IMI2 GA853989 - ERA4TB

European Regimen Accelerator for Tuberculosis

WP3 – *IN VIVO* PROFILING

D3.1. First Report on implementation of selected mouse models for in *vivo* evaluation of drug candidates.

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Definitions

Partners of ERA4TB are referred to herein according to the following codes:

Grant Agreement. The agreement signed between the beneficiaries and the IMI JU for the undertaking of the ERA4TB project.

Project. The sum of all activities carried out in the framework of the Grant Agreement.

Work plan. Schedule of tasks, deliverables, efforts, dates and responsibilities corresponding to the work to be carried out, as specified in Annex I to the Grant Agreement.

Consortium. The ERA4TB Consortium, comprising the legal entities signatories of the Grant Agreement.

Consortium Agreement. Agreement concluded amongst ERA4TB participants for the implementation of the Grant Agreement. Such an agreement shall not affect the parties' obligations to the Community and/or to one another arising from the Grant Agreement.

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1. Abstract

The main objective of WP3 (*in vivo* profiling) is to evaluate novel anti-tuberculosis (TB) compounds, singly or in combination under *in vivo* infection conditions, in selected animal models. This activity requires to establish and implement experimental animal models, starting with murine models. The foreseen delivery date for this first report on murine models was delayed due to the strong impact of the COVID-19 pandemic on the timetable for the implication of the different mouse models. However, in the meantime, some of the originally planned models have been established and first experiments have been undertaken.

Indeed, C3HeB/FeJ mouse breeding colonies have been set up and enlarged in two partner institutions (IPP and FZB) and after numerous discussions with EFPIA partner GSK and collaborating partner Anne Lenaerts (Colorado State University), initial experimental plans have been established for C3HeB/FeJ mouse benchmark experiments. Experiments using these mice have been undertaken or are currently underway. In these experiments, cultures of *Mycobacterium tuberculosis* (Mtb) strain Erdman are used, which have been prepared by pellicular growth by one of the EFPIA partners (GSK) and distributed to the concerned WP3 partners, where appropriates stock cultures for infection have been prepared. Preparations of this Mtb Erdman strain have also been used to infect BALB/c mice in acute and chronic infection models, which have been implemented at two partner institutions (SCI and IPP, respectively) and used for the initial drug testing experiments on compound MPL-447 supplied by TB Alliance (TBA), following an experimental plan that has been discussed and optimized in collaboration with the teams involved in WP5. In conclusion, we consider that deliverable D3.1 has been achieved, and that the corresponding BALB/c and C3HeB/FeJ mouse models are implemented and operational at the partner research institutions (IPP, SCI & FZB), as foreseen and described in the DoA.

2. Methods

a) Implementation of the C3HeB/FeJ mouse model

C3HeB/FeJ (also known as “Kramnik”) mice develop necrotic lung lesions following infection with Mtb, and thus mimic conditions that resemble to some extent those that are present in human TB patients. Three types of lesions can be observed, which are named type I, type II and type III lesions. As described in *Irwin et al., . 2015. Dis Model Mech. 8(6); 59, Irwin. et al., 2016 ACS Inf Dis 2(4): 251-67,* and *Ryan et al. 2014. Tuberculosis. 94(5):511-8,* type I granulomas correspond to encapsulated granulomas that are highly organized and show caseous necrosis and hypoxia, whereas type II granuloma correspond to fulminant neutrophilic alveolitis, with central necrosis, and type III granulomas correspond to lesions that are normally seen in BALB/ c mice and do not contain caseous necrosis and hypoxia. The initial experiments undertaken at IPP and FZB with C3HeB/FeJ mice inside appropriate BSL3 animal houses serve as benchmark experiments to establish the experimental conditions in which type I granulomas are most prevalent using the Mtb Erdman strain that was distributed to WP3 members. To monitor drug effectivity in plasma without endpoint CFU analysis, biomarkers such as neutrophil-derived ones are evaluated in Mtb-infected C3HeB/FeJ mice.

Experimental planning and data for the initial C3HeB/FeJ benchmarking mouse study (IPP):

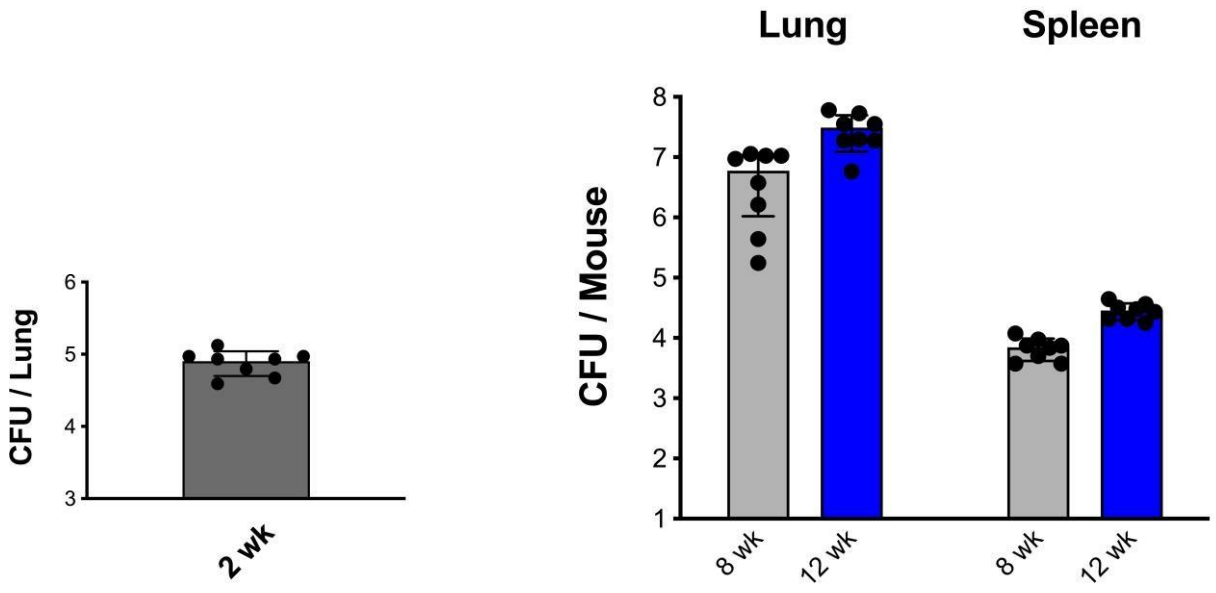
Experimental groups for set up the Kramnik model:

Group	Number of mice	Time after the infection	Output
1	3	(Not infected) 8 weeks	Histology
2	3	(Not infected) 12 weeks	Histology
3	5	1 day	CFU
4	8	2 weeks	CFU
5	8	8 weeks	CFU
6	8	12 weeks	CFU
7	8	8 weeks	Histology
8	8	12 weeks	Histology
TOTAL	51		

→ **Infection with Erdman strain by aerosol (45 mice)** : 08/02/21 (1 run) a suspension at a concentration of $1.5.10^5$ c/ml (+ 6 mice non infected as control for histology)

Results of CFU counting at different timepoints:

→ **Day 1 (5 mice)** : 09/02/21 CFU under limit of detection !! For 1 mouse : 15 Cfu



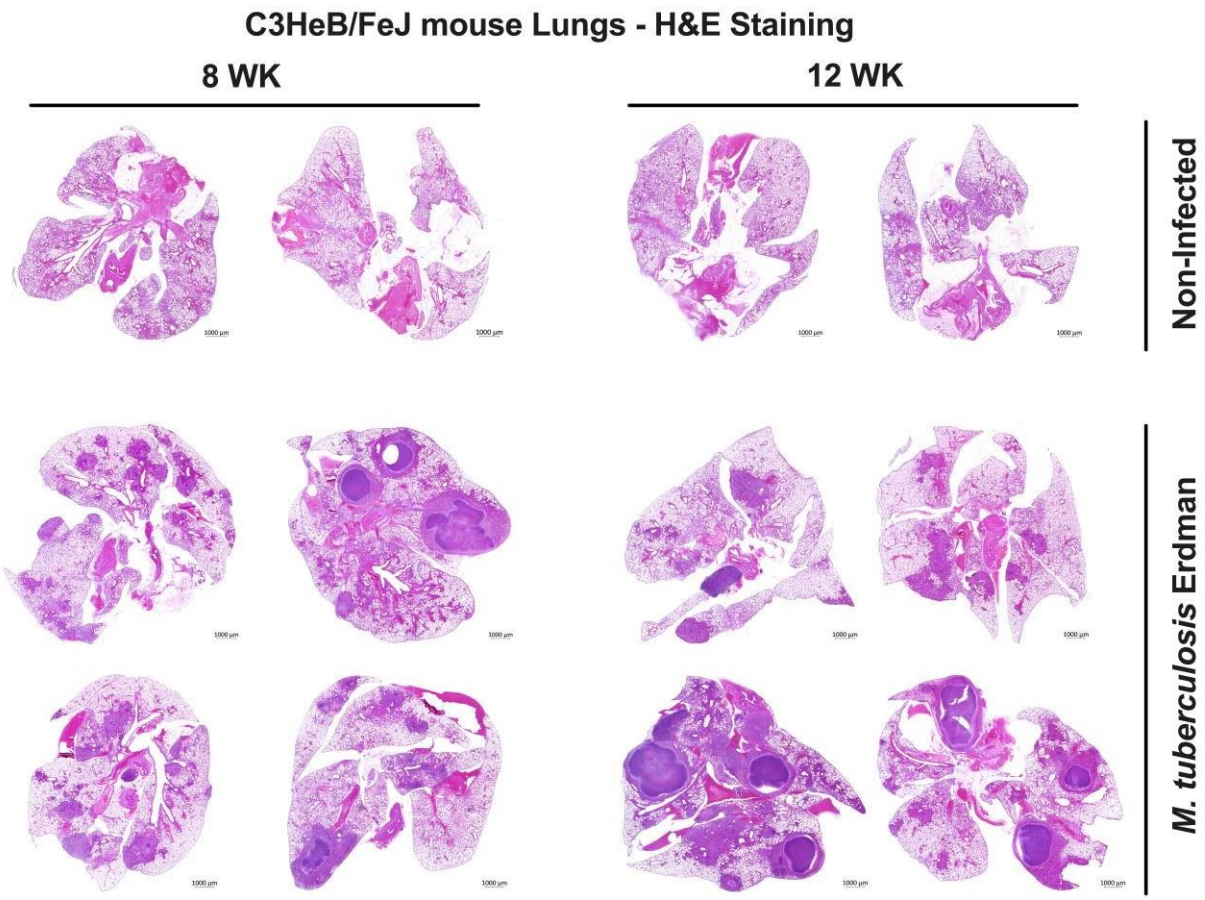
CFU counts shown for week 2 (8 mice) (22/02/21), week 8 (8 mice for CFU) (06/04/21) and week 12 (03/05/21).

CFU counting showed that despite the low infectious dose delivered to the lungs of the mice (as suggested by the day 1 control mouse results), all mice included in the experiment had been infected and developed increasing bacterial load over time.

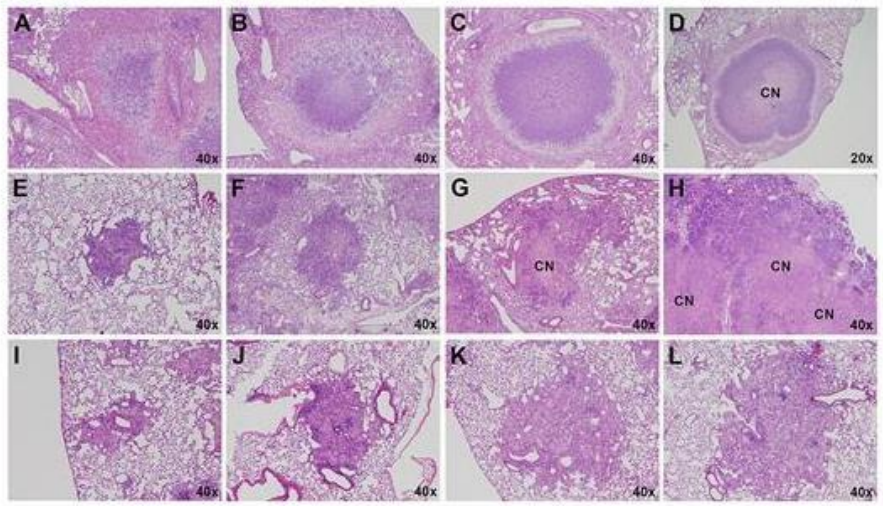
Results of histological examination of lungs:

Comparison of the obtained histological results (examples shown below) with previously published histological results obtained from C3HeB/FeJ mice (Irwin et al., *Dis Model Mech* (2015) 8 (6): 591–602).

suggested that after 8 or 12 weeks of infection, most infected C3HeB/FeJ mice developed type I granulomas, thereby validating the experimental protocol for follow -up studies involving treatment with selected anti-TB drug candidates.



Representative examples of results obtained in the current benchmark study, showing the lesions that occurred in the lungs of the C3H mice at 8 and 12 weeks.



Progression of Type I, II and III lesions over time. Each panel represents a single lesion (H&E-stained) obtained from an individual representative animal (*n*=5) euthanized at the time point defined here. Progression of Type I lesions from individual animals at 45 (A), 50 (B), 55 (C) and 61 (D) days following aerosol infection. Progression of Type II lesions from individual animals at 20 (E), 35 (F), 38 (G) and 40 (H) days following aerosol infection. Progression of Type III lesions from individual animals at 30 (I), 35 (J), 61 (K) and 75 (L) days following aerosol infection. CN, caseous necrosis.

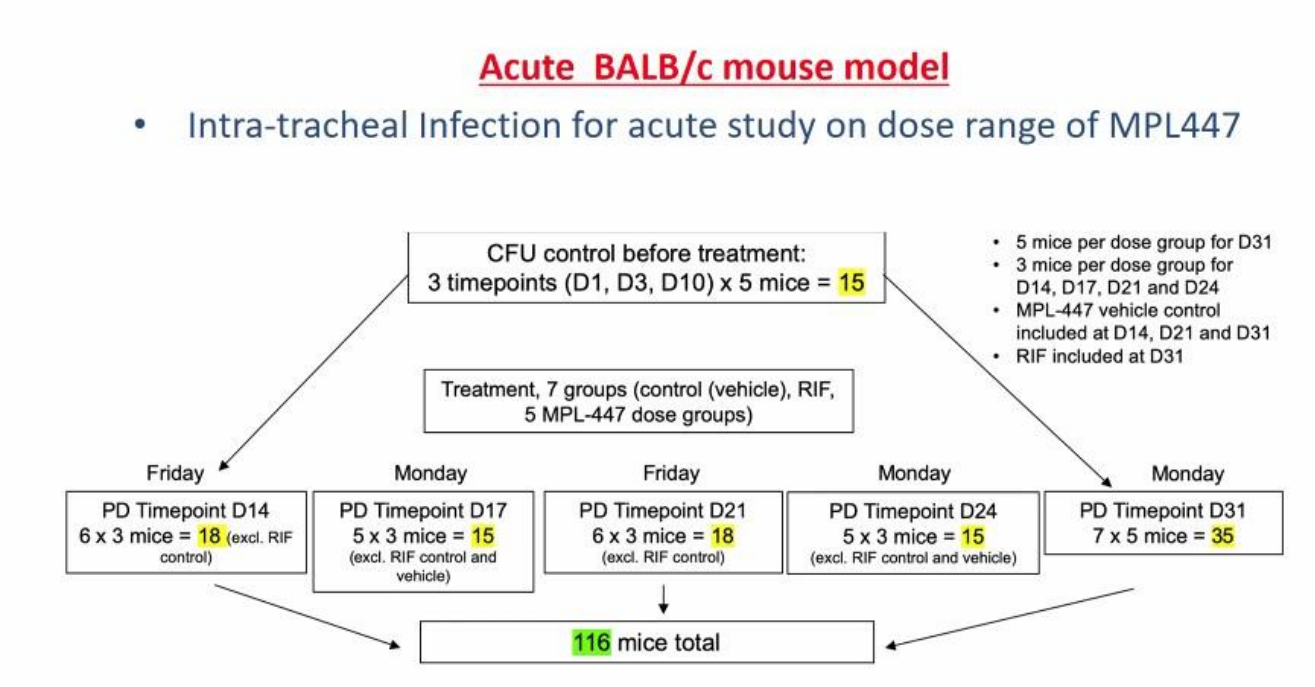
Results published by Irwin et al., *Dis Model Mech* (2015) 8 (6): 591–602) (for comparison)

Similar studies are currently underway at FZB

b) Implementation of the BALB/c acute and chronic mouse models

BALB/c mouse acute and chronic infection models are often used for the evaluation of novel TB drug candidates, and these models have been established within the framework of WP3 in the partner institutions IPP and SCI. For the infection of mice, the Mtb Erdman strain that was distributed to WP3 members was prepared and used in IPP and SCI. The experimental plan for testing MPL-447, a novel drug candidate provided by TBA, was elaborated in collaboration with the molecule owner (TBA) and a specific asset progression plan (APP) was presented to the steering committee of ERA4TB, and received its approval. The experimental plan was then further refined in collaboration with the team from WP5, and appropriate reporting spread sheets have been elaborated in collaboration with the team from WP1. The experiments for the acute BALB/c model on MPL-447 were undertaken by the team at SCI, using intratracheal infection of mice with Mtb Erdman, whereas experiments for the chronic BALB/c mouse model were undertaken at IPP, using an aerosol infection with Mtb strain Erdman.

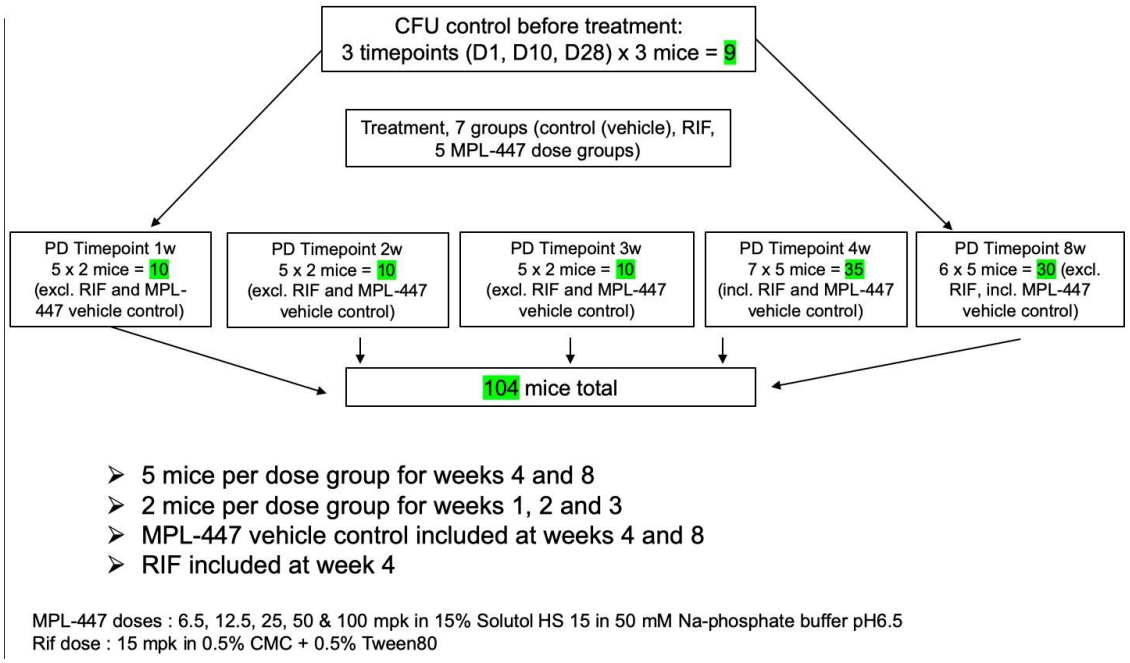
Following experimental plan for the Acute model was developed with help of the teams from WP5 and applied by the team of SCI:



CFU determination for PD analysis has been undertaken and provided to TBA and other partners of WP3. These data have also been uploaded to the ERA4TB portal.

Blood sampling has been made for PK analysis. Samples are currently stored at -80°C awaiting decontamination and preparation for being sent to CRO for PK analysis.

Following experimental plan for the Chronic model was developed with the help of the teams of WP5 and applied by the team of IPP:



- 5 mice per dose group for weeks 4 and 8
- 2 mice per dose group for weeks 1, 2 and 3
- MPL-447 vehicle control included at weeks 4 and 8
- RIF included at week 4

MPL-447 doses : 6.5, 12.5, 25, 50 & 100 mpk in 15% Solutol HS 15 in 50 mM Na-phosphate buffer pH6.5
Rif dose : 15 mpk in 0.5% CMC + 0.5% Tween80

A PK sampling protocol has been established with the help of the teams from WP5:

Sparse sampling after first dose (2 time-points, e.g. 1h (at t_{max}) and 24h post-dose (informs about elimination)

- 3 mice per dose group
- All MPL-447 dose groups to be sampled

Rich sampling in steady state (6 time-points)

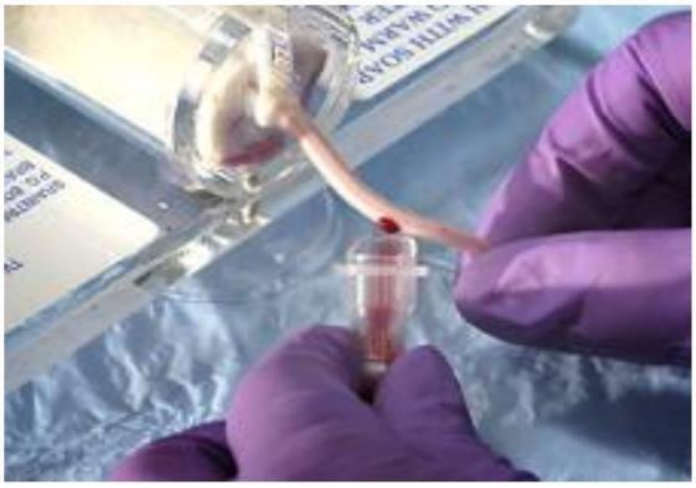
- pre-dose, 0.5, 1, 2, 4, 7 hours post-dose
- sampling spread across several days
- 9 mice per day to be sampled
- 2 samples per mouse

- 18 samples daily

  3 mice per dose group

Sparse rifampicin PK sampling in steady state as control (e.g. around t_{max} (2h) and 6h)

- 5 mice per time-point
- 10 samples



Example of tail vein sampling in BSL3 animal facility

CFU determination CFU determination for PD analysis has been undertaken and the results provided to TBA and other partners of WP3. These data will also be uploaded to the ERA4TB portal.

Blood sampling has been made for PK analysis. Samples are currently stored at -80°C awaiting decontamination and preparation for being sent to CRO for PK analysis.

Conclusion

This first report on the implementation of the acute and chronic BALB/c, and the C3HeB/FeJ mouse models, confirms that the mentioned models have been set up and are operational within the framework of WP3 activities. The models are now available for the different requests that have been or will be formulated by EFPIA & partners, and will be adapted to the specific needs of the requests. Samples taken from experiments using these models are used for analysis of PD and PK data, as well as for research on appropriate biomarkers of TB drug regimen efficacy.