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

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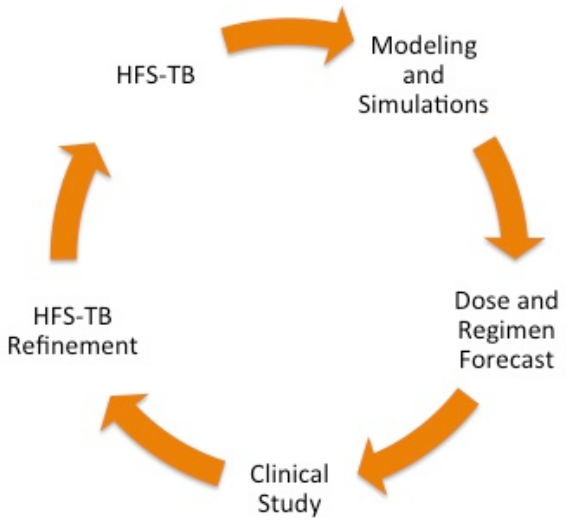
Issue #1: The Applicant is requested to summarise the minimum amount of in-vitro and human PK data (healthy subjects and/or infected patients) that is necessary before commencing use of the HFS-TB to identify i) PK/PD relationships and ii) initial doses and regimens. The Applicant should explain how additional data that emerge during the development programme can be factored in to refine or expand the use of the model. Provision of a diagrammatic algorithm as part of the response would be helpful.

Response: The **<u>minimum</u>** amount of information required is an estimate of the concentration-time profile of a single dose in humans. This can come either from early phase pharmacokinetic studies in patients, such as micro-dosing (so-called phase 0) typically executed in approximately 10 subjects, phase I studies typically performed in 20-100 subjects, or an estimate from animal models based on allometric scaling. With such data, dose-effect studies and dose-scheduling studies can be performed in the HFS-TB and integrated through Monte Carlo Simulation. The output based on these minimal inputs is (a) range of drug concentration exposure associated with optimal microbial kill, (b) range drug concentration exposure associated with resistance suppression, and (c) optimal dosing schedule and (d) covering ranges of drug exposure profiles encountered in humans. The same minimum data is also required for combination therapy.

Data emerging during development can be incorporated as part of an iterative process, starting with quantitative output from the HFS-TB indicating the range of drug exposures and range of bacterial responses, followed by modeling and simulation, prediction of clinical doses and optimal regimen, followed by Phase II clinical studies to assess microbial responses in sputum, and then feedback into the model to expand various aspects of it such as taking into account pharmacokinetic variability encountered in patients, special microbial populations (e.g., intracellular Mycobacterium tuberculosis), and the kill curves that were encountered in patients.



Issue #2: The Applicant should discuss whether and how the HFS-TB could take into account drug concentrations that may occur at the site(s) of infection and other factors that could affect drug activity and/or organism susceptibility (e.g. physiological conditions).

Response: The HFS-TB takes into account the drug penetration into the site of infection through modelling of minimal and maximal drug concentrations that may be achieved in patient blood or other body compartments. When concentration-time profiles at the site of infection are known (from clinical or preclinical data), these profiles are utilized in the HFS-TB. As regards to evaluating the impact of differing physiological conditions on drug exposure-response relationships, the HFS-TB is actually a collection of several systems whereby *M tuberculosis* is either: (a) in log-phase growth under ambient air, (b) semi-dormant under acidic conditions, (c) in a state of non-replicating persistence (NRP), or (d) residing in macrophages. These systems are thought to be representative of the various mycobacterial stages encountered in patients and can thus model the range of responses to drug concentrations that should be covered in patients to maximally affect all Mtb population types. Thus one of the advantages of this system is that these different simulated physiological conditions can be isolated and studied in relation to dose-effect and dose-scheduling and later integrated to arrive at optimal doses/drug exposure profiles that maximize microbial kill and minimize the occurrence of resistance. Moreover, *M tuberculosis* strains with differing drug susceptibilities (e.g., as measured by minimum inhibitory concentrations (MICs)), as well as different phylogenetic lineages, can also be examined in the HFS-TB.

Issue #3: The Applicant should further justify the ability of the HFS-TB to evaluate antibacterial activity against non-log phase organisms.

Response: See response to issue #2. Indeed, more experiments have been performed with non-log phase growth *M tuberculosis* compared to log-phase. This has been done in order to better mimic the bacterial populations that are thought to be present in lung lesions under different pH conditions as well as under hypoxia. For these studies, the pH and oxygen tension are measured throughout the experiment, the physiologic state of the bacteria (semi-dormant or NRP or intracellular) is confirmed using molecular methods when the bacteria are sampled, as is the lack of susceptibility to INH in the case of NRP. The concentration-time profiles to which these physiological states are exposed have been validated by measuring the actual concentrations achieved in each HFS-TB. While these physiological states are approximations, they nevertheless allow us to sample bacterial states with differential responses to anti-TB drugs and therefore allow establishment of ranges of dosing that have to be present to maximize killing.

Issue #4: The Applicant should discuss to what extent they perceive that the HFS-TB could i) minimise the need for non-clinical efficacy data ii) reduce the need for clinical dose-finding studies and iii) shorten the duration of the drug development programme.

Response: A primary purpose of non-clinical efficacy studies is to generate proof-of-concept (POC) of drug and regimen efficacy and sufficient evidence of a PK/PD relationship to support progression to clinical trials to further evaluate said drug and regimen. The HFS-TB is not intended to replace preclinical

and clinical studies but to help reduce their number and produce "portable endpoints" that can be assessed *in vitro*, animal studies and human patients (i.e. drug exposure profiles and corresponding bacterial responses). The CPTR team considers that the evidence generated to date supports the HFS-TB as a drug development tool for generation of initial non-clinical efficacy data and suggested doses in a cost-effective manner that are then evaluated in, and inform the efficient design of *in-vivo* studies in less tractable disease models to confirm proof of concept and PK/PD relationships. The HFS-TB may also have a prominent role in further iterations of non-clinical studies to fully delineate PK/PD relationships and optimize trial design. As it is perhaps the most tractable dynamic non-clinical PK/PD model, the HFS-TB is expected to minimize the need for extensive dose-finding studies in animal models and clinical trials and thereby shorten the duration of drug development programmes. Additionally, the use of HFS-TB may help design animal efficacy study regarding dose levels and schedules, and help preclinical combo-study design with optimal dose and dose schedules. Recent experiences in which repeated phase 2 dose-finding trials were required to identify the optimal doses of PA-824 and rifapentine illustrate that in the absence of hypothesis generating preclinical studies such as the HFS-TB, extensive iterative clinical trials are needed which prolong clinical development programs (Diacon AH, Dawson R, du Bois J, Narunsky K, Venter A, Donald PR, van Niekerk C, Erondu N, Ginsberg AM, Becker P, Spigelman MK. Phase II dose-ranging trial of the early bactericidal activity of PA-824. *Antimicrob. Agents Chemother*. 2012; 56(6):3027-31.) The fact that the optimal dosing strategies remain incompletely defined for 4 of the 5 drug classes most commonly used in TB treatment (i.e., isoniazid, rifamycins, pyrazinamide, fluoroquinolones) further illustrates the need for more effective delineation of PK/PD relationships and dose optimization in TB drug and regimen development. In this respect, several of the higher doses of standard drugs being studied in ongoing large clinical trials were actually first derived in the HFS-TB.

Issue #5: Based on the retrospective searches conducted the Applicant proposes that HFS-TB has a high predictive accuracy for clinical trial outcomes. The Applicant should summarize instances in which the predictions arising from use of the HFS-TB did not correlate well with clinical findings and discuss the possible reasons. In particular, to identify and discuss any instances in which the HFS-TB has underestimated or overestimated the mycobacterial responses in patients.

Response: First, the retrospective data was not used to calculate predictive accuracy and bias (i.e., systematic over-calling or under-calling in the prediction). The definition of prediction we proposed is based on comparing outcomes in clinical studies at a time t_2 to predictions at an earlier time (t_1) by the HFS-TB. The definition we chose depends on first identifying the error in estimating a quantity identified in the clinic (say dose =4.5g5g/day) at least 6 months after the HFS-TB prediction (say 4.0g/day0g) by simply subtracting the forecast value from the true value (0.5g) and dividing by the true value (clinical observation is the gold standard or true value). This is also used to calculate bias. The results are then summated for all studies, using weighting procedures that include quality of clinical study and size of clinical study (the larger the number of patients, the more accurate the calculation). What follows is the mathematical description of the same steps.

As described in the Briefing Book, error (E) was defined as the observed results in a clinical study at time T, minus the predicted value P:

E= T-P

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:

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

Accuracy (A) was defined as:

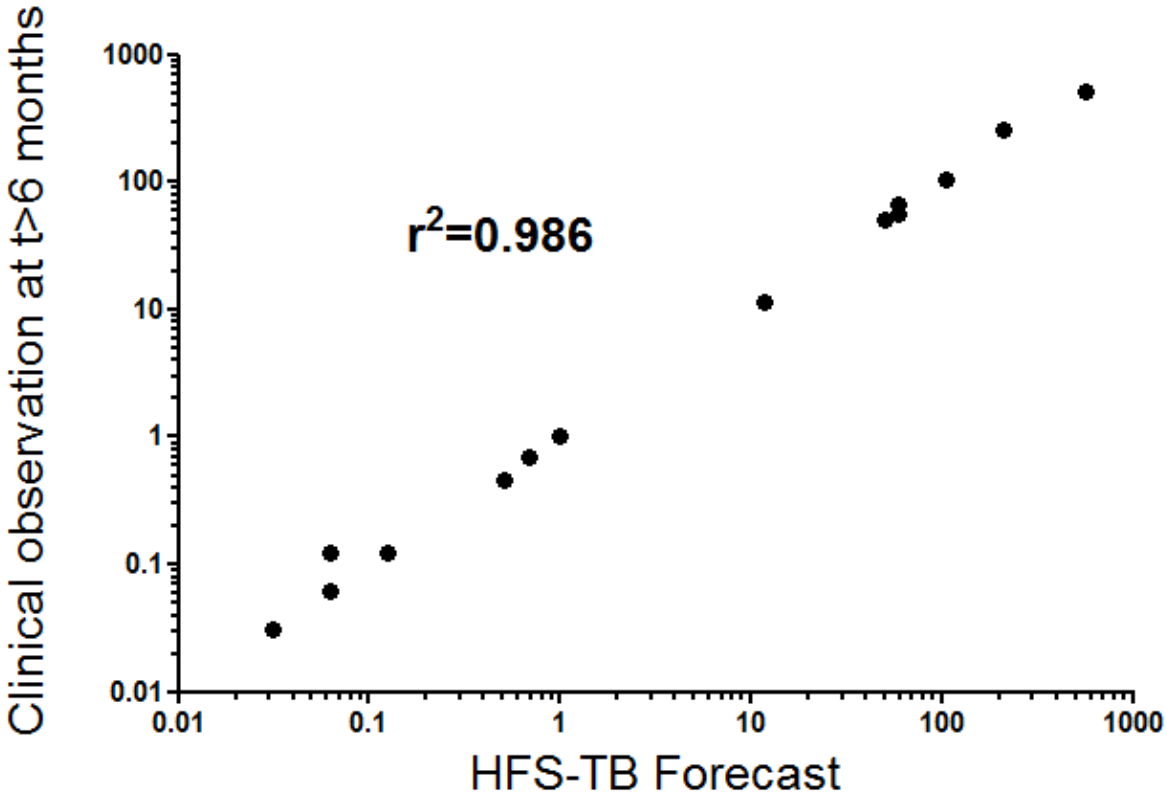
A=100%-MAPE

Bias (B) was defined as

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

As per these definitions, the predictive accuracy value was 94.4% (CI=84.3-99.9%), with a bias of 1.8% (CI=-13.7-6.2%). As can be seen in the graph below, the correlation between the HFS-TB-predicted versus clinically-observed results showed a determination coefficient of r^2=0.986.

HFS-TB predicted vs. Clinic Observed



In the analysis for predictive accuracy, the 94% accuracy figure means that the HFS-TB was accurate to within 94% of the value of a dose or optimal drug concentration, or susceptibility breakpoint. It does not mean the system was accurate 94% of the time. As an example, the HFS-TB and Monte Carlo Simulations (MCS) forecast that optimal pyrazinamide concentration at site of infection was an AUC/MIC of 209, or 11.7 if serum drug concentrations are used as an estimate of exposure. Four years later, a prospective clinical study found not only that AUC/MIC was the PK/PD driver for pyrazinamide, but that the optimal serum AUC/MIC was 11.3. In this case, this is 96.5% forecast accuracy. The bias in the HFS-TB forecasting has been calculated and is 1.8% (CI=-13.7-6.2%). Since this crosses "zero" it means there is very little over-prediction or under-prediction.

The question asked is about identifying and discussing any instances in which the HFS-TB has underestimated or overestimated the mycobacterial responses in patients based on the retrospective study. As discussed above, the CPTR team retained the definition of bias as pertaining to predictions in which a HFS-TB study made a prediction, and then at least 6 months later a clinical study published results pertaining to the prediction. For retrospective studies, the CPTR team prefers to classify those as correlations as opposed to predictions. An interesting example of the interaction of correlation and prediction is demonstrated in a set of innovative HFS-TB experiments published by Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. *J Infect Dis*. 2011 Dec 15;204(12):1951-9. This study made two thought provoking assertions based on HFS-TB findings and Monte Carlo Simulations. First, it showed that MDR-TB does not arise due to non-adherence. Next, it showed that the main driver of MDR-TB was pharmacokinetic variability. Then it predicted that in Western Cape in South Africa MDR-TB would arise in 0.68% of patients despite 100% adherence to standard dosing of the first-line regimen. This conflicted sharply with all prior studies up to that time, most of which had been based on retrospective analyses from observational studies. Indeed, this was the basis of directly observed treatment, short-course (DOTS) and appeared to be a robust concept. On the basis of the concept alone, the HFS-TB results appeared not to correlate with the accepted notion that missing doses of anti-TB drugs was directly correlated to poor treatment outcomes through the development of drug resistance and that assuring that patients take their drugs would result in favorable outcomes. Since that time however, several studies have been published, which include one meta-analysis showing that despite DOTS dramatically improving adherence in 8774 patients compared to self-administered therapy in 3708 patients, there was no difference in MDR-TB rates (Pasipanodya JG, Gumbo T. *Clin Infect Dis*. 2013 Jul;57(1):21-31). In a second meta-analysis of prospective clinical studies, pharmacokinetic variability for isoniazid alone was shown to be associated with MDR-TB and therapy failure (Pasipanodya JG, Srivastava S, Gumbo T. *Clin Infect Dis*. 2012 Jul.;55(2):169-77). In a prospective clinical study of 142 Western Cape patients, >91% of therapy failure and 100% of MDR-TB was explained by pharmacokinetic variability. Acquired drug resistance during the first 2 months when there was 100% adherence was 0.7%, similar to 0.68% predicted by HFS-TB two years earlier (Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. *J Infect Dis*. 2013 Nov 1;208(9):1464-73). In a recent large prospective epidemiology study in Morrocco, 91 cases who defaulted were compared 186 controls who did not (Cherkaoui I, Sabouni R, Ghali I, Kizub D, Billioux AC, Bennani K, Bourkadi JE, Benmamoun A, Lahlou O, Aouad RE, Dooley KE. *PLoS One*. 2014 Apr 3;9(4):e93574). Default was not associated with acquired

drug resistance. This interesting example shows that an initially perceived poor correlation between HFS-TB results and accepted notions based on conceptual understanding, proved to be an adequate correlation once data from high-quality clinical studies became available.

There is another experiment, which predicted that drug resistance to INH would arise in 80 hours, due to efflux pump induction and microbial kill of drug susceptible organism, leaving behind drug resistant subpopulation. In studies published up to that, and up to now, early bactericidal activity (EBA) studies have shown no emergence of isoniazid resistance in the sputum of patients that early. We believe that there are two reasons. First, EBA studies are not optimized to detect efflux pump induction, which is transient phenomenon and will disappear if MTB from sputum is first cultured on drug free media, and then tested for resistance. Second, we believe the standard breakpoint used to measure isoniazid is wrong, and thus misses low level resistance. Thus, it remains to be seen in the future if that HFS-TB study was correct or not. However, on the face of it based on the retrospective look it was incorrect based on the parameter of time to emergence of resistance for isoniazid.

Issue #6: The Applicant should present any available data that could further support the claim that the HFS-TB model can select dose regimens and drug combinations that are least likely to select for drug resistant strains. The answer should include a discussion of dose selection if the HFS predicts that much higher doses are needed to suppress the selection for resistant organisms vs. those needed for adequate efficacy.

Response:
Prediction of higher doses:

There are several studies that have demonstrated this. Indeed, the first such demonstration of this was with the HFS-TB in 2004. The first study involved moxifloxacin, which underwent dose-effect studies in the HFS-TB. It was demonstrated that there were drug exposures at which moxifloxacin killed optimally, based on inhibitory sigmoid E_{max} curves and steepest slopes. These exposures are easily achieved with the standard moxifloxacin dose of 400 mg. However, these very exposures were the ones that **amplified** for emergence of resistance. Instead, the concentrations that suppressed resistance were a moxifloxacin-free (non-protein-bound) area under the concentration-time curve from 0 to 24 h to the minimum inhibitory concentration of s 53. For patients taking moxifloxacin doses of 400, 600, or 800 mg/day, the calculated target-attainment rates to suppress drug resistance were 59%, 86%, and 93%, respectively. Thus, a moxifloxacin dose of 800 mg/day is likely to achieve proper M. tuberculosis microbial kill and to suppress drug resistance. However, tolerability of this higher dose remains to be fully evaluated.

A second study entitled “Pharmacodynamic evidence that ciprofloxacin failure against tuberculosis is not due to poor microbial kill but to rapid emergence of resistance”, it was demonstrated that the standard dose had excellent microbial kill effects, but drug resistance would arise within weeks. The study warned that this was likely to occur when ciprofloxacin and ofloxacin were used as second line agents in

multidrug resistance tuberculosis and that extra resistance was inevitable. This was one year prior to publications on XDR-TB.

The example of pyrazinamide exemplifying this point will also be shown during the team presentation.

Issue #7, Part A: The Applicant should provide any accessible additional data that concern the recent use of the HFS-TB to select dose regimens for clinical studies.

Response: Studies to inform dose regimens for Pulmonary TB are ongoing. High dose rifampin and moxifloxacin studies have been completed for tuberculous meningitis and showed survival superiority. The team is also aware of unpublished work with Sutezolid (oxazolidinone). CPTR continues to pursue these data under our CPTR secure data use agreement. TB related studies are not typically performed in-house at pharmaceutical companies, due to BSL-3 requirements.

Issue #7, Part B: The Applicant is requested to discuss how the various uses of the HFS-TB could be further evaluated prospectively.

Response: As part of a quality standards and best practice approach, CPTR will be conducting a more extensive literature search to include newer research and publications that have been published since the time of the data and analysis represented in the briefing book. Newer clinical studies will be incorporated into an updated literature search and analysis. This HFS-TB team intended to update the original search and analysis over-time as new publications and data emerged. These newer studies were published after our cut-off date for search criteria and were performed outside the USA and include newer drugs. For a regulatory qualification/fit for purpose submission for Scientific Opinion, it is expected that an updated literature search will be conducted.

As a recognized expert and lead for multiple publications on the HFS, Dr. Gumbo has been invited by the editors (Drs. Tanya Parish and David Roberts) of *Mycobacteria Protocols* to contribute a chapter on the HFS-TB to the forthcoming third edition. This chapter will enable a more detailed description of methodology than a manuscript form would allow. This will form the basis for the manual of procedures which will be part of the final qualification submission. In addition, a further series of experiments for moxifloxacin and PA-824 are being planned to assess the inter- and intra-lab variability.