TB DRUG DISCOVERY BIOASSAY RESOURCES

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Multiple UIC units support TB drug discovery

- **UIC**
  - **College of Pharmacy**
    - **Department of Pharmaceutical Sciences**
      - Role: Drug Metabolism
    - **Pharmacognosy Institute**
      - Role: Natural Products Isolation and Structure
  - **Research Resources Center**
    - **HTS Core**
      - Role: HTS & Crystal Structure
    - **Biophysics Core**
      - Role: Protein Production & Binding Assays
  - **Institute for Tuberculosis Research**
    - Role: In vitro & In vivo bioassays
## Challenges and Solutions in Early-Stage TB Drug Discovery

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

Phenotypic-based screening: hit ID

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

>85-90% ↓ luminescence

MIC
vs Mtb H₃⁷Rv
Microplate Alamar Blue Assay (MABA)

Cytotoxicity IC₅₀
vs Vero cells
Resazurin reduction

SI = IC₅₀/MIC > 10

In vitro profiling
### Phenotypic-based Screening: *In vitro* Hit Profiling

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

CFU in 24-well plates

10 days under <0.16% oxygen
No replication!

Low oxygen-adapted *M. tuberculosis* carrying luxABCDE

28h “recovery” in air

luminescence

2 x 10⁴

**MBC by CFU vs Bioluminescence Against Replicating M. tuberculosis luxABCDE**

- **Compound**
- **Plating on 7H11 agar plate**: 7/14 days, 14 days, 37 °C
- **Luminescence is read ~daily**
- **MBC**, **Time-kill information**

**Total time required**

- **42 days**
- **14 days**

**Figure 1**

- **Rufomycin (static)** MIC 0.02 ug/mL
  - Concentration ranges: 0.01, 0.025, 0.0625, 0.015625, 0.003906
  - Log RLU vs Time (days)

- **Eumycin (cidal)** MIC 0.07 ug/mL
  - Concentration ranges: 0.0, 0.025, 0.015625, 0.003906
  - Log RLU vs Time (days)
Post Antibiotic Effect Can Shed Light On Target Vulnerability
Activity Against Macrophage-Internalized Bioluminescent \( \textit{Mtb} \)

5x10^4 Macrophages seeded to 96 WP

\( \text{Infect with} \quad H37Rv\_\text{LuxABCDE} \quad 5x10^5 \)

\( \text{Treat with amikacin overnight} \)

Wash with PBS

Add test compounds

Incubate for 6-7 days

Bioluminescence

\[ \text{Conc (\text{\textmu M})} \]

\[ \begin{align*}
\text{Rifampicin} & : 2, 1, 0.5, 0.25, 0.13, 0.063, 0.031, 0.016, 0.008, \text{Bact cont} \\
\text{Bedaquiline} & : 2, 1, 0.5, 0.25, 0.13, 0.063, 0.031, 0.016, 0.008, \text{Bact cont} \\
\text{Amikacin} & : 34, 17, 8.5, 4.25, 2.13, 1.063, 0.53, 0.27, 0.13, \text{Bact cont}
\end{align*} \]
Phenotypic based screening: spectrum of activity

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

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

**Chronic infection model**
Tx begins Day 31

- qPCR w PMA dye to reduce time to data by 3 weeks
## Target profile of TB drug lead

<table>
<thead>
<tr>
<th>Assay</th>
<th>Ideal</th>
<th>Acceptable</th>
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<tbody>
<tr>
<td>MIC</td>
<td>&lt;0.1 uM</td>
<td>5 uM</td>
</tr>
<tr>
<td>Vero cytotoxicity IC\textsubscript{50}</td>
<td>&gt;100 uM</td>
<td>&gt;20 uM</td>
</tr>
<tr>
<td>Vero IC\textsubscript{50}/MIC</td>
<td>&gt;1000</td>
<td>&gt;50</td>
</tr>
<tr>
<td>LORA/MABA</td>
<td>&lt;5</td>
<td>Any</td>
</tr>
<tr>
<td>MBC/MIC</td>
<td>&lt;5</td>
<td>Any</td>
</tr>
<tr>
<td>EC\textsubscript{90} vs intramacrophage Mtb</td>
<td>&lt; 1 uM</td>
<td>&lt;20 uM</td>
</tr>
<tr>
<td>MIC vs H\textsubscript{37}Rv/drugR strains &amp; global clades</td>
<td>&lt;2x</td>
<td>&lt;8x</td>
</tr>
<tr>
<td>MIC vs G+, G-/MIC vs Mtb</td>
<td>&gt;50</td>
<td>&gt;10</td>
</tr>
<tr>
<td>MIC with serum or albumin</td>
<td>&lt;4</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Combinations (diaMOND)</td>
<td>Synergistic</td>
<td>Not antagonistic</td>
</tr>
<tr>
<td>Mouse infection model log\textsubscript{10} lung CFU reduction</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
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