Hollow Fiber System Model of Tuberculosis (HFS-TB) as an *in vitro* preclinical tool for optimization of dose selection and drug regimen in anti-TB drug development

Critical Path Institute, on behalf of Critical Path to Tuberculosis (TB) Drug Regimens Consortium (CPTR)

Document type:	Dossier submitted for Qualification Opinion
Document status:	FINAL
Release date:	24 February 2014
Procedure Number:	EMEA/H/SAB/049/1/QO/2014/SME
Number of pages:	42

CPTR Preclinical and Clinical Sciences Workgroup - Hollow Fiber System Sub-team Participants

Dr. Tawanda Gumbo, *MD – University of Texas Southwestern Medical Center*

Dr. Debra Hanna, *PhD – Critical Path Institute*

Dr. Klaus Romero, *MD, MS, FCP – Critical Path Institute*

Dr. Eric Nuermberger, *MD – Johns Hopkins University*

Dr. Khisi Mdluli, *PhD – Global TB Alliance*

Dr. Jotam Pasipanodya, *MD, Dr.PH – University of Texas Southwestern Medical Center*

Dr. Antonia Kolokathis, *MD – Critical Path Institute*

Dr. Elizabeth Walker, *PhD – Critical Path Institute*

Robin Keen – *Janssen Pharmaceuticals*

Dr. Koen Andries, *PhD – Janssen Pharmaceuticals*

Lindsay Lehmann, *MBA – Critical Path Institute*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Accuracy
ADR	Acquired drug resistance
AUC	Area under the curve
B	Bias
CFU	Colony forming unit
CI	Confidence interval
CPTR	Critical Path to Tuberculosis Drug Regimens Consortium
DDT	Drug Development Tool
E	Error
EMA	European Medicines Agency
FE	Forecasting error
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HFS-TB	Hollow fiber system model of TB
ICAAC	Interscience Conference on Antimicrobial Agents and Chemotherapy
IND	Investigational New Drug
MAPE	mean absolute percentage error
MDR-TB	Multi-drug resistant tuberculosis
MIC	Minimum inhibitory concentration
MSE	Mean squared error
Mtb	*Mycobacterium tuberculosis*
P	Value predicted at t_1
PCS-WB	CPTR Pre-Clinical & Clinical Sciences Workgroup
PD	Pharmacodynamics
PK	Pharmacokinetics
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
T	True value identified in clinical study at t_2
TB	Tuberculosis
TBCT 29	Tuberculosis Trials Consortium Study 29
USPHS	US Public Health Service Grading System
VXDS	Voluntary Exploratory Data Submission
XDR-TB	Extensive drug resistant

1. EXECUTIVE SUMMARY

The Critical Path to TB Drug Regimens (CPTR) initiative aims to develop consensus and build an evidence base to support potential new drug development tools for tuberculosis (TB) combination regimen development. One such tool, the **hollow fiber system model of TB** (HFS-TB) was developed 10 years ago and has been used to inform drug doses, select drug regimens and improve therapeutic strategies involving old as well as new antibiotics. The HFS-TB can be effectively utilized to mimic the pharmacokinetic disposition of antibiotics observed in TB patients and the metabolic and physiologic behavior of *Mycobacterium tuberculosis* (*Mtb*) populations commonly encountered in patients with pulmonary TB. The HFS-TB also mimics intracellular bacteria characteristics of disseminated TB and can quantify the sensitivity and resistance of these *Mtb* populations to various doses and combinations of antibiotic agents over time.

The CPTR Pre-Clinical & Clinical Sciences Workgroup (PCS-WG) requests an EMA Qualification Opinion for the following Context of Use:

- The HFS-TB can be used in anti-TB drug development programs as an additional and complementary tool to existing methodology to inform selection of dose and treatment regimen to maximize bactericidal effects and minimize emergence of drug resistance. HFS-TB can be used in submissions to EMA throughout the drug development process for a product, especially for more informed design of Phase I, Phase II and Phase III clinical studies.

The HFS-TB system provides essential pharmacologic and microbiologic information that cannot be obtained from any other single, commonly accepted tool. In addition, its use should significantly shorten the overall time to complete necessary preclinical and clinical pharmacology studies. In this dossier, we utilize high-quality evidence from the published literature demonstrating a high degree of predictive accuracy for, and strong correlation between, data generated by the HFS-TB and clinical trial results.

METHODS: This team performed comprehensive literature searches to identify all relevant publications that were used to perform our analyses.

Search A identified all HFS-TB studies and Monte Carlo simulation studies published in the literature that utilized the HFS-TB output to make therapeutic predictions. HFS-TB generated data from studies obtained in Search A were then compared to clinical data from studies obtained in Searches B and C.

Search B identified clinical studies that were used to examine therapeutic relevance of the HFS-TB output through descriptive correlations. For each correlation, it was required that these clinical studies were published prior to HFS-TB. Therefore they were not used for predictive accuracy assessment.

Search C identified clinical studies that were used to evaluate predictive or forecasting accuracy of the HFS-TB study output. For each predictive evaluation, it was required that the clinical study was published at least six months after the HFS-TB publication.

Standard evidence-based medicine criteria were used to evaluate the quality of clinical studies and are described in the Methods section (Section 5). Data and information were extracted from the relevant publications to enable several types of analyses. These analyses are described within the Results section of this document (Section 6).

RESULTS:

Search A identified 26 studies that reported the output of HFS-TB or used the HFS-TB output in Monte Carlo simulations.

Search B identified 17 clinical studies.

Search C identified 20 clinical studies.

Analyses Conducted Based upon Search Results

Analysis 1: *Mtb* kill rates in patient sputum, patterns of microbial kill, cessation of effect and time to emergence of drug resistance from the 17 clinical studies identified in Search B were compared to the same parameters in the 26 HFS-TB studies identified in Search A. Descriptive correlations demonstrated excellent concordance for these parameters for standard doses of isoniazid, rifampin, pyrazinamide, ethambutol, ciprofloxacin and moxifloxacin.

Analysis 2: Predictive accuracy was examined using 20 clinical studies identified in Search C, which were published at least 6 months after HFS-TB studies identified in Search A.

In Analysis 2a, the forecasting accuracy of the pharmacokinetic/pharmacodynamic (PK/PD) indices or dosing schedules associated with optimal microbial kill or resistance suppression was identified. The PK/PD indices associated with microbial kill and resistance suppression by rifampin, isoniazid, ethambutol and pyrazinamide were accurately predicted in the HFS-TB when compared with the clinical studies.

In Analysis 2b, the data from HFS-TB studies suggested new hypotheses relevant to therapeutic strategies and contradicted some accepted therapeutic strategies. These HFS-TB study data were then compared to clinical studies published at a later date. Six such hypotheses were subsequently confirmed by the results of the clinical studies.

In Analysis 2c, the quantitative predictive accuracy of several HFS-TB study generated parameters, including optimal drug doses and PK/PD exposure values, was calculated. HFS-TB study results were compared to results generated in clinical studies performed after the HFS-TB results were published. The forecasting accuracy rate was 94.4% (95% confidence interval [CI]: 84.3-99.9). The bias was 1.8% (CI:-13.7 to 6.2) and thus crossed zero. Therefore, it is proposed that the HFS-TB model is a drug development tool that is highly accurate for forecasting optimal drug exposures, drug doses, dosing schedules and appropriate drug combinations for anti-TB drugs/drug regimens.

CONCLUSION: These data demonstrate a validated forecasting accuracy for HFS-TB and support its utility as a valuable complementary, additional tool to existing methods for anti-TB drug dose selection and regimen design. HFS-TB outputs can facilitate drug development strategies and be useful for more informed design of Phase I, Phase II and Phase III clinical studies.

2. INTRODUCTION

TB has been a public health problem for millennia and it is currently estimated to be the cause of 3% of all deaths in low and middle-income countries primarily in Asia and Africa.[1] Additionally, there are an estimated 8.8 million new cases of TB each year worldwide.[2] While antibiotic therapy is available, the treatment is for six months in the simplest of cases and up to five years in multi-drug resistant (MDR-TB) and extensive drug resistant TB (XDR-TB) cases.[3-6] Resistance to antimicrobial agents is a widespread and an increasing problem. As an example, it is estimated that 50-70% of TB patients in India have MDR-TB.[4] Thus, an urgent need exists for new treatment regimens that will optimize antibacterial activity of currently available agents, cure patients in weeks rather than months, and reduce the number of patients with drug resistant disease. The CPTR initiative seeks to accelerate the development of a safer, faster-acting, shorter duration regimen for TB. One goal is to achieve more efficient design of clinical trials by the application of validated approaches that provide a more accurate understanding of PK/PD relationships for new TB drugs regimens.

3. QUESTION FOR THE AGENCY

Question: Does the Agency agree that the results presented here support our proposed COU for the Hollow Fiber System Model (HFS-TB) as an additional and complementary tool for use in anti-TB drug development for optimal selection of drug, dose and dosing schedules and for informing the development of potential combination therapy regimens?

CPTR Position: CPTR PCS-WG believes that the results from our data analysis conclusively establish the high predictive accuracy of HFS-TB output for clinical trial outcomes (Section 6.5). When used in an anti-TB drug development program, outputs from HFS-TB, as described in Section 4, can be run through Monte Carlo simulations for a given drug or drug combination to yield a quantitative understand of PK/PD relationships, expected dose-response curves for patients and expected rates of, and time to, resistance emergence for concentration-related resistance. These parameters can be used in conjunction with the overall preclinical study results to optimize choice of drug(s), dose(s), and dosing schedules.

4. BACKGROUND

4.1 Background of the CPTR Initiative

The CPTR initiative is a broad collaboration of pharmaceutical companies, government regulatory and multilateral agencies, academia, civil society advocates and non-government organizations that aim to accelerate the development of new, safe and highly effective TB treatment regimens with shorter therapy durations than the current standard of care. CPTR was formed through the collaboration and support of the Bill & Melinda Gates Foundation, the Global Alliance for TB Drug Development and the Critical Path Institute. The CPTR PCS-WG strives to identify, develop consensus around and build the evidence base to support potential new Drug Development Tools (DDTs) for TB medical product development.

4.2 The Hollow Fiber System of TB (HFS-TB)

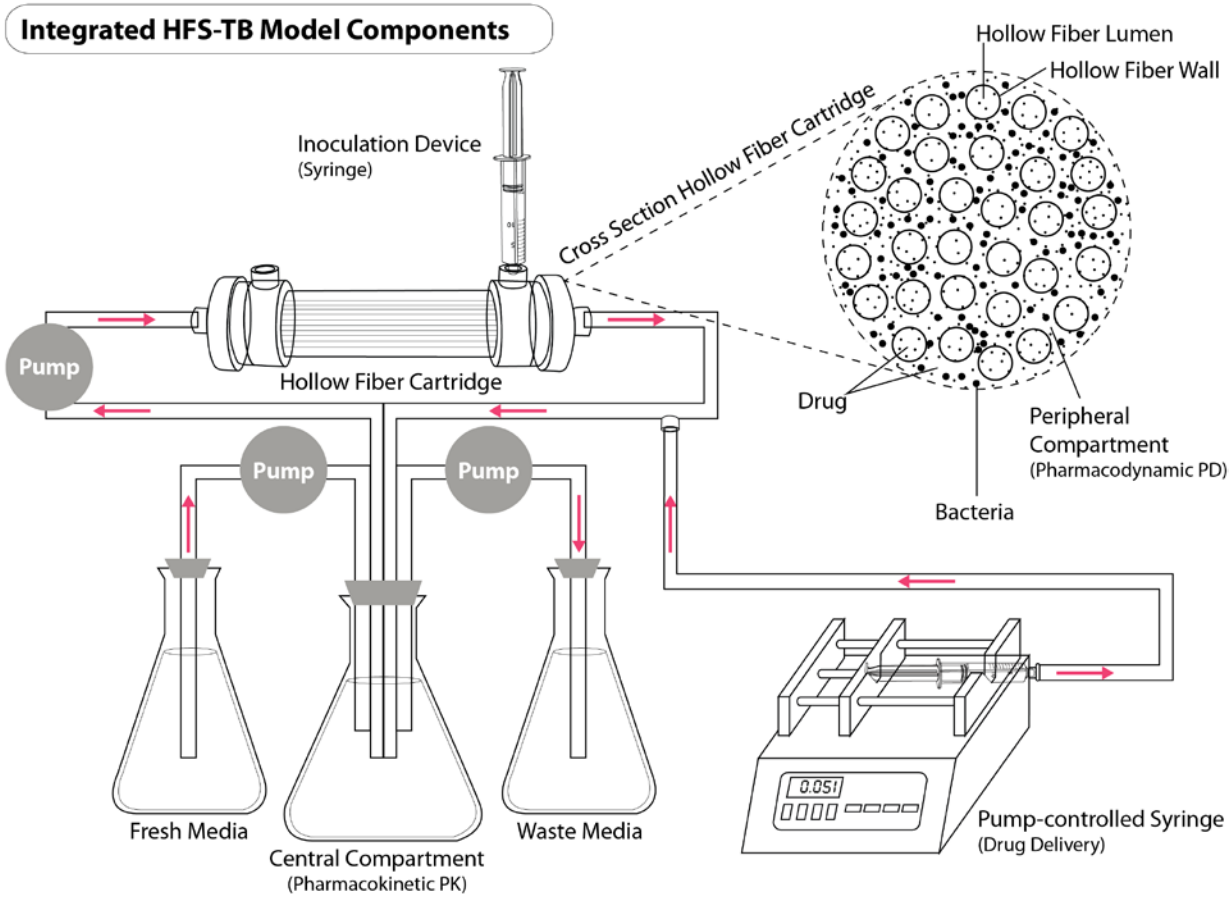
Since the time of both Robert Koch, who discovered *Mycobacterium tuberculosis* (*Mtb*), and his protégé Paul Ehrlich, who laid the foundation for modern chemotherapy and antibiotic drug development, animal models have been used extensively for TB drug development.[7,8] However,

these animal models have sometimes failed to predict clinical efficacy. In addition, the advent of antimicrobial PK/PD science required a tractable, patient-relevant model of pharmacokinetics. This model would allow repetitive sampling of bacteria during drug therapy, as is the standard in clinical trials, and would easily delineate differential effects of drug therapy on the various metabolic sub-populations of *Mtb*.

Approximately 10 years ago, the HFS-TB was developed by Gumbo et al. and first presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) [9-10]. Since that time, the model has been refined to become more sophisticated in its utility and application, which has expanded to include the ability to influence effective dose selection in clinical settings. The applicability of this tool and the utility of data produced by it are described below. The HFS-TB was developed not just to fill the gaps in knowledge associated with existing preclinical efficacy models, but also to offer more quantitative measurements and outputs than those provided by animal models. This is important, as quantitative PK/PD assessment is crucial to the development and execution of an integrated drug development process.

The HFS-TB was specifically designed to: (1) mimic the pharmacokinetic concentration-time profiles of antibiotics observed in TB patients in both plasma and at the site of infection, (2) mimic the metabolic and physiologic behavior of *Mtb* strains encountered in infected patients given that *Mtb* can exist in one of three metabolic states which impact the efficacy of drugs against the bacterium, (3) quantify the sensitivity and resistance of these *Mtb* strains to various doses and combinations of antibiotic agents over time and (4) perform PK/PD studies that will inform dose selection in clinical trials. The HFS-TB enables a quantitative understanding of the relationship between dynamic drug concentrations, as well as dynamic populations of drug-susceptible and drug-resistant *Mtb*, over time. HFS-TB assembly specifications can be found in the Amendment. The HFS-TB (Figure 1) consists of a “pharmacodynamic” compartment (also referred to as the peripheral compartment) and a pharmacokinetic (PK) compartment, which consists of a central compartment that allows drug to equilibrate with contents of the peripheral compartment via diffusion process across the hollow fibers. The peripheral (“pharmacodynamic”) compartment also houses either extracellular or intracellular *Mtb*, which can be maintained for several months.

Figure 1. Diagram of Hollow Fiber System for TB (HFS-TB)



Hollow fibers are semi-permeable capillary tubes, whose pore sizes can be varied depending on the type of study being performed. A fiber pore diameter of 42kDa has been commonly utilized in the TB model because it allows easy and rapid equilibration of small molecules across the hollow fibers, while preventing bacilli from distributing between compartments, or entering the hollow fiber lumina. The lumina of the hollow fibers is part of a continuous media flow path that includes tubing and the central reservoir. Drugs are administered via a computer-controlled syringe pump, with drug entering the central compartment via tubing in the flow path. Drug infusion rates are designed to mimic a desired peak concentration and time to peak concentration, as encountered in patients. Fresh media is introduced to the central compartment via tubing in the flow path and used media removed via the central reservoir to create a dilution system that allows the concentration of the drug to decline over time with the same half-life as observed in humans. The system allows the investigator to mimic varied concentration-time profiles and thus drug combination regimens. The actual drug or drug combination levels achieved are directly quantified by direct sampling of the central compartment via a stopcock mechanism in the flow path tubing at pre-specified times for measurement of achieved drug concentrations. The timing of sampling within a given study is determined by the PK profile of the drug(s) of interest. The actual drug concentrations observed are then utilized in mathematical *in silico* models, dose-finding studies and for extrapolation to the equivalent clinical doses in patients.

Bacilli (which are too large to cross the pores) are inoculated into the peripheral compartment via a syringe device (Figure 1). The inoculated bacteria could be at low pH when semi-dormant

bacilli are needed for evaluation, under anaerobic conditions to generate the non-replicating persister state, at ambient air and normal pH for log-phase growth bacteria, or within macrophages or neutrophils to represent the intracellular state. The growth media circulating in the system is selected to maintain a particular pH, or support human-derived cell lines such as macrophages. The system is incubated at 37ºC with oxygen and CO_2 content pre-specified according to the metabolic status of *Mtb* under study. The peripheral compartment is then sampled at pre-specified times, typically day 0, day 3, day 7 and then every seven days thereafter, similar to the sampling schedule typically used for determination of total bacterial burden in sputum in liquid cultures.

There are multiple outputs from the HFS-TB, including: (1) total bacterial colony forming unit (CFU) count; (2) drug-resistant *Mtb* CFU count; (3) drug concentration which can be modeled using compartmental PK analysis methods; (4) macrophage count (in some studies) and number of bacteria per macrophage; (5) RNA expression and (6) whole genome sequencing which can be performed on sampled material. All of these PD measures can be directly correlated with PK measurements taken at the same time point within a study. This is a significant advantage to *in vivo* model study methods. The last three types of PD measures allow a systems pharmacology-based approach that can be utilized in drug development.

4.3 Proposed Use of HFS-TB in Drug Development

The HFS-TB can: (1) mimic the concentration-time profiles of antibiotics observed in TB patients, (2) mimic the metabolic and physiologic behavior of *Mtb* populations commonly encountered in infected patients with pulmonary TB and the intracellular *Mtb* characteristic of disseminated TB and (3) quantify the sensitivity and resistance of these *Mtb* populations to various doses and combinations of antibiotic agents over time. When these outcomes are correctly achieved, the results can then be used in Monte Carlo simulations to identify (i) optimal doses of drugs, (ii) drug combinations which are most likely to achieve desired microbial outcomes, (iii) expected response rates from a drug or combination regimen, (iv) expected rates of and time to resistance emergence in patients and (v) susceptibility breakpoints based on a minimum inhibitory concentration (MIC) above which therapy by a specific drug will fail.

The HFS-TB is proposed for use in optimization of drug regimens and dose selection to maximize the bactericidal and sterilizing effect rates and minimize the emergence of resistance. When used early in the drug development cycle as a complementary and additional tool to existing methodologies, information regarding optimal dose selection, dosing schedules and potential combination therapies can be obtained. Additionally, the HFS-TB can be used in a post-approval setting to optimize currently used drug regimens (for both dose and dosing schedule) for drug-susceptible and drug-resistant TB. Therefore, the results obtained by the HFS-TB are expected to support trial design for Phase I, II, III and IV clinical trials.

Phase I dose ranging study design can be optimized by data from HFS-TB experiments especially when the PKs in humans can be predicted by data from preclinical studies. The HFS-TB is used to identify optimal PK/PD exposures associated with maximal and fastest bactericidal and sterilizing effect rates and resistance suppression. Monte Carlo simulations would then be used to identify optimal clinical dose. In appropriate circumstances the need for dose ranging study designs, which potentially expose some patients to suboptimal doses, might be avoided. In later-phase development, HFS-TB provides information to help identify the optimal doses and combinations for Phase II and III studies, the proportion of patients expected to have maximal response and the expected rates of acquired drug resistance.

4.4 Advantages of the HFS-TB

Animal models quickly became the preferred model in TB drug development following elegant work by Paul Ehrlich using various classes of chemotherapeutic agents at the turn of the last century. In the last 70 years, mice and guinea pigs have been used for anti-TB drug development with considerable success, but also with some notable failures. In the 1950s, the studies of Steenken and Wolinsky in guinea pigs demonstrated no effect of pyrazinamide, which led to its reduced use.[11] Fortunately, the studies of Yeager et al. in patients and Grumbach in mice, demonstrated pyrazinamide efficacy and it is now known to be essential for short course chemotherapy as demonstrated in clinical studies in East Africa by the British Medical Research Council. [12-14]

The utility of these *in vivo* models for new anti-TB regimens has been questioned, most recently by Mitchison and colleagues (renowned subject matter experts for *in vivo* model systems of *Mtb*). [15-18] The predictive accuracy of these *in vivo* models for reducing duration of therapy in moxifloxacin containing regimens (as a substitution for isoniazid) has been contested, as the preclinical results did not track with clinical findings for the regimen.[17,19,20] A more recent example of inconsistency between animal model data and clinical predictability is a mouse study that demonstrated that daily dosing of rifapentine would lead to cure of TB in three months or less in the standard regimen,[21] which led to the Tuberculosis Trials Consortium Study 29 (TBTC 29) of 531 patients. In TBTC 29, daily rifapentine in patients was no better than daily rifampin.[22] Moreover, the animal models have limited use in assessment of acquired drug resistance in combination regimens due to comparatively low bacterial burdens than achieved in humans. Furthermore, the lack of repetitive sampling of *Mtb* in mice or guinea pigs, which is the standard approach in clinical trials, limits extrapolation from these models for time-to-event outcomes and identification of the exact timing of drug resistance emergence. Finally, a formal study to examine how accurate these models are at quantitative forecasting has not been performed, thus the *in vivo* models cannot be used as baseline models.

The HFS-TB offers distinct advantages to current *in vivo* model systems for evaluating efficacy, resistance potential and dose determination as described below.

- **Simulation of human PK and PD**

The primary advantage of the HFS-TB is its capacity to simulate human PK/PD of a drug or drug combination. This is strengthened by the capability for iterative and repetitive sampling for quantitative measurement of both organism and drug concentration simultaneously. This is a distinct feature of this model as repetitive sampling of both drug and organism are not feasible in *in vivo* models of infection based on limitations of access to infection sites for both organism and drug. Therefore, providing a quantitative understanding of PK/PD relationships is the primary benefit of HFS-TB in the drug development process. This allows measurement of efficacy and resistance suppression providing a rational and efficient approach to explore novel combination therapies, which can then be directly translated, into more effective clinical trial designs.

- **Microbial response to drug(s)**

The HFS-TB has been used to determine the bactericidal and sterilizing effect (i.e., microbial kill) rates, likelihood of resistance emergence and effects of drug combinations, which are comparable to those effects in the sputum cultures of patients. The HFS-TB model has the advantage that the microbial sub-populations important in sterilizing effect (i.e., non-replicating persisters and semi-dormant bacilli) can be separately studied from log-phase growth sub-populations. This allows for more accurate identification of microbial kill rates, resistance

emergence within each sub-population and differential effects of antibiotics, all of importance in design of regimens that would shorten therapy duration. [23,24]

- **Complete eradication**

The ability to culture the entire contents (usually 20 mL of culture) of the peripheral compartment of the HFS-TB at the end of an experiment interval allows assessment of the potential for a compound's ability to completely eradicate *Mtb* at early time points such as one or two months. The data from this model can inform the likely time point in a clinical trial setting that can be considered for proof of efficacy rather than relying on relapse rates (as is current practice) in *in vivo* models of infection.

- **Assessment of resistance potential**

As cited above, the HFS-TB has the advantage of accurately mimicking human PK. This is an improvement to static *in vitro* models, which are typically employed to assess resistance potential for drugs or drug combinations. Drug instability issues are obviated in the dynamic HFS-TB model as well. As an example, rifampicin, a key component of the current standard of care for TB and the new anti-TB drug ertapenem, are unstable in static medium incubator conditions used to assess MIC or minimum bactericidal concentration profiles. These two aspects are important because the shape of the concentration-time curve of some anti-tuberculosis drugs (i.e., the stressor) is an important determinant of microbial effects such as acquired drug resistance. This reflects the evolutionary principle in which oscillations in the intensity of environmental stressors result in higher mutation rates than with constant stressor pressure.[25-28] Comprehensive understanding of concentration-effect profiles is needed to fully understand resistance potential. For the same reasons, the HFS-TB has advantages over *in vivo* models, (i.e. rodent models) in which the half-life of many drugs differ greatly from that in humans, thereby exposing *Mtb* to different shapes of the concentration-time curves, or intensity of the chemical stressor.

- **Repetitive sampling**

Repetitive sampling from the same system offers another advantage over *in vivo* models, which rely on terminal procedures to obtain samples to be cultured. Repetitive sampling vastly improves statistical power, time-to-event analysis and repeated event analysis.

- **Assessment of drug combinations**

Anti-TB drugs exhibit peak and area under the concentration-time curve (AUC) concentration-dependent synergy and antagonism in patients.[29] Two and three drug combinations, at different doses for each drug, can be performed in the HFS-TB allowing identification of dose and concentration-dependent synergy or antagonism.[30]

5. METHODS

Extensive evidence supporting the utility of HFS-TB to enable selection of dose and drug regimens in TB clinical trials has been published. A thorough literature search was designed to identify relevant published studies of HFS-TB experiments (Search A) and TB clinical trials (Search B and Search C). Information and data were extracted from these studies and used to establish **descriptive correlations** between HFS-TB outputs and clinical study observations (Analysis 1) and **predictive accuracy** of the HFS-TB to forecast clinically observed outcomes of drug treatment (Analysis 2).

5.1 Literature Search Strategies

In order to avoid potential publication bias, a search was reviewed by utilizing not only PubMed, EMBASE, ISI Web of Science and the Cochrane Libraries, but also the Grey literature (unpublished or un-indexed reports which can include conference proceedings, non-indexed journals, internal reports, pharmaceutical industry reports and student dissertations and theses). Conference presentations were also reviewed and accessed. In addition, CPTR members were in communication with colleagues in the pharmaceutical industry whose companies were involved in anti-TB drug development and researchers who do hollow fiber studies, to determine if they had performed HFS-TB studies. All experiments reported are included in Table 2 and all were used for further analyses.

5.1.1 Search A: HFS-TB experiments

The purpose of this search was to identify all published experiments using the HFS-TB. The data extracted from these publications were used to evaluate the correlation with clinical outcomes (Analysis 1) as well as to evaluate forecasting capability (Analysis 2). The team searched PubMed, EMBASE, ISI Web of Science and the Cochrane Libraries for studies published beginning January 1, 2000, the year experiments of the HFS-TB were first performed and December 31, 2012. The following medical subject heading terms and strategy were used: "hollow fiber" OR "hollow fibre" AND either "tuberculosis" OR "mycobacterium" OR "mycobacteria." Bibliographies of original articles, key reviews of TB PK/PD studies and consensus statements were also examined for additional relevant studies. A manual search of meeting abstracts was then conducted for the ICAAC, the annual Conference of the Infectious Diseases Society of America, the Gordon Research Conferences (Tuberculosis Drug Development) and the 1st to 5th International Workshops on Clinical Pharmacology of Tuberculosis Drugs. In addition, the team searched other literature sources via Inside Conferences, clinicaltrials.gov and Open Grey (System for Information on Grey Literature in Europe) at http://www.opengrey.eu. Where conference abstracts were found, but a full study was published later in scientific journals, the full study was taken as the main reference. There was no exclusion of articles by language.

5.1.2 Search B: Clinical TB studies performed prior to HFS-TB studies

The purpose of this search was to identify all clinical TB studies containing relevant data for our analyses examining correlation with output of the HFS-TB (Analysis 1). A PubMed search was conducted for clinical studies performed with anti-TB monotherapy or dual therapy, in which pharmacokinetics were documented or a dose-scheduling study design was employed, published from the beginning of chemotherapy from January 1, 1943[31-33] up to December 31, 2012. Search terms with either "isoniazid" or "rifampin" or "rifampicin" or "pyrazinamide" or "ethambutol" or "moxifloxacin" or "ciprofloxacin" or 'linezolid" AND "tuberculosis" were used and the results filtered to include only "clinical trials". The drugs for the search string were chosen based on results from the literature search for hollow fiber studies described above with the intent to correlate data from HFS-TB and clinical trials. In addition, PK/PD review articles identified using subject headings "pharmacokinetics-pharmacodynamics" or "PK/PD" AND "tuberculosis" were identified and read and their references manually examined.

5.1.3. Search C: Clinical TB studies performed at least 6 months after HFS-TB studies

The purpose of this search was to identify all clinical TB studies containing relevant data for our analyses examining predictive accuracy of the HFS-TB output (Analysis 2). Selection criteria

and search terms were as in Search B. However, the period used for this search was from January 1, 2003, the year the HFS-TB was first introduced at ICAAC, until December 31, 2012. To assess true predictivity of HFS-TB outputs, clinical studies were only chosen if they were published at least 6 months after publication of the HFS-TB that they were compared to. These studies were used in Analyses 2.

5.1.4 Assessment of quality of clinical studies

All publications of clinical studies retrieved through Search B and Search C were judged for quality of evidence in accordance with the scale described in Table 1, which is based on both the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria and those used by the Infectious Diseases of America-US Public Health Service Grading System (USPHS) for evidence based medicine decision making.[34,35] This method was adapted to determine the quality of evidence from the clinical study that supported or refuted the findings of the HFS-TB. Numeric estimates of parameter clinical values from key opinion leaders based on clinical experience, or reports of expert committees were NOT considered to be evidence.

Table 1. Quality of Evidence of Clinical Studies Used to Compare to Predictions

Quality Of Evidence Score	Criteria
1	Evidence From ≥1 Properly Randomized, Controlled Trial; Meta-Analysis Of Randomized, Controlled Trials That Followed PRISMA Recommendations
2	Evidence From ≥1 Well-Designed Prospective Clinical Trial, Without Randomization; From Prospective Cohort Or Case-Controlled Analytic Studies; Dramatic Experimental Study Results Of Uncontrolled Clinical Studies
3	Evidence From Multiple Time-Series; Evidence From Dramatic Epidemiological Data
4	Evidence From A Large Retrospective Case Series In Single Centre; Examination Of Clinical Isolates From Case Series

5.1.5 Analysis methodology of studies identified in Searches A to C.

5.1.5.1. Analysis 1: Descriptive correlation

The first analysis was descriptive and established the correlation between HFS-TB output (Search A) and observations from clinical studies published prior to the publication of HFS-TB studies (Search B) for equivalent treatment regimens. Specifically, kill rates in sputum of patients; patterns of microbial kill, cessation of effect and time to emergence of drug resistance were compared. This analysis is not considered predictive, but merely descriptive.

5.1.5.2. Analysis 2: Predictive accuracy

In order to minimize bias in this analysis, we examined all publications that documented qualitative or quantitative outputs of the HFS-TB in tandem with Monte Carlo simulations at

publication time = t_1 (Search A) and then searched the clinical literature published at least six months after at time t_2 years, i.e., $t_2 > (0.5+ t_1)$ years (Search C). Findings in clinical studies were considered the actual observations. By definition, a prediction is a forecast, in that the prediction is stated or declared at an earlier time point, and the observation confirming or refuting the prediction is made at a later time. Thus, true prediction occurs when a model's output is published, followed either by the explicit generation and publication of confirmatory clinical data or by the publication of independent clinical data that could be used for confirmatory purposes. This was the rationale for choosing clinical studies published at least 6 months (search C or observation of the truth) after publication of the HFS-TB in search A (i.e., the forecast). Steps for minimizing bias and for reporting systematic reviews outlined by the Cochrane collaboration, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [36] and GRADE Working Group (*http://www.gradeworkinggroup.org/publications/ JCE_series.htm*) [37] were applied.

Analysis 2a.

Search A identified some studies in which the HFS-TB made a prediction on PK/PD indices associated with optimal treatment effect. Search C identified some clinical studies performed after the publication of these HFS-TB studies that also examined PK/PD indices associated with optimal effect. We performed a qualitative analysis of forecasting accuracy based on comparison of HFS-TB-generated PK/PD indices associated with optimal treatment effect (AUC/MIC, peak/MIC, or % time above MIC [$\%T_{MIC}$]), with the findings from the high quality clinical studies.

Analysis 2b.

Search A identified some HFS-TB studies that generated new hypotheses relevant to therapeutic strategies and also challenged some long accepted strategies. Search C identified several studies that directly examined those hypotheses. We performed a qualitative assessment to determine the accuracy of the output from these HFS-TB studies by comparing them with the results of clinical studies published after the HFS-TB publications. We used the synthesis methods outlined by the Cochrane Collaboration for qualitative systematic analyses.

Analysis 2c.

Search A identified HFS-TB studies that made predictions of optimal PK/PD exposure values, optimal drug doses and MIC breakpoints associated with optimal clinical outcomes. Search C identified clinical studies that also examined these factors and were published after these HFS-TB studies. For the HFS-TB experiments that predicted drug concentrations associated with either optimal efficacy or emergence of drug resistance, susceptibility breakpoints, or proportions of patients achieving a particular response, pairs of the HFS-TB ± Monte Carlo simulations versus clinical data were examined. Both accuracy and bias were calculated and then summated. The ideal Drug Development Tool (DDT) would have an error of zero. As an example, if the DDT predicts the optimal exposure necessary for optimal kill of *Mtb* to be 15 mg/L at time t_1 and is followed by a clinical study at t_2 showing that exactly 15 mg/L of the drug were associated with optimal efficacy, then error is zero and accuracy 100%. Error (E) is the true value identified in clinical study at t_2 (T) minus value predicted at t_1 (P). In other words:

$$E = T - P \qquad (1)$$

For a number of trials or experiments *i* of up to *n*, this takes the form of the mean absolute percentage error (MAPE), which is given by:

$$\text{MAPE}=\frac{1}{n}*\left[\sum_{i=1}^{n}\left|\frac{T_i-P_i}{T_i}\right|*100\right] \quad (2)$$

If the MAPE is the forecasting error (FE), the accuracy (A) is defined by:

$$\text{A}=100\%-\text{FE} \quad (3)$$

If the forecast error is zero (i.e., T=P), then accuracy is 100%. If forecast error is 100%, then accuracy is zero. MAPE has the advantage of (1) making the units of the forecast variable unimportant, through normalizing different scales by dividing by the size of the true or observed value (T) and (2) allowing the comparison to a benchmark or pre-defined threshold. If the true value T is zero however, the problem of dividing by zero arises and we switch from MAPE to mean squared error (MSE).

In calculating the summary estimate of accuracy, weighting was performed. First, it was assumed that there is one true accuracy measure for the HFS-TB, thus weighting reverts to a fixed effects model. Weighting in a given clinical study was based on the size of the population sample, divided by the quality of evidence score provided in Table 1.

A second test characteristic of interest is bias. Bias (B) is the tendency of the DDT to overstate or understate the true value. As an example, one model may be biased towards forecasting emergence of acquired drug resistance compared to what is actually observed in patients later (B<0) while another may rarely show emergence of acquired drug resistance when clinical studies will show resistance emergence often (B>0).

$$\text{B}=\sum_{i=1}^{n}(T_i-P_i)/n \quad (4)$$

Weighting was as discussed above for accuracy. For the HFS-TB experiments that predicted drug concentrations associated with either optimal efficacy or emergence of drug resistance, susceptibility breakpoints, or proportions of patients achieving a particular response, pairs of the HFS-TB ± Monte Carlo simulations versus clinical data were examined. Both accuracy and bias were calculated and then summated.

While mice and guinea pigs have historically been utilized as animal models for TB, several considerations led us to exclude either as the "standard" tools for comparison to HFS-TB. First, dose-ranging and dose-scheduling PK/PD studies for sterilizing effect, for acquired drug resistance and for concentration-dependent synergy and antagonism in mice and guinea pigs are scant. Indeed, there have been vastly more PK/PD studies performed in the HFS-TB than in these animal models. Second, very few of the animal studies have employed proper mathematical simulation steps for dose selection in regimen design or for susceptibility breakpoint determination. Third, the accuracy of these animal models in quantitative forecasting has not been studied. To our knowledge, the same level of statistical rigor has not been applied to define the predictive accuracy of *in vivo* models to clinical outcome. Given the lack of a standard DDT for TB whose accuracy has been quantified, we examined what would happen if one had a completely unreliable tool. We assumed that randomly generated pairs of quantitative prediction at t_1 versus clinically generated values at t_2 would be the base model currently available for forecasting. A random number generator was utilized to generate actual or true value and predict value pairs for 1,000 experiments.

6. RESULTS

6.1 Literature Search A: HFS-TB experiments

Search A identified 26 studies involving the HFS-TB, which are shown in Table 2.[10,23-25,38-59].

Table 2. Hollow Fiber System Tool of TB (HFS-TB) Publications

Study Reference	Year	Regimen (Plus =In combination)	Findings/Conclusions
MONOTHERAPY			
Gumbo et al.[10]	2004	Moxifloxacin	• Biphasic kill • Acquired drug resistance (ADR) • First study to use mathematical models in PK/PD of anti-TB drugs • Identification of optimal moxifloxacin dose
Ginsburg et al.[50]	2004	Moxifloxacin	• Moxifloxacin post-antibiotic effect
Gumbo et al.[38]	2005	Ciprofloxacin	• Standard doses lead to emergence of ADR • Biphasic kill • Ciprofloxacin likely to be ineffective and should be replaced by moxifloxacin
Gumbo et al.[40]	2007	Isoniazid	• Use of slow and fast acetylator pharmacokinetics • First study to show role of efflux pumps in drug tolerance as part of ADR and why isoniazid effect ceases after 3 days
Gumbo et al.[41]	2007	Isoniazid	• Slow/rapid isoniazid pharmacokinetics mimicked • PK/PD indices identified; described by series of "U" shaped curves • 300mg/day inadequate for optimal kill in some ethnic populations
Gumbo et al.[39]	2007	Rifampin	• Rifamycin half-life has little relevance to efficacy • Rifampin efficacy measures such as resistance suppression and post-antibiotic effect are driven by peak/MIC • Microbial kill linked to AUC/MIC • Standard human doses are inadequate for resistance suppression and optimal microbial kill
Gumbo et al.[23]	2009	Pyrazinamide	• New *in vitro* model for examination of sterilizing effect • Derivation of quadratic function describing concentration vs. ADR • For optimal kill, doses >60mg/kg rather than current 15-30mg/kg identified in Monte Carlo Simulations
Musuka et al.[48]	2008	Thioridazine	• Efficacy is driven by peak/MIC and AUC/MIC • Observation of a wobbly PK/PD parameter • Efficacious doses are in range toxic to humans
Drusano et al.[51]	2012	Moxifloxacin	• Moxifloxacin kills *Mtb* in non-replicating persister (NRP) state at rates higher than expected
Drusano et al.[52]	2012	Rifampin	• *in vitro* HFS-TB model of NRP, recapitulated RIF relapse and failure rates observed in actual patients: 12.7% versus 14%
COMBINATION THERAPY			
Drusano et al.[24]	2010	Rifampin Plus Moxifloxacin	• Rifampin plus moxifloxacin synergistic in resistance suppression but antagonistic in microbial kill

Srivastava et al.[25]	2010	Ethambutol/ Isoniazid -Alone and in combination	• Ethambutol monotherapy and in combination with INH examined • Multiphase ethambutol/isoniazid pharmacokinetics mimicked • Demonstrated the sequence and role of efflux pumps on drug tolerance and in the emergence of multiple drug resistance • Ethambutol kill linked to AUC/MIC
Srivastava et al.[42]	2011	Rifampin Plus Isoniazid Plus Pyrazinamide	• Established that pharmacokinetic variability hypothesis of therapy failure and ADR • Predicted the proportions of patients who would have a two month sputum conversion rate and ADR despite 100% adherence • First *in vitro* study to interrogate public health policies used by TB programs
Srivastava et al.[44]	2011	Rifampin Plus Isoniazid	• Disproved the pharmacokinetic mismatch hypothesis • Deliberate mismatch of rifampin and isoniazid leads to improved microbial kill
Drusano G et al.[47]	2011	Rifampin Plus Moxifloxacin	• In combination therapy; 5/7 day regimen allowed emergence of moxifloxacin resistance while 7/7 day regimen did not
Srivastava et al.[49]	2011	1-,2-,3-drug combinations of rifampin, isoniazid and pyrazinamide compared to effect in 56 patients	• Microbial kill rates of optimal drug combinations in the *in vitro* HFS-TB model are the same • Pattern of ranking of regimens is the same as in patients in terms of microbial kill • Confirmation of the clinically accepted notion that rifampin and isoniazid in combination prevent resistance development to each other
Okusanya et al.[54]	2012	Rifampin/ Linezolid-Alone and in combination	• Rifampin and linezolid were additive, demonstrating potential use for sterilizing effect
Xue et al.[53]	2012	Rifampin/Linezolid/ PNU100480/PNU-101603 - Alone and in combination	• Kill effects in log-phase growth bacilli for PNU-100480 and its metabolite, PNU-101603 were additive; however, resistance emerged quickly for all single agents
Okusanya et al.[55]	2012	Rifampin/PNU-100480/ PNU-101603 –Alone, & combined	• PNU-101603 and not the parent compound (PNU-100480) enhanced the sterilizing effect of rifampin
Louie et al.[56]	2012	PNU-100480 Plus Rifampin	• PNU-100480 plus its metabolite (PNU-101603) together with RIF 600mg/day synergistically killed *Mtb* • The combination of PNU metabolite and rifampin prevented ADR to PNU or rifampin alone
Louie et al.[57]	2012	PNU-100480 Plus PNU-101603	• PNU-100480 at 600 mg q12h was as effective as a continuous infusion of 1200 mg/d. 600 mg q12h plus RIF 600 mg qd sterilized the HFS-TB and prevented ADR
Louie et al.[58]	2012	PNU100480/PNU-101603/ Rifampin, alone & combined	• PNU-100480 was synergistic with the major metabolite (PNU-101603) in killing *Mtb* but did not prevent ADR
MONTE CARLO SIMULATONS OF HFS-TB OUTPUT			

Gumbo T.[43]	2010	Rifampin, isoniazid, pyrazinamide, ethambutol, moxifloxacin	• Set new susceptibility breakpoints for rifampin, isoniazid and pyrazinamide and confirmed those for moxifloxacin and ethambutol
Jeena et al.[45]	2011	Isoniazid	• Selection of optimal isoniazid doses in TB meningitis, disseminated TB and pulmonary TB in children less than 10 years old
Goutelle et al.[46]	2009	Rifampin	• Selection of rifampin optimal dose for microbial kill and resistance suppression in pulmonary TB
Goutelle et al.[59]	2011	Rifampin	• Full mathematical model on the effect of rifampin from first day to last day of therapy suggested need to increase rifampin dose above 600 mg/day

6.2. Literature Search B: Clinical studies published before HFS-TB studies

A literature search for clinical studies published before the HFS-TB experiments identified in Search A, identified 17 clinical studies shown in Table 3. Data from these studies were used in Analysis 1.

Table 3. Clinical Studies Published Before HFS-TB Results Used In Descriptive Correlation.

Study reference	Year	Quality of Clinical Evidence
Sirgel et al.[60]	2000	1
Pasipanodya et al.[61]	2011	1
Donald et al.[62]	1997	1
Jindani et al.[63]	1980	1
Jindani et al.[64]	2003	1
Gangadharam et al.[65]	1961	1
Gangadharam et al.[66]	1961	1
Devadatta et al.[67]	1961	1
Selkon et al.[68]	1964	1
Selkon et al.[69]	1964	1
Anonymous.[70]	1976	1
Anonymous.[71]	1981	1
Yeager et al.[12]	1952	2
British Medical Research Council[72]	1969	1
Ginsburg et al.[73]	2003	4
Hesseling et al.[74]	2010	1
Visser et al.[75]	2011	1

6.3. Literature Search C: Clinical studies published after HFS-TB studies

A literature search for all relevant clinical studies published at least 6 months after the HFS-TB studies from Search A identified studies shown in Table 4. Data from these studies were used in Analysis 2.

Table 4. Clinical Studies Published After HFS-TB studies Used in Predictive and Forecasting Accuracy.

Study reference	Year	Quality of Clinical Evidence
Devasia et al.[76]	2009	2
Chang et al.[77]	2010	1
Gandhi et al.[78,79]	2006	3
Pillay et al.[79]	2007	3
Chatterjee et al.[80]	2013	2
Machado et al.[81]	2012	4
Louw et al.[82]	2011	4
Pasipanodya et al.[83]	2013	1
Pasipanodya et al.[84]	2012	1
Pasipanodya et al.[85]	2013	2
Chigutsa et al.[29]	2013	1
Peloquin et al.[86]	2008	2
Pasipanodya et al.[87]	2013	2
Gumbo et al.[88]	2013	1
Pranger et al.[100]	2011	4
Pranger et al.[101]	2011	4
Thwaites et al.[89]	2011	1
Williamson et al.[90]	2012	4

6.4 Analysis 1: Descriptive correlation between HFS-TB and clinical studies

Search A identified 26 HFS-TB experiments ± Monte Carlo simulations, while Search B identified clinical studies shown in Table 3. For any correlation made, the clinical data was required to have been published prior to the HFS-TB data to which it was compared. In addition, a comprehensive review performed in 2011 re-examined clinical studies published prior to the execution of HFS-TB experiments evaluating the same regimens.[61] These clinical studies incorporated drug concentrations or dose scheduling as part of their study design and several also utilized a new compartmental analysis method to define PK/PD parameters associated with the optimal effect of either monotherapy or dual therapy. Table 5 summarizes PK/PD correlations, comparisons of kill rates reported in HFS-TB studies from Search A with those from sputum in clinical trials from Search B and timing of resistance emergence in terms of kill rates in sputum patterns of microbial kill, cessation of effect and time to emergence of drug resistance. Table 5, which includes all studies identified, demonstrates an excellent correlation between the outcome of HFS-TB studies and clinical studies. These findings support the hypothesis that the HFS-TB ± Monte Carlo simulations is a DDT that accurately predicts bacterial kill rates obtained in clinical efficacy trials. For perspective, literature reports of mouse and guinea pig TB drug studies demonstrate limitations and lack of correlation to patient outcomes. Mouse PK/PD studies of isoniazid and rifampin were short duration (six days), bactericidal effect studies and did not reflect sterilizing activity. They found a predominantly AUC/MIC-driven microbial kill and provided no data on acquired drug resistance.[61,91-93] The mouse studies failed to predict the effect of peak/MIC ratio on long-term outcomes. In a murine study on moxifloxacin, a full dose-response curve could not be derived because higher doses that generate AUCs tolerable to humans were toxic to mice. [93]

Table 5. Correlation of HFS-TB Findings with Clinical Studies Published Prior to the HFS-TB Results

Drug	HFS-TB Estimate	Clinical Estimate (Sputum)
Isoniazid		
Standard dose early bactericidal effect study (log_{10} CFU/mL)		
Hong Kong [China] patients (95% CI)*	0.40 (0.23–0.69)[41]	0.37 (0.16–0.58)[60,61]
Cape Town [South Africa] patients (95% CI)*	0.60 (0.38–0.74)[41]	0.65 (0.43–0.87)[60,61]
Chennai [India] patients (95% CI) *	0.90 (0.40–0.93)[41]	0.94 (0.45–1.43)[60,61]
Maximal kill in inhibitory sigmoid E_{max} curve (log_{10} CFU/mL)	0.9±0.2[41]	0.6±0.2[61,62]
Hill-factor or slope in inhibitory sigmoid E_{max} curve	0.9±0.4[41]	1.0±0.41[61,62]
Time to cessation of effect	80hrs[40]	72hrs[63,64]
PK/PD parameter associated with optimal effect	AUC/MIC; Peak/MIC[41]	Peak/MIC, AUC/MIC[61,65-69]
Rifampin		
Standard dose early bactericidal effect (log_{10} CFU/mL)	0.28[39]	0.25 (-0.08 to 0.57)[60]
Microbial kill of standard dose day 3-6	0.28[39]	0.27 (0.06 to 0.47)[60]
PK/PD parameter associated with optimal effect	Peak/MIC;AUC/MIC[39]	Peak/MIC [61,70]
Ethambutol		
Maximal EBA (log_{10} CFU/mL) (95% CI) or SD	0.22 (0.14–0.29)[25]	0.26±0.12[63,64]
Sterilizing effect rate (log_{10} CFU/mL)	0.04 to 0.10[25]	0.1[63,64]
PK/PD parameter associated with optimal microbial kill	AUC/MIC vs. peak/MIC[25]	AUC/MIC[71]
Pyrazinamide[61]		
Kill rates on days 0-4 (log_{10} CFU/mL)	-0.1[23]	-0.1 ± 0.2[63,64]
Sterilizing effect kill rates: days 4-14 days (log_{10} CFU/mL)	0.09 to 0.01[23]	0.12 ± 0.05[63,64]
Time to emergence of resistance in monotherapy (weeks)	2-3[23]	2-3[12,61]
PK/PD parameter associated with optimal effect	AUC/MIC[23]	AUC/MIC[61,72]
Ciprofloxacin/moxifloxacin		
Time To Emergence Of Resistance (Days)	10-14[10,38]	10-13[73]
Pyrazinamide/isoniazid/rifampin combination		
% with 2-month sputum conversion in Western Cape, South Africa	58.3[42]	51-60[74,75]

*The differences in early bactericidal activity effect (EBA) was driven by modeling the effects of N-acetyl transferase 2 SNPs on isoniazid AUCs in based on frequencies in each population and then determining effect of standard dose based on the inhibitory sigmoid E_{max} of AUC/MIC versus EBA from the HFS-TB

6.5 Analysis 2: Predictive accuracy of the HFS-TB in forecasting

We identified all publications that documented qualitative or quantitative outputs of the HFS-TB in tandem with Monte Carlo simulations at publication time = t_1 in Search A (Table 2). For each comparison made to a HFS-TB study output, we identified relevant clinical studies published at least six months after at time t_2 years, i.e., $t_2 > (0.5+ t_1)$ years in Search C. Findings in clinical studies were considered the actual observations. These searches were used in three types of analyses for forecasting accuracy: (a) correct ranking of PK/PD indices which have relevance to dose scheduling, (b) accuracy in generating or refuting hypothesis with direct relevance to therapeutic strategies and (c) quantitative forecasting.

6.5.1. Analysis 2a: Forecasting accuracy in ranking of PK/PD indices or dosing schedules by HFS-TB

In order to select the best dose and dosing schedule, experiments are performed to rank the antimicrobial PK/PD parameters. Search A and Search C were used to determine how accurate the HFS-TB had been in predicting the PK/PD indices associated with optimal effect in patients. Results are shown in Table 6.

Table 6. Clinical Studies Supporting HFS-TB-Derived PK/PD Parameters or Dosing Schedule Based on Microbial Kill and Resistance Suppression

Drug	HFS-TB Microbial Kill	HFS-TB Resistance Suppression	Clinical Evidence of Efficacy in Combination Therapy
Isoniazid	AUC/MIC [41]	Peak/MIC; AUC/MIC [41]	Peak, AUC,MIC [29,85,87]
Rifampin	AUC/MIC [39]	Peak/MIC; AUC/MIC [39]	Peak, AUC, MIC [29,85,87]
Ethambutol	Peak/MIC or AUC/MIC [25,94]	$\%T_{MIC}$ [25]	Peak/MIC [29]
Pyrazinamide	AUC/MIC [23]	$\%T_{MIC}$ [23]	AUC/MIC [29,85,87]

If an antibiotic's optimal efficacy is linked to peak/MIC ratio, this means that for that drug the optimal way to dose it is to increase the peak as much as tolerated. In such a case, the elimination half-life is less relevant. This allows intermittent dosing. As an example, rifampicin's once-a-day dosing has been proven to be effective, despite its 3hr elimination half-life. On the other hand, if an antibiotic's effect is linked to the percentage of time that the concentration persists above MIC ($\%T_{MIC}$), the optimal dose scheduling strategy is based on frequent administration at relatively lower doses or linked to strategies aimed at increasing its elimination half-life. If a drug is AUC/MIC linked, specific dosing schedules become less critical, as long as the appropriate cumulative dose is given. In order to derive the optimal dose, an exposure-response relationship is derived, with exposure defined as the magnitude of either peak/MIC or $\%T_{MIC}$, or AUC/MIC (whichever is linked to that drug) in order to select the appropriate exposure associated with 80-90% of optimal effect. The appropriate exposure associated with 80-90% optimal effect is used to identify the optimal dose and the appropriate dosing schedule.

It is noteworthy that in the case of rifampin, the murine model of TB found a predominant role for AUC/MIC and not peak or peak/MIC.[91,92] In contrast, the HFS-TB tool found that peak/MIC was more important for post-antibiotic effect (i.e., microbial kill or inhibition that continues long after an antibiotic has been removed) and acquired drug resistance while AUC/MIC was more important for microbial kill. The HFS-TB tool also predicted that in long term studies (i.e., over many weeks to months), peak/MIC would play the predominant role.[39,61] Similarly, the murine model established a role for AUC/MIC and missed the role of peak or peak/MIC, while the HFS-TB had predicted both as important, especially for resistance and long-term outcomes.[41,61,92] For pyrazinamide, murine and guinea pig studies confirmed the AUC/MIC role first identified in the HFS-TB.[23,95] Table 5 demonstrates that these HFS-TB findings were borne out in combination therapy studies in patients which were published three to five years after the publication of the original HFS-TB studies. Table 6 provides evidence that the HFS-TB is an accurate DDT in identifying and ranking PK/PD parameters of clinical relevance for combination therapy.

6.5.2. Analysis 2b: Systematic review to establish predictive accuracy of accepted qualitative therapeutic notions overturned by the HFS-TB or new hypothesis otherwise unknown

Findings from Search A and Search C were also used in a systematic analysis, which followed both PRISMA and Cochrane Collaboration criteria, to establish the accuracy of the HFS-TB in establishing new therapeutic frameworks in TB and overturning some accepted notions found by the HFS-TB to be erroneous. As an example, some HFS-TB predictions were refuted using mouse and guinea pig models, or expert consensus.[96-98] These HFS-TB predictions were borne out by clinical studies published subsequent to the initial HFS-TB publications (Table 7).

Table 7. HFS-TB Accuracy in Therapeutic Hypotheses Verification

HFS- TB and Monte Carlo simulation Predictions at t_1	Clinical Study Findings at t_2
MOXI and CIPRO standard doses have a biphasic kill pattern due to differences in concentrations associated with optimal microbial kill and those associated with suppression of ADR, which led to ADR arising between day 10 and 14.[10,38]	i. Biphasic kill rate of Mtb and emergence of ADR with >10 days of monotherapy with MOXI, levofloxacin and CIPRO.[76] ii. Biphasic kill of MOXI administered for ≥10 days.[77]
CIPRO (and ofloxacin) used at standard doses with second line agents in MDR-TB will lead to further ADR within weeks [38]	Emergence of what was later termed XDR-TB when quinolones used with second line drugs in MDR-TB.[78,79]
Efflux pump derived multiple drug resistance to monotherapy as an early event leading to high level resistance chromosomal mutations; The "antibiotic resistance arrow of time"[25,99]	i. Transcriptional profile of sequential sputum isolates in patients who were in the process of developing MDR-TB from initially susceptible isolates, despite DOTS.[80] ii-iii. Demonstration that efflux pump inhibitors can reverse drug resistance in MDR-TB and XDR-TB clinical isolates[81,82]
Acquired drug resistance (ADR) and most microbiological failure is not due to poor compliance [42]	Meta-analysis of 10 prospective trials of patients on DOT (n=8774) versus SAT (n=3708): Incidence ratios for DOT versus SAT were 1.5 vs. 1.7% for microbiological failure, 3.7% vs. 2.3% for relapse and 1.5% vs. 0.9% for ADR.[83]
Drug resistance and therapy failure is driven by pharmacokinetic variability [42]	i. Meta-analysis of 13 randomized studies with 1631 rapid acetylator and 1751 slow acetylators. The rapid versus slow acetylator relative risk was 2.0 for microbiological failure and 2.0 for ADR, even in combination therapy.[84] ii. Clinical cohort of 142 patients demonstrated poor long-term outcomes such as relapse and therapy failure, as well as two month sputum conversion.[85]
Pharmacokinetic mismatch does not lead to emergence of isoniazid or rifampin resistance[44]	i-ii. Two clinical cohorts of 142 and 59 patients demonstrated no increased ADR with mismatch of pharmacokinetic profiles of rifampin and isoniazid (manuscript in preparation).

Table 7 demonstrates the capability of controlled experiments in the HFS-TB to predict clinical outcomes, even against several fundamental concepts that underpin anti-TB therapeutics such as directly observed short course therapy. In addition, they gave rise to a new paradigm for how drug resistance arises and the most important causes for therapy failure, especially the role of biological variability such as pharmacokinetic variability in initiating a set of events that start the bacteria on the way to the acquired drug resistance state.[50,104,105] These new concepts have direct

relevance in the design of new drug regimens and new dosing concepts that will minimize the emergence of drug resistance and therapy failure. As an example, they de-emphasize the necessity to develop new drug combinations in fixed dose formulations.

6.5.3 Analysis 2c: Quantitative accuracy of the HFS-TB in forecasting the clinical future

To evaluate the accuracy of **quantitative predictions** from the HFS-TB tool, the team selected publications that documented quantitative outputs of the HFS-TB in tandem with Monte Carlo simulations at publication time = t_1 (Search A). The team then performed a literature search (Search C) of clinical literature published at least 6 months after each HFS-TB study at time t_2 years, i.e., $t_2 > (0.5+ t_1)$ years. The findings at t_2 were considered valid. A quality of evidence score combined with number of patients in the clinical study was used for weighting. The team defined forecasting error (FE) as MAPE, the accuracy (A) 100% minus FE (equation #3). Similarly, the bias of the HFS-TB tool was calculated. However, to contextualize the approach it was compared to a model recognized to be completely random but utilized in 1,000 forecasting experiments.

The randomly generated pairs of predicted value versus actual values demonstrated that random prediction was 100% correct in 1.6% of experiments, while 6% were within 95% of the actual value (i.e., error less than 5%). Mean accuracy (95% CI) was 15.6% (8.7-22.5). The mean bias (B) was close to 0% at -01% (-2.5 to 2.2). Thus, in the case of the random model, there was no significant bias in either direction. In summary, even randomly generating figures has some forecasting accuracy better than zero. This means that a truly useful model would have to vastly beat the rate of 15.6% and be outside the upper 95% confidence bounds of 22.5%.

Eight clinical studies at t_2 examining 14 therapeutic parameters forecast by the HFS-TB studies with Monte Carlo simulations at t_1 and are shown in Table 8.[10,23,25,29,42,43,85-87,89,90,94,100,101] The 95% confidence interval for predictive accuracy was 84.3-99.9%, while the bias was -13.7 to 6.2% and thus crossed zero. Compared to the random forecasting model, the HFS-TB was several folds more accurate. Indeed, one of the reasons it was only in the lower 90% was because of MIC breakpoints which change by a scale of two. A miss of one tube dilution, considered to be acceptable in the clinical laboratory, leads to an accuracy of only 50%.

Table 8. Quantitative Accuracy of the HFS-TB Tool

Drug	Parameter	HFS-TB	Clinical observation	Number of patients	Weighting %	Weighted accuracy	Weighted bias (%)
Pyrazinamide	Optimal AUC/MIC[23,85]	209	258	142	13.4	10.9	2.6
Isoniazid	Optimal AUC/MIC[23,85]	567	520	142	13.4	12.2	-1.2
Ethambutol	Optimal peak/MIC[25,29,94]	0.51	0.46	59	11.2	9.9	-1.2
Moxifloxacin	AUC/MIC at Dose of 400 mg/day[10,86]	59	66	9	0.9	0.8	0.1
Moxifloxacin	AUC/MIC at Dose of 400 mg/day[10,100,101]	59	56	9	0.4	0.4	-0.0
Moxifloxacin	Optimal AUC/MIC[10,89]	106	106	61	11.5	11.5	0
Pyrazinamide	Optimal AUC/MIC[23,29]	11.7	11.3	59	11.2	10.8	-0.4
Rifampin	Breakpoint MIC (mg/L)[43,87]	0.0625	0.125	36	3.4	1.7	1.7
Rifampin	Breakpoint MIC (mg/L)[43,90]	0.0625	0.0625	52	2.2	2.5	0
Isoniaid	Lower level resistance breakpoint MIC (mg/L)[43,87]	0.0312	0.0312	36	3.4	3.4	0
Isoniazid	Lower level resistance breakpoint MIC[43]	0.125	0.125	36	3.4	3.4	0
Pyrazinamide	Breakpoint MIC[43,87]	50	50	59	11.2	11.2	0
Moxifloxacin	Breakpoint MIC[43,100,101]	1	1	16	0.8	0.8	0
% of patients	ADR in Western Cape[42,85]	0.68	0.7	142	13.4	13.0	0.4
All	**SUMMARY**				**100**	**94.43**	**1.8%**

7. SUMMARY

Several levels of evidence have been presented to demonstrate the accuracy of the HFS-TB tool using clinical studies as the final arbiter of predictive accuracy. Analysis 1 demonstrated an excellent correlation between the HFS-TB and clinical observations for bactericidal effect and sterilizing effect patterns, rates of kill, dose-response patterns, time to emergence of resistance and PK/PD parameters associated with long term outcome. Next, evidence was presented showing that HFS-TB correctly predicted the PK/PD parameters of standard drugs that drive bactericidal effect, sterilizing effect, acquired drug resistance and long-term outcomes, based on combination therapy clinical studies published after the publication of the HFS-TB data (Analysis 2a). In addition we presented evidence of new hypotheses generated by HFS-TB relevant to DDT, overturning some accepted beliefs founded by *in vivo* data, all of which has been confirmed in subsequent high quality clinical studies (Analysis 2b). Finally, evidence was presented demonstrating that the HFS-TB tool has a quantitative predictive accuracy of 94% in forecasting optimal drug exposures, susceptibility breakpoints, rates and time to emergence of resistance and doses to be used in drug regimens to treat TB (Analysis 2c).

The data presented here support the current utility of the HFS-TB as a valuable tool for anti-TB drug dose selection and regimen design with a validated forecasting accuracy, which could facilitate submissions to EMA throughout the drug development process and be useful for better design of Phase I, Phase II and Phase III clinical studies.

8. REFERENCES

1. **Christopher J. L. Murray, C.J.L., and A.D. Lopez**. 1996. Global Burden of Disease: A comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Harvard School of Public Health, Cambridge, MA.

2. **World Health Organization**. Tuberculosis factsheet N 104. 2011. World Health Organization.

3. **World Health Organization**. Treatment of tuberculosis: Guidelines. Fourth edition. WHO/HTM/TB/2009.420. 2010. Geneva, World Health Organization. 11-13-2010.

4. **World Health Organization**. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. WHO/HTM/TB/2010.3. 2010. Geneva, World Health Organization.

5. **Blumberg, H. M., W. J. Burman, R. E. Chaisson, C. L. Daley, S. C. Etkind, L. N. Friedman, P. Fujiwara, M. Grzemska, P. C. Hopewell, M. D. Iseman, R. M. Jasmer, V. Koppaka, R. I. Menzies, R. J. O'Brien, R. R. Reves, L. B. Reichman, P. M. Simone, J. R. Starke, and A. A. Vernon**. 2003. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. Am.J.Respir.Crit Care Med. **167**:603-662.

6. **Ahuja, S. D., D. Ashkin, M. Avendano, R. Banerjee, M. Bauer, J. N. Bayona, M. C. Becerra, A. Benedetti, M. Burgos, R. Centis, E. D. Chan, C. Y. Chiang, H. Cox, L. D'Ambrosio, K. DeRiemer, N. H. Dung, D. Enarson, D. Falzon, K. Flanagan, J. Flood, M. L. Garcia-Garcia, N. Gandhi, R. M. Granich, M. G. Hollm-Delgado, T. H. Holtz, M. D. Iseman, L. G. Jarlsberg, S. Keshavjee, H. R. Kim, W. J. Koh, J. Lancaster, C. Lange, W. C. de Lange, V. Leimane, C. C. Leung, J. Li, D. Menzies, G. B. Migliori, S. P. Mishustin, C. D. Mitnick, M. Narita, P. O'Riordan, M. Pai, D. Palmero, S. K. Park, G. Pasvol, J. Pena, C. Perez-Guzman, M. I. Quelapio, A. Ponce-de-Leon, V. Riekstina, J. Robert, S. Royce, H. S. Schaaf, K. J. Seung, L. Shah, T. S. Shim, S. S. Shin, Y. Shiraishi, J. Sifuentes-Osornio, G. Sotgiu, M. J. Strand, P. Tabarsi, T. E. Tupasi, A. R. van, M. Van der Walt, T. S. van der Werf, M. H. Vargas, P. Viiklepp, J. Westenhouse, W. W. Yew, and J. J. Yim**. 2012. Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients. PLoS Med **9**:e1001300. doi:10.1371/journal.pmed.1001300 [doi];PMEDICINE-D-11-02356 [pii].

7. **Koch, R.** 1882. Die atiologie der tuberkulose. Berliner Klinischen Wocheschrift **15**:221-230.

8. **Ehrlich, P.** 1908. Ueber moderne chemotherapie. Tagung der Deutschen Dermatologischen Gesellschaft.

9. **Gumbo, T., Louie, A., Deziel, M., and Drusano, G**. Pharmacodynamic driven dosing of Rifampin in a hollow fiber system model of tuberculosis. 43rd Interscience Conference for Antimicrobial Agents and Chemotherapy. Abstracts of the 43rd Interscience Conference for Antimicrobial Agents and Chemotherapy . 2003. Washington, DC, American Society for Microbiology.

10. **Gumbo, T., A. Louie, M. R. Deziel, L. M. Parsons, M. Salfinger, and G. L. Drusano**. 2004. Selection of a moxifloxacin dose that suppresses drug resistance in *Mycobacterium tuberculosis*, by use of an *in vitro* pharmacodynamic infection model and mathematical modeling. J.Infect.Dis. **190**:1642-1651.

11. **Steenken, W., Jr. and E. Wolinsky**. 1954. The antituberculous activity of pyrazinamide *in vitro* and in the guinea pig. Am.Rev.Tuberc. **70**:367-369.

12. **Yeager, R. L., W. G. Munroe, and F. I. Dessau**. 1952. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. Am.Rev.Tuberc. **65**:523-546.

13. **British Medical Research Council**. 1974. Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Third report. East African-British Medical Research Councils. Lancet **2**:237-240.

14. **Grumbach, F.** 1958. Activity of pyrazinamide against experimental tuberculosis. Ann.Inst.Pasteur (Paris) **94**:694-708.

15. **Coates, A. R., Y. Hu, A. Jindani, and D. A. Mitchison**. 2013. Contradictory results with high-dosage rifamycin in mice and humans. Antimicrob.Agents Chemother. **57**:1103.

16. **Davies, G. R., A. S. Pym, D. A. Mitchison, E. L. Nuermberger, and J. H. Grosset**. 2007. Evaluation of new antituberculosis drugs in mouse models. Antimicrob.Agents Chemother. **51**:403-404.

17. **Mitchison, D. A. and K. C. Chang**. 2009. Experimental models of tuberculosis: can we trust the mouse? Am.J Respir.Crit Care Med. **180**:201-202.

18. **Mitchison, D. A.** 2010. Activity of pyrazinamide in the guinea pig model of tuberculosis. Antimicrob.Agents Chemother. **54**:5420.

19. **Nuermberger, E. L., T. Yoshimatsu, S. Tyagi, R. J. O'Brien, A. N. Vernon, R. E. Chaisson, W. R. Bishai, and J. H. Grosset**. 2004. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. Am.J.Respir.Crit Care Med. **169**:421-426.

20. **Dorman, S. E., J. L. Johnson, S. Goldberg, G. Muzanye, N. Padayatchi, L. Bozeman, C. M. Heilig, J. Bernardo, S. Choudhri, J. H. Grosset, E. Guy, P. Guyadeen, M. C. Leus, G. Maltas, D. Menzies, E. L. Nuermberger, M. Villarino, A. Vernon, and R. E. Chaisson**. 2009. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. Am.J Respir.Crit Care Med. **180**:273-280.

21. **Rosenthal, I. M., M. Zhang, K. N. Williams, C. A. Peloquin, S. Tyagi, A. A. Vernon, W. R. Bishai, R. E. Chaisson, J. H. Grosset, and E. L. Nuermberger**. 2007. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. PLoS.Med. **4**:e344.

22. **Dorman, S. E., S. Goldberg, J. E. Stout, G. Muzanyi, J. L. Johnson, M. Weiner, L. Bozeman, C. M. Heilig, P. J. Feng, R. Moro, M. Narita, P. Nahid, S. Ray, E. Bates,**

B. Haile, E. L. Nuermberger, A. Vernon, and N. W. Schluger. 2012. Substitution of rifapentine for rifampin during intensive phase treatment of pulmonary tuberculosis: study 29 of the tuberculosis trials consortium. J.Infect.Dis. **206**:1030-1040.

23. **Gumbo, T., C. S. Siyambalapitiyage Dona, C. Meek, and R. Leff**. 2009. Pharmacokinetics-Pharmacodynamics of pyrazinamide in a novel *in vitro* model of tuberculosis for sterilizing effect: A paradigm for faster assessment of new antituberculosis drugs. Antimicrob.Agents Chemother. **53**:3197-3204.

24. **Drusano, G. L., N. Sgambati, A. Eichas, D. L. Brown, R. Kulawy, and A. Louie**. 2010. The Combination of Rifampin plus Moxifloxacin Is Synergistic for Suppression of Resistance but Antagonistic for Cell Kill of Mycobacterium tuberculosis as Determined in a Hollow-Fiber Infection Model. MBio. **1**. doi:10.1128/mBio.00139-10 [doi].

25. **Srivastava, S., S. Musuka, C. Sherman, C. Meek, R. Leff, and T. Gumbo**. 2010. Efflux-pump-derived multiple drug resistance to ethambutol monotherapy in *Mycobacterium tuberculosis* and the pharmacokinetics and pharmacodynamics of ethambutol. J Infect Dis **201**:1225-1231.

26. **Taddei, F., M. Vulic, M. Radman, and I. Matic**. 1997. Genetic variability and adaptation to stress. EXS **83**:271-290.

27. **Taddei, F., M. Radman, J. Maynard-Smith, B. Toupance, P. H. Gouyon, and B. Godelle**. 1997. Role of mutator alleles in adaptive evolution. Nature **387**:700-702.

28. **Matic, I., F. Taddei, and M. Radman**. 2004. Survival versus maintenance of genetic stability: a conflict of priorities during stress. Res.Microbiol. **155**:337-341.

29. **Chigutsa, E., J. G. Pasipanodya, F. A. Sirgel, T. Gumbo, and H. McIlleron**. 2013. Modeling the non-linear effects of drug exposure on sterilizing effect rates in pulmonary tuberculosis. Submitted.

30. **Srivastava, S., Sherman, C., and Gumbo, T**. Bactericidal and Sterilizing Activities of Anti-Tuberculosis Drugs During the First 28 Days: A Prequel. 53rd Interscience Conference of Antimicrobial Agents and Chemotherapy , A-1569. 9-13-1023. Washington, DC.

31. **Lehmann, J.** 1946. Para-aminosalicylic acid in the treatment of tuberculosis. Lancet **1**:15.

32. **Schatz, A., E. Bugie, and S. A. Waksman**. 1944. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc.Soc.Exper.Biol.& Med **55**:66-69.

33. **Smith, D. G. and S. A. Waksman**. 1947. Tuberculostatic and Tuberculocidal Properties of Streptomycin. J Bacteriol. **54**:253-261.

34. **Canadian Task Force on the Periodic Health Examination**. 1979. The periodic health examination. Canadian Task Force on the Periodic Health Examination. Can.Med Assoc.J **121**:1193-1254.

35. **Khan, A. R., S. Khan, V. Zimmerman, L. M. Baddour, and I. M. Tleyjeh**. 2010. Quality and strength of evidence of the Infectious Diseases Society of America clinical practice guidelines. Clin Infect Dis **51**:1147-1156.

36. **Moher, D., A. Liberati, J. Tetzlaff, and D. G. Altman**. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ **339**:b2535.

37. **Balshem, H., M. Helfand, H. J. Schunemann, A. D. Oxman, R. Kunz, J. Brozek, G. E. Vist, Y. Falck-Ytter, J. Meerpohl, S. Norris, and G. H. Guyatt**. 2011. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. **64**:401-406.

38. **Gumbo, T., A. Louie, M. R. Deziel, and G. L. Drusano**. 2005. Pharmacodynamic evidence that ciprofloxacin failure against tuberculosis is not due to poor microbial kill but to rapid emergence of resistance. Antimicrob.Agents Chemother. **49**:3178-3181.

39. **Gumbo, T., A. Louie, M. R. Deziel, W. Liu, L. M. Parsons, M. Salfinger, and G. L. Drusano**. 2007. Concentration-dependent *Mycobacterium tuberculosis* killing and prevention of resistance by rifampin. Antimicrob.Agents Chemother. **51**:3781-3788.

40. **Gumbo, T., A. Louie, W. Liu, P. G. Ambrose, S. M. Bhavnani, D. Brown, and G. L. Drusano**. 2007. Isoniazid's bactericidal activity ceases because of the emergence of resistance, not depletion of *Mycobacterium tuberculosis* in the log phase of growth. J.Infect.Dis. **195**:194-201.

41. **Gumbo, T., A. Louie, W. Liu, D. Brown, P. G. Ambrose, S. M. Bhavnani, and G. L. Drusano**. 2007. Isoniazid bactericidal activity and resistance emergence: integrating pharmacodynamics and pharmacogenomics to predict efficacy in different ethnic populations. Antimicrob.Agents Chemother. **51**:2329-2336.

42. **Srivastava, S., J. G. Pasipanodya, C. Meek, R. Leff, and T. Gumbo**. 2011. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect.Dis. **204**:1951-1959.

43. **Gumbo, T.** 2010. New susceptibility breakpoints for first-line antituberculosis drugs based on antimicrobial pharmacokinetic/pharmacodynamic science and population pharmacokinetic variability. Antimicrob.Agents Chemother. **54**:1484-1491.

44. **Srivastava, S., C. Sherman, C. Meek, R. Leff, and T. Gumbo**. 2011. Pharmacokinetic Mismatch Does Not Lead to Emergence of Isoniazid- or Rifampin-Resistant Mycobacterium tuberculosis but to Better Antimicrobial Effect: a New Paradigm for Antituberculosis Drug Scheduling. Antimicrob.Agents Chemother. **55**:5085-5089.

45. **Jeena, P. M., W. R. Bishai, J. G. Pasipanodya, and T. Gumbo**. 2011. In silico children and the glass mouse model: clinical trial simulations to identify and individualize optimal isoniazid doses in children with tuberculosis. Antimicrob.Agents Chemother. **55**:539-545.

46. **Goutelle, S., L. Bourguignon, P. H. Maire, G. M. Van, J. E. Conte, Jr., and R. W. Jelliffe**. 2009. Population modeling and Monte Carlo simulation study of the pharmacokinetics and antituberculosis pharmacodynamics of rifampin in lungs. Antimicrob.Agents Chemother. **53**:2974-2981.

47. **Drusano, G. L., N. Sgambati, A. Eichas, D. Brown, R. Kulawy, and A. Louie**. 2011. Effect of Administration of Moxifloxacin plus Rifampin against Mycobacterium tuberculosis for 7 of 7 Days versus 5 of 7 Days in an *in vitro* Pharmacodynamic System. MBio. **2**. doi:mBio.00108-11 [pii];10.1128/mBio.00108-11 [doi].

48. **Musuka, S., S. Srivastava, C. S. Siyambalapitiyage Dona, C. Meek, R. Leff, J. G. Pasipanodya, and T. Gumbo**. 2013. Thioridazine Exhibits Wobbly Pharmacokinetic-Pharmacodynamic Parameters During Treatment of Tuberculosis: A Theoretical Basis of Shorter Duration Curative Monotherapy with Congeners. Antimicrob.Agents Chemother. Published ahead of print 16 September 2013 , doi:10.1128/AAC.00829-13

49. **Srivastava, S., C. Sherman, C. Meek, R. Leff, and T. Gumbo**. 2012. Hollow Fiber Model to Study Bactericidal and Sterilizing Effect of Multiple Drugs Within Two Months: Implications for Design of New Clinical Regimens. Submitted.

50. **Ginsburg, A. S., J. Lee, S. C. Woolwine, J. H. Grosset, F. M. Hamzeh, and W. R. Bishai**. 2005. Modeling *in vivo* pharmacokinetics and pharmacodynamics of moxifloxacin therapy for Mycobacterium tuberculosis infection by using a novel cartridge system. Antimicrob.Agents Chemother. **49**:853-856.

51. **Drusano, G., Sgambati, N., Stewart, A., Brown, D., Kulawy, R., and Louie, A**. Exposure-Response of Mycobacterium tuberculosis (*Mtb*) in the State of Wayne-Hayes Level II Anaerobiosis to Moxifloxacin (M). Inter-Science Conference on Anti-microbials And Chemotherapy . 2012. **A1-604/58**

52. **Drusano, G., Sgambati, N., Stewart, A., Brown, D., Kulawy, R., and Louie, A**. Prediction of Clinical Outcome from Hollow Fiber Infection Model (HFIM) Data for Rifampin (RIF) Therapy of M. tuberculosis (*Mtb*) in Non-Replicative Persister (NRP) Phase. Inter-Science Conference on Anti-microbials And Chemotherapy . 2012. **A1-603/57**

53. **Xue, B., Okusanya, O. O., Zhu, T., van der Graaf, P. H., Buli, C. C., Bhavnani, S. M., Ambrose, P. G., and Forrest, A**. Pharmacokinetic-Pharmacodynamic (PK-PD) Analysis Evaluating the Effects of Rifampin (RIF), Linezolid (LZD), PNU-100480 (Sutezolid, U-480), and Its Metabolite PNU-101603 (U-603), as Single Agents, Against Mycobacterium tuberculosis (*Mtb*) in Log-Phase Growth Using Data from a Hollow-Fiber Infection Model (HFIM). Inter-Science Conference on Anti-microbials And Chemotherapy. 2012.

54. **Okusanya, O. O., Xue, B., Zhu, T., van der Graaf, P. H., Bhavnani, S. M., Ambrose, P. G., and Forrest, A**. Pharnacokinetic-Pharamcodynamic (PK-PD) Analysis Evaluating the Effects of Rifampin (RIF) and Linezolid (LZD), Alone amd In Combination, Against Mycobacterium tuberculosis (*Mtb*) in the Non-Replicating Persister (NRP) State Using Data from a Hollow-Fiber Infection Model (HFIM). Inter-Science Conference on Anti-microbials And Chemotherapy. 2012.

55. **Okusanya, O. O., Xue, B., Zhu, T., van der Graaf, P. H., Bulik, C. C., Bhavnani, S. M., Ambrose, P. G., and Forrest, A.** Pharmacokinetic-Pharmacodynamic (PK-PD) Analysis Evaluating the Effects of Rifampin (RIF), PNU-100480 (Sutezolid, U-480), and Its Metabolite PNU-101603 (U-603), Alone and In Combination, Against Mycobacterium tuberculosis (*Mtb*) in the Non-Replicating Persister (NRP) State Using

Data from a Hollow-Fiber Infection Model (HFIM). Inter-Science Conference on Anti-microbials And Chemotherapy. 2012.

56. **Louie, A., Brown, D., Swift, M., Files, K., and Drusano, G**. Clinical Doses of PNU-100480 (U, Sutezolid) Plus Rifampin (R) Synergistically Kills Mycobacterium tuberculosis (*Mtb*) while Linezolid (L) Plus R Does Not. Inter-Science Conference on Anti-microbials And Chemotherapy. 2012. E-1993.

57. **Louie, A., Brown, D., Files, K., Swift, M., Fikes, S., and Drusano, G.** Pharmacodynamics Of PNU-100480 (U, Sutezolid), A New Oxazolidinone, In Combination With Its Active Metabolite In The Killing Of Mycobacterium tuberculosis (*Mtb*) In An *in vitro* Hollow Fiber Infection Model (HFIM). Inter-Science Conference on Anti-microbials And Chemotherapy . 2012. **A-1265.**

58. **Louie, A., Eichas, A., Files, K., Swift, M., Bahniuk, N., Kulawy, R., Brown, D., and Drusano, G**. Activities of PNU 100480 (PNU 480) Alone, PNU 480 Plus Its Major Metabolite PNU 101603 (PNU 1603), and PNU 480 Plus PNU 1603 In Combination with Rifampin (RIF) Against Mycobacterium tuberculosis (*Mtb*). Inter-Science Conference on Anti-microbials And Chemotherapy. 2012. **A1-1737**

59. **Goutelle, S., L. Bourguignon, R. W. Jelliffe, J. E. Conte, Jr., and P. Maire**. 2011. Mathematical modeling of pulmonary tuberculosis therapy: Insights from a prototype model with rifampin. J.Theor.Biol. **282**:80-92.

60. **Sirgel, F. A., P. R. Donald, J. Odhiambo, W. Githui, K. C. Umapathy, C. N. Paramasivan, C. M. Tam, K. M. Kam, C. W. Lam, K. M. Sole, and D. A. Mitchison**. 2000. A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. J Antimicrob.Chemother. **45**:859-870.

61. **Pasipanodya, J. and T. Gumbo**. 2011. An oracle: antituberculosis pharmacokinetics-pharmacodynamics, clinical correlation, and clinical trial simulations to predict the future. Antimicrob.Agents Chemother. **55**:24-34.

62. **Donald, P. R., F. A. Sirgel, F. J. Botha, H. I. Seifart, D. P. Parkin, M. L. Vandenplas, B. W. Van de Wal, J. S. Maritz, and D. A. Mitchison**. 1997. The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. Am.J Respir.Crit Care Med. **156**:895-900.

63. **Jindani, A., V. R. Aber, E. A. Edwards, and D. A. Mitchison**. 1980. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am.Rev.Respir.Dis. **121**:939-949.

64. **Jindani, A., C. J. Dore, and D. A. Mitchison**. 2003. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. Am.J.Respir.Crit Care Med. **167**:1348-1354.

65. **Gangadharam, P. R., A. L. Bhatia, S. Radhakrishna, and J. B. Selkon**. 1961. Rate of inactivation of isoniazid in South Indian patients with pulmonary tuberculosis. Bull.World Health Organ **25**:765-777.

66. **Gangadharam, P. R., S. Devadatta, W. Fox, C. N. Nair, and J. B. Selkon**. 1961. Rate of inactivation of isoniazid in South Indian patients with pulmonary tuberculosis. 3.

Serum concentrations of isoniazid produced by three regimens of isoniazid alone and one of isoniazid plus PAS. Bull.World Health Organ **25**:793-806.

67. **Devadatta, S., A. L. Bhatia, R. H. Andrews, W. Fox, D. A. Mitchison, S. Radhakrishna, C. V. Ramakrishnan, J. B. Selkon, and S. Velu**. 1961. Response of patients infected with isoniazid-resistant tubercle bacilli to treatment with isoniazid plus PAS or isoniazid alone. Bull.World Health Organ **25**:807-829.

68. **Selkon, J. B., W. Fox, P. R. Gangadharam, K. Ramachandran, C. V. Ramakrishnan, and S. Velu**. 1961. Rate of inactivation of isoniazid in South Indian patients with pulmonary tuberculosis. 2. Clinical implications in the treatment of pulmonary tuberculosis with isoniazid either alone or in combination with PAS. Bull.World Health Organ **25**:779-792.

69. **Selkon, J. B., S. Devadatta, K. G. Kulkarni, D. A. Mitchison, A. S. Narayana, C. N. Nair, and K. Ramachandran**. 1964. The emergence of isoniazid-resistant cultures in patients with pulmonary tuberculosis during treatment with isoniazid alone or isoniazid plus PAS. Bulletin of the World Health Organization **31**:294.

70. **Anonymous**. 1976. A comparative study of daily followed by twice- or once-weekly regimens of ethambutol and rifampicin in the retreatment of patients with pulmonary tuberculosis: second report. Tubercle. **57**:105-113.

71. **Anonymous**. 1981. Ethambutol plus isoniazid for the treatment of pulmonary tuberculosis--a controlled trial of our regimens. Tubercle. **62**:13-29.

72. **British Medical Research Council**. 1969. A controlled comparison of four regimens of streptomycin plus pyrazinamide in the retreatment of pulmonary tuberculosis. Tubercle. **50**:81-114.

73. **Ginsburg, A. S., S. C. Woolwine, N. Hooper, W. H. Benjamin, Jr., W. R. Bishai, S. E. Dorman, and T. R. Sterling**. 2003. The rapid development of fluoroquinolone resistance in *M. tuberculosis*. N.Engl.J.Med. **349**:1977-1978.

74. **Hesseling, A. C., G. Walzl, D. A. Enarson, N. M. Carroll, K. Duncan, P. T. Lukey, C. Lombard, P. R. Donald, K. A. Lawrence, R. P. Gie, P. D. van Helden, and N. Beyers**. 2010. Baseline sputum time to detection predicts month two culture conversion and relapse in non-HIV-infected patients. Int.J Tuberc.Lung Dis **14**:560-570.

75. **Visser, M. E., H. M. Grewal, E. C. Swart, M. A. Dhansay, G. Walzl, S. Swanevelder, C. Lombard, and G. Maartens**. 2011. The effect of vitamin A and zinc supplementation on treatment outcomes in pulmonary tuberculosis: a randomized controlled trial. Am.J.Clin.Nutr. **93**:93-100.

76. **Devasia, R. A., A. Blackman, T. Gebretsadik, M. Griffin, A. Shintani, C. May, T. Smith, N. Hooper, F. Maruri, J. Warkentin, E. Mitchel, and T. R. Sterling**. 2009. Fluoroquinolone resistance in *Mycobacterium tuberculosis*: the effect of duration and timing of fluoroquinolone exposure. Am.J Respir.Crit Care Med **180**:365-370.

77. **Chang, K. C., C. C. Leung, W. W. Yew, T. Y. Lau, W. M. Leung, C. M. Tam, H. C. Lam, P. S. Tse, M. Y. Wong, S. N. Lee, K. I. Wat, and Y. H. Ma**. 2010. Newer fluoroquinolones for treating respiratory infection: do they mask tuberculosis? Eur.Respir.J **35**:606-613.

78. **Gandhi, N. R., A. Moll, A. W. Sturm, R. Pawinski, T. Govender, U. Lalloo, K. Zeller, J. Andrews, and G. Friedland**. 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet **368**:1575-1580.

79. **Pillay, M. and A. W. Sturm**. 2007. Evolution of the extensively drug-resistant F15/LAM4/KZN strain of Mycobacterium tuberculosis in KwaZulu-Natal, South Africa. Clin.Infect Dis **45**:1409-1414.

80. **Chatterjee, A., D. Saranath, P. Bhatter, and N. Mistry**. 2013. Global Transcriptional Profiling of Longitudinal Clinical Isolates of Mycobacterium tuberculosis Exhibiting Rapid Accumulation of Drug Resistance. PLoS ONE. **8**:e54717.

81. **Machado, D., I. Couto, J. Perdigao, L. Rodrigues, I. Portugal, P. Baptista, B. Veigas, L. Amaral, and M. Viveiros**. 2012. Contribution of Efflux to the Emergence of Isoniazid and Multidrug Resistance in Mycobacterium tuberculosis. PLoS.One. **7**:e34538.

82. **Louw, G. E., R. M. Warren, N. C. Gey van Pittius, R. Leon, A. Jimenez, R. Hernandez-Pando, C. R. McEvoy, M. Grobbelaar, M. Murray, P. D. van Helden, and T. C. Victor**. 2011. Rifampicin reduces susceptibility to ofloxacin in rifampicin-resistant Mycobacterium tuberculosis through efflux. Am.J Respir.Crit Care Med. **184**:269-276.

83. **Pasipanodya, J. G. and T. Gumbo**. 2013. A Meta-Analysis of Self-Administered vs Directly Observed Therapy Effect on Microbiologic Failure, Relapse, and Acquired Drug Resistance in Tuberculosis Patients. Clin Infect Dis **57**:21-31.

84. **Pasipanodya, J. G., S. Srivastava, and T. Gumbo**. 2012. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect.Dis **55**:169-177.

85. **Pasipanodya, J., H. McIlleron, A. Burger, P. A. Wash, P. Smith, and T. Gumbo**. 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J.Infect.Dis. First published online July 29, 2013 doi: 10.1093/infdis/jit352

86. **Peloquin, C. A., D. J. Hadad, L. P. Molino, M. Palaci, W. H. Boom, R. Dietze, and J. L. Johnson**. 2008. Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. Antimicrob.Agents Chemother. **52**:852-857.

87. **Pasipanodya, J., Gumbo, T., and McIlleron, H**. Clinical validation of new rifampin and isoniazid susceptibility breakpoints derived from engineering simulations of pharmacokinetic-pharmacodynamic hollow fiber model results. 53rd Inter-Science Conference on Anti-microbials And Chemotherapy . 9-12-2013. Denver, CO.

88. **Gumbo, T., E. Chigutsa, J. G. Pasipanodya, M. Visser, H. McIlleron, and F. A. Sirgel**. 2013. The pyrazinamide susceptibility breakpoint above which combination therapy fails. Submitted.

89. **Thwaites, G. E., S. M. Bhavnani, T. T. Chau, J. P. Hammel, M. E. Torok, S. A. Van Wart, P. P. Mai, D. K. Reynolds, M. Caws, N. T. Dung, T. T. Hien, R. Kulawy, J. Farrar, and P. G. Ambrose**. 2011. Randomized pharmacokinetic and pharmacodynamic comparison of fluoroquinolones for tuberculous meningitis. Antimicrob.Agents Chemother. **55**:3244-3253.

90. **Williamson, D. A., S. A. Roberts, J. E. Bower, R. Vaughan, S. Newton, O. Lowe, C. A. Lewis, and J. T. Freeman**. 2012. Clinical failures associated with rpoB mutations in phenotypically occult multidrug-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis **16**:216-220.

91. **Jayaram, R., S. Gaonkar, P. Kaur, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharat, R. K. Shandil, E. Kantharaj, and V. Balasubramanian**. 2003. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. Antimicrob.Agents Chemother. **47**:2118-2124.

92. **Jayaram, R., R. K. Shandil, S. Gaonkar, P. Kaur, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharath, E. Kantharaj, and V. Balasubramanian**. 2004. Isoniazid pharmacokinetics-pharmacodynamics in an aerosol infection model of tuberculosis. Antimicrob.Agents Chemother. **48**:2951-2957.

93. **Shandil, R. K., R. Jayaram, P. Kaur, S. Gaonkar, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharath, and V. Balasubramanian**. 2007. Moxifloxacin, ofloxacin, sparfloxacin, and ciprofloxacin against *Mycobacterium tuberculosis*: evaluation of *in vitro* and pharmacodynamic indices that best predict *in vivo* efficacy. Antimicrob.Agents Chemother. **51**:576-582.

94. **Deshpande, D., S. Srivastava, C. Meek, R. Leff, and T. Gumbo**. 2010. Ethambutol optimal clinical dose and susceptibility breakpoint identification by use of a novel pharmacokinetic-pharmacodynamic model of disseminated intracellular *Mycobacterium avium*. Antimicrob.Agents Chemother. **54**:1728-1733.

95. **Ahmad, Z., M. M. Fraig, G. P. Bisson, E. L. Nuermberger, J. H. Grosset, and P. C. Karakousis**. 2011. Dose-dependent activity of pyrazinamide in animal models of intracellular and extracellular tuberculosis infections. Antimicrob.Agents Chemother. **55**:1527-1532.

96. **Mitchison, D. A., A. Jindani, G. R. Davies, and F. Sirgel**. 2007. Isoniazid activity is terminated by bacterial persistence. J.Infect.Dis. **195**:1871-1872.

97. **Wallis, R. S., M. Palaci, and K. Eisenach**. 2007. Persistence, not resistance, is the cause of loss of isoniazid effect. J Infect.Dis. **195**:1870-1871.

98. **Ahmad, Z., L. G. Klinkenberg, M. L. Pinn, M. M. Fraig, C. A. Peloquin, W. R. Bishai, E. L. Nuermberger, J. H. Grosset, and P. C. Karakousis**. 2009. Biphasic kill

curve of isoniazid reveals the presence of drug-tolerant, not drug-resistant, Mycobacterium tuberculosis in the guinea pig. J Infect Dis **200**:1136-1143.

99. **Schmalstieg, A. M., S. Srivastava, S. Belkaya, D. Deshpande, C. Meek, R. Leff, N. S. van Oers, and T. Gumbo**. 2012. The antibiotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. Antimicrob.Agents Chemother. **56**:4806-4815.

100. **Pranger, A. D., A. R. van, R. E. Aarnoutse, S. D. van, D. R. Uges, J. G. Kosterink, T. S. van der Werf, and J. W. Alffenaar**. 2011. Evaluation of moxifloxacin for the treatment of tuberculosis: 3 years of experience. Eur.Respir.J. **38**:888-894.

101. **Pranger, A. D., J. G. Kosterink, A. R. van, R. E. Aarnoutse, T. S. van der Werf, D. R. Uges, and J. W. Alffenaar**. 2011. Limited-sampling strategies for therapeutic drug monitoring of moxifloxacin in patients with tuberculosis. Ther.Drug Monit. **33**:350-354.

102. **Macgowan, A. P., R. Reynolds, A. R. Noel, and K. E. Bowker**. 2009. Bacterial strain-to-strain variation in pharmacodynamic index magnitude, a hitherto unconsidered factor in establishing antibiotic clinical breakpoints. Antimicrob.Agents Chemother. **53**:5181-5184.

103. **Ford, C. B., R. R. Shah, M. K. Maeda, S. Gagneux, M. B. Murray, T. Cohen, J. C. Johnston, J. ardy, M. ipsitch, and S. M. ortune**. 2013. *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug resistant tuberculosis. Nat.Genet.

104. **de Steenwinkel, J. E., M. T. Ten Kate, G. J. de Knegt, K. Kremer, R. E. Aarnoutse, M. J. Boeree, H. A. Verbrugh, S. D. van, and I. A. Bakker-Woudenberg**. 2012. Drug Susceptibility of Mycobacterium tuberculosis Beijing Genotype and Association with MDR TB. Emerg.Infect.Dis. **18**:660-663.

105. **Gumbo, T.** 2013. Biological variability and the emergence of multidrug-resistant tuberculosis. Nat.Genet. **45**:720-721.

106. **Davies, G. R. and E. L. Nuermberger**. 2008. Pharmacokinetics and pharmacodynamics in the development of anti-tuberculosis drugs. Tuberculosis (Edinb.) **88 Suppl 1**:S65-S74.

107. **Budha, N. R., R. B. Lee, J. G. Hurdle, R. E. Lee, and B. Meibohm**. 2009. A simple *in vitro* PK/PD model system to determine time-kill curves of drugs against *Mycobacteria*. Tuberculosis (Edinb.) **89**:378-385.

108. **Tam, V. H., A. Louie, M. R.Deziel, W. Liu, G. L. Drusano**. 2007. The Relationship between Quinolone Exposures and Resistance Amplification Is Characterized by an Inverted U: a New Paradigm for Optimizing Pharmacodynamics To Counterselect Resistance. *Antimicrob Agents Chemother*. **51**:744–747.

9. AMENDMENT

After EMA's review of the briefing document and in response to their queries and requests for information (received on December 17, 2013), we have provided information and additional details as points of clarity in this amendment.

Question 1

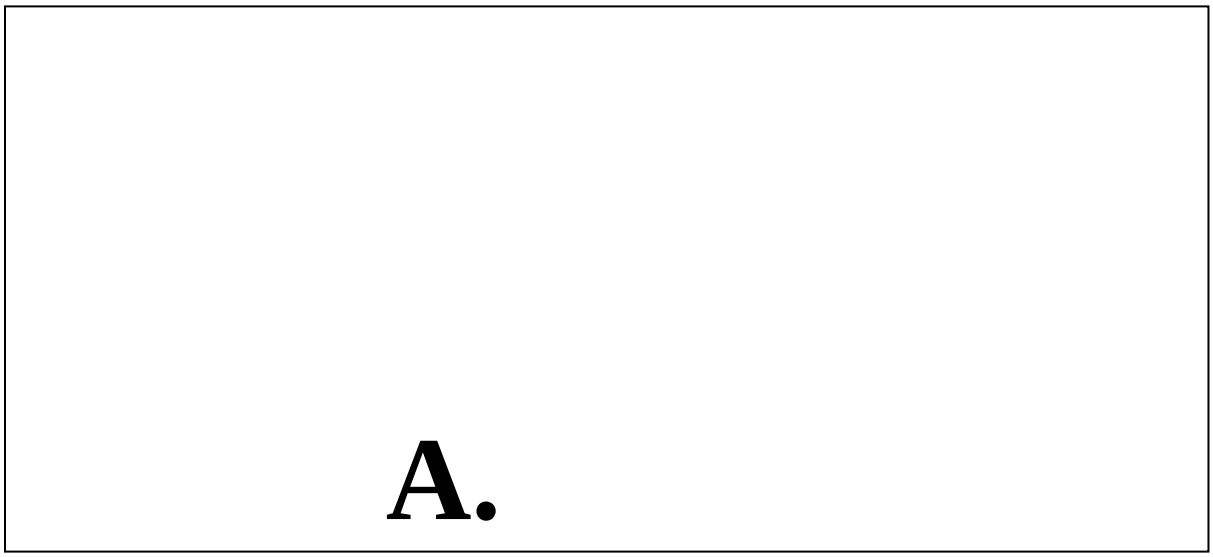
> *Please provide additional evidence that the HFS-TB can support all the stated claims. For regulatory purposes it is recommended to build you briefing package around the proposed claims:*
>
> *a. mimic the concentration-time profiles of antibiotics observed in TB patients*
>
> *b. mimic the metabolic and physiologic behavior of Mtb populations commonly encountered in infected patients with pulmonary TB and the intracellular Mtb characteristic of disseminated TB and*
>
> *c. quantify the sensitivity and resistance of these Mtb populations to various doses and combinations of antibiotic agents over time. When these outcomes are correctly achieved, the results can then be used in Monte Carlo simulations to identify (i) optimal doses of drugs, (ii) drug combinations which are most likely to achieve desired microbial outcomes, (iii) expected response rates from a drug or combination regimen, (iv) expected rates of and time to resistance emergence in patients and (v) susceptibility breakpoints based on a minimum inhibitory concentration (MIC) above which therapy by a specific drug will fail.*

Our response to the agency:

Response to question 1a: All the HFS-TB studies reported in Table 2 had drug concentrations measured from the central compartment at several time points from 24-48h. From these data actual concentration-time profiles were constructed. Reports had graphs showing the whole concentration-time profile to demonstrate what was actually achieved in each experiment and/or reported HFS-TB compartmental pharmacokinetic parameters (clearance, volume of distribution and area under the concentration-time curve).

Examples of published concentration-time profiles are shown in Figure 2 below. Thus, the concentration-time profiles achieved in the HFS-TB and drug compartmental pharmacokinetic parameter estimates were reported to document that these accurately mimicked human pharmacokinetics.

Figure 2. Antibiotic concentration-time profiles achieved in the HFS-TB.



A. Standard multidrug regimen. Error bars are standard deviations of replicates from two experiments.
B. The triphasic pharmacokinetic profile of ethambutol achieved in the HFS-TB.

Response to question 1b: All HFS studies were reported based upon the exact metabolic profile of *M. tuberculosis* they used. In the early years, the log-phase growth experiments, representing the majority of studies, used changes in CFU/mL, and provided growth curves of the bacteria sampled from drug treated systems, as well as non-treated controls in which log-phase growth curves are derived. Growth rates of 0.069 (0.059–0.079) $\log_{10}$ CFU/mL/day have been recorded. In addition, the mathematical models that use the observed bacteria burden lead to calculations of the exact growth rate constants for drug susceptible and drug-resistant subpopulations, which demonstrated continued log-phase growth. For semi-dormant bacteria at low pH, the pH in the system is measured at intervals. As an example, one study reported "Measurement of the pH at the start of each experiment, as well as every 7 days for up to 28 days, demonstrated no pH change in either the external or the peripheral compartment."[23] In addition, growth rates of the semi-dormant bacteria are also calculated, based on observed bacterial burden, and grow at a rate 10-fold slower than log-phase. For non-replicating persistent bacteria, several parameters are monitored to validate both hypoxia and metabolic phenotype. In one report in the published studies "O_2 tensions in the liquid medium were monitored at least 5 days/week. There were 36/53 (68%) measurements over the course of the experiment that were 10 parts per billion (ppb) or lower. The highest (one occasion) was 14 ppb. We also examined the organisms for the ability to be stained with Nile red as an indicator of the NRP state. This is shown in Fig. 4A and B. The organisms under anaerobiosis demonstrated clear staining with Nile red, whereas log-phase organisms did not. It has also been demonstrated previously (7) that organisms in the NRP state due to Wayne-Hayes level II anaerobiosis produce a 16-kDa protein (α-crystalline protein). Given the documentation of O2 tension, Nile red staining, and production of α-crystalline protein, we conclude that the organisms studied were in the NRP state due to Wayne-Hayes level II anaerobiosis."[24]

Response to 1c: Similarly, all HFS-TB studies in Table 2 reported repetitive sampling of the peripheral compartment and quantification of the colony forming units per milliliter (CFU/mL) at several time points during the study. Thus they all reported microbial kill rates (bactericidal

and sterilizing effects). In addition, 19 of the 22 HFS-TB used the samples to quantify the CFU/mL of drug resistant sub-populations at various pre-specified sampling time points. These later data were used to assess the relationship between drug concentration and the emergence of ADR.

The steps describing how these data are used, in response to 1c (i-v) are given in our response to question #3 below.

Question 2

> *Please provide more details on the Hollow Fiber System platform. Are there any specifications that should be respected? Are the platforms used in your literature reviews comparable?*

Our response to the agency:

HFS-TB assembly specifications are listed below. To ensure further laboratory knowledge, the team will also be developing a HFS-TB Methods Manual that will be published and utilized for appropriate laboratory training. The central aspects of the HFS-TB include (a) the cartridge assembly, (b) the tubing connected to and from the cartridge assembly, (c) the central reservoir (d) fresh media which is delivered via tubing and a mechanical pump, (e) used media also removed via tubing and a mechanical pump and (f) the syringe pump. Each of the components is purchased from vendors who manufacture via good manufacturing practices and are required components of the HFS-TB assembly.

The cartridge assembly consists of the hollow fiber cartridge and flexible tubing with valves that ensure one-directional flow. The cartridge assembly is manufactured by one of three independent commercial companies using good manufacturing practices. Material used to make the membranes of the cartridges is pre-specified depending on the drugs being studied. Commonly used materials include cellulosic, polysulfone, polypropylene, coated polypropylene, polyethylene, and coated polypropylene, most HFS-TB systems use cellulosic or polysulfone membranes. The molecular weight cut-off of the membranes is specified for each experiment, and includes typical molecular weight cut-offs which are 5, 10, 20, 30, and 42kD; with 42kD being the most commonly used. The cartridges carry 20 mL of peripheral compartment space volume and the hollow fibers have a surface area of 3,000 cm^2. Tubing, central reservoir bottles, mechanical and syringe pumps are purchased from several vendors that supply standard laboratory supplies.

The HFS-TB is assembled in each research laboratory with all components manufactured elsewhere. In a typical experiment, flow rates for circulation within the central compartment are 5-120 ml/min and the rate of addition of fresh media varies from 0.3 ml/min up to 2ml/min. The exact rates and the volume of media in the central reservoir vary depending on the antibiotic half-life in patients. All systems are assembled to run with standard power outlets, in a CO_2 incubator and at 37^0C.

Additionally, a systematic comparison of performance of the HFS-TB between laboratories for factors such as the metabolic status of *Mtb*, recapitulation of human pharmacokinetics, quantitation of drug-resistant and drug-susceptible microbial populations and output of mathematical models will be performed as part of a newly funded effort. With this, the reproducibility of results between laboratories can be quantified. In order to make this comparison, HFS-TB studies will be designed and performed to specification by more than one laboratory. The results of these studies will be used to calculate between laboratory coefficients of variability.

Question 3

Please provide more details on the MC simulation methodology to define an optimal dose/regimen. E.g. target attainment, number of simulated individuals, PK model (model building and evaluation) and good M&S practices.

Our response to the agency:

The relationship between drug-exposures achieved and drug-susceptible and drug-resistant subpopulations sizes at various time points were used to model the relationship between drug exposure measures versus size of bacterial population in standard Hill-type equations (inhibitory sigmoid E_{max} model). For drug-resistant subpopulation relationship to drug concentrations, the inverted "U" shaped curve (hormesis) of Tam et al has been used, as well as the quadratic function of Gumbo et al.[23,108] In addition, this output has been used for modeling and simulation for a system of inhomogeneous differential equations that relate drug concentration and drug-susceptible/drug-resistant subpopulations over time. An example of utilizing microbial and pharmacokinetic output in these equations, and the final model output and predictions, are shown in Gumbo et al.'s "Selection of a Moxifloxacin Dose That Suppresses Drug Resistance in Mycobacterium tuberculosis, by Use of an In Vitro Pharmacodynamic Infection Model and Mathematical Modeling", which is the first published paper on the HFS-TB.[10] Thus a variety of approaches is used to calculate drug exposures associated with optimal microbial kill suppression of drug resistance from HFS-TB outputs.

Once the drug exposures associated with optimal outcome are identified, they are used to identify doses that would achieve this result in patients, as well as to identify preliminary susceptibility breakpoints. First, the drug exposure associated with optimal kill and suppression of drug resistance is identified in the HFS-TB. This could be an AUC/MIC value of 209 for pyrazinamide or a rifampin peak/MIC concentration of 175.[23, 39] Population pharmacokinetic parameter estimates of these drugs and their variance parameters for a specific population of patients (for example Western cape Province of South Africa) are used as prior data (i.e., domain of inputs). Thus, the full covariance matrix from the population modeling analysis is embedded into a particular sub-routine depending on the software. As is typical in the modeling and simulation literature, the size of individuals simulated is usually ≥10,000, in order to stabilize the variance in the tails of the distribution. Also incorporated at this stage are multimodal distributions of PK parameters for those drugs for which certain single nucleotide polymorphisms (SNPs) are associated with clinically meaningful PK variability (e.g., isoniazid). The output is a distribution of clearance values, peak concentrations, AUCs and concentration time profiles. The most adequate distributions are chosen based on the ability to closely recapitulate the original pharmacokinetic parameters. These simulations are performed for several ascending doses of each drug. Next, the distribution of the drug MICs are taken into account and AUC/MIC, peak/MIC, percent of time concentration persists above MIC is calculated. The proportion of patients who achieve or exceed the target drug exposure identified as optimal for microbial kill or resistance suppression is then calculated. The weighted average population target attainment probability (TAP) for a particular clinical drug dose is then determined based on the formula:

$$\sum_{i=1}^{n}(\boldsymbol{TAP} * \boldsymbol{MIC} \quad \boldsymbol{distribution})$$

where, *i* is the proportion of isolates at the lowest MIC and *n* is the proportion of isolates at the highest MIC. If the target attainment probability is sub-optimal for a particular dose, a higher dose is chosen and the whole process repeated, until an optimum dosing regimen is attained. External validation is performed by also examining the predicted effects of a dose with known clinical outcomes; TAP should be similar (very close) to the proportion of patients known to respond to that dose. Gumbo et al.'s paper, "Pharmacokinetics-Pharmacodynamics of Pyrazinamide in a Novel *In Vitro* Model of Tuberculosis for Sterilizing Effect: a Paradigm for Faster Assessment of New Antituberculosis Drugs", examines such simulations for dose selection, starting with HFS-TB output.[23] A similar process is followed for selection of MIC to be used for susceptibility breakpoint. The maximum tolerated dose is utilized in these simulations, and the MIC above which >10% of subjects attain drug exposures below those known to be optimal is chosen as susceptibility breakpoint. T. Gumbo's publication, "New Susceptibility Breakpoints for First-Line Antituberculosis Drugs Based on Antimicrobial Pharmacokinetic/Pharmacodynamic Science and Population Pharmacokinetic Variability", is an example of use of HFS-TB output data in simulations to identify susceptibility breakpoints.[43]

Question 4

Please include protocols/plans/reports for the conducted searches (A, B, C) and analyses 1, 2 (2a-2b-2c).

Our response to the agency:

A description of the literature search strategy is described in section 5.1 in the briefing document. References have been provided in the briefing document for the conducted searches and corresponding analyses. There are no other protocols or plans for this study.

Question 5

In the searches performed you refer to correlation and predictive evaluations based on the publication date of the studies. I understand that your reference to HFS-TB publication refers to the date where the analysis of a specific drug with the platform was published. Are there any cases where the publication dates for the HFS-TB and Clinical studies are not concordant with the completion dates?

Our response to the agency:

Yes, there may be clinical study completion dates that are not concordant with the publication dates. However, the goal of prediction is based on the transfer of information through time, and is dependent on the release of date of the completed analysis, not the data-collection completion date.

Question 6

How do you address publication bias?

Our response to the agency:

Publication bias is addressed on p. 14, section 5.1.

Question 7

Did you perform any in house experiments?

Our response to the agency:

The analyses performed and described in this briefing document were based on data derived from the published literature. We have received an award with funding to conduct in-house experiments and will be further investigating new treatment regimens with the HFS-TB and with new emerging clinical data.