RIFASHORT: Persister Study

University of Leicester: Mr J Decker, Dr N Garton, Dr G Mukamolova, Prof M Barer
University of Sussex: Dr S Waddell
St Georges University: D J Dhillon, Prof T Harrison, Dr A Jindani
What are “Persisters”?

“The sub-population of bacteria, either in vivo or in vitro, that are able to resist conventionally lethal levels of antibiotics”
Basic Mechanisms of Chemotherapy

D. A. Mitchison, M.B.*

SPECIAL BACTERIAL POPULATIONS

Figure 2. Hypothesis of special populations of the bacterial population in lesions killed by different drugs.
## Differentially Culturable Bacteria

<table>
<thead>
<tr>
<th>Colony Forming Units (CFU)</th>
<th>Most Probable Number (MPN)</th>
<th>Most Probable Number + Culture Supernatant (MPN_CSN)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solid Media</strong></td>
<td><strong>Liquid Media</strong></td>
<td><strong>Liquid Media</strong></td>
</tr>
<tr>
<td>Middlebrook 7H10, 7H11, Löwenstein–Jensen</td>
<td>Middlebrook 7H9 (MGIT), Youmans, Sautons</td>
<td>Middlebrook 7H9 + Culture Supernatant</td>
</tr>
</tbody>
</table>
Culture Supernatant Dependant (CSN_D) Mycobacteria

[A.K.A Resuscitation Promoting Factor Dependant (RPF_D) and Culture Filtrate Dependant (CF_D) Mycobacteria]

- Tolerant to multiple classes of antibiotics vs platable mycobacteria (RIF, INH, STR).
- Been demonstrated to cause relapse in the cornel mouse model.
- The numbers in sputum vary between patients but often account for >90% of the total culturable bacteria vs growth on 7H9/7H10.

References:
Bacteria per ml of sputum (Log10)

- Colony Forming Units (Middlebrook 7H10+)
- Most Probable Number (Middlebrook 7H9+)
- Most Probable Number + Culture Supernatant

LoD
RI = Log10(MPN_CS) – Log10(CFU)
Lipid Body (LB) containing Mycobacteria

- In response to stresses such as NO, nutrient limitation and hypoxia, Mtb form large intracellular lipid incisions.
- Lipid bodies formation can induce antimicrobial tolerance.
- High LB levels 3-4 weeks into treatment has been correlated with unfavourable outcome.


Sloan, D. 2015 Pharmacodynamic Modeling of Bacillary Elimination Rates and Detection of Bacterial Lipid Bodies in Sputum to Predict and Understand Outcomes in Treatment of Pulmonary Tuberculosis. *Clin Inf Dis v61* 

Persister Study

Validation Study
Validations that LB levels 4 weeks into treatment is predictive of unfavourable outcome

Case-Control Study
To determine if persister determinants can predict treatment failure and/or relapse.

Correlation Study
Validation Study Recruitment
Case - Control Study Recruitment
Correlation Study Recruitment

Correlation Study Recruitment

Botswana  Uganda  Target
Additional Persister Studies

The Consortium for TB Biomarkers (CTB2) Persister Study

Aim:
To determine if persister determinants can predict treatment failure and/or relapse.
Resuscitation Index (RI) is calculated as

\[ RI = \log_{10}(\text{MPN}_{\text{CSN}}) - \log_{10}(\text{CFU}) \]
Resusitation Index increases over time

Day on HRZE

Resusitation Index

-4

-2

0

2

4

-4

-2

0

2

4

5

10

15

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Summary

- The RIFASHORT: Persister Study is up and running!
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Correlation Study

Aims:
- To determine if transcriptional signature can be used to predict response to treatment.
- To discover potential persister transcription signatures.
- To address if Lipid Body and CSN_D phenotypes are correlated
Case-control Study

Aims
- To determine if persister determinants can predict treatment failure and/or relapse.
- To determine if treatment with high dose RIF targets persister sub-populations.
Validation Study

Aim
- To validate the previous observation that high lipid body levels one month post treatment is a strong predictor of unfavourable outcome.