduces cure rates considerably, attaining ADR suppression targets is crucial [43, 44]. For pulmonary tuberculosis, the levofloxacin dose that is able to achieve suppression of ADR was 1,500 mg/day or around 20 mg/kg/day, as shown in **Figure 4**. Thus, the inferiority of levofloxacin to moxifloxacin when added to amikacin, ethionamide and pyrazinamide demonstrated in mice could be due to a differential dose issue. At high enough doses, levofloxacin could be equivalent to moxifloxacin [10, 11]. In the case of TBM, the dose that would suppress ADR was higher than what is currently known to be tolerated by patients. However, given the lower bacterial burden in CSF compared to pulmonary cavities, the PK/PD goal of therapy in TBM may not be

284 resistance suppression but the EC_{80}. In that case, the levofloxacin dose of 1500 mg/day
285 could still be used for TBM.
286
287 Third, we identified the MIC above which levofloxacin therapy is expected to fail, in MCE.
288 We identified this as an MIC of 0.5 mg/L for both MGIT and agar dilution using
289 Middlebrook 7H11. At one dilution higher (1 mg/L), one would need to double the dose
290 to achieve the same TAP. Since, unlike gatifloxacin, exposures associated with the EC_{50}
291 and EC_{80} actually amplify ADR, we did not examine the effect of higher doses such as
292 3,000 mg/day at higher MICs. The doses would be several times above the standard,
293 and the safety is unclear, as is the effect given resistance amplification. Thus, we did not
294 propose a susceptible dose dependent zone for levofloxacin.
295
296 Our study has a few limitations. First, the levofloxacin concentrations of patients with
297 MDR pulmonary TB that we utilized as an external check on pharmacokinetic modeling
298 were from a retrospective study. Moreover, except for levofloxacin, peak concentrations
299 of other drugs that comprised the MDR-TB regimen were not measured and therefore,
300 would make it difficult to predict outcomes. However, the observations of low
301 levofloxacin concentrations associated with unfavorable outcomes in patients are similar
302 to those derived from the modeling exercise and thus likely true. Second, the clinical
303 data set utilized for external validation of modeling for TBM was relatively small.
304 However, the high rate of failure, and use of the index of disability-free survival, made it
305 possible to model, despite the small sample size. On the other hand, disability-free
306 survival cannot be solely attributable to TBM, and the effect of other factors such as
307 penetration of companion antituberculosis compounds into the CSF, will be important to
308 consider. Yet, both model predicted and clinically observed unfavorable outcomes were

associated with low CSF exposures and identified similarly high doses for success, and therefore, despite these limitations, are likely to be true.

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; J.P. and T.G. performed Probit and CART analysis of clinical data; S.G.M., C.P., S.B., P. A., G.T., S.K.H., performed the clinical studies and/or analyses; 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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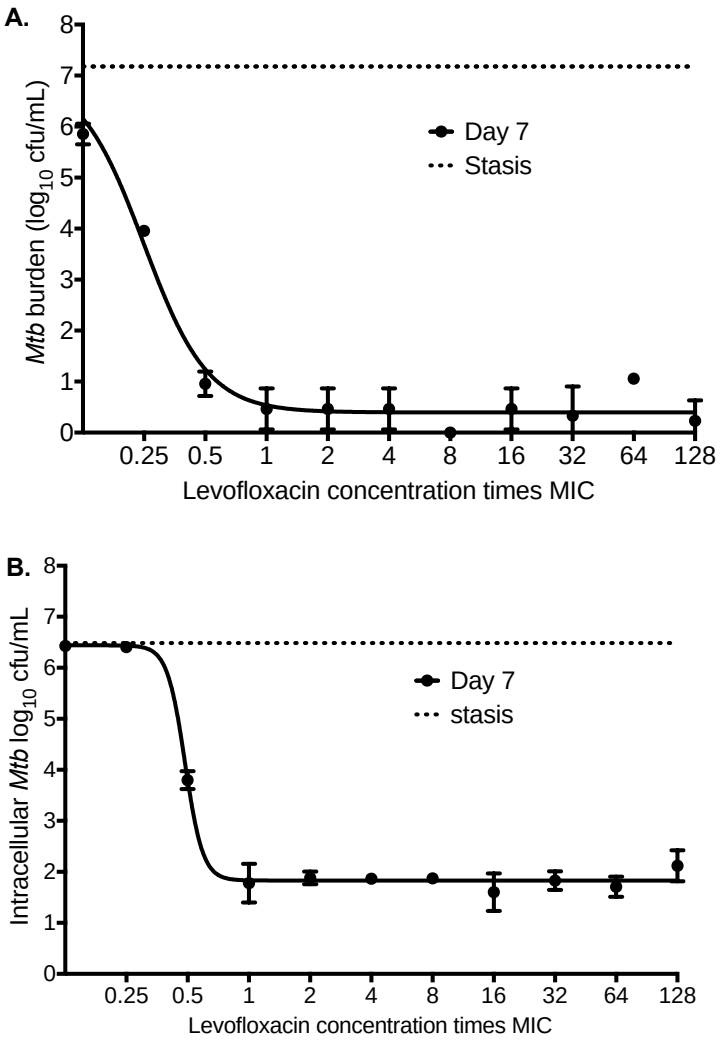
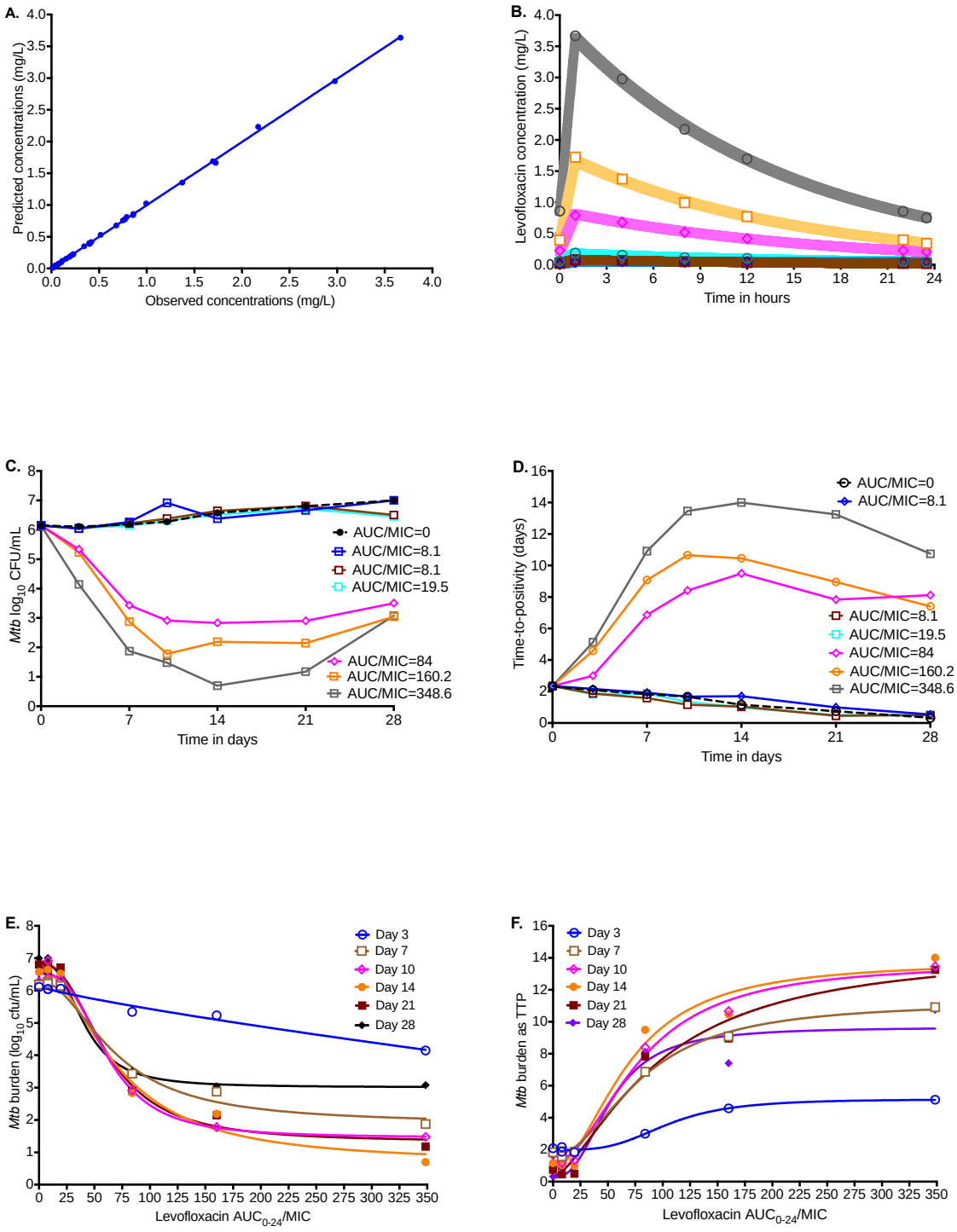
Figure 1. Levofloxacin concentration-effect against *Mtb*



Mtb cultures were coincubated for 7 days, with three replicates for each drug concentration. Shown are mean values; error bars are for standard deviation. The stasis line indicates bacterial burden at the start of treatment. Each drug concentration is expressed as a multiple of MIC. **(A)** Levofloxacin effect against extracellular log-phase growth *Mtb* plateaus demonstrated a steep decline in bacterial burden below MIC, with no increased killing at the concentration of 1 times MIC or above. Maximal kill (E_{max}) was >7 $\log_{10}$ CFU/mL below stasis. (**B**) We infected activated THP-1 cells and co-incubated them with levofloxacin for 7 days. The concentration-effect pattern was similar to that

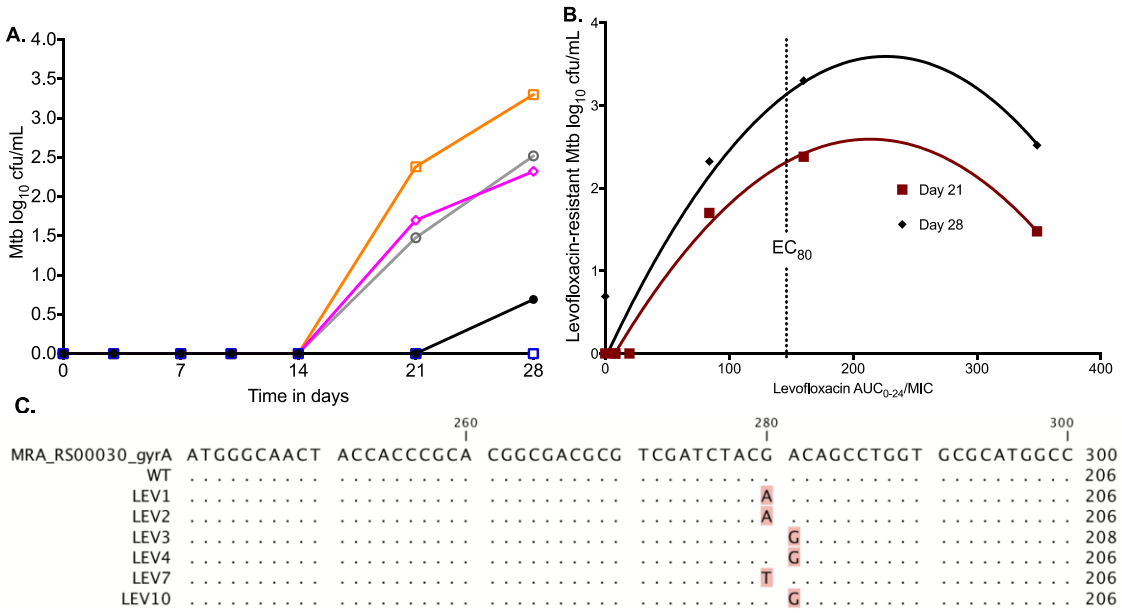
seen with extracellular *Mtb* co-incubation, with the difference that microbial kill below stasis was 2 $\log_{10}$ CFU/mL less than against extracellular bacilli, which means less efficacy against intracellular bacilli. A lower maximal kill of intracellular versus extracellular Mtb has also been seen with other quinolones such as moxifloxacin.

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



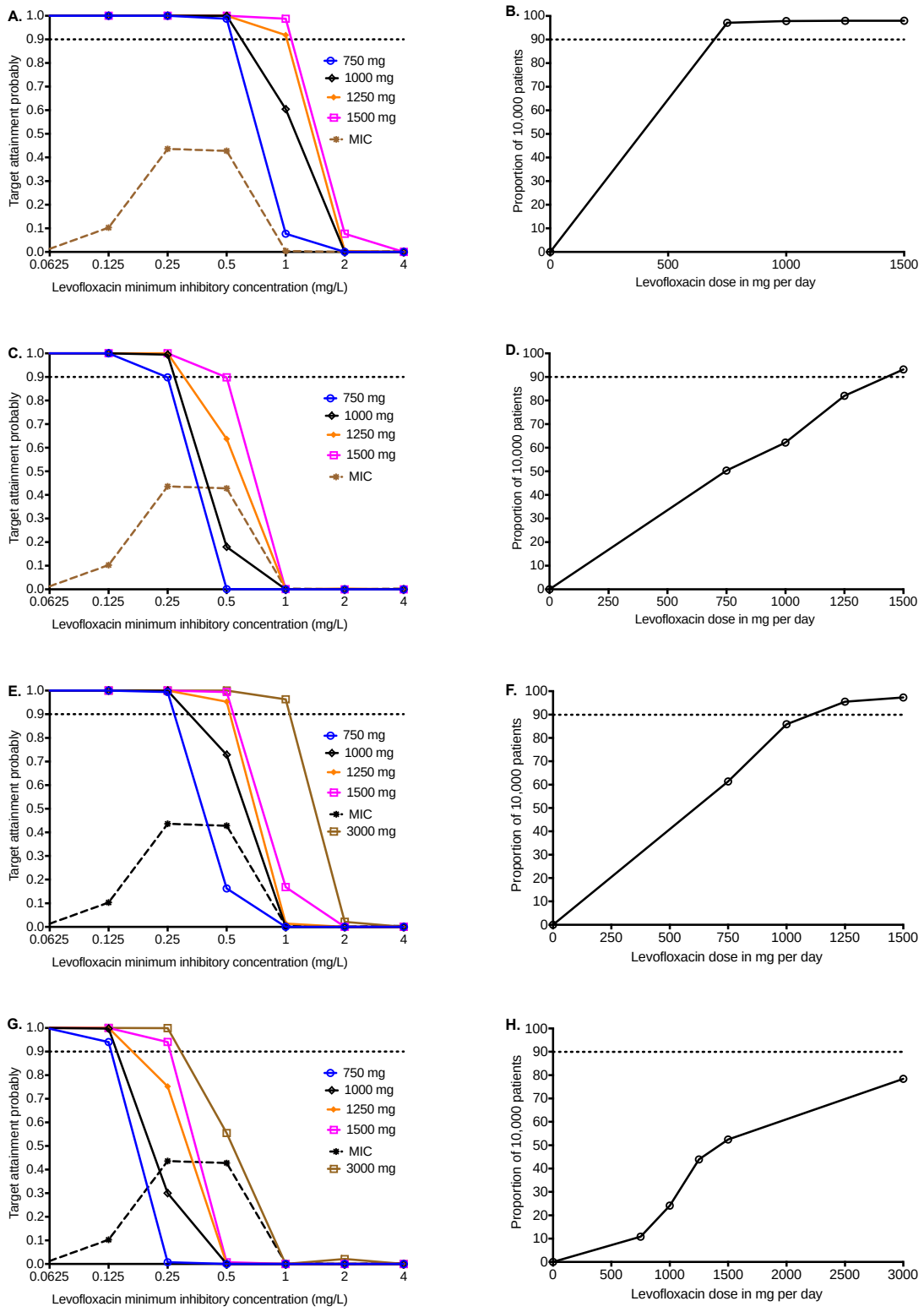
A. Model predicted versus observed levofloxacin concentrations in the hollow fiber system. **B.** Levofloxacin concentrations achieved in each HFS-TB at each time point were used for pharmacokinetic modeling. The shaded area is the 95% confidence interval for the pharmacokinetic model derived concentrations for each dose; the observed concentrations show that the model described the data well. **C.** Time change in CFU/mL with duration of therapy shows that the exposures could be separated into two groups. The highest exposure achieved near sterilization on day 14, followed by rebound on day 21. **D.** Time-to-positivity (TTP) shows the same pattern. **E.** The inhibitory sigmoid E_{max} model curves are shown as CFU/mL on each sampling day versus AUC/MIC. The figure shows that the EC_{50} did not change significantly with increasing duration of therapy. **F.** Inhibitory sigmoid E_{max} modeling using TTP as measure of bacterial burden shows a higher EC_{50} than was obtained with CFU/mL.

Figure 3. Effect of exposure on acquired resistance in the hollow fiber system and analysis of whole genome sequencing



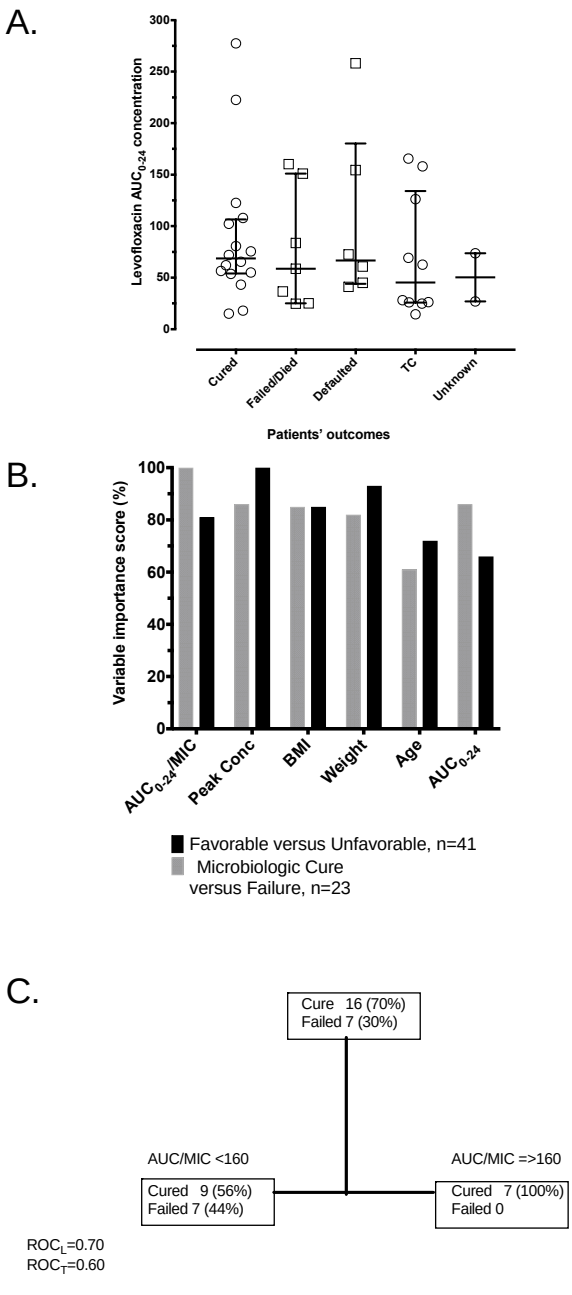
(A) Shows that resistance (log_{10} CFU/mL) arose in the three of the levofloxacin treated systems on day 21, under the three highest $AUC_{0\text{-}24}$/MIC exposures of 84 (magenta diamonds), 160.2 (orange squares), 348.6 (grey open circles), which were higher than non-treated controls (black solid circles). On day 28, the size of the drug-resistant subpopulation CFU/mL to the total CFU/mL was 6.56% for the $AUC_{0\text{-}24}$/MIC of 84, 100% for $AUC_{0\text{-}24}$/MIC of 160.2, and 27.5% for the $AUC_{0\text{-}24}$/MIC of 348.6. **(B)** The relationship between size of drug-resistance subpopulation and exposure shows that the EC_{80} was associated resistance amplification. The suppression of resistance occurs at a much higher exposure and can be read off the graph as 360. **(C)** The Sanger sequence alignment which compares wild type (WT) to resistant strains shows nucleotide changes in the levofloxacin resistant isolates at positions 280 and 281.

Figure 4. Monte Carlo Experiments of different levofloxacin doses for pulmonary and meningeal TB



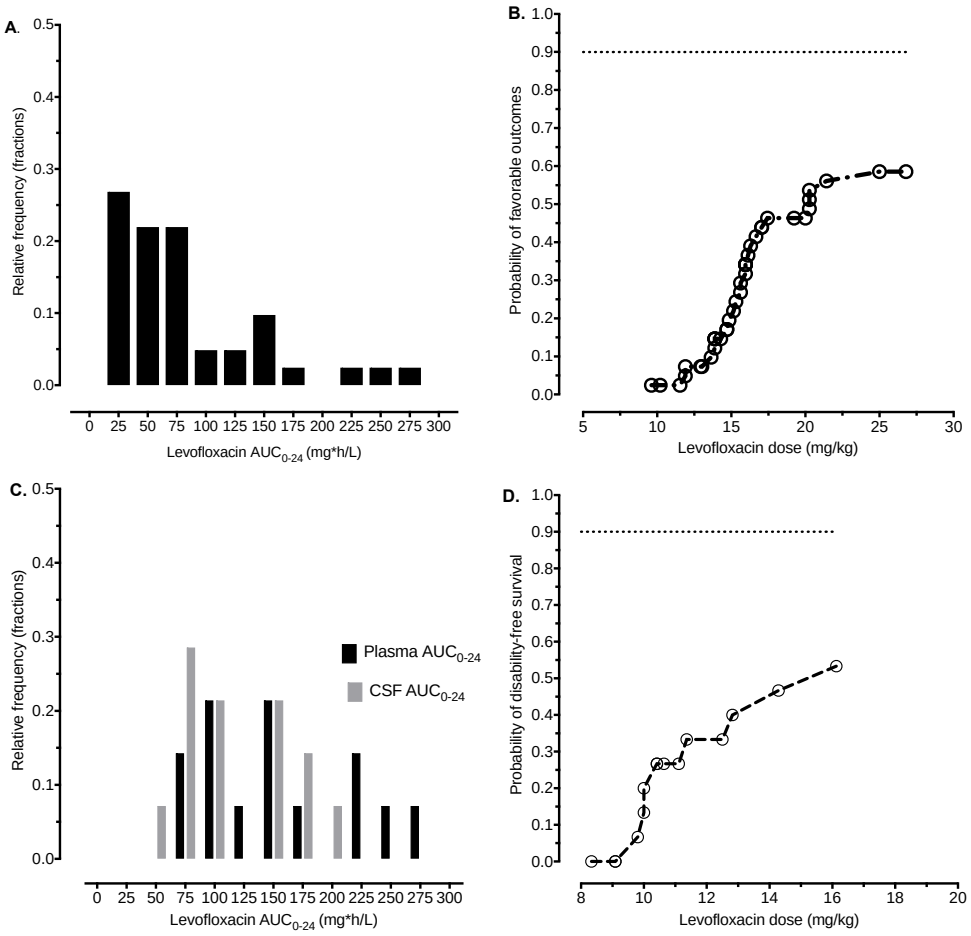
A. Target attainment probability (TAP) for the EC_{80} exposure in pulmonary cavities. At a dose of 1,000 mg, TAP falls below 90% above an MIC of 0.5 mg/L. **B. P**roportion of 10,000 patients treated with different doses who achieve the EC_{80} in pulmonary cavities, reveals an optimal dose of 1,250 mg. **C.** TAP of different doses in achieving exposure associated with resistance suppression in pulmonary cavities. At a dose of 1,500 mg, the TAP falls below 90% above an MIC of 0.5 mg/L. **D.** The levofloxacin dose that would achieve the exposure associated with resistance suppression in >90% of patients with pulmonary cavities was 1,500 mg each day. **E-F** shows performance of different doses in achieving the EC_{80} in the CSF. The TAP fell below 90% above an MIC of 0.5 mg/L for all doses, except 3,000 mg a day. **G-H.** TAP of different doses in achieving exposure associated with resistance suppression in the CSF precipitously drops above an MIC of 0.25 mg/L.

Figure 5. AI derived predictors of outcomes in patients



A. There was no statistically significant difference in the distribution of the median levofloxacin $AUC_{0\text{-}24}$s by treatment outcome using standard statistical inferences. **B** Ranking of important variables from two random forest models based on two different definitions of outcome. **C.** Representative tree used to generate the random forest model output.

Figure 6. Probit regression in patients treated with levofloxacin-based regimens



A. AUCs achieved in the blood of patients with pulmonary tuberculosis in Tanzania. **B.** The probit model of levofloxacin dose mg/kg for patients with pulmonary MDR-TB from Tanzania. The curve flattened out at 60% probability of good output for pulmonary MDR-TB, which is problem. **C.** Distributions of AUCs in the blood and cerebrospinal fluid of Vietnamese patients with meningitis. **D.** Probit model in patients with drug-susceptible tuberculosis who also received rifampin for tuberculous meningitis.

Table 1. Levofloxacin pharmacokinetic parameters and variances

	Domain of input (mean ±SD) [6, 29]	**10,000 simulated subjects** (mean ±SD)	**FDA package insert** (mean ±SD)
Clearance in L/hr	7.63 ±3.55	7.61±1.88	8.58±1.74
Volume in L	81.21±41.66	81.33±6.50	100±16
Absorption constant (hr^{-1})	5.96±2.38	5.95±1.54	-
$AUC_{0\text{-}24}$ (mg*h/L) for 750 mg	-	87.13±15.6	90.7±17.6
Peak (mg/L) for 750 mg	-	8.56±2.55	8.6±1.9

Supplementary Table 1. Inhibitory sigmoid E_{max} model fits for levofloxacin effect in the HFS-TB

Model parameter	Day 7	Day 10	Day 14	Day 21	Day 28
Log_{10} cfu/ml-based					
E_{con} (log_{10} CFU/mL)	6.31±0.19	6.53±0.18	6.68±0.31	6.84±1.89	6.87±0.15
E_{max} (log_{10} CFU/mL)	4.42±0.50	5.07±0.38	6.00±0.86	5.52±0.42	3.86±0.26
H	2.00±0.70	2.85±1.21	1.91±0.80	2.44±0.78	2.74±0.70
EC_{50} (AUC_{0-24}/MIC)	67.89±11.44	61.18±9.63	70.11±14.58	61.16±8.59	41.33±7.92
R^2	0.990	0.993	0.986	0.994	0.993
TTP-based					
E_{con} (days)	1.65±0.21	1.26±0.47	0.95±0.83	0.33±0.89	0.27±0.86
E_{max} (days)	9.57±0.66	12.43±1.44	12.83±2.20	14.19±3.90	9.39±1.65
H	2.05±0.44	2.04±0.74	2.00±1.07	1.54±0.84	2.52±1.76
EC_{50} (AUC_{0-24}/MIC)	80.13±6.78	78.26±11.46	68.7±17.42	95.16±35.73	52.13±21.55
R^2	0.997	0.991	0.978	0.976	0.959

Table 2. Effect of levofloxacin, moxifloxacin, and gatifloxacin in HFS-TB and MCE from different studies with similar experimental design

	Moxifloxacin	Gatifloxacin	Levofloxacin
Maximal kill (log_{10} CFU/ml/day)	0.57	0.68	0.61
Time to 1% ADR (days)	10	21	21
% population resistant at end of experiment	100	5	100
AUC/MIC resistance suppression	53	184	360
EC_{80}	56	184	146
MCE-derived dose (mg/day): resistance suppression			
Pulmonary tuberculosis	800	800	1500
Meningeal tuberculosis	-	1,200	>3,000