evotec

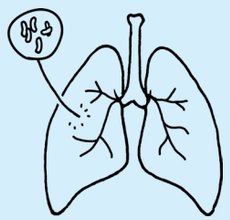
#RESEARCHNEVERSTOPS

Tuberculosis at Evotec

An Expert, End-to-End Industrial Platform

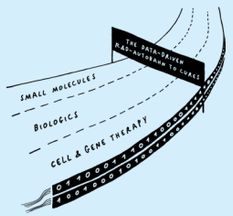
Partner for Global Impact against TB

TB integrated R&D: innovation and power at controlled timelines and costs



Breadth of *M.tb* platforms, under one roof

- *M.tb* capabilities to support concept to candidate; Pathogenic *M.tb* used – BSL3 facilities
- BSL3 HTS, gold standard *in vitro* and *in vivo* models
- Support for targeting bacteria or host, modality-agnostic
- Clinical development support – dose/regimen selection through animal models, hollow-fiber systems, modelling



Evotec's integrated approach

- *M.tb* platforms operate at industrial scale
- Supported by Evotec quality and data management
- Access to all Evotec capabilities
- Flexible, bespoke project design with seamless integration

Credentials from discovery to the clinic

- Team of multi-discipline, seasoned TB specialists including global leaders in the field
- Proven success in TB drug and vaccine discovery, translational science and clinical development
- Multi-year partnerships with BMGF, TB Alliance

An experienced team for expert project design, execution and outcomes

World leading expertise

Name	Florian Von Groote Bidlingmaier	Anna Upton	Pia Thommes	Francesca Bernardini	Eric Bacque	Pascale Lejeune	Guillaume Mondésert	Mike Bodkin
Title	EVP, Head Global Health and Clinical Dev	SVP Infectious Diseases	VP Anti-infectives Virology	VP, *in vitro* Biology, AMR TA Leader	VP, Head of Chemistry	VP Head Translational Biology	VP, Head of High Throughput Biology & Screening	EVP, Head in silico R&D
Ex-perience	>10 years Pharma & clinical	>15 years Not-for-profit & Biotech	>25 years Pharma & Biotech	>19 years Pharma & Biotech	>20 years Pharma	>20 years Pharma	>20 years Pharma	>20 years Pharma
Expertise	TASK Applied Science	TB Alliance	Euprotec, KuDOS, GSK, Astra Zeneca	Arpida, Polyphor, Debiopharm	Sanofi, Aventis, Rhone-Poulenc Rorer	Sanofi, Bayer	Sanofi, Microcide	Lilly

An experienced team for expert project design, execution and outcomes

Scientific team and Project Leaders

Name	Christine Roubert	Alastair Parkes	Evelyne Fontaine	Aurélie Ray	Stephanie Sans	Sylvie Sordello	Emilie Huc-Claustre
Title	Principle Scientist Infectious Diseases & TB & Global Health TA Leader	VP Head Medicinal Chemistry	Senior Research Scientist, Medicinal Chemistry	Senior Research Scientist, *In vitro* Biology	Research Scientist, *in vitro* Biology	Principal Scientist, Translational Biology	Research Scientist, *In vivo* Pharmacology
Experience	>15 years in Tb Immunology	>20 years Drug Discovery	>25 years Drug Discovery	>15 years in Tb Immunology	>20 years industry & Biotech	>20 years Pharma, Biotech & CRO	>10 academic research
Former education / companies	PhD Cellular and Molecular Neurobiology, Sanofi	PhD Organic Chemistry, GSK	PhD Organic Chemistry, Sanofi	PhD Immunology, Trudeau Institute, Transgene	Isoprim Biotech, Sanofi	PhD human Physiopathology, Sanofi, Physiogenex, Vibiosphen	PhD Biochemistry
Name	**Corinne Lafon**	**Bastien Cautain**	**Fabrizio Simonetti**	**Stephanie Sandiford**	**Kirsty Skinner**	**Tara Langley**	**-**
Title	Group Leader, Screening	Team Leader, Screening	Research Scientist, Screening	Senior Scientist II, Infectious Diseases	Senior Scientist II, Infectious Diseases	Senior Scientist II, Infectious Diseases	-
Experience	>20 years Pharmaceuticals	>15 years Pharma & CRO	>10 years academia	>10 years academia & industry	>8 years academia & industry	>9 years industry & Biotech	-
Former education / companies	PhD cellular Biology, Sanofi	PhD Human Physiopathology, Elli Lilly, Medina Foundation	PhD Cellular & Molecular Biology	PhD Medical Microbiology	PhD Microbiology	PhD virology	-

Proven impact against TB

Through experience, expertise and partnering excellence


nature medicine

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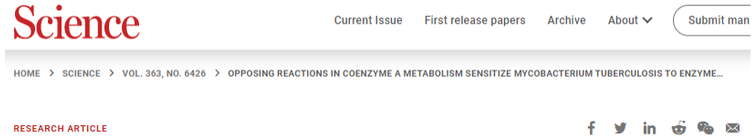
Comment | Published: 05 July 2021

The Tuberculosis Drug Accelerator at year 10: what have we learned?


AMERICAN SOCIETY FOR MICROBIOLOGY | mBio® AN OPEN ACCESS JOURNAL PUBLISHED BY THE AMERICAN SOCIETY FOR MICROBIOLOGY

mBio. 2019 Jul-Aug; 10(4): e01405-19. PMCID: PMC6747715
Published online 2019 Jul 9. doi: 10.1128/mBio.01405-19 PMID: 31289182

Bactericidal Disruption of Magnesium Metallostasis in *Mycobacterium tuberculosis* Is Counteracted by Mutations in the Metal Ion Transporter CorA


Science

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HOME > SCIENCE > VOL. 363, NO. 6426 > OPPOSING REACTIONS IN COENZYME A METABOLISM SENSITIZE MYCOBACTERIUM TUBERCULOSIS TO ENZYME...

RESEARCH ARTICLE

Opposing reactions in coenzyme A metabolism sensitize *Mycobacterium tuberculosis* to enzyme inhibition


ACS Publications | ACS INFECTIOUS DISEASES

This Journal | Search | Submit a manuscript

ACS Infect Dis. 2022 Mar 11; 8(3): 557–573. PMCID: PMC8922279
Published online 2022 Feb 22. doi: 10.1021/acsinfecdis.1c00570 PMID: 35192346

Identification of β-Lactams Active against *Mycobacterium tuberculosis* by a Consortium of Pharmaceutical Companies and Academic Institutions


HHS Public Access
Author manuscript
Peer-reviewed and accepted for publication

About author manuscripts | Submit a manuscript

ACS Infect Dis. Author manuscript; available in PMC 2020 May 21. PMCID: PMC7241432
Published in final edited form as: NIHMSID: NIHMS1588217
ACS Infect Dis. 2019 Aug 9; 5(8): 1433–1445. PMID: 31184461
Published online 2019 Jun 11. doi: 10.1021/acsinfecdis.9b00112

Dual-Pharmacophore Pyrithione-Containing Cephalosporins Kill Both Replicating and Nonreplicating *Mycobacterium tuberculosis*


frontiers in Immunology

Front Immunol. 2021; 12: 668060. PMCID: PMC8284339
Published online 2021 Jun 28. doi: 10.3389/fimmu.2021.668060 PMID: 34276658

Integrative Analysis of Human Macrophage Inflammatory Response Related to *Mycobacterium tuberculosis* Virulence


ACS Publications | JOURNAL OF MEDICINAL CHEMISTRY

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J Med Chem. 2022 Feb 10; 65(3): 1996–2022. PMCID: PMC8842310
Published online 2022 Jan 19. doi: 10.1021/acs.jmedchem.1c01565 PMID: 35044775

In Vitro and In Vivo Inhibition of the *Mycobacterium tuberculosis* Phosphopantetheinyl Transferase PptT by Amidinoureas


ChemMedChem
An Official Journal of the EFMC

Chemistry Europe
European Chemical Societies Publishing

Research Article | Open Access

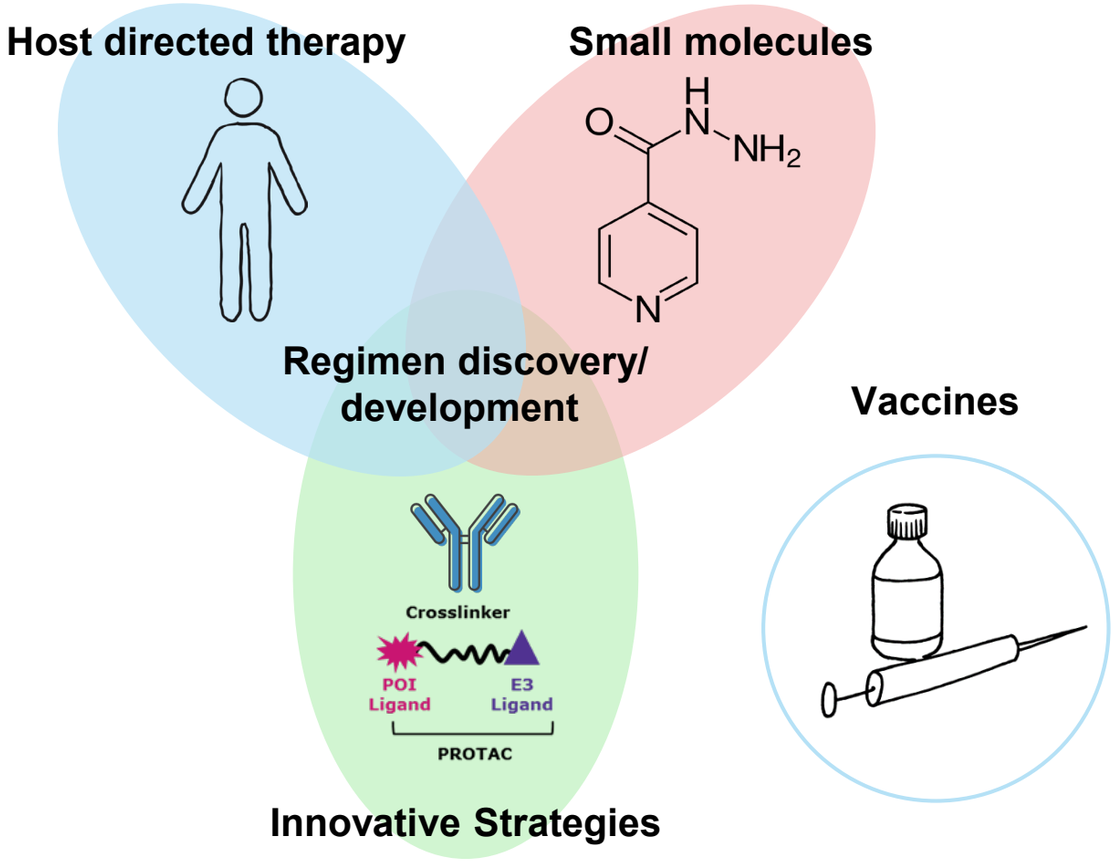
The Discovery and Structure-Activity Evaluation of (+)-Floyocidin B and Synthetic Analogs

Yolanda Kleiner, Dr. Christoph Pöverlein, Jannike Klädtke, Dr. Michael Kurz, Henrik F. König, Dr. Jonathan Becker, Dr. Sanja Mihajlovic, Dr. Florian Zubeil, Dr. Michael Marner ... See all authors

First published: 26 October 2021 | https://doi.org/10.1002/cmdc.202100644

An expert end-to-end TB platform within a global organisation

From high quality standard TB models to innovative and bespoke assay development



Supporting a wide range of TB R&D:

- Small molecules against replicating/non-replicating *M. tb*:
  - BLS3 HTS for cell-based approaches in *M. tb*
  - Gold standard and innovative *in vitro M. tb* assays
  - Murine TB models for each stage of discovery/development
- Host-directed approaches:
  - *M. tb* intra-macrophage assays, binding assays
  - Murine TB models with immune marker readouts
- Regimen discovery/development
  - *In vitro* combination studies, including hollow fibre
  - *In vivo* combinations – relapsing mouse model
- **Most importantly:**
  - **Custom assay-development or adaptation, to support individual project needs**

Tuberculosis disease overview

Host cells and environments for *Mycobacterium tuberculosis (M. tb)*

- TB transmission occurs by airborne particles
- Once inhaled, the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract and bronchi to reach the alveoli of the lungs (TB is primarily a lung infection)
- Following colonisation, an inflammatory cellular infiltrate triggers, in the lungs, the formation of granulomas

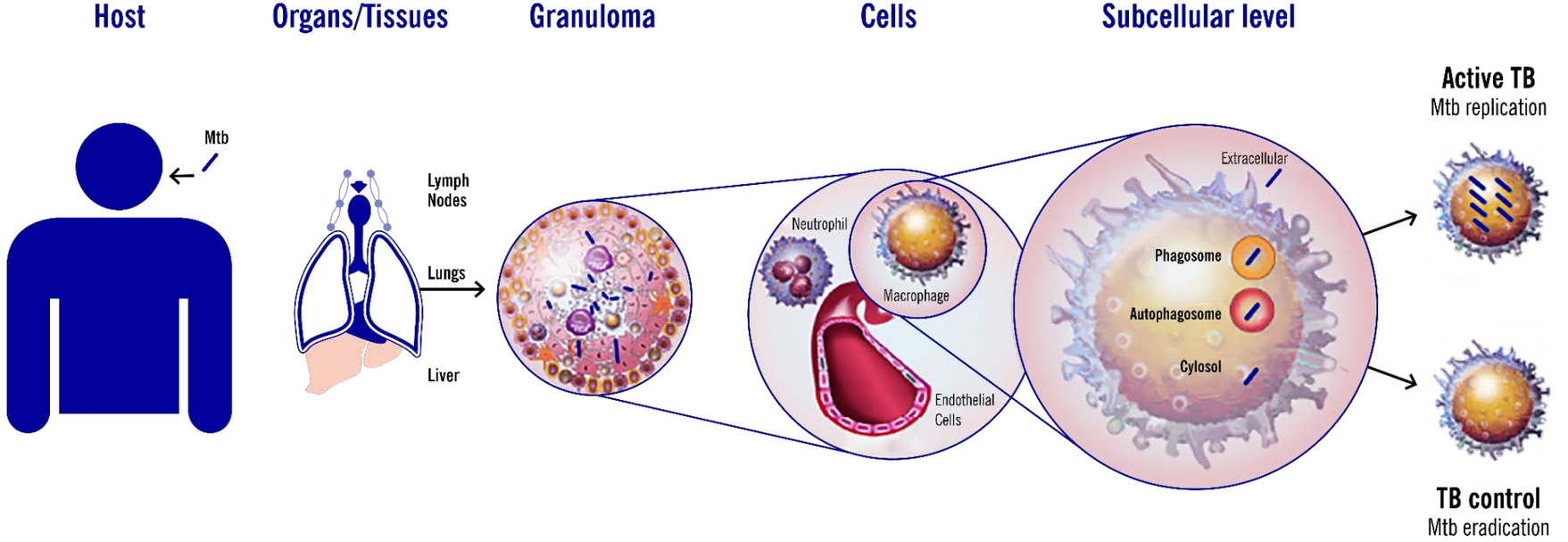
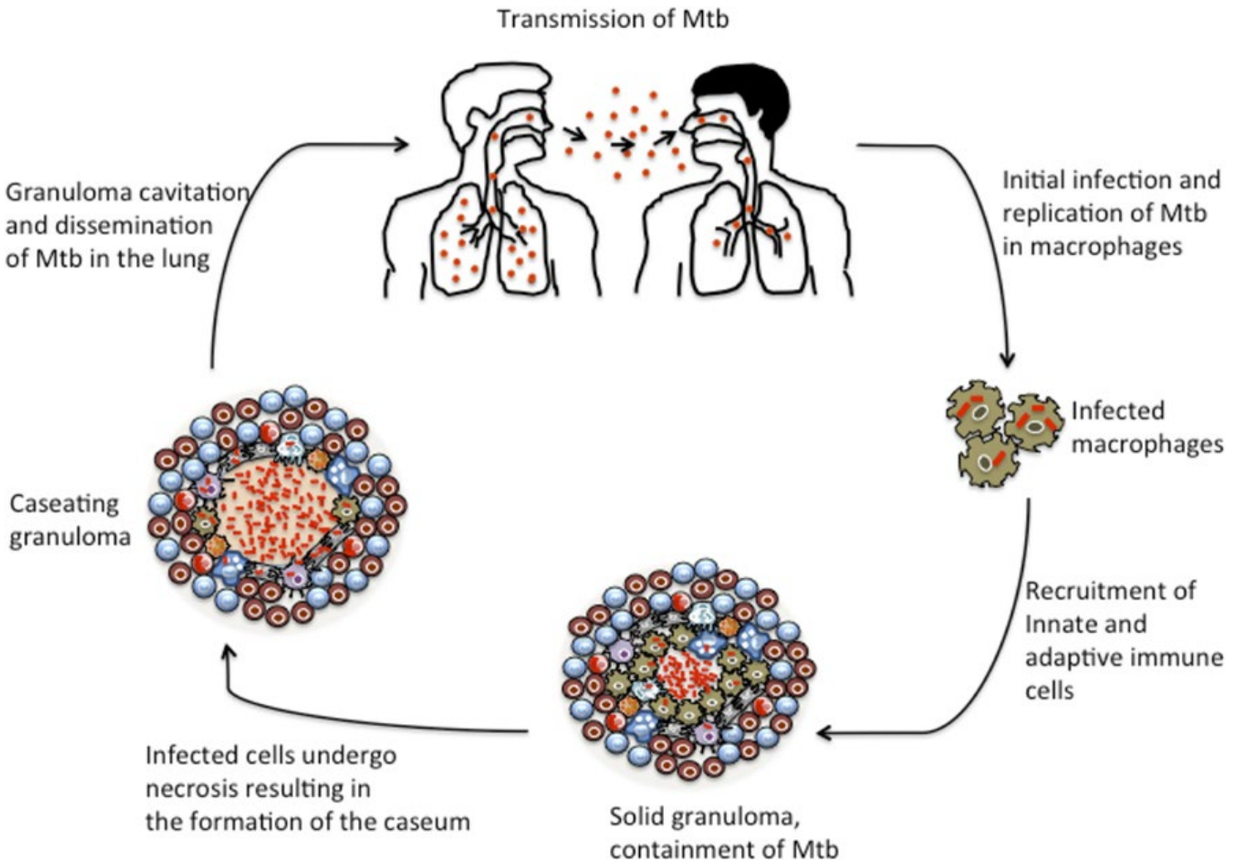


Image adapted from Bussi et gutierrez, FEMS, 2019

A range of *in vitro* and *in vivo* assays to reflect the heterogeneous TB lesion environment

Accessing the full anti-*M. tb* profile, to better predict performance as a novel therapeutic



***In vitro* assays on extracellular bacteria:**

- Replicating *M. tb*
- Non-replicating *M. tb*

***In vitro* assays on intracellular bacteria:**

- Replicating *M. tb*
- Non-replicating *M. tb*

***In vivo* models reflecting:**

- Acute phase of infection
- Chronic phase of infection
- *M. tb* in necrotic granulomas
- Cure/relapse following combination treatment

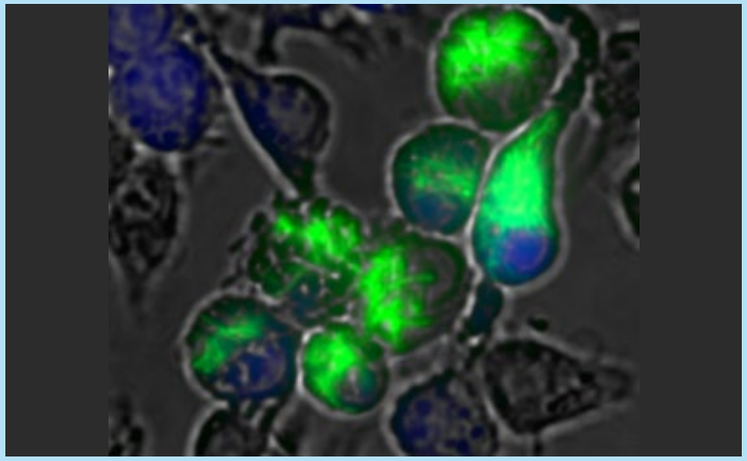
Reference (Figure): Cambier, C.J., et al. Cell. 2014; doi:10.1016/j.cell.2014.11.024

TB Research – from Discovery Biology to Clinic

Seamless program progression from discovery to development

Discovery biology

- Assay development
- Target and MoA identification
- MoR determination
- Omics and sequencing technologies
- Antibody research

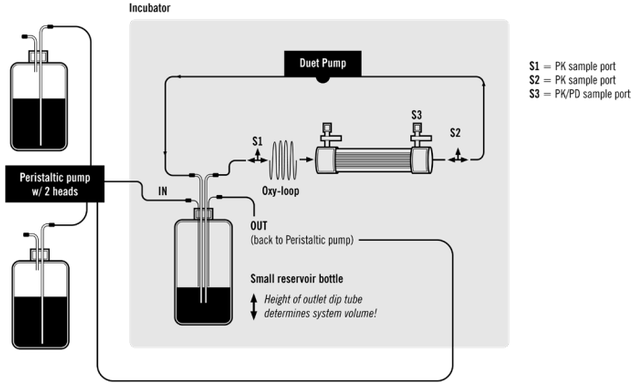


Integrated Drug Discovery to Phase 1

- Target/Whole cell screening (*M. tb*)
- Anti-*M. tb in vitro* activity, profiling
- Natural products drug discovery
- Med chem, comp chem and structure based drug design
- Highly efficient DMTA cycles
- State of the art DMPK, *in vivo* pharm
- Clinical-enabling – INDiGO
- Phase 1

Translational microbiology and PK/PD

- *In vivo* microbiology for efficacy profiling
- *In vivo* and *in vitro* PK/PD platforms including Hollow Fibre systems
- Mathematical modelling and simulations
- Translation of discovery data to the clinical setting



TB Integrated R&D – Small molecules

Support from concept to clinic

Hit identification

- BSL3 MTS/HTS
- Assay development and miniaturization
- Support for back-screening and hit expansion
- MICs and MBCs for hit profiling

Hit to lead

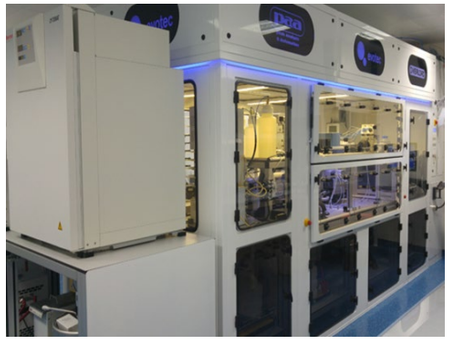
- MICs to support SAR – replicating, non-replicating and intracellular *M. tb*
- Mode of action and resistance
- Highly Acute (PoC) and Acute mouse models

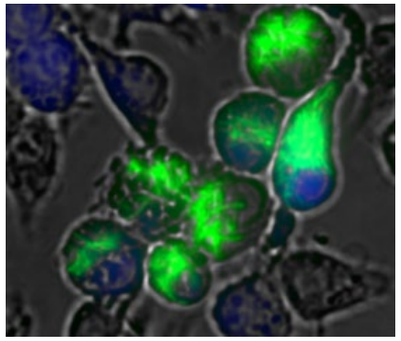
Lead optimisation

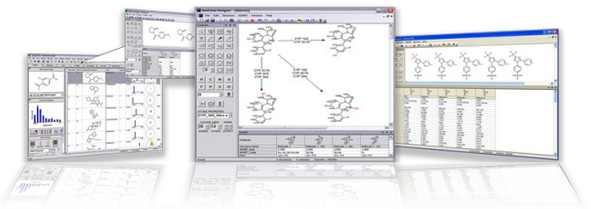
- Beyond SAR support: MBCs, time kill curves, inoculum/serum effect for in-depth profiling
- Acute and chronic mouse models, including Kramnik model (necrotic granuloma)

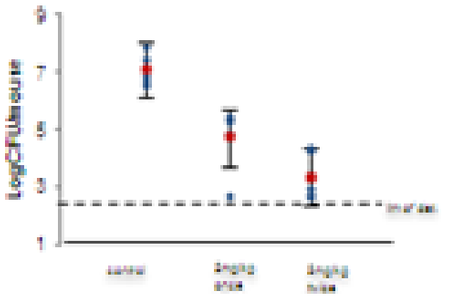
Development

- Hollow Fibre Infection Model (HFIM) (H37Ra) – monotherapy or combination PK/PD and resistance studies
- 14-day mouse model combination studies – kill kinetic, time to cure





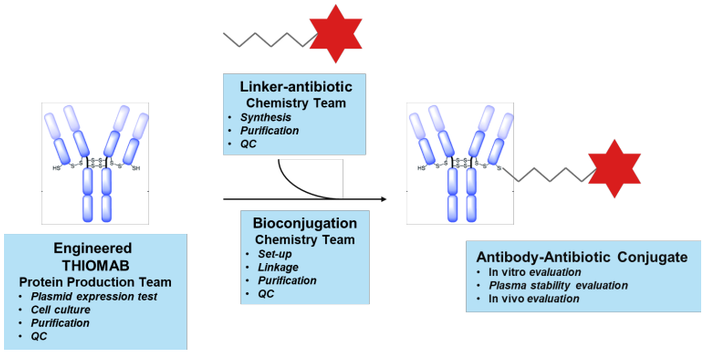




TB Integrated R&D – Antibody-drug conjugate

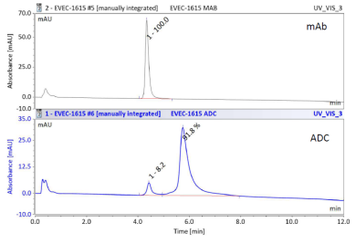
From concept to candidate “under-one-roof”

Biosynthesis and characterisation of an antibiotic antibody conjugated product

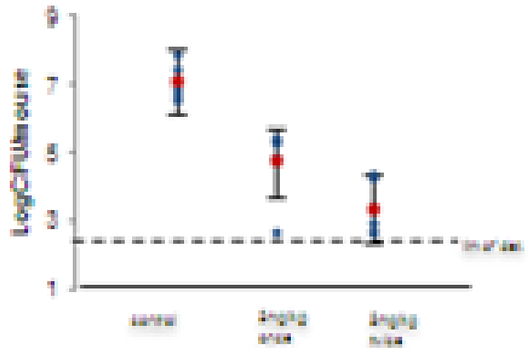


Antibody production, payload synthesis and bio-conjugation

- Evaluation of binding
- Enzymatic release assay
- *In vitro* infection experiments to assess *M. tb* growth inhibition



Plasma stability

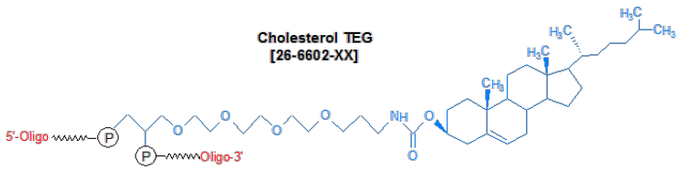


In vivo efficacy study

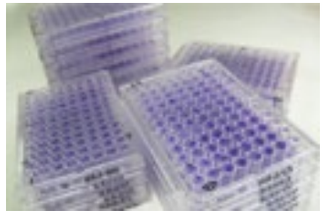
TB Integrated R&D – Oligonucleotides

From concept to candidate “under-one-roof”

Synthesis and efficacy study of oligonucleotides

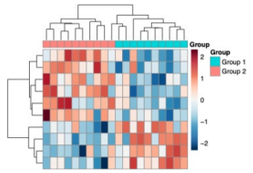


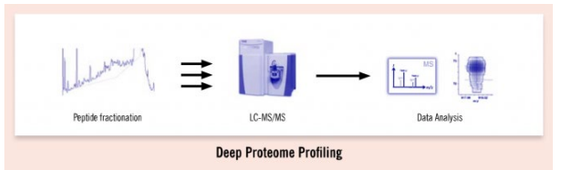
Custom oligonucleotides synthesis, conjugation with tag as cholesterol tag (low, medium or large scale production)



In vitro
Activity study

- Uptake evaluation in different media conditions (Flow cytometry and confocal microscopy)
- Growth inhibition assay (standard and intramacrophagic)
- Synergy with standard Tb drugs

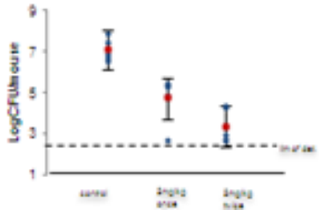




In vitro
Mode of action study

Omics on *M. tb*

- qRT-PCR
- RNAseq
- Proteomics analysis

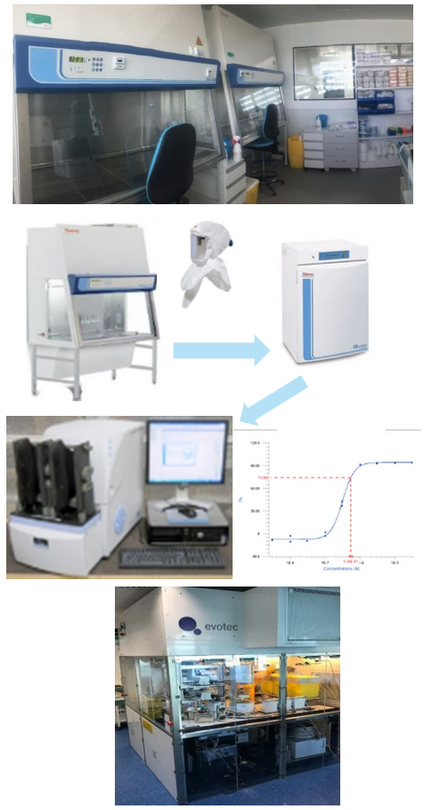


In vivo
efficacy study

- Intranasal administration
- CFU in lung and spleen

Anti-TB *in vitro* platforms – Broad capabilities, from HTS to HFIM

Evaluation and characterisation of anti-TB activity from early discovery to development



HTS screening platform	• BSL3 screening capabilities for MTS/HTS • Assay development and miniaturization • Support for back-screening and hit expansion
***In vitro* activity testing**	• Virulent and attenuated *M. tb* – BSL3 or BSL2 • MICs to support SAR – replicating, non-replicating and intracellular *M. tb* • Readouts – CFU, absorbance, luminescence, fluorescence
Anti-*M.tb* activity profile and MoA studies	• MBCs, time kill curves, inoculum/serum effect for in-depth profiling • MoA studies and Mode of resistance studies including mutant generation and characterisation • Bespoke assay development or assay transfer
Advanced micro-biology and *in vitro* PKPD	Hollow Fibre Infection Model (HFIM) for TB – H37Ra • Single drug or drug combination PK/PD; resistance studies • Evaluation against replicating, semi-dormant *M. tb* • Intracellular system under development • Mathematical modelling support • Significant capacity – up to 34 cartridges in parallel

HTS – High throughput screening
HFIM – Hollow Fibre Infection Model

Anti-*M. tb* activity testing – Assays to support SAR

Ready to go assays modeling TB niches

Assay	*M. tb* status	Comments	Throughput
MIC	Replicating	Measure of growth inhibition Bactericidal activity can be determined by CARA assay	80 cpds, DR, duplicate, 3 weeks[1)]
MIC in cholesterol medium	Replicating		80 cpds, DR, duplicate, 3 weeks[1)]
MEC	Non replicating	Measure of bactericidal activity	80 cpds, DR, duplicate, 3 weeks[1)]
MIC *M. tb* infected-THP1 cells	Replicating, intracellular	Measure of intracellular growth inhibition	160 cpds, DR, duplicate, 6 weeks
MEC *M. tb* infected-foamy THP1 cells	Non replicating, intracellular	Measure of intracellular bactericidal activity in foamy / hypoxic THP1	40 cpds, DR, duplicate, 8 weeks
Whole cell accumulation assay	Replicating	Relative quantification of compounds inside the bacteria by Mass spectrometry	From 8 to 16 cpds, in duplicate, 4 weeks

[1)] If assay ready plates (ARP) used, throughput can be increased to 160 cpds

Minimal Inhibitory Concentration (MIC) determination

Replicating extracellular *M. tb*

Highly robust MABA assay to determine the lowest antibiotic concentration that inhibits the growth of *M. tb* H37Rv (MIC).

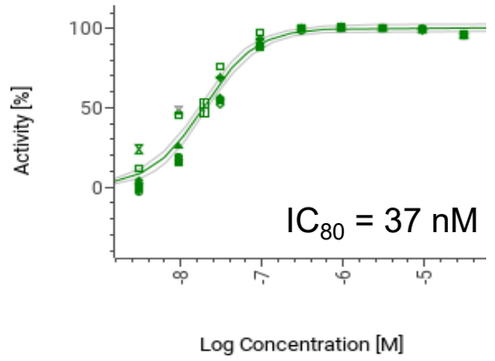
BSL2 Lab

- Compound dilution
- Dose response in 96 well-plates
- Compound distribution in 7H9 / casitone / catalase / BSA / palmitate medium

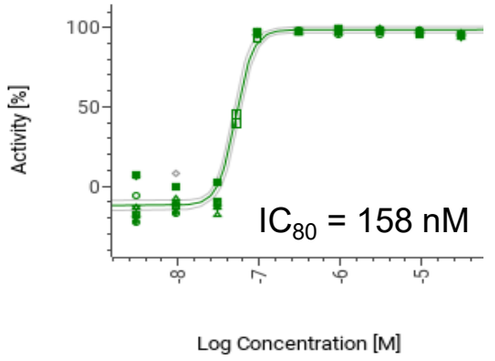
BSL3 Lab

M. tb addition, H37Rv, Black 96 well-plates → Incubation, 6 days, 37°C, 5% CO_2 → Alamar Blue® addition, Incubation 24h → Fluorescence readout

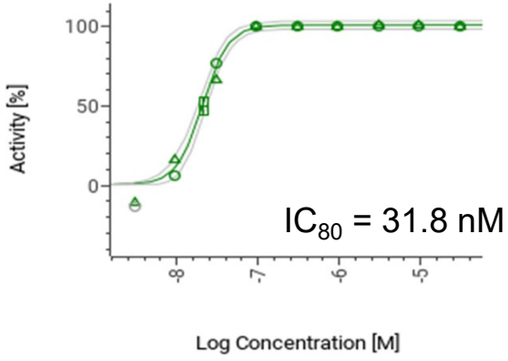
Rifampicin



Isoniazide



Bedaquiline



Growth inhibition curve for MIC_{80} determination

MIC_{80}: Lowest antibiotic concentration that inhibits 80% of the growth of *Mtb* H37Rv

Minimal Effective Concentration (MEC) determination

Non-replicating extracellular *M. tb*

ATP-based assay to determine the lowest antibiotic concentration that affects viability of non replicative *M. tb* H37Rv.

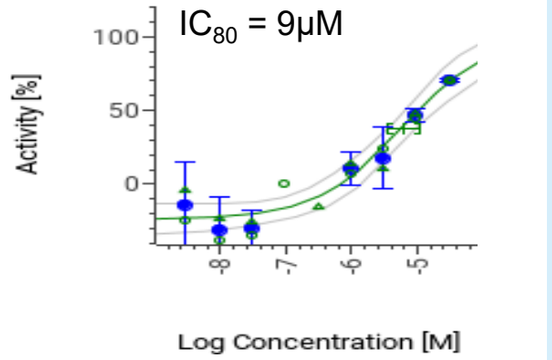
BSL2 Lab

- Compound dilution
- Dose response in 96 well-plates
- Compound distribution in **PBS**

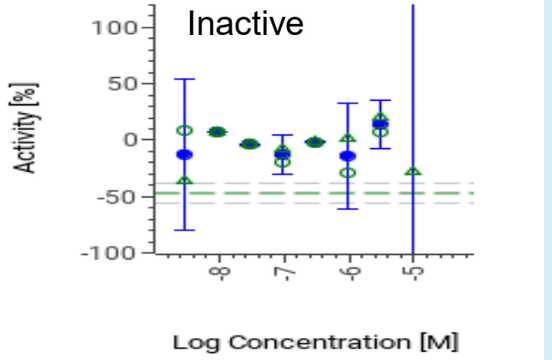
BSL3 Lab

M. tb H37Rv stationary phase culture addition, White 96 wells plates → Incubation, 7 days, 37°C, 5% CO_2 → BacTiter-Glo™ addition → ATP readout (IC_{80})[1)]

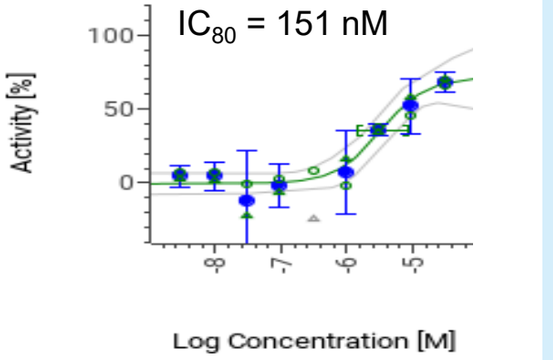
Rifampicin



Ethambutol



Bedaquiline



Growth inhibition curve for MEC_{80} determination

MEC_{80}: Lowest antibiotic concentration that decreases viability by 80% of *Mtb* H37Rv

[1)] additional read out: Miniaturized CFU

Minimal Inhibitory Concentration (MIC) in cholesterol

Replicating extracellular *M. tb* in cholesterol media

MABA assay to identify small molecules that inhibits *M. tb* growth in medium containing cholesterol as the principle carbon source[1)]

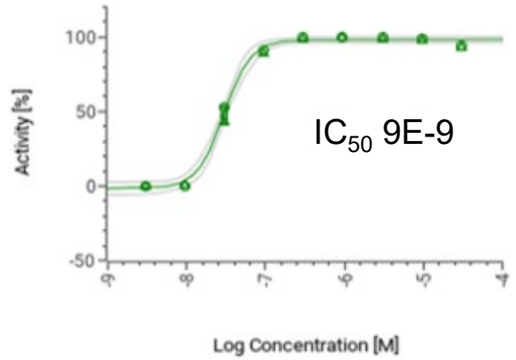
BSL2 Lab

- Compound dilution
- Dose response in 96 well-plates
- Compound distribution in **cholesterol medium**

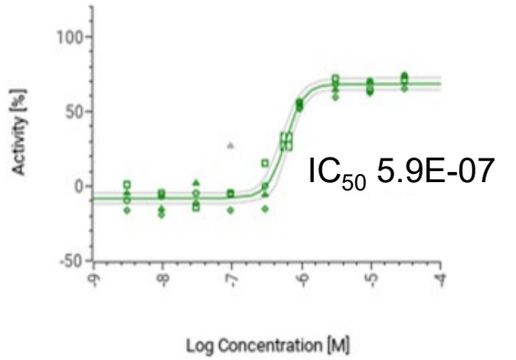
BSL3 Lab

Fresh *M. tb* culture in acetate medium (10 days) →  → Incubation, 12 days in cholesterol medium, 37°C, 5% CO_2 → Alamar Blue® addition, Incubation 24h → Fluorescence readout

Rifampicin



Cholesterol cpd 1



	IC_{80} in Cholesterol medium (M)	IC_{80} in Palmitate medium (M)
Rifampicin	9.15E-09	6.68E-08
Isoniazide	9.03E-08	1.67E-07
Cholesterol cpd 1	5.99E-07	> 3E-5
Cholesterol cpd 2	2.89E-08	> 3E-5

Growth inhibition curve for MIC_{80} determination

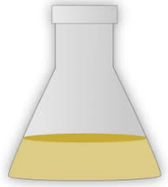
MIC_{80}: Lowest antibiotic concentration that inhibits 80% of the growth of *Mtb* H37Rv

[1)] a critical carbon source during infection

MIC and Minimal Bactericidal Concentration (MBC) in cholesterol, using Charcoal Agar Resazurin Assay (CARA*)

Replicating extracellular *M. tb* in cholesterol media

***Mtb* H37Rv culture, in acetate medium**
10 days

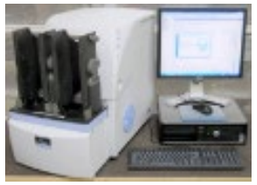


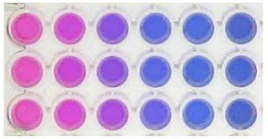
***Mtb* addition, H37Rv, in cholesterol medium**
Black 96 well-plates

Incubation, 9 days,
37°C, 5% CO_2

Alamar Blue® addition, **Incubation 24h**

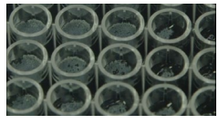
Fluorescence readout





Transfer 10 µl in **Charcoal Agar 96 well plate**

Incubation 7 days



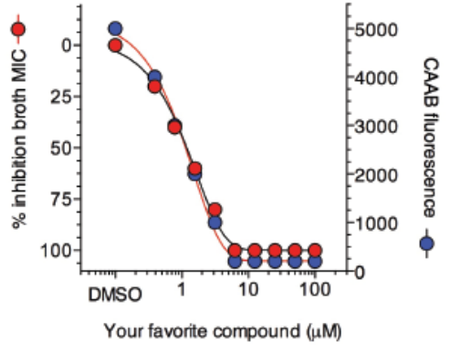
Alamar Blue® addition, **Incubation 1h**

Fluorescence readout

Charcoal Agar Resazurin Assay (CARA)

Minimal Inhibitory Concentration (MIC_{90}) determination

MIC_{90}=CARA-MBC_{99}



Minimal Bactericidal Concentration (CARA-MBC>99) determination

2 different readouts

* Gold B, 2016 Dec 14.

Intra-macrophage Minimal Inhibitory Concentration (MIC) Determination

Replicating intracellular *M. tb*

Highly robust infection assay to determine the lowest antibiotic concentration that inhibits the growth of *M. tb* H37Rv inside macrophages (Intra MIC).

BSL2 Lab

- Assay ready plate
- Dose response in 384 well-plates

BSL3 Lab

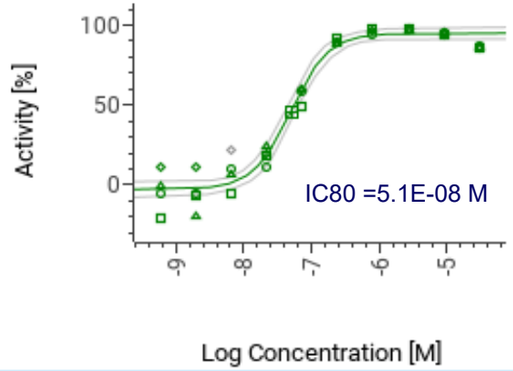
Infection of THP1 cells with H37Rv-GFP in presence of PMA.

After several washing steps, cells are seeded in 384 Microplates containing compounds in dose response

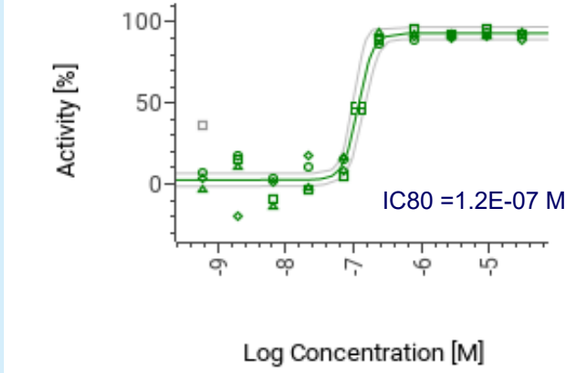
Incubation 4 days

- Cell fixation with PFA and cell staining Draq5
- High content imaging (Arrayscan Cellomics or Operetta)

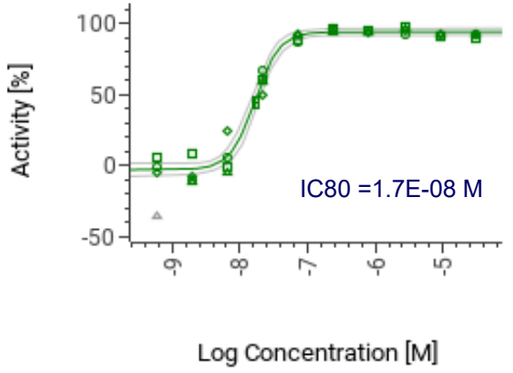
Rifampicin



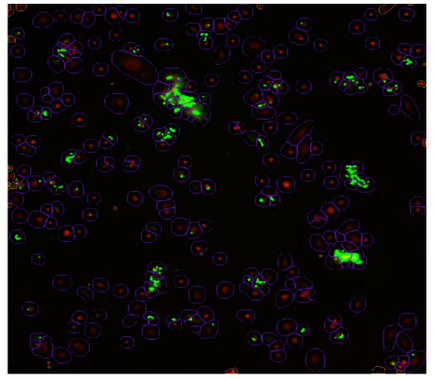
Isoniazide



Bedaquiline



Growth inhibition curve for intra-macrophage MIC_{80} determination



Intra-foamy Macrophage Minimal Effective Concentration (MEC) determination

Non-replicating intracellular *M. tb*

Infection assay run under hypoxia to determine the lowest antibiotic concentration that affects the viability of non replicating *M. tb* H37Rv inside foamy macrophages (Intra- foamy MEC).

BSL2 Lab

- Assay ready plate
- Dose response in 96 well-plates

BSL3 Lab

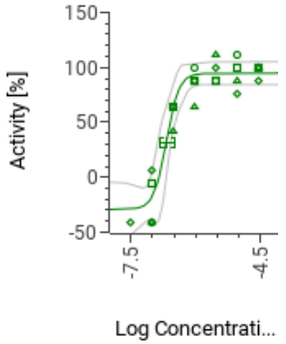
Infection of PMA differentiated-THP1 cells with **H37Rv LuxABCDE**

After several washing steps, cells are seeded in 96 wells plate containing cpds in dose response

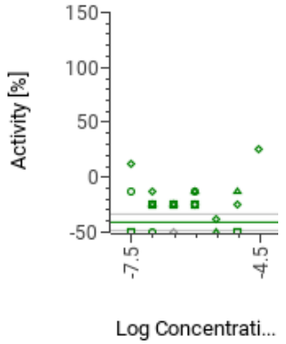
Incubation 4 days under hypoxia

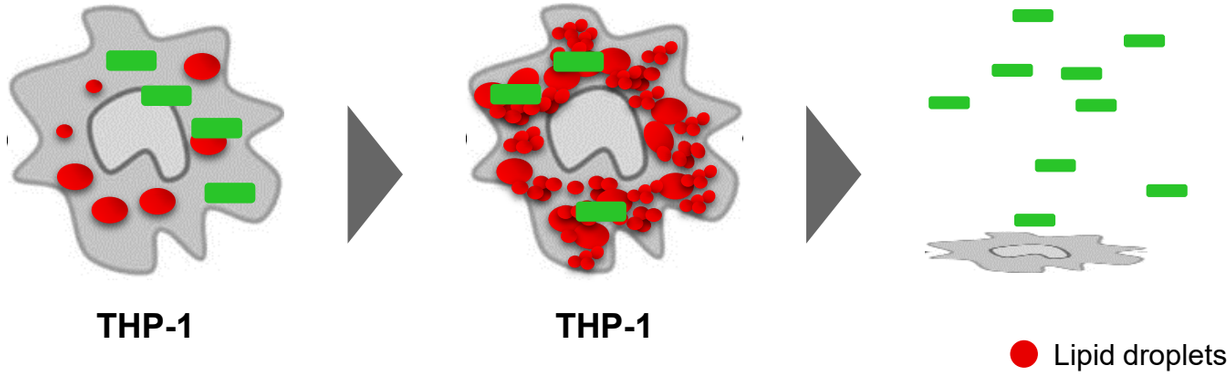
Regrowth phase of 7 days and RLU read-out

Rifampicin



Ethambutol





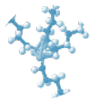
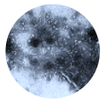
Growth inhibition curve for intra-foamy macrophage MEC_{80} determination

Whole-cell accumulation assay in *M. tb* H37Rv

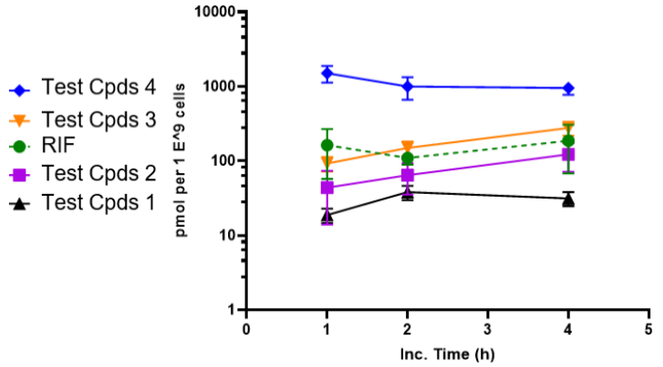
Replicating extracellular *M. tb*

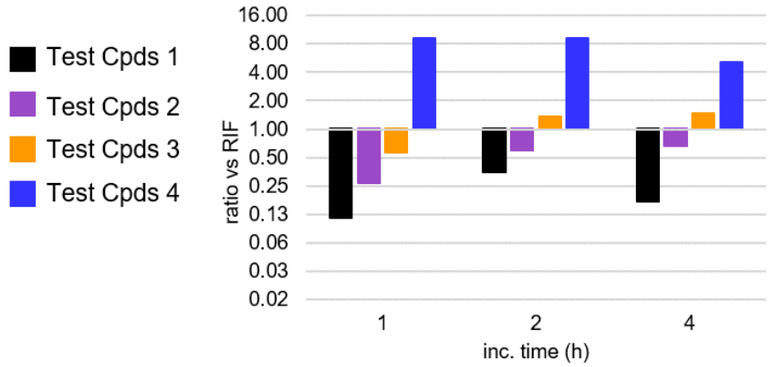
A direct assay to evaluate intra-*M. tb* compound concentration by mass spectrometry, independent of antibacterial activity

BSL3 Lab

Culture growth until exponential phase and transfer to 96-well plate	Add cpds	Cell lysis after several washing step	Preparation for MS	LC-MS measurement / data analysis





- **Rank compounds in a series** or compare different chemical series with respect to accumulation within *Mtb*
- **Support SAR**, especially in the context of IC_{80}, target potency and intra-*Mtb* metabolism data

Anti-*M. tb* activity profiling and MoA studies

Ready to go assays for further activity characterisation

Assay	*M. tb* status	Comments
MIC with 25% serum	Replicating	Measure of growth inhibition in presence of serum from various species
MBC	Replicating	Minimal Bactericidal Concentration
Inoculum effect	Slowly replicating	Measure of growth inhibition with an increasing inoculum (at high inoculum, bacteria enter a non-replicating state)
MIC on solid medium	Replicating	Measure of growth inhibition on 7H11
Time Kill Curve	Replicating	Monitor bacterial growth and death over time
Mutant generation	Replicating	Mutant characterisation including whole genome sequencing and analysis also available. MIC on solid medium recommended prior to start

Minimal Bactericidal Concentration (MBC) determination

Replicating extracellular *M. tb*

BSL2 Lab

- Compound dilution
- Dose response
- 96 well-plates

Compound distribution in 7H9 / casitone / BSA / palmitate medium

BSL3 Lab

M. tb **H37Rv** addition to 96 well-plates

Incubation, **6 days**, 37°C, 5% CO_2 → Alamar Blue® addition, Incubation 24h → **Fluorescence readout**

7 days

Incubation, **11 days**, 37°C, 5% CO_2 → 50µl from wells spread on agar plates → Incubation, **3 weeks**, 37°C, 5% CO_2 / **CFU counts**

32 days

MBC: Lowest antibiotic concentration required to kill 2 logs of the initial bacterial inoculum
CFU: Colony-Forming Unit

Time Kill Curve (CFU)

Replicating extracellular *M. tb*

The Time Kill Curve (TKC) is used to determine the bactericidal or bacteriostatic activity, over time, of antibiotics

BSL3 Lab

5 weeks

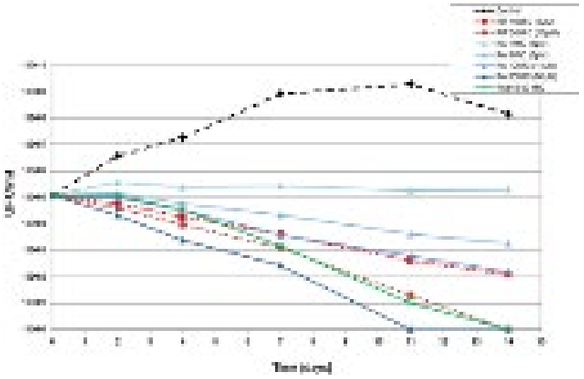
H37Rv
7H9 / OADC / Glycerol / Tween medium

Compound (6conc) **+ reference + control** in DMSO

Incubation: 37°C, shaker 150rpm

At days 2, 4, 7,11, 14 for each condition

- 0.5 ml are collected
- Centrifuged
- Washed
- Diluted
- Dilution are spread on Agar plates



Incubation, **3 weeks,**
37°C, 5% CO_2

CFU count

CFU: Colony-Forming Unit

Solid Medium Minimal Inhibitory Concentration determination

Replicating extracellular *M. tb*

Determination of the lowest compound concentration that inhibits the growth of *M. tb* H37Rv on 7H11 agar plates. The MIC value on solid medium is used as a starting point concentration to determine the frequency of resistance.

BSL2 Lab

Preparation of 6-well plates with 7H11 containing antibiotics at different concentrations (2-fold serial dilutions)

Example drug distribution

BSL3 Lab

From a cryotube, preparation of two bacterial suspensions at 3.10^6 CFU/ml and 30000 CFU / ml

Add 40 µl of each suspension to all wells of the same series dropping 4 spots of 10 µl each.

Incubation
3 weeks 37°C

CFU count
Solid MIC determination

Frequency of Resistance (FoR) determination

Replicating extracellular *M. tb*

BSL2 Lab

Preparation of 7H11 agar plates containing compounds (2X, 4X, 8X and 16X MIC)

BSL3 Lab

24 cultures of *M. tb* H37Rv > OD=0.9

10^8 CFU spread on:

-2X solid MIC (10X)

-4X solid MIC (10X)

-8X solid MIC (10X)

10^9 CFU spread on:

-2X solid MIC (10X)

-4X solid MIC (10X)

-8X solid MIC (10X)

4 cultures used for numeration

Resistant clones isolation and characterisation (stability, DNA extraction, whole genome sequencing, cross resistance assessment)

Timeline: 2 months 6 months

Anti-*M. tb* activity characterisation

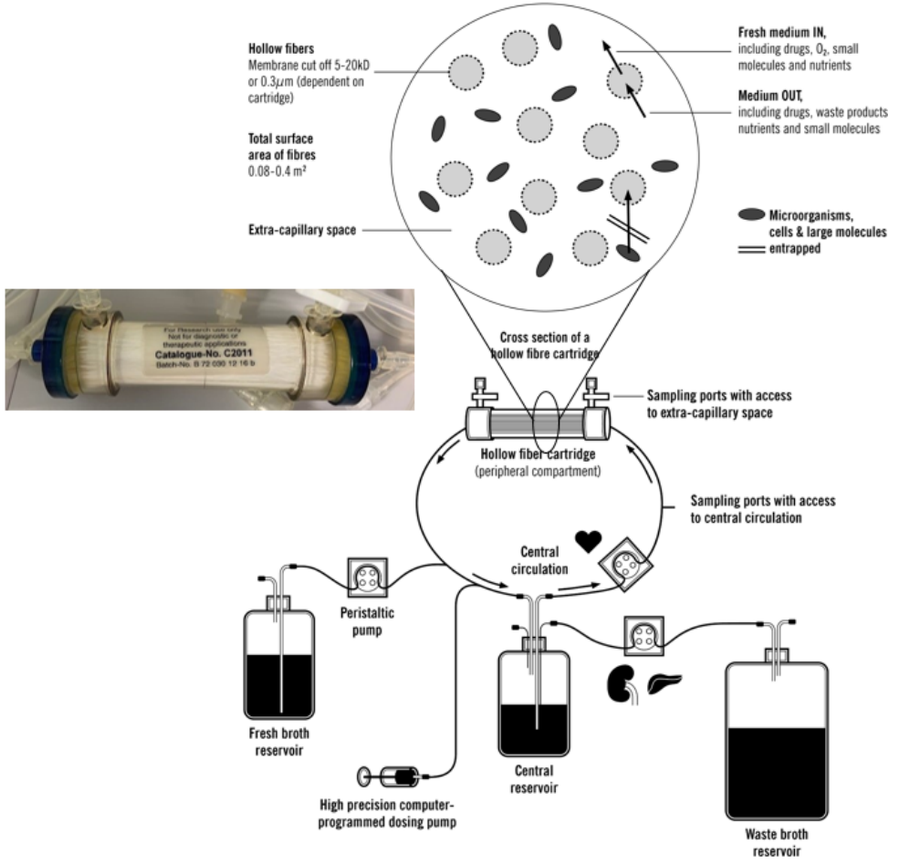
Tailored service adapted to the project

Technology available	Possible assays
Purification of RNA using Maxwell equipment	Bacterial or host RNA for RNAseq, dual RNAseq, DigitalPCR
Confocal microscopy	Bacterial imaging (uptake of fluorescent compounds), infected cell imaging (phagolysosome fusion etc)
Flow cytometry	Antibody binding, fluorescent compound uptake etc
BACTEC MGIT	Whole blood assay
Mesoscale	Cytokine profiling

Hollow Fibre Infection Model using *M. tb* H37Ra

Significant expertise in advanced PK/PD

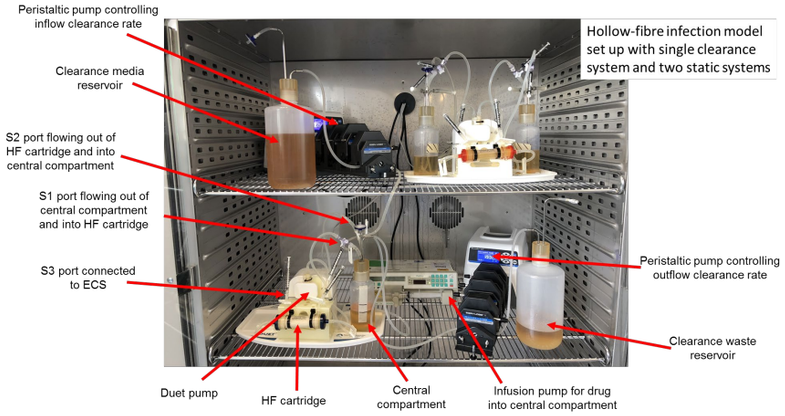
- The Hollow Fibre Infection Model (HFIM) provides an *in vitro* method of assessing the impact of drug exposure(s) on a cell or combination of cells (eukaryote or prokaryote)
- The HFIM is the most capable *in vitro* model for evaluating PK/PD indices and optimising dosing regimens for bacterial killing and suppressing the amplification of drug resistant mutant subpopulations
- Combinations of up to 4 drugs have been used in the system in models lasting up to 42 days; experience with “difficult” drugs requiring optimisation of HFIM system parameters
  - Standard of care in TB therapy: HRZE combination
- Assessment of multiple combinations of drugs against *Mtb* in different growth phases under BSL2 containment
  - Replicative, Semi-dormant (grown in low pH), Intracellular infection models (currently being developed using THP-1 cells)
- System can also be applied to determine resistance development
- Mathematical modelling support including population PK profiling
- **HFIM study designs can be tailored to specific requirements or bespoke client protocols**



Hollow Fibre Infection Model

Capabilities, capacity and experience

- Containment level 2 laboratory space dedicated to running HFIM studies
- Up to 34 hollow fibre cartridges[1)] can be operated in parallel for different organisms, using variable drug infusion and clearance rates, with study durations ranging from hours to 6 weeks
- Dedicated team of scientists trained to operate the system
- Full range of microbiology support including alternative endpoints, whole-genome sequencing and bioinformatics services to support resistance characterisation studies, and mechanism of action determination
- Bioanalysis facilities for LC-MS analysis of PK samples
- A dedicated PK/PD modelling team
- **Capacity still increasing**



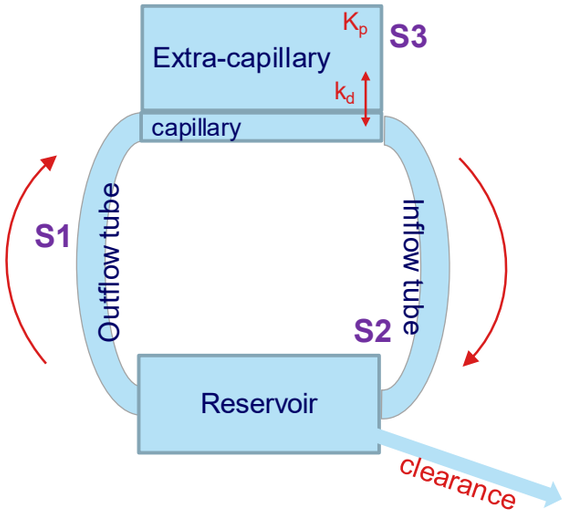
[1)] Depending on model type and duration

Hollow Fibre Infection Model

Mathematical modelling overview

Mathematical modelling support to Hollow Fibre systems provides:

- Experimental design for single- and multiple-drug experiments
  - Generation of infusion parameters (duration, rates, frequency) to reproduce specific PK profile
- Mathematical models for interpretation of pharmacokinetic and pharmacodynamic data
- PK/PD analysis



Parameters of the model

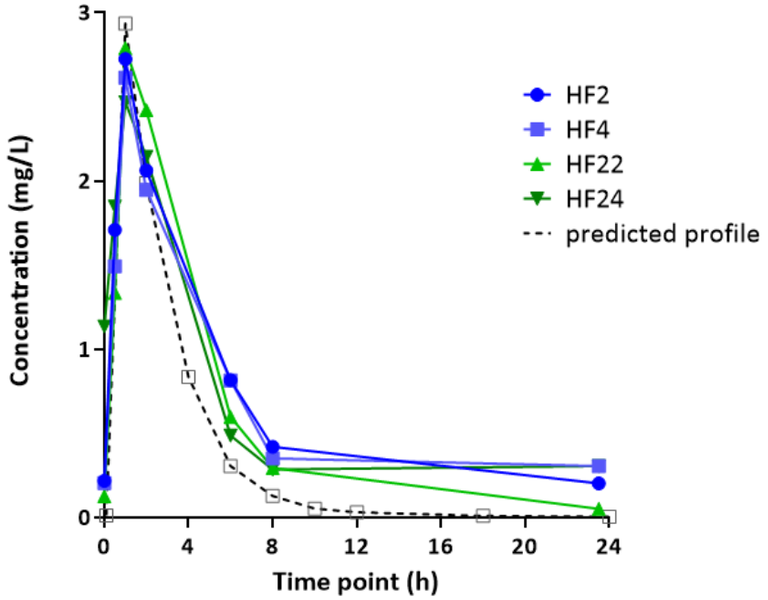
- Parameters related to physical system
  - Volumes – determined by set-up
  - Flow-rate
  - Clearance – variable, dependent on experiment
- Parameters related to the compound – optimised using experimental data
  - Partition coefficient (K_p)
  - Diffusion constant (k_d) for partitioning between capillary and extra-capillary compartments

Hollow Fibre Mathematical Model

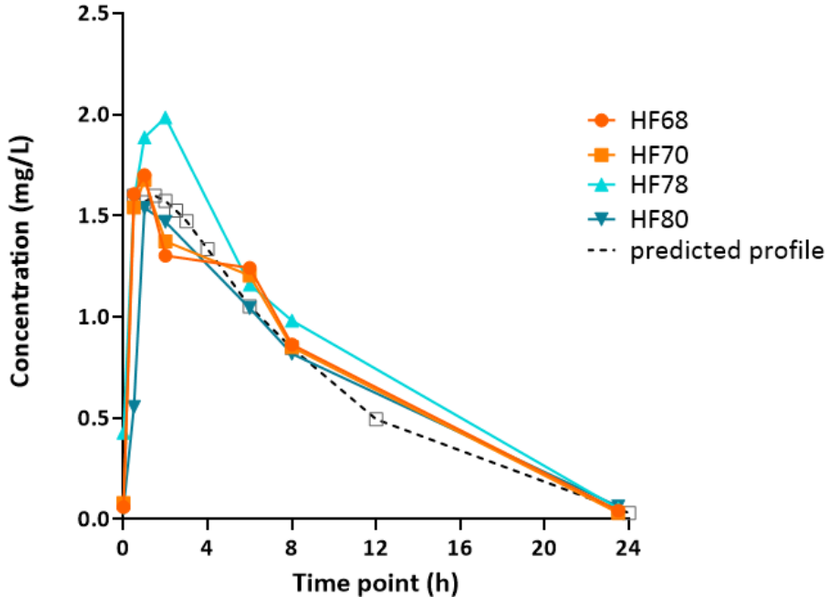
Advanced experimental design – Simulation of arbitrary PK profiles

Design of experiments matching arbitrary PK profiles with high degree of accuracy

Drug 1 – Match human PO dose profile



Drug 2 – Match human PO dose profile



Pre-clinical *in vivo* Pharmacology – Tuberculosis

From proof of concept studies to candidate nomination

- Pre-clinical drug discovery team advising on your project for the most efficient way to reach your objectives
- Propose the most suitable *in vivo* models for POC studies, PK/PD studies or efficacy studies
- Build together a tailored approach for your drug discovery project including
  - Formulation of the drug in accordance with route of administration
  - PK studies in rodent species (infected or not)
  - Selection or tailoring of PK/PD models in accordance with *in vitro* assays and identification of pharmacodynamics biomarkers
  - Efficacy studies with optimised dosing regimen and suitable study endpoints (bacteria burden, survival, relapse, biomarkers)
- Process of continuous and interactive exchanges for flexibility, decision making to optimised timelines and process

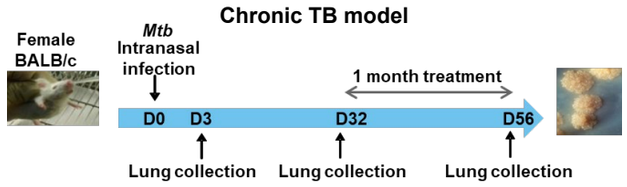
- **Sampling to support analysis for complete evaluation of drug**
  - Blood micro-sampling
  - Organ collection (lung and spleen)
- **Broad range of sample analysis**
  - Gene/mRNA
  - Flow cytometry
  - Histology/IHC
  - Mass spectrometry (DMPK and metabolite follow up)
- **Custom assay development**
  - Protein analysis (ELISA or MSD assay)

TB *in vivo* models (BSL3)

Bespoke *in vivo* studies in gold standard TB models

Study design

- BALB/c or C3HeB-FeJ mice
- Infection intranasal with *M. tb* H37rv
- Dose range
- Monotherapy or combination
- Bespoke dosing regimen
- Treatment duration up to 3 months
- Study duration up to 6 months

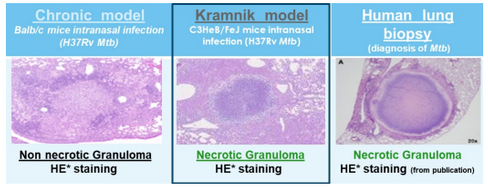


1 Replicating phenotype: monotherapy
- Highly Acute TB model (early PoC)
- Acute TB model (confirmation)

2 Replicating phenotype: monotherapy or combination
- 14-day TB model with kinetics analysis
- 14-day TB model with/or without relapse

3 Slow and non replicating phenotype: monotherapy or combination
- Chronic TB model: non necrotic granuloma

4 Different phenotypes: monotherapy or combination
- Kramnik TB model: necrotic granuloma

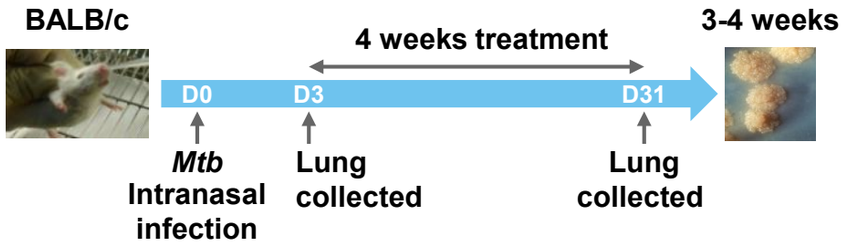


- **Standard endpoints**
  - Bacterial burden (CFU/tissue)
    - MED and MBD (minimum effective and bactericidal dose)
    - Early Bactericidal Activity (EBA)
    - Relapse Mouse Model (RMM)
  - Survival
- **Additional endpoints**
  - Histopathology and *M. tb* staining
  - Immune response: FACS/cytokines
  - Blood micro-sampling for bioanalysis
- **Mechanism of action**
  - Molecular biology: RNA seq or RT-PCR

Acute TB mouse model

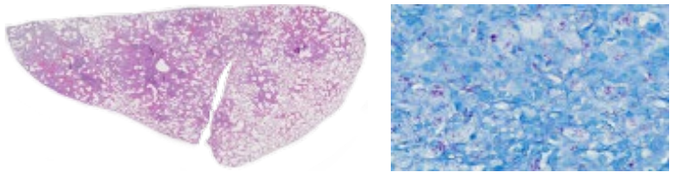
To determine activity against replicating phenotypes of *M. tb*

Acute model schedule



Replicating bacteria extra or intracellular

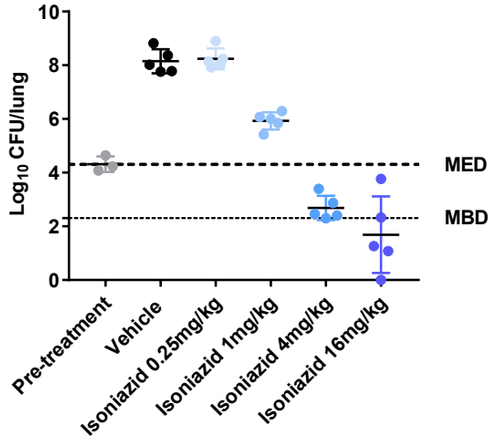
Histologic characterisation

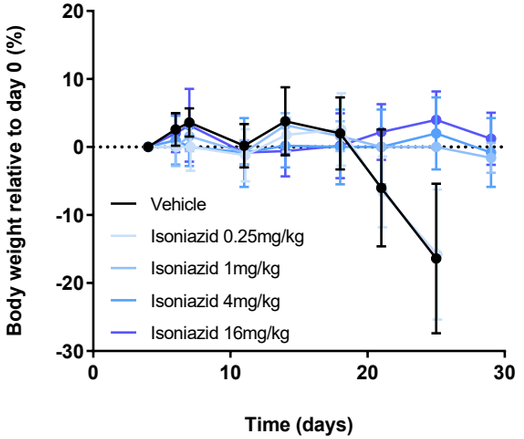


HE coloration

Ziehl-Neelsen staining

Endpoints survival, body weight and CFU/lung





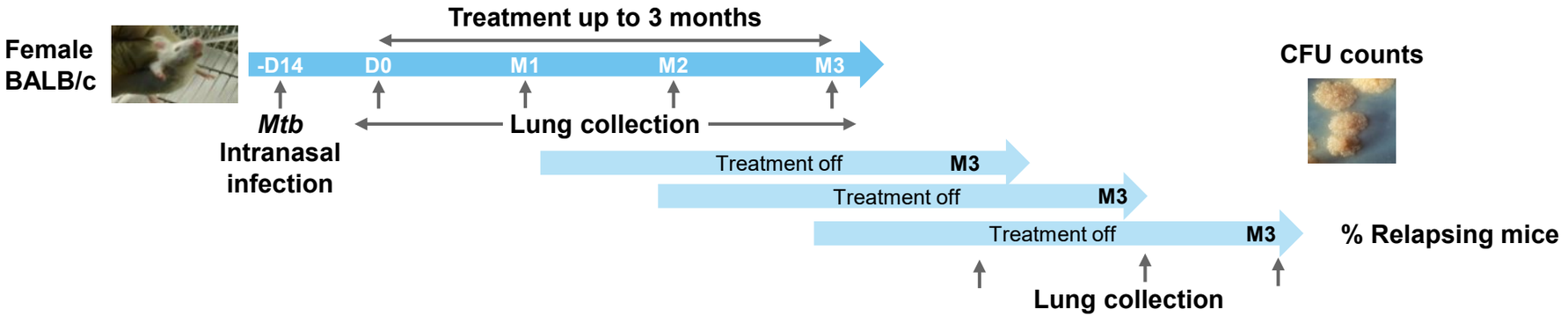
Model validated with compound reference

Compounds	MED	MBD
Rifampicin (R)	40 mg/kg	160 mg/kg
Isoniazid (H)	1-4 mg/kg	4-16 mg/kg

14-day infection TB mouse models

To determine bactericidal and sterilising activity contribution to drug combinations

14-day model schedule Relapsing Mouse Model



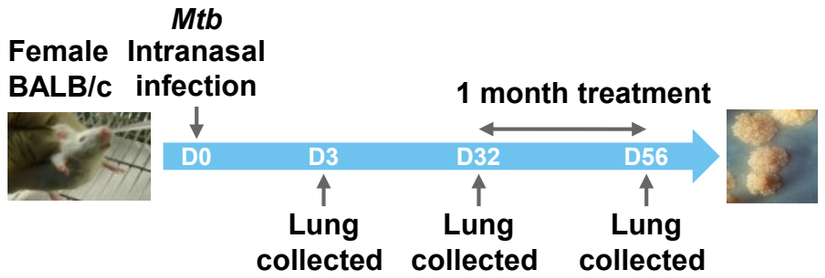
RMM validated using 2 reference treatments: BPaMZ and RHZ

CFU/lung and % relapse	Log_{10} CFU / tissue (Mean +/- SEM) at			% Relapse (positive mice / total) at 3 month post end treatment	
Drug Regimen	**Day -13**	**Day 0**	**2 months**	**1 month**	**2 months**
No treatment	4.77 +/-0.14	7.68 +/-0.04	–	–	–
B_{25} Pa_{50} M_{100} Z_{150}	–	–	0.68 +/-0.68	57% (8/14)	0% (0/15)
B_{25} Pa_{100} M_{100} Z_{150}	–	–	1.58 +/-0.18	27% (4/15)	0% (0/15)
R_{10} H_{25} Z_{150}	–	–	1.87 +/-0.14	–	100% (15/15)

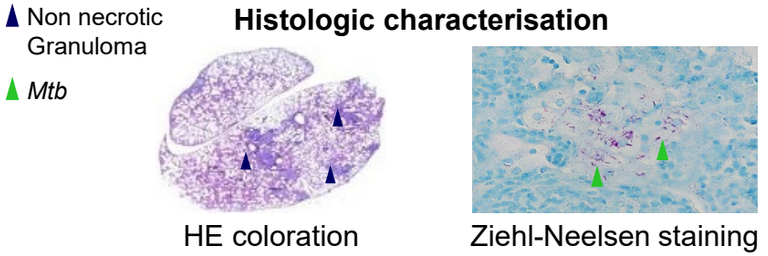
Chronic TB mouse model

To determine activity against slowly and non replicating *M. tb* phenotypes

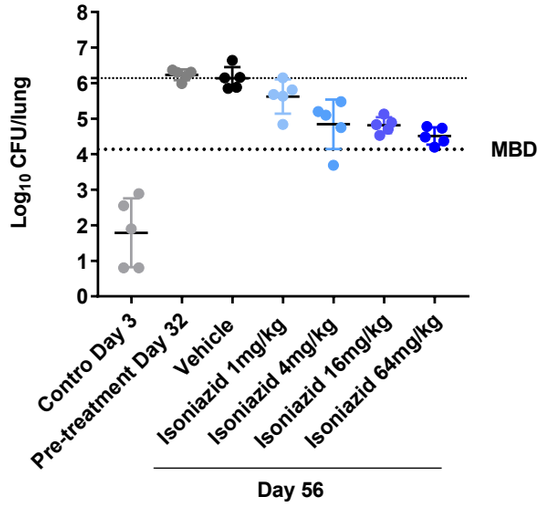
Chronic model schedule



Non or slow replicative bacteria in non necrotic granuloma



Endpoints CFU/lung: validated with references

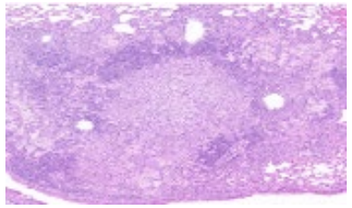
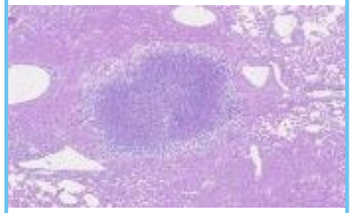
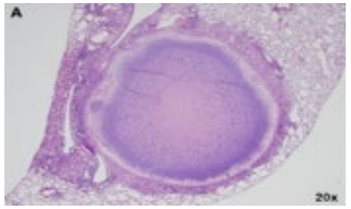


Compounds	Efficacy: 2 Log_{10} CFU reduction MBD
Rifampicin	10 mg/kg
Pyrazinamide	150 mg/kg
Isoniazid	NA

Kramnik TB mouse model

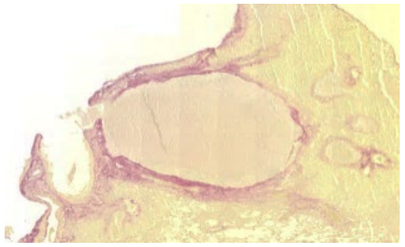
To determine activity against diverse *M. tb* phenotypes and necrotic granuloma distribution

Granuloma status in different chronic models

Chronic model Balb/c mice intranasal infection (H37Rv *Mtb*)	**Kramnik model** C3HeB/FeJ mice intranasal infection (H37Rv *Mtb*)	**Human lung biopsy** (diagnosis of *Mtb*)
		
Non necrotic Granuloma HE[1)] staining	**Necrotic Granuloma** HE[1)] staining	**Necrotic Granuloma** HE[1)] staining (from publication)

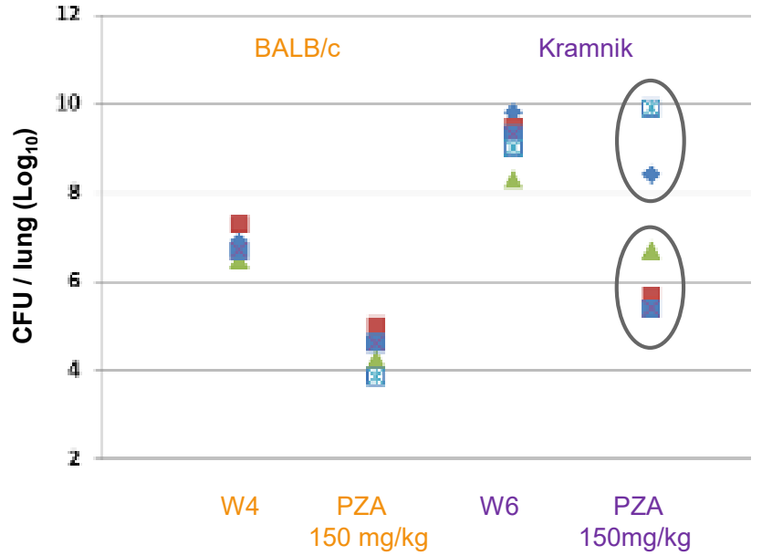
Necrotic granuloma development in Kramnik model from 6 weeks onwards

Necrotic granuloma



Red sirius = Fibrosis

Example of PZA efficacy in BALB/c and Kramnik chronic models



[1)] HE: Hematoxylin and Eosine

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