TB DRUG DISCOVERY BIOASSAY RESOURCES

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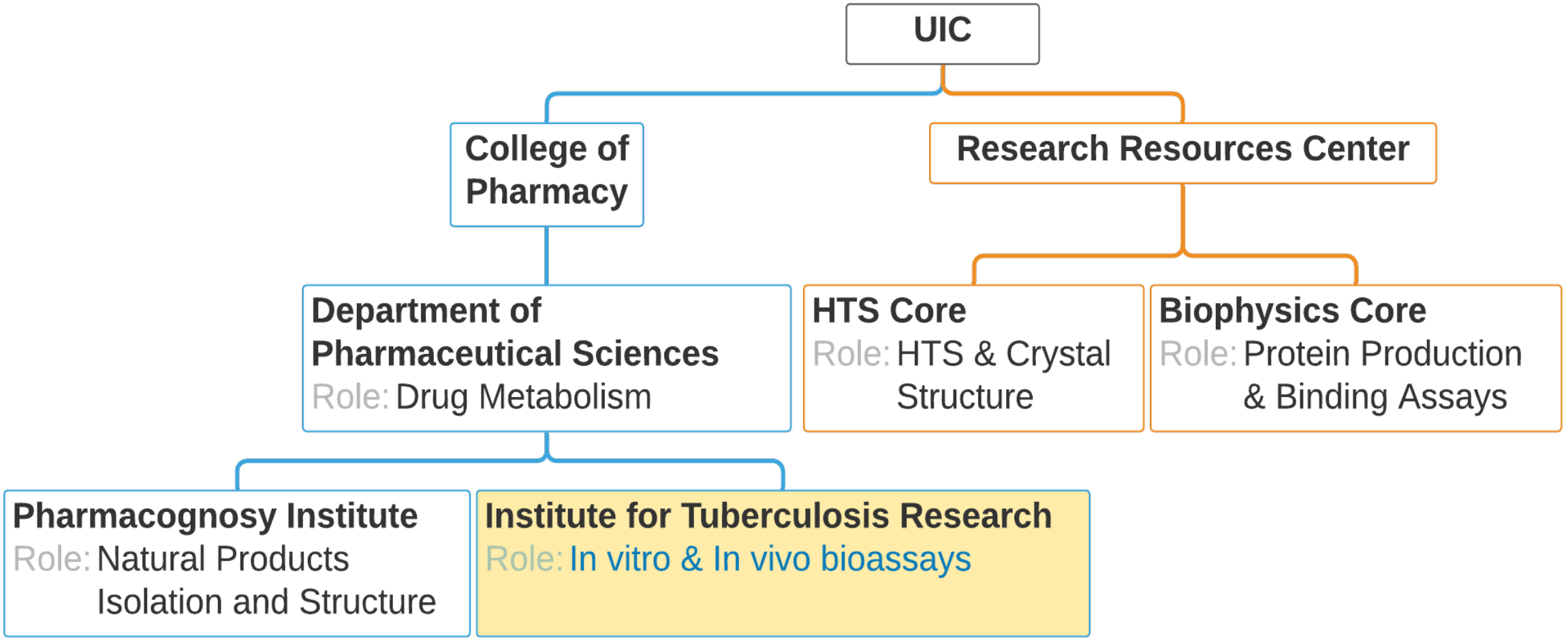
Multiple UIC units support TB drug discovery



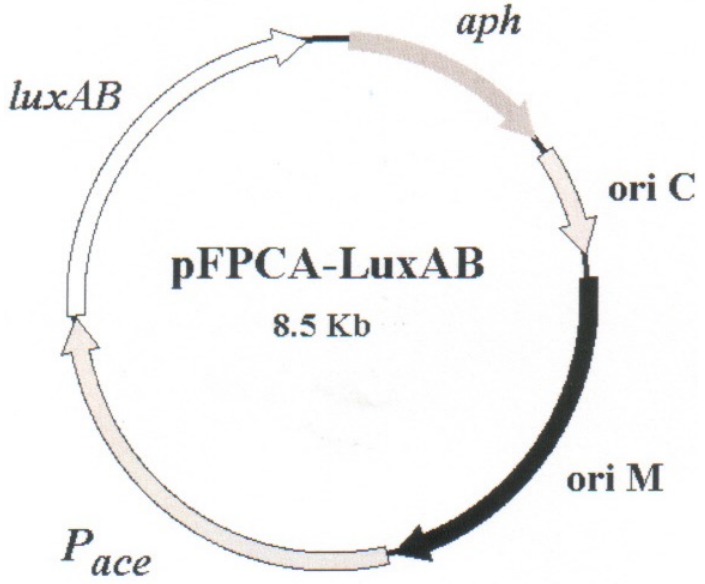
Challenges and Solutions in Early-Stage TB Drug Discovery

Challenge	Solution
Virulence of *M. tuberculosis* (*Mtb*) + lack of avirulent surrogate species with similar drug susceptibility	Use virulent but drug-sensitive *Mtb* Powered Air Purifying Respirators (PAPR) Biosafety Level 3 lab
Slow growth of *Mtb*, 3 weeks for colony formation	Metabolic surrogates of viability: • Resazurin reduction (Microplate Alamar Blue Assay; MABA) • Luciferase reporter genes • Intracellular ATP
Early ID of treatment shortening potential	Determine killing of non-growing culture by Low Oxygen Recovery Assay (LORA)
Mouse models take 1.5 – 2.5 months	qPCR reduces time by 3 weeks

1st & 2nd Generation Bacterial Luciferase Reporters

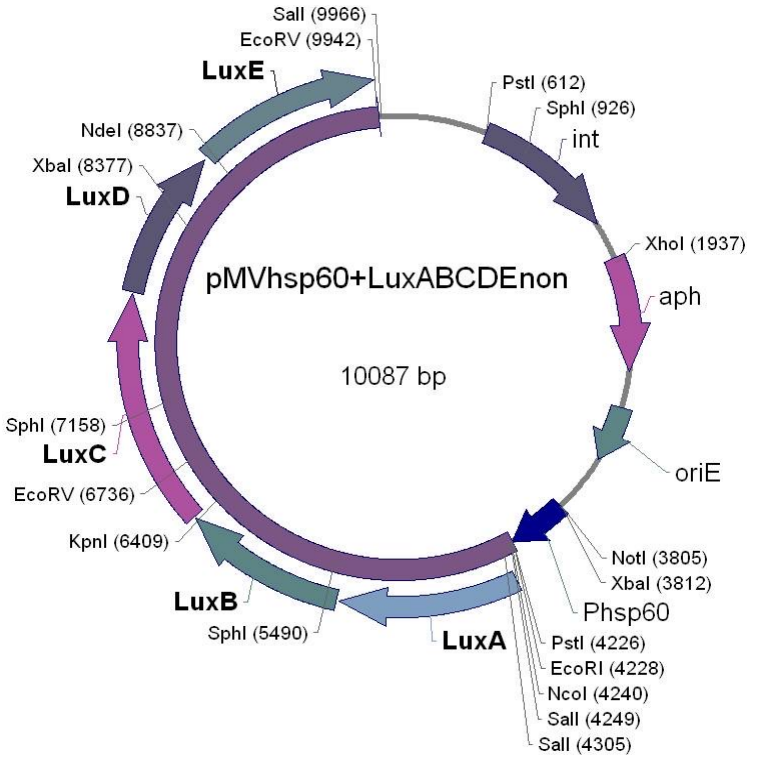
First Generation

- Plasmid-borne
- Requires substrate (n-decanal)
- End-point assay



Second Generation

- Integrated into chromosome
- Codes for both enzyme and substrate
- Enables Kinetic Assays

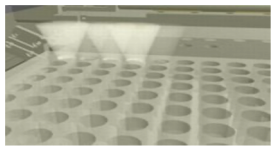


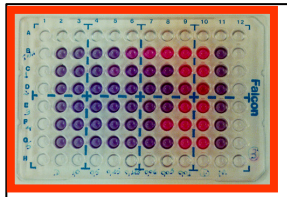
Andreu N, Zelmer A, Fletcher T, Elkington PT, Ward TH, et al. (2010) Optimisation of Bioluminescent Reporters for Use with Mycobacteria. PLOS ONE 5(5): e10777.

Phenotypic-based screening: hit ID

HTS
vs Mtb luxABCDE
96 or 384 well
100K samples/month

>85-90% ↓ luminescence





MIC
vs *Mtb* $H_{37}Rv$
Microplate Alamar Blue Assay (MABA)

Cytotoxicity IC_{50}
vs Vero cells
Resazurin reduction

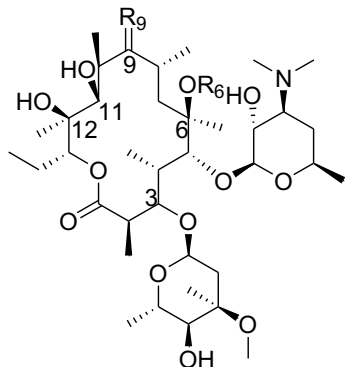
SI = IC_{50}/MIC >10

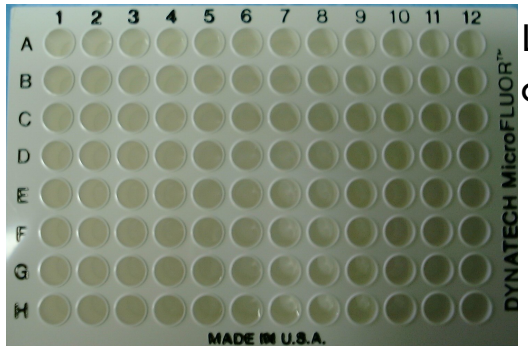
In vitro profiling

Phenotypic-based Screening: *In vitro* Hit Profiling

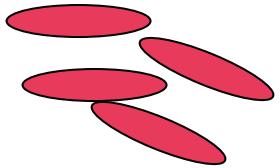
Property	Assay
Potential to shorten treatment	MIC vs non-replicating *Mtb* culture (LORA)
Killing effect (vs. growth inhibition only)	Minimum bactericidal concentration (MBC)
Persistent suppression of growth following compound clearance	Post Antibiotic Effect (PAE)
Ability to kill bacteria within host macrophage	Intramacrophage activity (EC90)
Synergy or antagonism in combination with established or experimental TB drugs	diaMOND to determine FICs
Frequency of mutation to resistance and target identification	Selection of resistant mutants and WGS

Non-Replicating *Mtb* luxABCDE: Low Oxygen Recovery Assay (LORA)

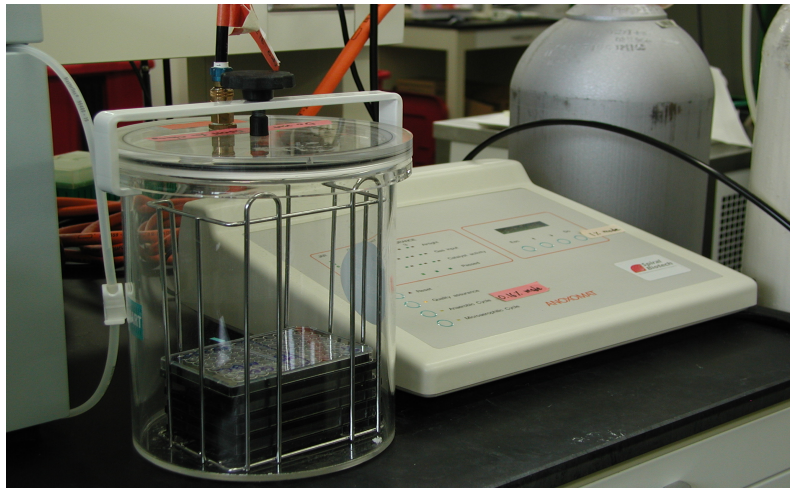




Low oxygen-adapted *M. tuberculosis* carrying luxABCDE



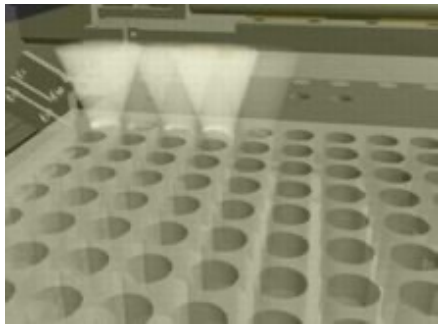
2 x 10^4



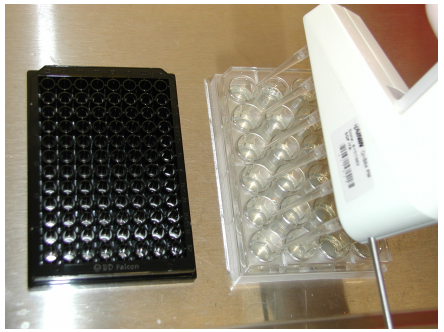
10 days under <0.16% oxygen
No replication!

28h "recovery" in air

luminescence



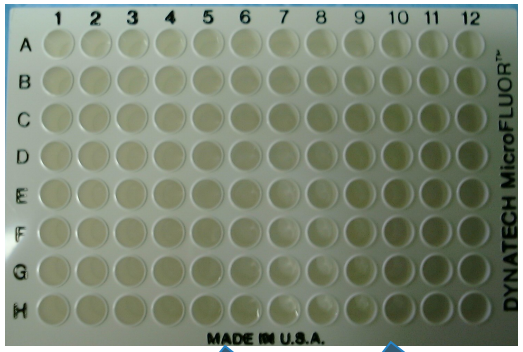
CFU in 24-well plates

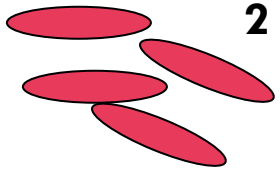


Cho S, Lee HS, Franzblau S. Microplate Alamar Blue Assay (MABA) and Low Oxygen Recovery Assay (LORA) for Mycobacterium tuberculosis. Methods Mol Biol. 2015;1285:281-92. PubMed PMID: 25779323.

MBC by CFU vs Bioluminescence Against Replicating M. tuberculosis luxABCDE

Compound





2 x 10^5 RLU

7/14 days

14 days, 37 °C

Plating on 7H11 agar plate

Luminescence is read ~daily

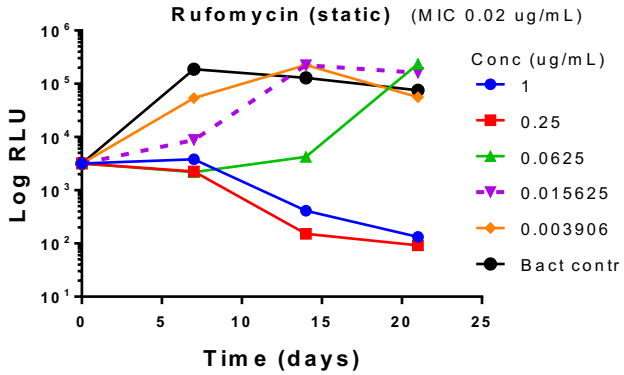
4 weeks

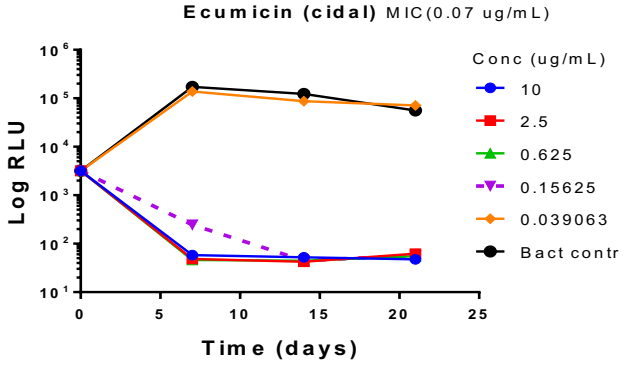
MBC

MBC, Time-kill information

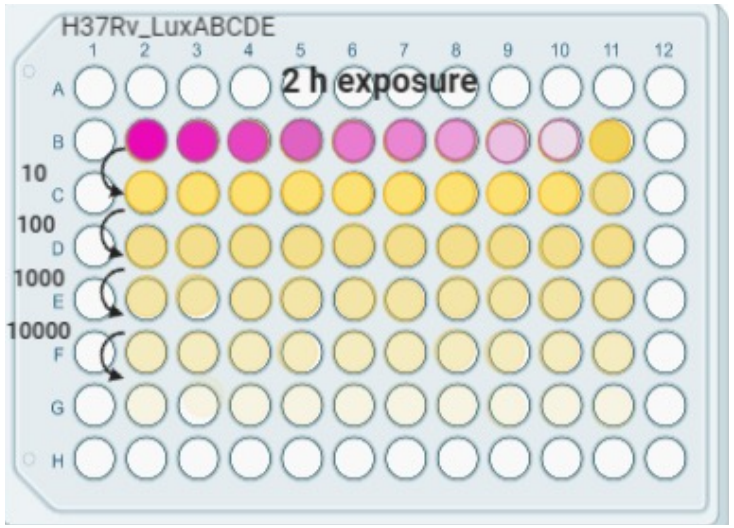
Total time required

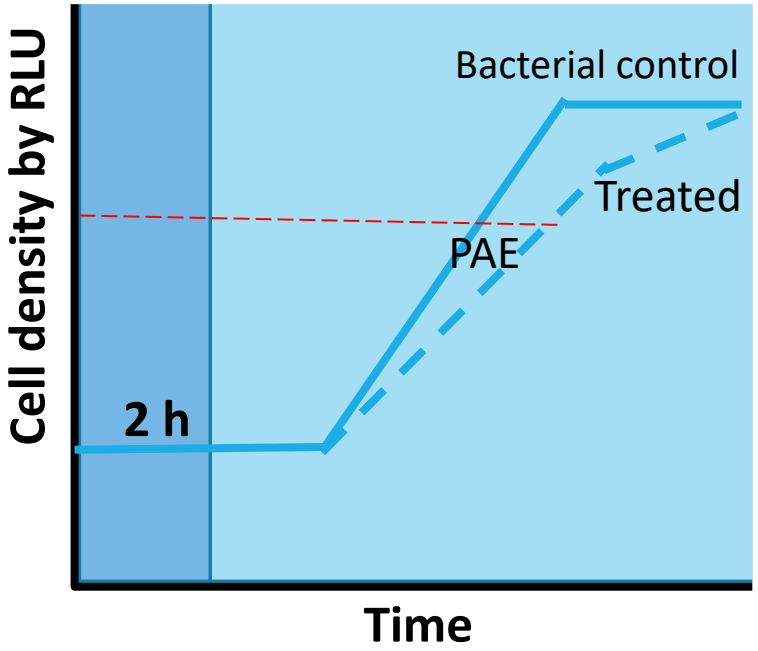
42 days	14 days

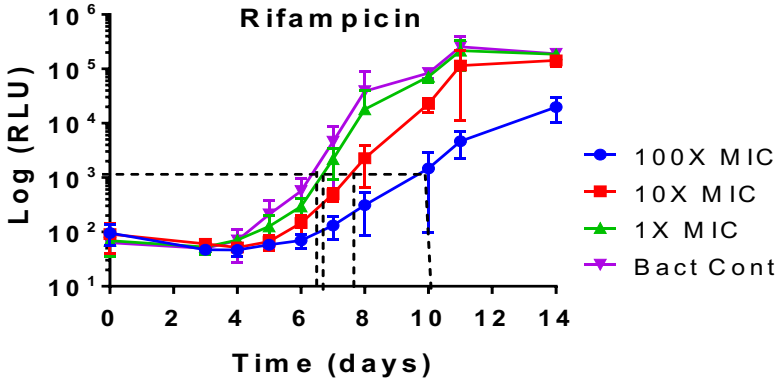


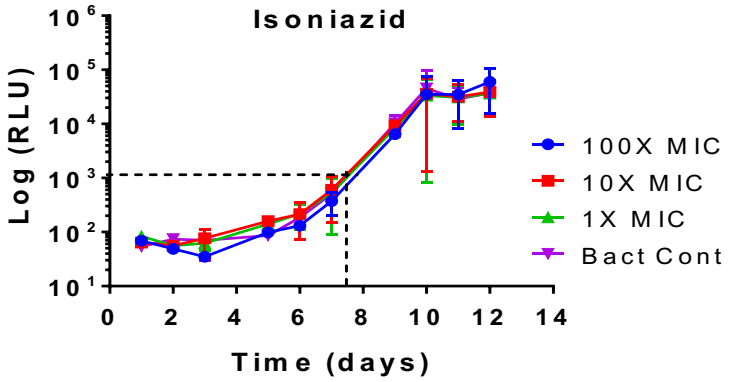


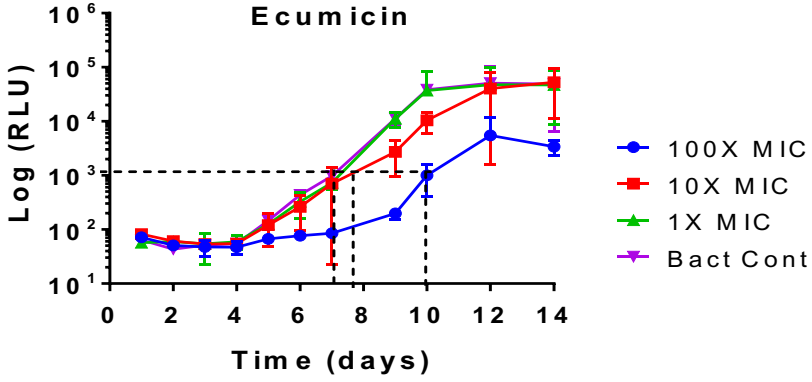
Post Antibiotic Effect Can Shed Light On Target Vulnerability

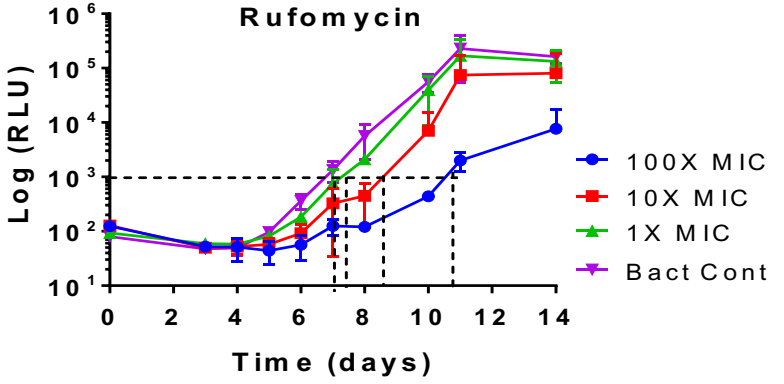






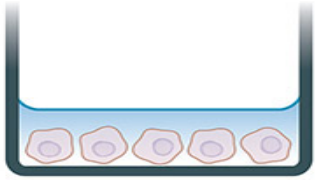




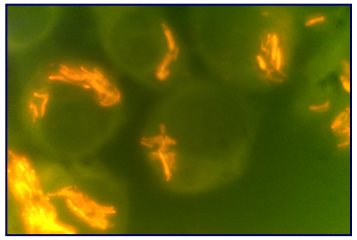


Activity Against Macrophage-Internalized Bioluminescent *Mtb*

$5x10^4$ Macrophages seeded to 96 WP



Infect with H37Rv_LuxABCDE $5x10^5$

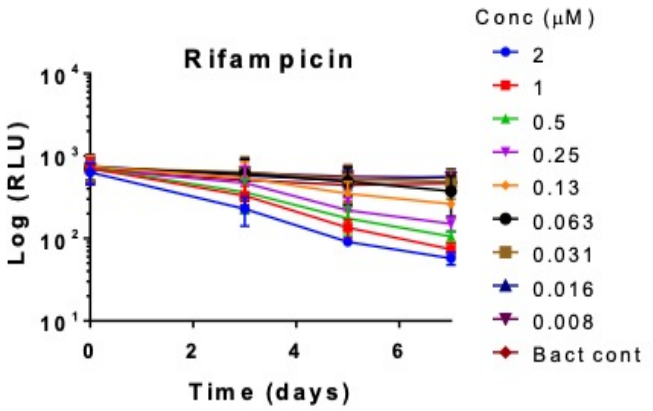


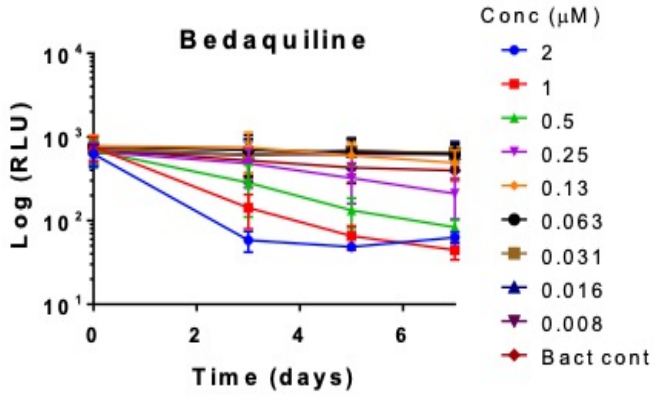
Treat with amikacin overnight

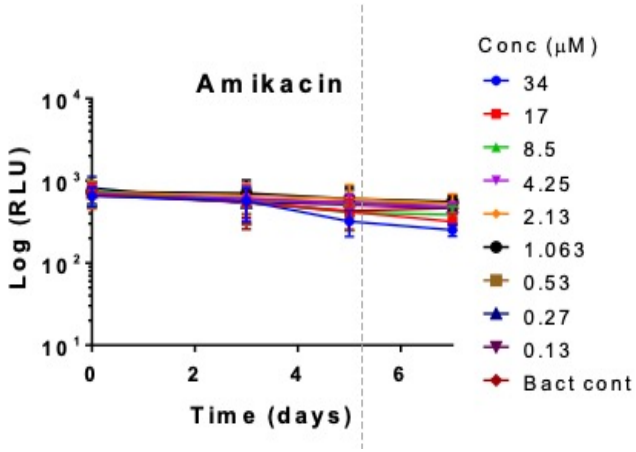
Wash with PBS
Add test compounds

Incubate for 6-7 days

Bioluminescence







Phenotypic based screening: spectrum of activity

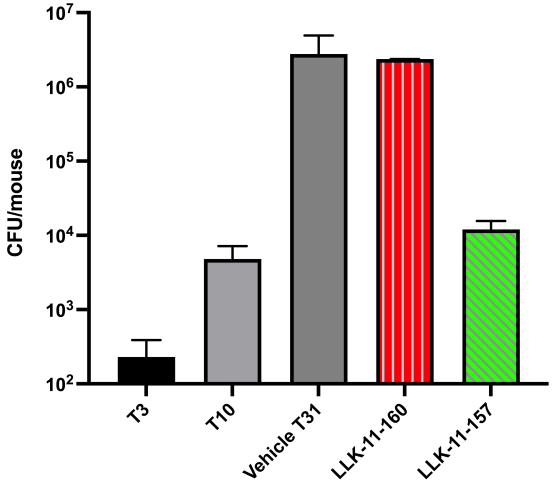
Property	MIC vs
Cross-resistance to existing TB drugs	H37Rv-isogenic strains mono-resistant to INH, rifampin, ethambutol, kanamycin, bedaquiline, moxifloxacin, etc.
Effective against clinical isolates from different geographical regions	6 global clade representatives
Broad or narrow spectrum anti-mycobacterial activity	M. abscessus, M. avium, M. ulcerans, M. bovis, etc.
Broad or narrow spectrum antibacterial activity	ESKAPE panel of 4 Gram - and 2 Gram + bacteria

Efficacy in Acute and Chronic Mouse Infection Models of TB

Aerosol infection of ~100 female BALB/c mice with low dose of M. tuberculosis Erdman

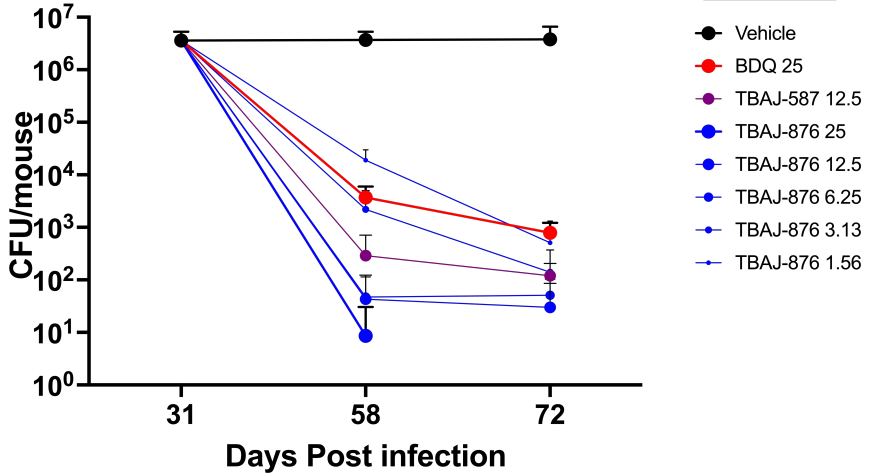
Acute infection model

Tx begins Day 10



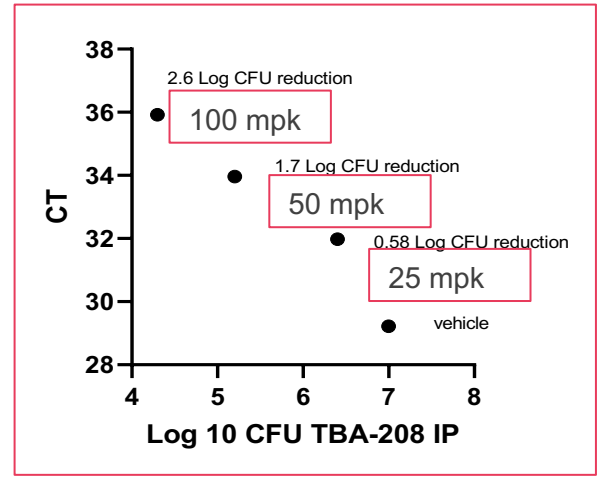
Chronic infection model

Tx begins Day 31



1) Mice sacrificed 3 days after final dose
2) Lung homogenates serially diluted & plated
3) Colonies counted after 3 weeks incubation

qPCR w PMA dye to reduce time to data by 3 weeks



Target profile of TB drug lead

Assay	Ideal	Acceptable
MIC	<0.1 uM	5 uM
Vero cytotoxicity IC_{50}	>100 uM	>20 uM
Vero IC_{50}/MIC	>1000	>50
LORA/MABA	<5	Any
MBC/MIC	<5	Any
EC_{90} vs intramacrophage *Mtb*	< 1 uM	<20 uM
MIC vs H_{37}Rv/drugR strains & global clades	<2x	<8x
MIC vs G+, G-/MIC vs *Mtb*	>50	>10
MIC ⬆ with serum or albumin	<4	<20
Combinations (diaMOND)	Synergistic	Not antagonistic
Mouse infection model log_{10} lung CFU reduction	3	1