

IMI1 Final Project Report Public Summary

Project Acronym: PREDICT-TB

Project Title: Model-based preclinical development of anti-tuberculosis drug combinations

Grant Agreement: 115337

Project Duration: 01/05/2012 - 31/10/2017

1. Executive summary

1.1. Project rationale and overall objectives of the project

Until 2013, no new drugs for therapy of tuberculosis (TB) had been developed and registered since the 1970s. Though successful treatment of TB relies on combinations of at least three drugs, many of the scientific and regulatory pathways to product registration, even for single agents, in this therapeutic area remained unclear. Despite a widespread desire for faster and more reliable development of combination regimens in TB, little data was available on how this might be practically achieved. Selection and optimization of drug combinations for clinical development was dependent on preclinical systems which did not fully capture *in vivo* pharmacodynamics of the heterogeneous states of *Mycobacterium tuberculosis* (MTB). The predictive power of these diverse systems for clinical trial outcomes was uncertain, escalating risks during the clinical development phase. PreDiCT-TB [<http://www.predict-tb.eu/>] implemented a comprehensive model-based approach to addressing this problem, generating a unique preclinical dataset evaluating a defined set of drugs and combinations across as broad a range of preclinical systems as possible and by synthesizing and integrating this preclinical information with published and individual patient data from clinical trials. The strategic aim of the consortium was to produce pharmacometric and statistical modelling frameworks and optimized decision pathways for development of combination regimens to facilitate successful progression of the most promising drugs and combinations to innovative early phase and ultimately pivotal clinical trials.

1.2. Overall deliverables of the project

- Evaluation of a defined set of anti-tuberculosis drugs and combinations, using a suite of novel and enhanced preclinical experimental systems that collectively better reflect the full range of physiological states of *Mycobacterium tuberculosis* (*Mtb*) likely to be encountered *in vivo*.
- Development of pharmacodynamic biomarkers for use in preclinical and clinical development that do not depend on growth of organisms, that more accurately reflect lethal action of drugs, and that can capture heterogeneity of this action at a single cell level.
- Comprehensive synthesis of published data from clinical trials of tuberculosis treatment and assembly of a database of individual patient data to calibrate the results of preclinical experiments and support translational modelling activities [<https://c-path.org/programs/tb-pacts/>]
- A quantitative modelling framework which will incorporate recommendations for study design, mathematical approaches for describing representative data from pre-clinical experiments and clinical trials and methods for model-based translational level bridging.
- A library of code [<http://www.ddmore.eu/>] for PKPD disease models which makes explicit use of preclinical and clinical data in modelling, prediction and simulation.
- Recommendations for optimized preclinical development pathways including assay selection, conditions and experimental design based on the performance of the panel of preclinical systems evaluated in PreDiCT-TB and for more reliable preclinical-clinical transitioning of compounds and combinations.

1.3. Summary of progress in final year

During the final 18-month period covered by this report, progress against the workplan outlined in version 1.3 of the Description of Work has been as projected and there have been no major deviations, with all deliverables scheduled being completed. Over the whole project, 98% of deliverables and all the milestones have been completed. With the agreement of the steering committee, limited redistribution of funds to two consortium partners (UCL, UoS) for additional work was finalised in early 2017 while an agreement on return of overpaid funds to the consortium from another partner (MCSN) was implemented, which is projected to be completed in September 2018. Overall spending of the consortium remained within the originally-requested amount.

Experimental work was continued by selected partners in WPs 1, 2 and 3 according to their available resources and was completed within the agreed extended timeline of six months. Data from these experiments was transferred in time for analyses to be completed by WP5, where appropriate.

Collection of individual patient data within WP4 was closed in January 2017 with a total of 31 clinical trials ultimately available for use by WP5. The systematic reviews of clinical trial data were completed and the results made available for combined analyses of preclinical and clinical outcomes. Previously identified risks to data sustainability were addressed by initiation of successful collaborations with the eTRIKS and ELIXIR consortia [<https://www.etriks.org/>] for preclinical datasets and with Critical Path to TB Regimens (CPTR) [<https://c-path.org/programs/tb-pacts/>] for clinical datasets.

Complementary pharmacometric, statistical and mathematical approaches to modelling of preclinical data and clinical trial simulation to aid design of future trials were finalised by WP5 partners and these tools prepared in formats suitable for redistribution or interactive use.

Plans for dissemination and sustainability were developed by WP6 in collaboration with partners and discussed with all steering committee members at the final general assembly meeting in Paris in May 2017. Supported by additional institutional funding from ULIV, collation of the ensemble of modelling analyses and completion of the final strategic outputs including recommendations for a revised preclinical and early clinical development pathway are in preparation for publication and presentation at a number of planned key fora in 2018 including a WHO workshop on innovation in clinical trials and the EMA Working Party on Infectious Diseases.

1.4. Significant achievements in the final year

Since May 2016, the PreDiCT-TB experimental partners have completed their round 2 experiments, resulting in one of the largest and most diverse databases of preclinical data on combinations of anti-tuberculosis drugs available. The sustainability and availability of this data is likely to be assured through engagement with the eTRIKS and ELIXIR consortia. In addition, the project was able to incorporate additional preclinical datasets from non-PreDiCT-TB investigators into the analysis, expanding the scope and reach of the resource.

Key biomarkers (molecular bacillary load assay, resuscitation-promotion factor culture filtrate) developed by the consortium have been optimised and made available on a non-commercial basis to other researchers in the field in a standardised format. They have been successfully applied to selected

preclinical models within the consortium but also to clinical specimens in projects supported by the PanACEA [<http://panacea-tb.net/>] and InterTB [<http://www.ipc.nxgenomics.org/intertb.htm>] clinical trial consortia.

The consortium successfully completed its systematic overview of Phase II (133 trials, 37,173 participants) and Phase III (174 trials, 39,123 participants) outcomes in clinical trials of tuberculosis treatment and assembly of individual patient datasets (31 trials, 15,088 participants), the majority of which have been transferred to the WHO supported TB-PACTS platform, creating the first viable and sustainable clinical trials repository in this area.

Clinical trial simulation tools developed by PreDiCT-TB have been evaluated against realistic clinical trial scenarios derived from the clinical trial reviews and database and will be made available for future use by drug developers through interactive web-based tools, while suitable code from the underlying models has been deposited with DDMoRe (<http://repository.ddmore.eu/models>).

The consortium began to implement its dissemination strategy for the final outputs of the work with important presentations highlighting the work of PreDiCT-TB at the European Medicines Agency (November 2016), Gordon Research Conference on TB Drug Development (July 2017) and a WHO-sponsored symposium at the 48th Union World Conference on Lung Health (November 2017). These activities will continue during 2018 as the final outputs are published and presented as detailed in section 5 of this report.

1.5. Scientific and technical results of the project

The results of the consortium's scientific activities are broadly summarised here for each work package and partner and are outlined in more detail in the deliverable reports deposited in SOFIA.

1.5.1 WP1 – *in vitro* models

The objective of WP1 was to develop *in vitro* laboratory tests which could predict the outcome of clinical trials with new combination regimens for TB.

First, WP1 partners used a range of *in vitro* laboratory tests (for example different growth rates in chemostats, growth inside mammalian cells, and non-replicating models) to test single TB drugs and then different combinations. Then they tested new TB drugs in combination with older drugs.

The WP1 participants have now characterised many different drug combinations that consisted of old and new drugs in a wide range of culture conditions. Interestingly no convincing evidence of synergy with any combination (new or old) was found. A significant increase in colony forming units was observed for some experimental systems when post drug exposure cultures were treated with Resuscitation Promoting Factors (RPFs, isolated from culture filtrates of *Mtb*). Demonstration of killing of RPF-dependant, dormant *Mtb* cells may be important in discovery assays such as the ones outlined in WP1. It may help to explain why many individual models are poorly predictive of human TB trials with any regimen, since the post experimental culture techniques used are not demonstrating the killing (or not) of these dormant sub-populations. They thus may well be useful in the prediction of the clinical outcome of new treatment regimens, although this work has not been definitively presented in this project deliverables. More work is needed and several grants from other bodies have already been awarded to WP1 Participants to continue this work.

Finally, *in silico* modelling of WP1 work was undertaken by WP5, which crystallises the potential value of WP1 work in the prediction of early stage clinical trials. Modelling of late stage relapse in humans continues despite the formal end to the consortium's funding given the importance of this topic. Activities in WP1 can be grouped according to the type of assay as follows:

Models of actively growing organisms

These models captured the responses of *Mtb* in conventional liquid culture and examined the impact of strain, growth rate and mechanism of action on pharmacodynamics.

- EMC performed time-kill experiments in liquid culture using both H37Rv and an East Asian clinical strain over 6 days and at reduced pH for combinations containing pyrazinamide. 12 single agents and an extensive set of combinations of 2, 3 and 4 of these agents at clinically relevant concentrations were evaluated.
- UCL evaluated the transcriptomic response of actively growing *M. bovis* BCG to a panel of nine single drugs, identifying a four-gene subset capable of reliably distinguishing mechanisms of drug action.
- PHE investigated the effects of growth rate on the efficacy of current antibiotic combinations, over a time-course, using controlled and defined chemostat cultures.

Models of slow/non-growing organisms

These *in vitro* models used modified growth conditions or organisms to approximate the physiological state of *in vivo* *Mtb* and study its impact on pharmacodynamics.

- SGUL used the Hu-Coates model of 100-day old stationary phase broth culture of H37Rv to evaluate the activity of nine single agents and selected pairwise combinations and the impact of addition of resuscitation promotion factors on the results. Rifamycins, bedaquiline and moxifloxacin were most active in this model, but there were few indications of departures from additivity.
- EPFLa studied the impact of nutrient limitation and growth rate on antibiotic killing on organisms in custom-built microfluidic devices, suggesting that these two factors may be independent contributors to antibiotic tolerance.
- EPFLb created a LUX-expressing streptomycin-dependent SS18b strain to perform intensive time-kill assays in media with different carbon sources with 11 single agents. The results were replicated using alternative readouts (REMA and BacTitreGlo). Cell-wall active agents demonstrated characteristic media-dependant pharmacodynamic patterns while clinically sterilizing drugs exerting the largest effects with time-dependent behaviour in some cases
- ULEIC employed a novel sputum cidal assay using patient specimens to evaluate the activity of 12 single agents and selected drug combinations. Rifamycins and fluoroquinolones were most active in this system while complete sterilization was observed for combinations of drugs.

- PHE achieved very slow controlled growth rates in chemostats and investigated the effects of growth rate on the efficacy of current antibiotic combinations in terms of mutation rates and transcriptional changes. Slow growers and fast growers responded differently in their genotypic and phenotypic responses.

Models incorporating immune effector cells

In vitro or *ex vivo* models were used to evaluate drug efficacy in the presence of immune pressure, specifically in the intracellular environment of phagocytic cells in variable states of activation and in co-culture with lymphocytes.

- GSK performed a high-content imaging assay using *Mtb* Erdman and the human THP-1 cell line to measure the MIC₉₀ of intracellular organisms over 4 days for 13 specified single agents.
- LSTM developed a high-content imaging assay using *Mtb* H37Rv-GFP and human THP-1 cells to measure elimination of extracellular and intracellular organisms over 5 days for six drugs including first-line drugs and fluoroquinolones.
- ULIV developed a modified *ex vivo* bactericidal assay using whole blood from healthy human volunteers with optimised incubation conditions and an automated read-out from the MGIT liquid culture system, which was found to be more reliable than alternative fluorescence readouts with GFP or mCherry strains of H37Rv. 10 individual drugs and 10 priority combinations were evaluated which broadly reproduced the rank order observed in clinical trials, but demonstrated less than additive effects of pyrazinamide.
- MPI used a high-content imaging assay based on FL83B hepatocytes to identify distinct phenotypes of the endosomal system associated with set of 9 single agents and their combinatorial pairs and triples. The most striking finding of the final chemi-genomic analysis was the association of bedaquiline with markers of endosomal activation.
- EPFLa developed novel microfluidic cell-trapping devices for use with time-lapse video measurements of replication of *Mtb* within motile phagocytes.

1.5.2 WP2 in vivo models

Activities in WP2 may best be grouped and summarised by model species:

1.5.2.1 Zebrafish models

These models enabled large numbers of drugs and drug combinations to be screened for *in vivo* efficacy, generating rich datasets for modelling in WP5. The zebrafish systems have been used extensively in WP3 to generate new technologies for drug screening.

ZF Screens (Partner 15)

- Developed and optimised a high-throughput, automated system for drug efficacy testing using robotic infection of larvae with mCherry-labeled *M. marinum* E11 combined with a COPAS sorting system to select homogeneously infected larvae and to dynamically monitor disease progression. Optimisation included a comparison of the source of the zebrafish.
- 13 different drugs were tested and 34 different combinations compared. Rb (100 µM), B (10 µM) and Rp (100 µM) were found to be the most effective single drugs, whereas mixtures of H(400 µM), Z(50 µM), E(100 µM) and either Rp (50 or 100 µM) or R (50 µM) were found to be the most effective combinations.

VUMC (Partner 13)

- Optimised a short-duration zebrafish larval screening system using humanised doses of drugs and multiple read-outs of bacterial growth. 16 round 1 combinations were screened and the data were analysed in WP5 showing that RHZE, RHZS, HS and HE were the best combinations. 42 round 2 combinations were tested and modelling showed the best combinations to be RHZM₅₀, Rb₂₀HZE, R₅₀HZE, Rp₁₀HZE and RHZB₁.
- Developed a non-growing zebrafish embryo infection model based upon stress (by starvation) induction of Rpf mutants of *M. marinum*. This model is currently prepared to be used to test the drug efficacy of compounds.

1.5.2.2 Mouse models

Several mouse models were used or developed which span multiple aspects of TB disease with the aim to generate drug efficacy data which are relevant to a spectrum of clinical read-outs from early bactericidal activity (EBA) to prevention of relapse trials.

GSK

- Established a short assay for the evaluation of the antitubercular efficacy in the acute phase of the *Mtb* infection in mice (C57BL/7 strain). Compounds from round 1 and 2 were evaluated in monotherapy. Blood exposure to get 90% of the maximum effect was determined for each compound and compared with the efficacious human blood exposure reached in the clinical practice or determined by clinical trials for new compounds.
- A new relapse model based on the efficacy of compounds on the acute phase has been set up. Different combinations were tested and the time needed to reach the cure of 80% of the population (T80) was obtained and compared. Combinations were ranked from lower to higher T80 (faster to slower combinations to achieve cure): RbHZE < RpHZE < RHZE < RMZE. This is partially in agreement with clinical trial results, where RHZE < RMZE, but RbHZE and RPHZE were very similar to RHZE. Furthermore, we could not confirm that the addition of Z to RH shortens the time of administration needed to reach a cure of the patients. High dose of R was able to sterilize mice.

EMC

- Developed and validated a model using BALB/c mice challenged with a Beijing strain of *Mtb* challenge to assess EBA in combination with pharmacokinetic information in a relatively short course experiment.
- Developed a new relapse assessment method (in collaboration with WP5) to provide a more 'data rich' comparison method of the efficacy and time to cure of the different regimens.

SGUL

- Tested first round drugs in the Cornell mouse model and showed that RH, RZ and RHZ regimens had similar bactericidal and sterilizing activities, HZ had reduced activity whereas a higher dose of R had enhanced activity with rapid clearance and a zero-relapse rate. Resuscitation promoting factor (RPF) was applied to the model to identify RPF-dependent persister organisms. In a modification to the model using a *hspX Mtb* mutant, treatment resulted in a more rapid clearance of bacillary load and a lower relapse rate than WT strain.
- Second round drugs testing in the Cornell model showed that substitution of R to Rb or E to O in the standard regimen failed to influence bactericidal and sterilizing activities but substitution of H with M resulted in significant bactericidal activity although RPF-dependent persisters were still present. A regimen with B replacing E showed bactericidal and sterilizing activity.

EPFL

- Established a model of latency using the non-replicating strain of *Mtb* (streptomycin (S)-starved 18b). The potency of B was confirmed in this model. B was more efficacious than a regimen of RHZ. B displayed no antagonism with Z or with delamanid (D).
- D was not active in the model when administered singly but no antagonism was detected in combination therapies, i.e. D + Z and D + B.

Janssen

- Aimed to back-validate the REMox non-inferiority clinical trial in a Swiss mouse model. Non-inferiority was not shown for 2MRZE/2MR and 2HRZM/2HRM. After extended evaluation of study designs, it was concluded that non-inferiority experiments in mice would require unrealistically large group sizes to reach sufficient statistical power and significance.
- An inferiority trial was designed which used 89 animals per arm to demonstrate inferiority with 80% power. However, all of the regimens achieved 0% relapse and it was not possible to prove that the four month 2HRZM/2HRM regimen, or the four month 2REZM/2RM regimen, was inferior to the six month standard of care (2HRZE/4HR).

MPIIB

- Established a *Nos2*^{-/-} mouse model with patient-like pathology in principle which correctly predicted clinical outcomes of R, Rb, M, and potentially linezolid. Therefore, this model could be used as a predictive TB drug development tool.
- Developed a humanized mouse model which effectively recapitulated key features of the human immune response and the corresponding pathology upon *Mtb* infection. This model could be of particular value for testing very promising pre-clinical drug candidates.

1.5.2.3 Guinea pig models

These were used by 2 partners (IP and HPA, now PHE) to test the efficacy of round 1 and 2 drugs alone and in combination. Novel, enriched sampling, study designs were proposed by WP5 and implemented by both partners. An initial study testing single drugs was able to demonstrate similarity in the data obtained at both sites and thus different combinations were tested by IP and PHE to maximise the number of tests performed in the model. The data from both sites were provided to WP5 for modelling which generated an overall ranking of the regimens in the guinea pig.

PHE

- Round 1 and 2 combinations of HRZE, HRZ, HRZM and MRZE were tested along with each of the single drugs using doses that were optimised by PK studies to achieve similar exposures to humans. The M-containing regimens were the most effective, showing early bactericidal activity.

IP

- Round 1 combinations of HRZ, RZ, HR and HZ were tested along with each individual drug and Rp and B were tested as single drugs. As with the PHE studies, the doses of drug were optimised for human exposures in satellite PK studies.
- An important reduction of the number of CFU were observed for both B and Rp during the 6 weeks of treatment with an efficacy comparable to HRZ.

1.5.2.4 Non-human primate model

This system was used to test drug activity in a pre-clinical model that most closely resembles human TB. **PHE** provided the expertise in macaque models of TB and advanced imaging techniques to measure disease progression. This was combined with a critical input from ULIV (WP5) who devised innovative study designs and provided analysis of outcomes.

- PK studies were performed with H, R, Z, E and M to optimise the exposures as single drugs and the doses were then adjusted following PK studies using different doses and combinations of drugs.
- A Pigeon double cross-over design was used to compare HR, HZ and HRZ in cynomolgus macaques infected with *M. tuberculosis* H37Rv. Differences between the regimens did not reach statistical significance, possibly because the burden of disease was low but the study was

highly successful in demonstrating feasibility (tolerability of regimens, delivery of complex dosing schedule, CT imaging to measure disease).

- A two period AB/BA design was used to compare HRZ with HE in cynomolgus macaques infected with *Mtb* Erdman in order to increase the disease burden prior to treatment. The study achieved the aims with respect to successful delivery and the data were provided to WP5.

1.5.2.5 WP2 Bioanalytical methods and provision of PK analysis – critical to the success of WP2 was the expertise and facilities at GSK which enabled harmonised analysis of drug PK across all of the mammalian models. Bioanalytical methods were established for quantitation of round 1 and round 2 drugs in blood and plasma of the different species (mouse, guinea pig and macaque) at the DMPK Unit, GlaxoSmithKline R&D, Spain. GSK provided blood or plasma levels of the different anti-TB drugs for the WP2 mammalian models, from both non-infected and infected samples. The corresponding PK analysis of the drugs levels was performed by WP5.

1.5.2.6 WP2 Inter-workpackage interactions – the *in vivo* models of WP2 were extremely important for providing materials with which to evaluate new technologies developed in WP3, for example the Molecular Bacterial Load (MBL) assay which was tech-transferred for use in zebrafish, mouse and guinea pig models. In some cases, the WP3 technologies were enabling for the development of the *in vivo* model itself, e.g. the zebrafish systems, or for providing an important read-out of drug efficacy such as the use of RPF to identify persister organisms. A new CT imaging analysis has also been established under a collaboration with WP3 to determine the infection load in the lungs of *Mtb* infected mice, this read-out is very useful since a longitudinal study may be performed in single mice, similar to what can be done in infected patients. This CT analysis was also applied to images from NHP studies, demonstrating utility across species. The most significant interaction with WP2 was that with WP5 who provided innovative study designs and data analysis. This input had a variety of benefits to the partners in WP2 which included:

- improved animal welfare (by reducing the numbers of animals or the length of experiments)
- increased efficiency by enabling more data to be obtained within the budget
- Improve relevance of animal data since the integration from data of different models may improve the predictivity of the pre-clinical data package

1.5.3 WP3 – Enabling technologies

Work package 3 was established to develop cross-cutting technologies that would assist in the development of new anti-tuberculosis drugs. During the life of this project a number of innovations were created that will allow generation of new knowledge about how to treat tuberculosis, together with methods that will speed development of new drugs in the pre-clinical and clinical phases of development.

Successes occurred in four different key areas: methods to detect dormant mycobacteria, tools to evaluate mycobacteria in different cell states, better methods for following the treatment response and new approaches for the screening for new anti-tuberculosis drugs. Specifically:

- EPFLb created an innovative set of mycobacterial green fluorescent protein constructs tagged to critical components of mycobacterial metabolism. These tools were linked to innovative microfluidic devices that assisted the study of antibiotic effect on mycobacteria.
- PHE developed a method that applies flow cytometry and fluorescent dyes for determining drug activity against *M. tuberculosis*. This method has potential in capturing information about cell populations that were unable to grow under standard conditions could also provide insights into the mode of action of the drugs
- UCL developed methodologies to adapt the molecular bacterial load assay to test samples from animal models
- USTAN also developed the MBLA further by redesigning the assay to make the system more stable for use in clinical trials settings. They also created a series of standard operating procedures for all of the major real time PCR platforms.
- USTAN developed an innovative methodology for detecting the cell state of mycobacteria in tissue samples using Raman spectroscopy. This allowed the cell state, associated with phenotypic resistance, to be determined in infected tissues. This was complemented with significant improvements in staining methods to identify dormant bacteria in clinical samples.
- UC3M in collaboration with GSK, developed a whole animal imaging system capable of delivering CT images of animals under treatment with anti-tuberculosis drugs
- ULEIC created more stabilised resuscitation factor for use in experiments together with protocols for its use, distributing it to partners when requested.
- VUCM developed a new method to screen novel drugs using the zebra fish model.
- ZFS also worked on the zebrafish model and developed imaging systems that could rapidly quantify the fish that were applied to drug screening.

1.5.4 WP4 Clinical Data

WP4 was tasked with identifying, summarising and obtaining individual patient datasets from all clinical trials relevant to the set of drugs evaluated by the consortium. The key scientific achievements include:

- Contributing to the development, adoption of the CDISC TB Data Standard [<https://www.cdisc.org/>].
- Construction of the PreDiCT-TB clinical trials database (31 trials, 15088 participants, 69 drug combinations), which formed the basis for translational modelling activities in WP5. The bulk of the database has now been transferred to the WHO sponsored TB-PACTS platform supported by CPTR.

- Systematic review of reporting of Phase II endpoints, highlighting broad diversity of outcomes used/reported and informing protocol development for the subsequent review of Phase II outcomes.
- Systematic review of Phase II outcomes, which was the first comprehensive overview of all early phase clinical trials in tuberculosis and highlighted an important translational disconnect between Phase IIA and IIB trials as well as the need for harmonisation of outcomes and provision of individual patient data.
- Systematic review of reporting of Phase III endpoints, which identified a need properly to classify on- and post-treatment outcomes and report them clearly as a combined measure which influenced protocol development for the subsequent review of Phase III outcomes.
- Systematic review of Phase III outcomes, which is the first comprehensive summary of the long-term performance of anti-TB drugs, examining the interaction between drug potency and regimen duration
- Meta-regression analyses focussing on the utility of two-month culture conversion and time-to-event outcomes as a predictor of treatment failure and relapse which supported clinical trials simulation efforts in WP5.

1.5.5 WP5 *in silico* modelling

WP5 was responsible for collation and translation of preclinical and clinical data into useful models for drug development. This required a high level of co-operation both intra- and inter-WP (with all the other WPs and with the majority of partners within the consortium connected). Their key results may be summarised as follows:

- Input to improved experimental designs for WP1 and WP2 during the first and second rounds of experiments, as well as publications describing optimization of experimental study designs for preclinical experiments in TB. This work included optimised PK sampling schemes, improved scheduling for bacteriological samples and innovative designs for animal experiments including duration-randomised and cross-over designs (UU, ULIV)
- Characterization of human and animal pharmacokinetics using population and naïve-pooled approaches. The models were used to confirm or update dosing recommendations for preclinical experiments and to establish pharmacokinetic-pharmacodynamic relationships to inform clinical trial simulation (UU, ULIV)
- Development of a method for studying pharmacokinetic interactions in man which has been referenced in an FDA guideline (UU)
- Development of a general pharmacodynamic interaction (GPDI) method for flexible and accurate characterisation of pharmacodynamic interactions between two or more drugs which was successfully applied to intensively sampled *in vitro* and *in vivo* data (UU)

- Prediction of early phase clinical data using complementary clinical trial simulation approaches and preclinical translational frameworks (UU, ULIV, GSK)
- Development of agent-based models that include immunology, mycobacterial cell state and pharmacokinetics and are capable of mimicking relapse (or can be developed into a relapse model) (USTAN)
- Development of a “virtual lung” through a network model that includes pathology, microbiology and pharmacokinetic components (USTAN)
- Unified analysis of suitable preclinical and clinical datasets using empirical longitudinal and time-to-event methods (ULIV)
- Clarification and evaluation of statistical relationships between preclinical and clinical results using a hierarchical meta-regression approach. These analyses formed the basis of a formal quantitative assessment of the relative performance of preclinical systems in predicting clinical trial outcomes (ULIV)
- Development of a method for clinical trial simulation with uncertainty to guide development decisions based on probability of success in Phase III. This approach utilised priors reflecting preclinical effect sizes in simulations of early phase trials linked to prediction of long-term outcomes through the results of meta-regression analyses in WP4 (ULIV)

1.5.6 WP6 – Project Management

Annual reports were completed on time and consortium meetings were held regularly throughout the 5 years of the project. In addition, the Paris 2017 meeting represented the final General Assembly for the project and therefore WP6 will have met all its objectives with the submission of this final report. This culmination builds on the successful interim meeting in Brussels with central IMI staff and external evaluators, delivery of a mid-project DoW revision subsequent to that meeting and conclusion of agreement on a 6-month no-cost extension. WP6 also successfully managed strategic co-ordination of activities with Critical Path to TB Regimens under the existing memorandum of understanding, which added significant value and impact to the project. Although no specific deliverable has been defined, significant work on sustainability of databases, via WP4 and WP7, and coordination of dissemination activities has been led by WP6. Finally, WP6 managed the interactions with both the Ethics Advisory Board (meetings three times a year, with regular attendance at the general assembly) and Scientific Advisory Board (yearly report, plus *ad hoc* calls as needed).

1.5.7 WP7 – Data Management

The infrastructure and tools provided by WP7 to support data management within the consortium were an essential component of the workflow supporting the project. The key achievements may be summarised as follows:

- Implementation of a data management plan to collect, curate, store, and provide access to pre-clinical and clinical datasets from PreDiCT-TB partners and external partners/institutions where appropriate.

- Development and finalisation of a project-specific data governance framework with provision for rigorous anonymization procedures and tracking and control of access to clinical datasets by partners.
- Establishment of a tranSMART server, hosted by ULIV, to store consortium and other curated data including metadata annotations on the context, for example experimental conditions for preclinical experiments, protocols and data dictionaries for clinical trials
- Collection of 31 individual patient datasets from Phase II and III clinical trials which were anonymized and curated to CDISC standard as appropriate
- Collection of 16 WP1 datasets and curation (format and content) and their integration into tranSMART data model
- Collection of 15 WP2 datasets and curation (format and content) and their integration into tranSMART data model
- Investigation of contribution of automated data curation tools to facilitate and enhance the curation process (Imperial College and eTriks e-Harmonization system)
- Development of an interactive analytics interface to perform simple automated analyses on datasets directly in tranSMART (J&J)
- Identification of sustainability solutions for preclinical data (ELIXIR) and clinical data (CPTR TB-PACTS platform) with successful negotiation of legal frameworks and governance arrangements for these resources with both external and consortium partners

1.6. Potential impact, main dissemination activities and exploitation of results

Please explain how the project scientific/technical outputs contribute to the overall IMI objectives:

- to provide socio-economic benefits for European citizens,
- to contribute to the health of European citizens,
- to increase the competitiveness of Europe and help to establish Europe as the most attractive place for biopharmaceutical research and development.

Please outline how the project outputs have/will have the potential to be rapidly and broadly spread and taken up within the scientific/industrial community and healthcare professionals.

PreDiCT-TB represented one of the largest single investments in preclinical research in tuberculosis treatment made by the European Union in the last decade and addressed an important translational gap within Europe between the drug discovery work of the NM4TB and MM4TB [<http://www.mm4tb.org/>] consortia and the support for innovative clinical development provided by the EDCTP-funded PanACEA consortium. The consortium brought together many of the leading academic groups in Europe in this therapeutic area, together with three of the most active industrial

partners in tuberculosis drug development globally. TB is a global public health problem and an important target for EU international development assistance since rates remain high in many EU nations. Given the high risk and costs of drug development in tuberculosis and the framing of the ultimate market for such drugs by the public sector, partnerships like PreDiCT-TB have been essential to maintaining the engagement of industry in tackling the need for new drugs for tuberculosis. PreDiCT-TB was able to effectively project European expertise in forming true partnerships with organisations in the US which have benefited from historically high levels of funding, particularly TB Alliance [<https://www.tballiance.org/>], CPTA and NIAID [<https://www.niaid.nih.gov/>] and with well-established US investigators. These international collaborations and the strength of the scientific network formed by ex-PreDiCT-TB partners, together with the data resources, experimental and pharmacometric tools and regulatory proposals generated by the consortium, represents an important upgrading of European capability and contribution to drug development in tuberculosis, facilitating and helping to de-risk future industrial investments in this therapeutic area within the EU and beyond. This goal has been furthered by PreDiCT-TB's promotion of an open model of innovation which has, as far as possible, minimised barriers to rapid adoption of the approaches and technologies the consortium has fostered. The overall strategy for impact and dissemination is outlined in section 5 while selected specific outputs are outlined by workpackage below:

1.6.1 WP1

Since WP1 participants have now characterised many different combinations of old and new drugs in a wide range of optimised *in vitro* culture techniques these techniques can now be transferred to any discovery/development pathways that scientific/industrial partners care to build.

We believe that use of Resuscitation Promoting Factors (RPFs) demonstrating killing of RPF-dependant dormant cells and/or the MBLA assay may be essential in these discovery assays to maximise understanding of individual drugs and combinations. The knowledge, consumables and SOPs for both techniques are now more easily available from the inventors than prior to the consortium. Finally modelling of WP1 work crystallises the potential value of *in vitro* work in the prediction of early stage clinical trial outcomes but the need to analyse beyond the initial experimental results.

The raw experimental data and modelling code for this work will be freely available via the ELIXIR & DDMoRe platforms thus future TB scientists have the ability to build on these data & experimental designs without repeating our experiments. Of course, work will be published and presented at scientific meetings and final publications will follow, ensuring dissemination to the scientific community.

1.6.2 WP2

The studies conducted in WP2, with significant contribution from WP3 and 5, have generated new *in vivo* models for drug evaluation as well as generating novel ways of describing the pre-clinical efficacy of drugs which have great potential to influence future TB drug discovery and development.

The innovative approach to experimental design of both pharmacokinetic and pharmacodynamic experiments based on a more sophisticated approach to statistical analysis has generated considerable interest among external collaborating investigators contributing datasets to WP2, particularly the use of longitudinal data analysis and duration-randomised relapse experiments in mice. The ability to reduce the numbers of animals required and to perform contemporaneous bioanalysis of drugs on small amounts of whole blood obtained in a non-lethal manner, offer significant benefits in terms of

animal welfare as well as experimental efficiency. Dissemination of these methods through publications, presentations and scientific collaboration could rapidly impact conduct of such experiments in the TB field.

The comprehensive preclinical database of animal experiments generated by WP2 has in addition set a precedent analogous to that of clinical trials whereby the conduct of preclinical studies and the information that they generate should ultimately be subject to systematic review and cumulative meta-analysis which may help to better understand the performance of drugs and regimens in animal models, the variability between different species and groups and the predictive value against clinical outcomes, as pioneered in other therapeutic areas by the CAMARADES collaboration [<http://www.dcn.ed.ac.uk/camarades/default.htm>].

Finally, the strong integration of the academic and industry partners that was achieved in WP2 will assist in adoption by industry of these concepts generated in the project such as improved methods for study designs, new *in vivo* models and read-outs, and helping to elaborate a modified critical path for the discovery of new antitubercular drugs.

1.6.3 WP3

The biomarkers developed by WP3 were adopted by a number of groups within and outside the consortium during the life of the project and their evaluation in both a preclinical and clinical context in new settings is ongoing. The ability offered by these new biomarkers to distinguish directly or indirectly between different mycobacterial states is of widespread applicability and interest.

MBLA has been a major success for the group. Not only has it been adapted to make it more widely applicable to a range of diagnostic platforms and experimental contexts, with support from EDCTP, it has been trialled in the field in the context of a clinical trial (TB-MAMS 01, PanBioME Study) [<http://panacea-tb.net/>]. The technology has been made available in kit form on an at-cost basis by USTAN and has been requested by a number of research groups.

Standardised preparation of culture filtrate derived resuscitation promotion factors by ULEIC enabled evaluation of this technology by a broader group of investigators particularly WP2 groups such as SGUL and EMC performing relapse experiments in mice. This development of the assay has resulted in new research collaborations within and outside Europe, most notably a clinical sub-study of the InterTB RIFASHORT Phase III trial [<http://www.ipc.nxgenomics.org/intertb.htm>].

The flow cytometric methods developed by PHE for use in the chemostat have been adopted and further developed by ULIV in collaboration with investigators at the University of Cape Town for evaluation of phenotypic antibiotic tolerance in a set of clinical strains derived from the KDH-TB clinical study.

1.6.4 WP4

The fully comprehensive and systematic overview of the efficacy of anti-TB drugs and combinations undertaken by WP4 has provided a benchmark and ongoing resources for several key constituencies in TB drug development and deployment, which have the potential to influence these stakeholders within a short time-frame and impact the success of future drug development efforts within Europe and beyond.

Firstly, the reviews have provided a unique resource for drug developers, policy-makers and regulatory agencies in understanding the key problems and obstacles in TB clinical trials as well as the value of currently suggested surrogate endpoints. Results from the reviews have already been presented at the EMA consultation on TB drug development guidance in November 2016, Gordon conference on TB drug development July 2017, WHO Taskforce on New Drugs in September 2017 and IUATLD Meeting in October 2017.

Second, the reviews have facilitated formal, unbiased comparisons with the results of preclinical assay systems based on ranking and absolute or relative measures of effect enabling these models to be broadly calibrated against performance in clinical studies and building confidence in their usefulness in development. This quantitative analysis approach forms part of the basis for assessment of the value of such systems in proposals for an updated preclinical and early clinical development pathway to be discussed at meetings with WHO and EMA later in 2018.

Third, much of the individual patient data from clinical trials assembled by PreDiCT-TB has been transferred for curation to the WHO-sponsored TB-PACTS platform supported by CPATH. This database represents the first viable clinical trials repository in this therapeutic area and has supported the research efforts of both PreDiCT-TB and TB-ReFLECT, results from which have already begun to influence TB research and policy. TB-PACTS has thus demonstrated its value for evidence synthesis and methodological research and its international reach, which will benefit TB researchers, policy-makers and patients alike.

1.6.5 WP5

The model-informed approach to drug development that was implemented by WP5 was the first comprehensive effort to apply modern pharmacometric modelling and simulation techniques to translational problems in tuberculosis and has broken new ground in the field. This would not have been possible without the extensive data resources generated or assembled by the other WPs in the consortium. Furthermore, the interactions between experimental and modelling groups within the consortium and with external partners have generated positive working relationships which will facilitate the continuing use of pharmacometric approaches in the future. This is an approach that is also gaining increasing visibility internationally through the collaborations of PreDiCT-TB with CPTR and NIAID.

Elements of the translational modelling framework have been published and presented at numerous influential fora including the Norwegian Medicines Agency in November 2017, an American Society of Clinical Pharmacology and Therapeutics webinar in October 2017, a WHO-sponsored seminar at the annual meeting of the International Union against Tuberculosis and Lung Disease in October 2017, while methods for characterising pharmacokinetic interactions have been referenced in FDA guidance on this topic.

Deposition of model code in commonly used languages for pharmacometrics (NMTran, R, MATLAB, C++) in the DDMoRe web-based repository ensures the ready availability of code and reproducibility of the results in publications for any groups interested in adopting these approaches. Web-based demonstrations of alternative simulation approaches to planning of early phase clinical trials have also

been developed for interactive use by non-pharmacometricians based on the Shiny suite of tools in Rstudio.

1.6.6 WP6

Not applicable to this section.

1.6.7 WP7

The data management framework created by WP7 will facilitate re-use of data resources generated by PreDiCT-TB after they have been transferred for long-term curation in the ELIXIR and TB-PACTS platforms. For clinical datasets, adoption and adaptation of the CDISC controlled vocabularies including version 2 of the TB data standard and linkage to a data dictionary (where available) will ensure the fidelity and sustainability of these data. The 21 datasets transferred to the TB-PACTS platform will ensure that a viable repository for tuberculosis clinical trials data is established that enjoys the long-term support of all major stakeholders and facilitates large-scale individual patient data analyses to support policy making and planning of future clinical trials. Similarly, early access of external researchers to preclinical datasets on the ELIXIR platform from November 2018 onward will help to further exploration and adoption of the PreDiCT-TB approach outside the consortium's original partners. In addition, WP7 has invested effort in understanding and developing best practices for data-sharing and governance in TB research, has already shared some of these experiences publicly at the InterTB conference in October 2016 and plans to publish an opinion piece to disseminate this learning in 2018.

1.7. Lessons learned and further opportunities for research

Please indicate how the collaboration in a public private partnership (PPP) has been an added value to achieve the objectives of the project.

Sustained funding of the magnitude granted to PreDiCT-TB is rarely achieved in the TB therapeutics area. The economies of scale achieved in a consortium of more than 20 partners have been incredibly powerful for all the participants; both EFPIA & academic. The openness achieved from the outset has been critical in achieving many of the consortium's goals not only in a timely fashion but in a manner that generated enduring internal and external collaborations beyond the end of the project. This openness was, in part, achieved due to the formal consortium structure with shared funding binding us to the DoW and its deliverables. In a non-PPP setting a looser collaboration may not have had this ability to communicate and drive common goals as efficiently. The scale of PreDiCT-TB also enabled some specialist services to partners such as bioanalysis and statistical support to be provided in a highly cost-effective and consistent way. With such a critical mass of scientists and companies we were also able to engage effectively with other important stakeholders in the TB therapeutics area such as CPTR, NIAID, TBA, TBDA & BMGF [<https://www.gatesfoundation.org/>], as well as key regulatory agencies such as the EMA [<http://www.ema.europa.eu/ema/>].

WP1 and 2 benefited from synergistic interactions between academics and EFPIA [<https://efpia.eu/>] partners that would never have happened without this IMI project. Specifically, academics gaining an understanding of how industry approaches drug discovery at scale and then in candidate progression through the early life cycle management has been essential when considering how to hone experimental design. Linking a specific model to a particular bacterial state has also been important in knowing how model results might inform an understanding of a molecule's activity in vivo. The

industrial partners have learnt about the breadth of models available to them in the TB academic community for assessment of lead and candidate molecules. These were not known prior to the project as many groups were not active specifically in drug discovery development (e.g. they were active in immunology, vaccine or host response) and had not tested pathogen response to drugs in combination previously. In addition, industrial partners have been able to select certain models (e.g. single cell confocal microscopy) to bring in-house whilst accepting other models may be better used in a collaborative externalised way in future.

In WP3 the interaction between academic groups and industry was less well established with the identified lead commercial partner withdrawing its support early in the process. Through the initiative of the academic groups, however, interaction with commercial and pharma groups has been sustained, or is being developed; e.g., MBLA is currently being developed for application on a commercial molecular assay platform.

The effort to assemble a significant body of individual patient data for use by WP4 and WP5 was greatly facilitated by the participation of the EFPIA partners, who contributed their expertise in the area of Open Data as well as a number of important clinical trial datasets. This involvement was crucial in establishing confidence in, and the credibility of, a general platform for data-sharing in TB clinical trials, incorporating best-practice procedures for data anonymisation and standardisation. The success of the PreDiCT-TB and TB-PACTS databases has ensured the engagement of many different stakeholders which bodes well for its long-term sustainability and continued relevance internationally to academic, pharmaceutical and non-governmental researchers.

The dialogue within WP5 between pharmaceutical and academic partners in creating the workplan for a translational PK-PD modelling framework in tuberculosis was extremely valuable in ensuring that the consortium addressed the key disconnects in development and the needs of drug developers. The mix of quantitative skills and approaches developed in WP5 was also enhanced by the interactions between the academic and industrial partners. The development of interactive software tools for clinical trial simulation in the later phases of the project also engaged pharmacometricians and clinical trialists from both the academic and industrial sides, ensuring the relevance of these efforts to current and future development programmes.

In WP7 it was noted that whilst the expertise and mind set are different between academic, SME and EFPIA partners, they are in fact complementary. This facilitated work in developing a data management system appropriate to the goals of the project. The availability of different legal and technical perspectives enabled the consortium to create a data-sharing and governance approach which embraced best practices whilst innovating, against a background of evolving guidance and law. The focus on the consortium's concrete goals in relation to data was invaluable in driving the internal conversation on how to practically apply these principles and the solutions that were ultimately found. It is hard to see how this degree of progress could have been made without the range of expertise among the academic and industrial partners and the ethical advisory board (EAB).

From your experience, please propose any recommendations/ solutions which could be useful for a PPP.

Many general lessons are of relevance to any PPP, irrespective of disease, even perhaps in a non-medical space. Some are IMI-specific, but others involve project governance, finance control, sustainability planning, Big Data governance (EU vs RoW), and Funding. Specifically:

- i. Pharmaceutical companies and funding cycles – IMI projects are long, with most being 4 to 6 years to achieve the higher aims necessary to address “bottlenecks” in the pharmaceutical industry; the bedrock of IMI. However, the reality is that many companies change at a rate that is faster than this. Internal discovery funding cycles are often only 2-3 years. Our experience with the withdrawal of some proposed partners prior to the grant agreement sign-off and, within the project, unexpected company restructuring, highlights the complex nature of industry involvement in both the disease area and project in general.
- ii. Project Governance – the set-up and governance of IMI projects is likely to have many similarities across the portfolio. We believe that IMI project structure (e.g. scientific guidance, ethical input, internal committee structure) could be shared prior to any kick-off meeting with more guidance to teams about what does and doesn’t work, frequency of meetings and committee make-up. This needs to be judged by how many consortia leaders had previously led, plus the specifics of the project aims.
- iii. Finance Control – redistribution of funds across the project from under-spenders to those whose ideas developed during the project was very challenging. We believe that IMI ought to ensure that projects have this flexibility built into governance and that partners know if they are not delivering work required (due to poor performance, not unforeseen scientific issues) and/or fail to provide sufficient information to understand their spend, monies can be redistributed at the discretion of the co-ordinator with the support of the steering committee.
- iv. DoW rewrite – all projects mature in their approach and thinking about a disease and science moves on at pace outside of the consortium. The process to react to this and achieve sign-off of our DoW rewrite at IMI was cumbersome and slow. Any way this can be improved would be welcome.
- v. Annual Meetings – These are time consuming to arrange and some partners required internal funding (e.g. for rooms, lunches etc) which ought to be pointed out and funded prior to the budget finalisation. We think a funding request for annual meeting activities from the leading partner should also include travel for advisory board members.
- vi. Sustainability – this should be a mandatory section in the DoW and appropriately funded, with suggested early and specific links to other IMI projects that are funded to support these activities.
- vii. Regulatory Plans - Regulatory impact can only happen if this is prospectively factored in to the deliverables and funding plan.

We also received specific feedback from individual WPs:

WP2:

Early ‘linking of partners’ so partners can team up as soon as possible and streamline protocols at an early stage – which prevents (logistic) limitations in a later phase of the project. This requires multiple

face to face meetings early in the project (we had 3 in the first 12 months) thus travel budgets should reflect this.

WP3:

For this work package where tools were being produced improved linkage with industrial partners might have facilitated a more rapid and comprehensive uptake of innovations. This WP did not have industrial leadership and suffered for that. Co-leadership of all WPs could have been a requirement of the governance structure.

WP4:

Positive engagement of industrial partners with the Open Data agenda can have an entraining effect on data-sharing by academic researchers and enable creation of data resources which can have a valuable impact on understanding how to overcome clinical drug development challenges. In the TB therapeutic area, a collaborative approach with openness to contribution and sharing of data from global sources resulted in a more successful and ultimately sustainable resource. Creating the right legal and ethical framework for sharing of individual patient data internationally is time-consuming and benefits from the expertise and experience of both academic and industrial partners. Looking outside of TB was beneficial for us. We had a good conversation with WWARN (see <http://www.wwarn.org/>) early in the project but now IMI could facilitate this more internally.

WP5:

A clear understanding of the agenda and roadmap for translational modelling activities is critically important to effectively address the problems of drug developers. While it is helpful to conceive these within a broader conceptual framework, it is also important to identify the key tools that are needed for clinical trial simulation to provide answers to development questions as realistically as possible. However, these can in principle be implemented using different technical approaches and modular or even parallel modelling activities can be a successful strategy in a PPP.

WP7:

It may be better for IMI projects to define a “data governance” work package rather than a “data management” work package. This approach may have helped to anticipate some of the post-project data custodianship issues e.g. transfer of data to third parties, definition and funding of data stewardship role. With the availability of the eTRIKS/ELIXIR and DDMoRe consortia we feel that there is appropriate data management support for future projects now.

In view of your project achievements, please provide your views on potential new research to further advance the field.

The profile and success of PreDiCT-TB has demonstrated the value of a more integrated approach to evaluation of development efforts in tuberculosis and the model of open collaboration adopted has facilitated broader discussions about how to maintain and extend the momentum of the initiative. The resources developed within PreDiCT-TB were developed with sustainability and future extensions of the research in mind, allowing for expansion of the databases to novel drugs and combinations targeting drug sensitive and drug resistant disease. Informal discussions involving PreDiCT-TB, CPT, EFPIA and the Bill & Melinda Gates Foundation (BMGF) are already exploring the possibility of linking

the new development methodology and tools to the outputs of the BMGF-funded TB Drug Accelerator with the vision of creating a truly comprehensive and global collaboration that would support all phases from discovery to early clinical trials. From an EU perspective, IMI and the EDCTP-supported PanACEA consortium can and should play a critical role in the formation and achievement of this vision. IMI is the funding body that sits at the interface of all these stakeholders and EFPIA members, allowing them to drive what is needed to bring new medicines from discovery to development in TB, by focusing on the remaining issues with the roadblocks identified in this project.

Biomarkers developed by the consortium, in particular MBLA, are now being employed in academic clinical trials and pre-clinical studies. It would be a major point of success if this tool could be further developed on a commercial platform and gain approval by EMA and FDA as part of the tuberculosis drug development pathway.

In light of recent clinical trial activity in MDR-TB it would be logical to extend the work of WP4 to include treatment for drug-resistant tuberculosis and to consider whether single-arm “trials” or high-quality observational cohort data could also be usefully included in the analysis. Since time-to-event analyses were hampered by inconsistent reporting, additional availability of individual patient data in the future may enable a more extensive analysis of these outcomes, which may offer advantages over culture conversion at 8 weeks in comparing regimens.

Although clinical trial simulation tools have been developed for Phase IIB trials, prediction of treatment failure and relapse at different durations in Phase III based on empirical approaches remains imperfect. Future work based on larger individual patient datasets, better biomarkers and more mechanistic approaches may improve the performance of such models. A more complete *in silico* model including *in vitro* and *in vivo* data able to predict more precisely cure in humans is still needed. However, in order to achieve this, continued close collaboration between modellers and experimentalists will be essential to break down disciplinary barriers and fill remaining gaps in knowledge.