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Product Development Scientific Support Department

# Qualification opinion

In-vitro hollow fiber system model of tuberculosis (HSF-TB)

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1 Last day of relevant Committee meeting.
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## Background information as submitted by the applicant[i]

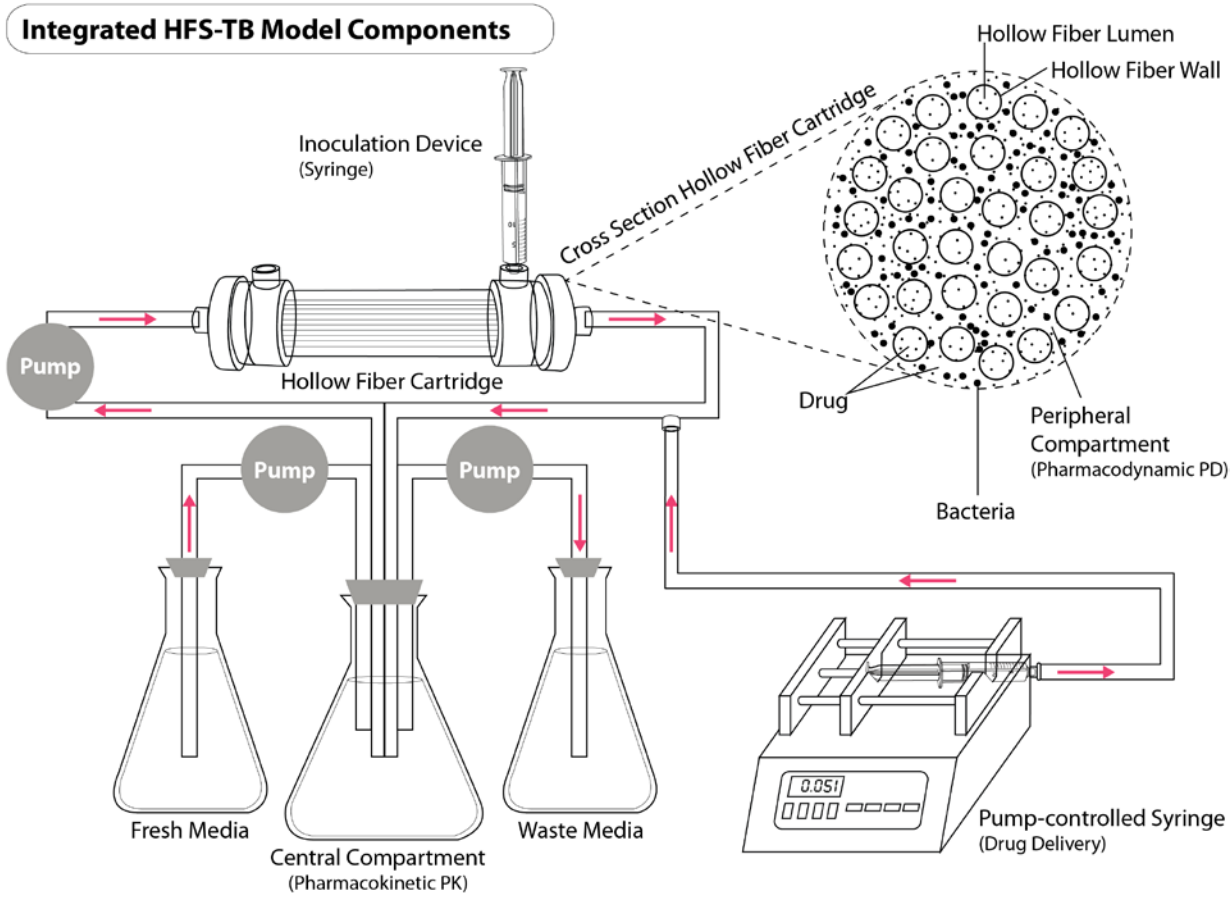
*Background of the CPTR initiative*
The CPTR initiative is a broad collaboration of pharmaceutical companies, government regulatory and multilateral agencies, academia, civil society advocates and non-government organizations that aim to accelerate the development of new, safe and highly effective TB 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.

*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. 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). 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 37°C 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.

*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.

*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. 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.

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*). 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. 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, 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. 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.

- 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. 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. 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.

**Summary of the qualification exercise as submitted by the applicant**

*Methods*

The sponsor 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) of the final dossier submitted. Data and information were extracted from the relevant publications to enable several types of analyses. These analyses are described within the results section of the final dossier submitted (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.

**Scientific Discussion based on the qualification team assessment**
Hollow fiber systems are already in use, not only for the in-vitro study of antimycobacterial products, but also for antivirals and for antibiotics acting against different bacterial species. The system is not intended to replace other experiments or clinical trials, and it should not be a mandatory system for implementation. Although expected to give supportive evidence to a marketing authorisation application, the system should not be considered as pivotal to obtaining a marketing authorisation. It will provide however important information with regard to dose selection, mechanism of action etc. Most likely the data from the HFS-TB will be used in a regulatory context to support clinical trial applications and scientific advice discussions where much of justification for the proof of concept and the dosing regimen may well depend on the data obtained with this model.

The meta-analyses provided by the consortium combined with the totality of evidence in scientific literature and the cases seen in regulatory submissions support the qualification of the HFS-TB system.

Some points of criticism on the qualification exercise that need further follow up from the Consortium are provided below:

- The data presented by the applicant are based on a relatively small number of published studies. Although it remains possible that all studies were not identified by the searches and that in particular non-published materials may not have been accessible, it appears that reasonable efforts were done to avoid bias in the selection of sources. No raw data were presented and all analyses were retrospective. Despite the fact that confirmatory clinical studies should have been published at least 6 months after the predictive HFS-TB study before being used this does not unequivocally reassure that both studies were independent from each other. Also the presented analyses include different PK/PD indices and clinical outcomes. It might be interesting when more data become available to present separate analyses per PK/PD indices - clinical outcomes combinations. Additional prospective data with hypotheses to be tested clearly formulated on beforehand would be of value to strengthen the predictive value of the model. The applicant indicated that two such prospective studies are currently conducted. One study is to establish the use of Moxifloxacin/rifampin/pyrazinamide + isoniazid or ethambutol. The other study will investigate PA824 to document dose scheduling to induce microbial kill and prevent resistance. Both will address log-phase growth, semi-dormant state and intracellular Mycobacteria. An evaluation of the hypotheses tested with the raw data being generated would be welcomed. Sometime has elapsed since the last literature searches were performed by the Consortium and perhaps a new search could identify new data that could be useful to better determine the value of the system. As it can be anticipated that more data will become available independently from the Consortium, it would be advisable to attempt collecting these in order to integrate them in further analyses. The consortium is encouraged to submit new data as indicated above in a follow up qualification opinion.

- It is considered very important to fully appreciate instances in which HFS-TB can over- or under-estimate anti-Mtb activity or fail to predict the clinical situation and to understand the reasons why this may occur (e.g. on the one hand the HFS-TB operates in an “immunosuppressed” state but on the other hand it may not accurately mimic local factors that could impact on antibacterial activity at the site of infection). Thus, it is necessary to consider whether the HFS-TB could lead to a decision not to develop a promising agent because the HFS-TB has underestimated its activity or could inadvertently over-estimate the activity of a regimen against fully susceptible (DS-TB) or MDR/XDR-Tb. It is unlikely that cases of underestimation will be known because they will likely result in premature termination of development of a new product or a particular treatment regimen will not be pursued in a clinical setting. It would however be problematic if useful treatments would not reach clinical practice if the reason would be a shortcoming of the HFS-TB. Although cases of overestimation are not reported for the time being they may exist and early recognition of such over-estimated activity would be needed for the benefit of the patient in clinical trial. When more data become available a better estimate of false negatives and false positives observed with the system could be given. Therefore it remains important that data on this aspect would be regularly updated also including examples that are not published but known to the Consortium. It would also be important to explain failures of the model to predict clinical outcome, because it would allow a

better understanding of the appropriate use of the model, and may indicate those conditions and purposes when it should not be used.

- An important asset of the system is that it can mimic the pharmacokinetic behaviour of a substance in humans. It is however not clear at the moment how the pharmacokinetics of combinations will be mimicked at the same time for all mono-components, in particular if their PK properties are markedly different. This will however need to be considered on a case by case basis and can probably not be addressed in a general way. Another remaining concern is the way the intracellular exposure to anti-Mtb substances is controlled in order to mimic exposure in patients at the site of the infection. If the HFS-TB model is being used, information should at least be available about the extent at which concentrations at the infection site and in particular at intracellular (granuloma) sites are mimicked on a case by case basis. The applicant has indicated that dynamic determinations of concentration-time profiles inside macrophages are possible and mentioned an example with moxifloxacin. The control of concentration-time curves intracellularly could also be systematically addressed in future work.

- The HFS-TB is a dynamic system that allows researchers to study relevant Mtb strains under different metabolic conditions. Organisms in log-phase growth, dormant state or non-replicating persistors can be studied separately. The HFS-TB system can also be used to study the intracellular state of Mtb within macrophages or neutrophils. This possibility is considered an important advantage of the system. It should be documented to a sufficient extent that the organisms were indeed in the desired metabolic state during the experiment.

- Operational characteristics and reproducibility from laboratory to laboratory are important aspects for a validated method. It is acknowledged that to some extent a case by case adaptation may need to be applied. It is also recognised that usually methods to determine pharmacodynamic actions may be less standardised than for instance safety pharmacology and toxicology studies. Irrespective of the experimental procedures and materials the prediction of the doses and regimens to be used clinically should be comparable. It is clear that at least sufficient controls must be built into tests run with HFS-TB and that within a laboratory procedures and materials should be well described and justified for the purpose of the study. Nevertheless, the applicant will make a standard operating procedures lab manual available and a limited series of experiments with Moxifloxacin and PA824 in triplicate are being planned to assess inter- and intra-laboratory variability. This is a welcomed standardisation that may contribute to wider usage of the HFS-TB system. The consortium is encouraged to submit these data in a follow up qualification opinion.

Regardless of the limitations of the data submitted, the totality of evidence in scientific literature and the cases seen in regulatory submissions support the qualification of the HFS-TB system.

**CHMP Qualification Opinion**

The HFS-TB is qualified to 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, including combination of 2 or more anti-Mtb drugs, to maximize bactericidal effects and minimize emergence of drug resistance. HFS-TB can be used in regulatory submissions throughout the drug development process for a product, especially for more informed design and interpretation of Phase I, Phase II and Phase III clinical studies. It should be noted that the qualification opinion does not mandate the use of the HFS-TB or exclude the use of alternative methods in the confined setting.

More specifically CHMP recommends that the HFS-TB may be useful as follows:

- To provide preliminary proof of concept for developing a specific drug or combination to treat tuberculosis
- To select the pharmacodynamic target (e.g. T/MIC, AUC/MIC)
- To provide data to support PK/PD analyses leading to initial dose selection for non-clinical and clinical studies, with the aim of limiting the number of regimens that are to be tested in vivo; it is anticipated that HFS-TB may be used to limit doses tested both in single drug and combination regimen studies in vivo
- To assist in confirming dose regimens for later clinical trials taking into account the accumulated human PK data in healthy volunteers and then patients as well as available information on exposure-response relationships

Annexes

- Applicant submission – Request for CHMP Qualification Opinion
- Applicant submission – Response to Questions raised by the qualification team
- Applicant submission – Discussion Meeting for HFS-TB Qualification Opinion Request (Slides)

[i] All annexes mentioned under the Applicant's position refer to the documentation submitted with the request.