Systematic Analysis of Hollow Fiber Model of Tuberculosis Experiments

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Background. The in vitro hollow fiber system model of tuberculosis (HFS-TB), in tandem with Monte Carlo experiments, was introduced more than a decade ago. Since then, it has been used to perform a large number of tuberculosis pharmacokinetics/pharmacodynamics (PK/PD) studies that have not been subjected to systematic analysis.

Methods. We performed a literature search to identify all HFS-TB experiments published between 1 January 2000 and 31 December 2012. There was no exclusion of articles by language. Bias minimization was according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Steps for reporting systematic reviews were followed.

Results. There were 22 HFS-TB studies published, of which 12 were combination therapy studies and 10 were monotherapy studies. There were 4 stand-alone Monte Carlo experiments that utilized quantitative output from the HFS-TB. All experiments reported drug pharmacokinetics, which recapitulated those encountered in humans. HFS-TB studies included log-phase growth studies under ambient air, semidormant bacteria at pH 5.8, and nonreplicating persisters at low oxygen tension of ≤10 parts per billion. The studies identified antibiotic exposures associated with optimal kill of Mycobacterium tuberculosis and suppression of acquired drug resistance (ADR) and informed predictions about optimal clinical doses, expected performance of standard doses and regimens in patients, and expected rates of ADR, as well as a proposal of new susceptibility breakpoints.

Conclusions. The HFS-TB model offers the ability to perform PK/PD studies including humanlike drug exposures, to identify bactericidal and sterilizing effect rates, and to identify exposures associated with suppression of drug resistance. Because of the ability to perform repetitive sampling from the same unit over time, the HFS-TB vastly improves statistical power and facilitates the execution of time-to-event analyses and repeated event analyses, as well as dynamic system pharmacology mathematical models.

Keywords. tuberculosis; PK/PD; hollow fiber system; quantitative drug development tool; Monte Carlo experiments.

There are an estimated 8.8 million new cases of tuberculosis each year worldwide. Although antibiotic therapy is available, the treatment is 6 months in duration for the simplest of cases and 2 or more years in multidrug-resistant (MDR) or extensively drug-resistant (XDR) cases [1,2]. Thus, an urgent need exists for new treatment regimens that will optimize antibiotic activity of currently available agents, cure patients in weeks rather than months, and reduce the number of patients with drug-resistant disease. To achieve this, nonclinical drug development tools (DDTs) with demonstrated predictive accuracy for clinical and microbial outcomes are needed. Since the time of Robert Koch and Paul Ehrlich, animal models have been the preferred nonclinical DDTs for tuberculosis. The in vitro hollow fiber system model of tuberculosis (HFS-TB) was introduced as a DDT for tuberculosis at the Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC) in Chicago in September 2003 (abstract A-1156), followed by a journal publication in 2004 [3].
The operational details for the HFS-TB have been described in detail elsewhere [3–5]. In brief, the HFS-TB was designed to give a quantitative understanding of the relationship between dynamic drug concentrations, as well as dynamic populations of drug-susceptible and drug-resistant *Mycobacterium tuberculosis* over time. The drug concentration-time profiles are designed to mimic those in humans, whereas the conditions in which *M. tuberculosis* is cultured are designed to mimic certain stress conditions encountered in vivo as well as normal in vitro culture conditions to identify differences in antibiotic effect that may translate to specific bacterial subpopulations. Thus, the HFS-TB consists of a pharmacodynamic compartment (also referred to as the peripheral compartment) and a pharmacokinetic compartment that consists of a central compartment that allows drug to equilibrate with the contents of the peripheral compartment via diffusion across semipermeable membranes called hollow fibers. The peripheral (“pharmacodynamic”) compartment also houses *M. tuberculosis*, which can be maintained for several months. Over the past decade, more experiments of pharmacokinetics/pharmacodynamics (PK/PD) of antituberculosis drugs have been performed with the HFS-TB model than with animal studies, based on an in-depth landscape analysis we performed in the past [4, 6]. Starting with the first HFS-TB study, Ulam and Metropolis’s Monte Carlo experiments were applied to the quantitative output of the model to bridge results from the laboratory to populations of patients [3, 7–9]. Based on these laboratory and simulation experiments, the Critical Path to Tuberculosis Drug Regimens initiated a regulatory pathway for qualification of the HFS-TB, including for voluntary exploratory data submission at the Food and Drug Administration and a submission for a qualification opinion at the European Medicines Agency. As part of the data submission, we performed a systematic analysis of all HFS-TB experiments performed up to the end of 2012. This manuscript presents a formal examination of that systematic analysis.

**METHODS**

**Objective**

The objective of the present study was to perform a systematic analysis of all HFS-TB laboratory experiments and Monte Carlo simulations based on the output of the HFS-TB model, to evaluate the predictive capability of the HFS-TB, the types of quantitative output from the model, and its advantages.

**Literature Search Strategy and Selected Studies**

We performed a literature search to identify all HFS-TB experiments that were published, or presented at international scientific meetings. Two authors (T. G., J. G. P.) searched PubMed, Embase, ISI Web of Science, and the Cochrane Libraries for studies published beginning 1 January 2000, the year that the first HFS-TB experiments were performed, through 31 December 2012. The Medical Subject Heading terms and strategy used included hollow fiber OR hollow fibre AND either tuberculosis OR mycobacterium OR mycobacteria. Bibliographies of original articles, key reviews of tuberculosis PK/PD studies, and consensus statements were also examined for additional relevant studies. A manual search of meeting abstracts was then conducted for ICAAC, the annual conference of the Infectious Diseases Society of America, the Gordon Research Conferences (Tuberculosis Drug Development), and the first to fifth 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; http://www.opengrey.eu). Where conference abstracts were found, but a full study was published later in a scientific journal, the full study was taken as the main reference (and thus the final publication date may be after 31 December 2012). HFS-TB or Monte Carlo simulation experiments in which the quantitative output from the HFS-TB was used met inclusion criteria. Two authors (T. G., J. G. P.) independently extracted the data into the prespecified table format. Consensus was reached for study inclusion after the discussion of each study. There was no exclusion of articles by language. Bias minimization was according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Steps for reporting systematic reviews were followed.

**Data Synthesis and Analysis**

Findings of the initial search were presented to the full group of investigators who examined them and sought further publications where they could be found. A systematic synthesis of the extracted data was then performed with a focus on hypotheses examined, number of drugs or regimens tested, qualitative and quantitative output of each experiment, and conclusions drawn from the experiments by the authors.

**RESULTS**

The literature search identified 26 HFS-TB studies, including 4 Monte Carlo simulations based on HFS-TB output [3, 10–36]. The full list of studies, characteristics, and drugs studied are shown in Table 1. The majority of studies were combination therapy HFS-TB studies (12 studies), 10 were monotherapy studies, and 4 studies used Monte Carlo methods. While experiments during the first 1–2 years lasted for about 14 days of therapy initially with log-phase growth cultures, the experiments were soon extended to at least 28 days of therapy and 56 days for sterilizing effect. The longest therapy duration was 6 months with moxifloxacin [37]. Several study designs were employed including (1) dose-effect studies (10 studies), (2) dose-scheduling...
Table 1. Publications on the Hollow Fiber System Model of Tuberculosis

<table>
<thead>
<tr>
<th>Study Reference</th>
<th>Regimen/Drug</th>
<th>Mycobacterium tuberculosis Physiologic State</th>
<th>Findings and Conclusions</th>
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<tbody>
<tr>
<td>Monotherapy studies</td>
<td></td>
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<tr>
<td>Gumbo et al [3]</td>
<td>Moxifloxacin</td>
<td>Log-phase growth</td>
<td>Biphasic kill; ADR; first study to use systems/ mathematical models in PK/PD of antituberculosis drugs; identification of optimal moxifloxacin dose</td>
</tr>
<tr>
<td>Gumbo et al [13]</td>
<td>Ciprofloxacin</td>
<td>Log-phase growth</td>
<td>Standard doses lead to rapid emergence of quinolone resistance; biphasic kill; ciprofloxacin likely to be ineffective and should be replaced by moxifloxacin</td>
</tr>
<tr>
<td>Gumbo et al [15]</td>
<td>Isoniazid</td>
<td>Log-phase growth</td>
<td>Use of slow and fast acetylator pharmacokinetics; first study to show role of efflux pumps in drug tolerance as part of ADR and why INH effect ceases after 3 d</td>
</tr>
<tr>
<td>Gumbo et al [10]</td>
<td>Isoniazid</td>
<td>Log-phase growth</td>
<td>Slow/rapid INH pharmacokinetics mimicked; INH PK/PD indices identified; ADR described by series of U-shaped curves; 300 mg/d inadequate for optimal kill in some ethnic populations</td>
</tr>
<tr>
<td>Gumbo et al [14]</td>
<td>Rifampin</td>
<td>Log-phase growth</td>
<td>Rifamycin half-life has little relevance to efficacy; RIF efficacy measures such as resistance suppression and postantibiotic effect are driven by peak/MIC; Microbial kill linked to AUC/MIC; standard human doses are inadequate for ADR suppression and optimal microbial kill</td>
</tr>
<tr>
<td>Gumbo et al [11]</td>
<td>Pyrazinamide</td>
<td>Semidormant/acidic</td>
<td>New in vitro model for examination of sterilizing effect; derivation of quadratic function describing drug concentration vs ADR; for optimal kill, doses &gt;60 mg/kg rather than current 15–30 mg/kg identified in Monte Carlo simulations</td>
</tr>
<tr>
<td>Musuka et al [16]</td>
<td>Thioridazine</td>
<td>Semidormant and log-phase growth</td>
<td>Efficacy is driven by peak/MIC and AUC/MIC; observation of wobbly PK/PD parameters; efficacious doses are in ranges toxic to humans</td>
</tr>
<tr>
<td>Drusano et al [33]</td>
<td>Moxifloxacin</td>
<td>Nonreplicating persisters/ hypoxia</td>
<td>Moxifloxacin kills Mtb in NRP state at rates higher than expected</td>
</tr>
<tr>
<td>Drusano et al [34]</td>
<td>Rifampin</td>
<td>Nonreplicating persisters/ hypoxia</td>
<td>In vitro HFS-TB model of NRP, recapitulated RIF relapse, and failure rates observed in actual patients: 12.7% vs 14%</td>
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<tr>
<td>Combination therapy studies</td>
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<tr>
<td>Drusano et al [12]</td>
<td>Rifampin plus moxifloxacin</td>
<td>Nonreplicating persisters/ hypoxia and log-phase growth</td>
<td>RIF plus moxifloxacin synergistic in resistance suppression but antagonistic in microbial kill</td>
</tr>
<tr>
<td>Srivastava et al [21]</td>
<td>Ethambutol/isoniazid (alone and in combination sequentially)</td>
<td>Log-phase growth</td>
<td>Ethambutol monotherapy and in combination with INH examined; Multiphase ethambutol/INH 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</td>
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<tr>
<td>Srivastava et al [29]</td>
<td>Rifampin plus isoniazid plus pyrazinamide</td>
<td>Semidormant/acidic and log-phase growth</td>
<td>Established the PK variability hypothesis of therapy failure and ADR; predicted the proportions of patients who would have a 2-mo sputum conversion rate and ADR despite 100% adherence; first in vitro study to interrogate public health policies used by tuberculosis programs</td>
</tr>
<tr>
<td>Srivastava et al [19]</td>
<td>Rifampin plus isoniazid</td>
<td>Semidormant/acidic and log-phase growth</td>
<td>Disproved the PK mismatch hypothesis; deliberate mismatch of RIF and INH leads to improved microbial kill</td>
</tr>
<tr>
<td>Drusano G et al [22]</td>
<td>Rifampin plus moxifloxacin</td>
<td>Log-phase growth</td>
<td>5/7-d regimen allowed emergence of moxifloxacin resistance while 7/7-d regimen did not</td>
</tr>
<tr>
<td>Srivastava et al [36]</td>
<td>1-, 2-, 3-drug combinations of rifampin, isoniazid, and pyrazinamide compared to effect in 56 patients</td>
<td>Semidormant/acidic and log-phase growth</td>
<td>Microbial kill rates of optimal drug combinations in the in vitro HFS-TB model are the same; pattern and ranking of regimens is the same as in patients in terms of microbial kill; confirmation of the clinically accepted notion that RIF and INH in combination prevent resistance development to each other</td>
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</tbody>
</table>
(aka dose fractionation) studies (4 studies), and (3) antibiotic sequencing studies (2 studies). The HFS-TB studies were performed in 4 laboratories in Texas, Maryland, and New York.

Table 1 also shows the different culture conditions in the peripheral (pharmacodynamic) compartment that were used in the published studies. Figure 1 shows the number of published studies for log-phase growth bacteria under ambient air (log-phase growth), semidormant bacilli under acidic conditions (pH 5.8), or nonreplicating persisters at an oxygen tension of 10 parts per billion or lower [11, 12]. The bacterial growth rates in these conditions, as measured by a live/dead cell viability assay, were 60.72 (95% confidence interval [CI], 33.76–87.68) relative fluorescence units (RFU)/hour for log-phase growth bacilli; 7.48 (95% CI, 3.06–11.90) RFU/hour for semidormant bacilli; and 4.72 (95% CI, 3.56–10.01) RFU/hour for nonreplicating persisters. Quantitation of growth rates using colony-forming units (CFU) per mL on Middlebrook 7H10 agar were 0.069 (95% CI, 0.059–0.079) log10 CFU/mL/day for log-phase growth bacilli; 0.013 (95% CI, 0.007–0.018) log10 CFU/mL/day for semidormant bacilli; and 0.005 (95% CI, 0.001–0.009) log10 CFU/mL/day for nonreplicating persisters [16].

All 22 HFS-TB experiments reported actual drug exposures that were measured in the HFS-TB and not merely the intended drug exposures. The studies measured drug concentrations in the central compartment from at least 6 sampling time points in a 24-hour dosing period and up to 12 time points in 48-hour dosing intervals. An example with moxifloxacin is shown in Figure 2. Reports included concentration-time profiles encountered, compartmental pharmacokinetic parameters of the drugs, peak concentration, peak to minimum inhibitory concentration (MIC), area under the concentration-time curve (AUC), AUC/MIC, and percentage of time that the concentration exceeded

### Table 1 continued.

<table>
<thead>
<tr>
<th>Study Reference</th>
<th>Regimen/Drug</th>
<th>Mycobacterium tuberculosis Physiologic State</th>
<th>Findings and Conclusions</th>
</tr>
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<tbody>
<tr>
<td>Okusanya et al [24]</td>
<td>Rifampin/linezolid (alone and in combination)</td>
<td>Nonreplicating persisters/hypoxia</td>
<td>Rif and linezolid were additive, demonstrating potential use for sterilizing effect</td>
</tr>
<tr>
<td>Xue et al [28]</td>
<td>Rifampin/linezolid/ PNU100480/PNU-101603 (alone and in combination)</td>
<td>Log-phase growth</td>
<td>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</td>
</tr>
<tr>
<td>Okusanya et al [25]</td>
<td>Rifampin/PNU-100480/ PNU-101603 (alone, and combined)</td>
<td>Nonreplicating persisters/hypoxia</td>
<td>PNU-101603 and not the parent compound (PNU-100480) enhanced the sterilizing effect of Rif</td>
</tr>
<tr>
<td>Louie et al [23]</td>
<td>PNU-100480 plus Rifampin</td>
<td>Log-phase growth</td>
<td>PNU-100480 plus its metabolite (PNU-101603) together with Rif 600 mg/day synergistically killed Mtb; The combination of PNU metabolite and Rif prevented ADR to PNU or Rif alone</td>
</tr>
<tr>
<td>Louie et al [31]</td>
<td>PNU-100480 plus PNU-101603</td>
<td>Log-phase growth</td>
<td>PNU-100480 at 600 mg every 12 h was as effective as a continuous infusion of 1200 mg/d. 600 mg every 12 h plus Rif 600 mg once daily sterilized the HFS-TB and prevented ADR</td>
</tr>
<tr>
<td>Louie et al [32]</td>
<td>PNU100480/PNU-101603/ rifampin, alone and combined</td>
<td>Log-phase growth</td>
<td>PNU-100480 was synergistic with the major metabolite (PNU-101603) in killing Mtb but did not prevent ADR</td>
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Monte Carlo simulation studies

- Gumbo T [17]: Rifampin, isoniazid, pyrazinamide, ethambutol, moxifloxacin
- Not applicable
- Set new susceptibility breakpoints for Rif, INH, and pyrazinamide and confirmed those for moxifloxacin and ethambutol

- Jeena et al [18]: Isoniazid
- Not applicable
- Selection of optimal isoniazid doses in tuberculosis meningitis, disseminated tuberculosis, and pulmonary tuberculosis in children <10 years old

- Goutelle et al [26]: Rifampin
- Not applicable
- Selection of Rif optimal dose for microbial kill and resistance suppression in pulmonary tuberculosis

- Goutelle et al [27]: Rifampin
- Not applicable
- Full mathematical model on the effect of Rif from first day to last day of therapy suggested need to increase Rif dose above 600 mg/d

Abbreviations: ADR, acquired drug resistance; AUC, area under the concentration-time curve; HFS-TB, hollow fiber system model of tuberculosis; INH, isoniazid; MIC, minimum inhibitory concentration; Mtb, Mycobacterium tuberculosis; NRP, nonreplicating persister; PK/PD, pharmacokinetics/pharmacodynamics; RIF, rifampin.
MIC. The calculated pharmacokinetic parameters mimicked those in patients to a very high degree in both monotherapy studies and combination therapy studies.

All 22 studies employed repetitive sampling of the pharmacodynamic, or peripheral, compartment starting just prior to first drug infusion and then on days 3 (some studies), 7, and 10 (some studies), and then every 7 days after day 7. The HFS-TB output included total \(M. \text{tuberculosis}\) burden (log\(_{10}\) CFU/mL), the log\(_{10}\) CFU/mL of a subpopulation resistant to a drug (selected by growth on antibiotic supplemented Middlebrook 7H10 agar), and the log\(_{10}\) CFU/mL of drug-resistant subpopulations on agar supplemented with efflux pump inhibitors (reserpine, thioridazine, and verapamil) in addition to drug. These samples were used to calculate rates of bactericidal effect, rates of emergence of drug resistance, and rates of emergence of efflux pump–related resistance and tolerance. These outputs were also examined using the Hill equation to calculate drug exposure associated with optimal microbial kill or in the quadratic function model for relationship between drug exposure and drug-resistant subpopulation to identify exposure associated with suppression of drug resistance [11]. These optimal exposures are AUC/MIC, or peak/MIC or percentage of time above MIC values that become useful as targets for dose-finding studies in the clinic. These exposure targets constituted primary predictions from HFS-TB experiments, to be tested for accuracy later in human clinical trials.

Drug concentrations, total bacillary population, and drug-susceptible and drug-resistant subpopulations were co-modeled in a series of inhomogeneous differential equations reflecting change with time based on repetitive sampling [3, 14, 15]. These are in essence systems equations and allow the HFS-TB quantitative output to be integrated into systems pharmacology and engineering simulations [39, 40].

The HFS-TB was used to perform computer-aided clinical trial simulations based on Monte Carlo experiments. Some studies utilized HFS-TB output such as AUC/MIC and peak/MIC relationships to predict the effect of different clinical antibiotic doses in achieving optimal exposures, to identify rates of sterilizing effect in sputum, and to predict the proportion of patients on standard triple therapy who would develop acquired drug resistance (ADR) as a result of pharmacokinetic variability [3, 10, 11, 29]. Four were stand-alone Monte Carlo experiments that examined target attainment for different doses in patients based on optimal exposure targets identified in the HFS-TB or derivation of new drug resistance breakpoints [17, 18, 26, 27].

DISCUSSION

Over the last decade, 22 HFS-TB experiments have been published. The studies demonstrate several advantages of the HFS-TB compared to current preclinical DDTs for evaluating efficacy, resistance potential, and dose determination. First, the HFS-TB has the capacity to simulate human PK/PD of a drug or drug combination. This is strengthened by the capability for iterative and repetitive sampling for simultaneous quantitative measurement of both organism and drug concentrations in the HFS-TB. 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 that can then be directly translated into more effective clinical trial designs.

Second, the HFS-TB has been used to determine bactericidal and sterilizing effect (ie, microbial kill) rates, likelihood of resistance emergence, and effects of drug combinations, which can be compared to those in sputum cultures of patients. The HFS-TB model has the advantage that the microbial subpopulations important in sterilizing effect (ie, nonreplicating persisters and semidormant bacilli) can be studied separately from log-phase growth subpopulations. This allows for more accurate identification of microbial kill rates, resistance emergence within each subpopulation, and differential effects of antibiotics, which are all important in the design of regimens that would shorten therapy duration.

Third, the ability to culture the entire contents of the peripheral (pharmacodynamic) 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 *M. tuberculosis* at early time points such as 1 or 2 months. This is because specific populations of nonreplicating persisters and semidormant bacilli are separately inoculated into the HFS-TB and kept in that state under antibiotic therapy. The kill rates of those specific subpopulations can then be followed with repetitive sampling until extinction of the subpopulation (ie, total sterilization) as documented by cultures and molecular methods (for noncultivable bacilli). When new regimens are advanced to phase 3 trials, a decision must be made about the treatment duration necessary to reach an acceptable cure rate. Such decisions are often informed by in vivo efficacy studies comparing candidate regimens to the standard of care using the proportion of animals relapsing after different treatment durations, an endpoint used because it is not currently possible to reliably quantify the viable but noncultivable bacterial population present at the end of treatment. Culture- and non-culture-based methods of quantifying a similar population in the HFS-TB model could enable better prediction of the treatment duration necessary for cure and thereby inform time points for evaluating proof of efficacy in clinical trials. By not relying on relapse after treatment completion, such HFS-TB experiments could be 3 or more months shorter than in vivo experiments studying sterilizing activity.

Fourth, the ability to recapitulate human pharmacokinetics in the HFS-TB is an improvement on standard static concentration in vitro models or serial passaging, typically employed to assess resistance potential for drugs or drug combinations. Drug instability or degradation issues, inherent with some small molecules such as rifampin and carbapenems, for example, are obviated in the dynamic HFS-TB model. Moreover, the actual shape of the concentration-time curve of some antituberculosis drugs (ie, the stressor) is an important determinant of microbial effects such as ADR. The HFS-TB model also overcomes the effect of interspecies differences in systemic clearance of drugs and volume of distribution (hence exact shape of concentration-time profile) between some in vivo models and humans, which could have a bearing on ADR and microbial kill for some drugs [13, 21, 30, 41, 42].

Fifth, repetitive sampling from the same HFS-TB unit offers another advantage over in vivo models, which rely on terminal procedures to obtain samples to be cultured. The HFS-TB allows repetitive measurement of total bacillary burden, drug-resistant subpopulations, and drug concentrations over the course of the experiment. This vastly improves statistical power, facilitates the execution of time-to-event analyses and repeated event analysis, and uniquely allows for the construction of dynamic system pharmacology models. It should be noted that in the clinic, patients undergo repetitive sampling of sputum (a form of liquid culture), which with technology such as the Mycobacteria Growth Indicator Tube liquid cultures routinely employed in clinical practice, allow estimation of *M. tuberculosis* burden via use of the time-to-positivity index [43, 44].

Sixth, antituberculosis drugs exhibit peak and AUC concentration-dependent synergy and antagonism in patients [45, 46]. HFS-TB experiments with 2–3 drug combinations, at different doses for each drug and with different dosing order or drug sequencing permutations, and enables identification of dose- and concentration-dependent synergy or antagonism [12, 19–25, 28, 29, 32, 36]. This facilitates the design of optimal drug combination regimens, at the correct doses, which have the potential to shorten therapy duration.

Finally, the 26 HFS-TB studies and Monte Carlo simulations made a number of predictions (Table 1). The first were predictions of optimal drug concentration exposures associated with optimal efficacy for standard therapy drugs as well as for experimental drugs. The second set of predictions was related to concentrations associated with resistance suppression and the proportion of patients likely to develop ADR at particular doses in particular combinations. The third set of predictions involved the best dosing schedule, hence the PK/PD driver of efficacy of the different drugs. The fourth set of predictions was the proportions of patients likely to develop ADR at a particular AUC/MIC or peak/MIC given a particular dose of drug and given the expected pharmacokinetic and MIC variability. The fifth set of predictions focused on drug resistance breakpoints that could differentiate patients likely to respond to therapy, which essentially overhauled currently accepted breakpoints. The accuracy of these predictions is examined in an accompanying article [47].

It should be stated that, like all systematic analyses, it is possible that some influential studies were missed in this report. As an example, HFS-TB studies performed by the pharmaceutical industry may not have been published and thus the results were...
not known at the time of publication. Such studies would expand the extent of HFS-TB publications evaluated, but would be unlikely to change the conclusions. In addition, the HFS-TB does not incorporate an immune system, and thus does not take into account the role of the immune system in bacterial clearance. This means that the drug exposures associated with maximal effect derived in the system are conservative. Finally, to support the extensive use of the HFS-TB, it will be important to identify the extent of interlaboratory variability related to quantitative outputs to further support broad application. This is being addressed by ongoing studies.

In summary, we found that the HFS-TB model offers the ability to perform humanlike PK/PD studies, to identify bacterial and sterilizing effect rates, and to identify exposures associated with drug resistance suppression. Repetitive sampling from the same unit allows for better statistical analyses, and construction of systems pharmacology mathematical models.

Notes

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Potential conflicts of interest. T. G. founded Jacaranda Biomed Inc, and is also a consultant for Astellas Pharma USA for antifungal compounds. All other authors report no potential conflicts.

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

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