

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.**

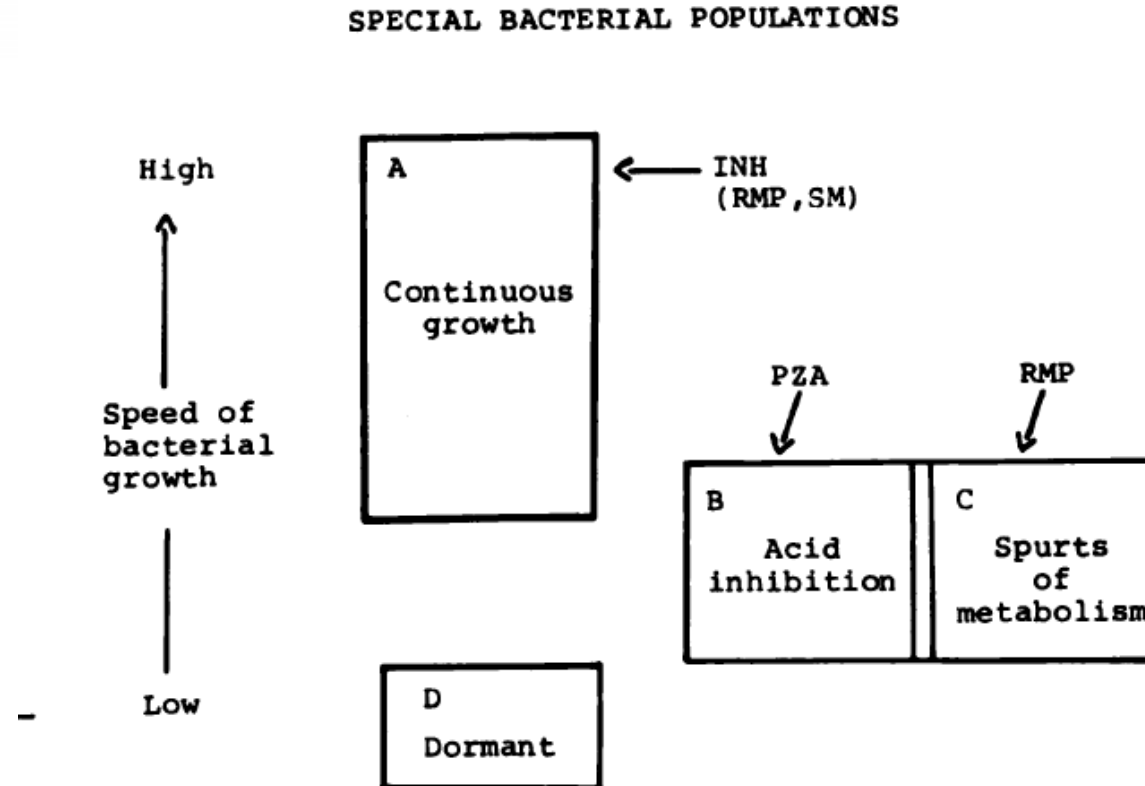
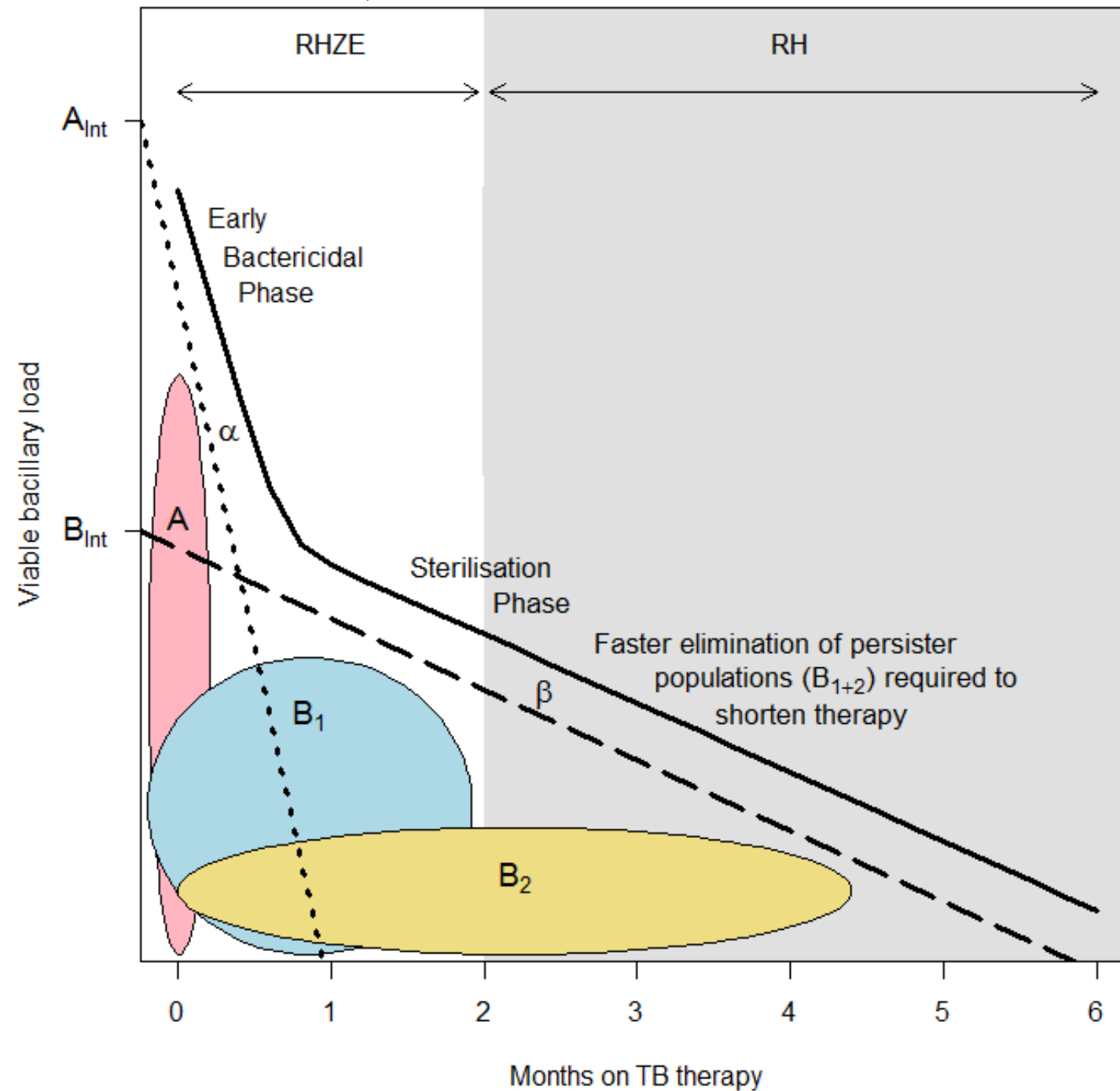
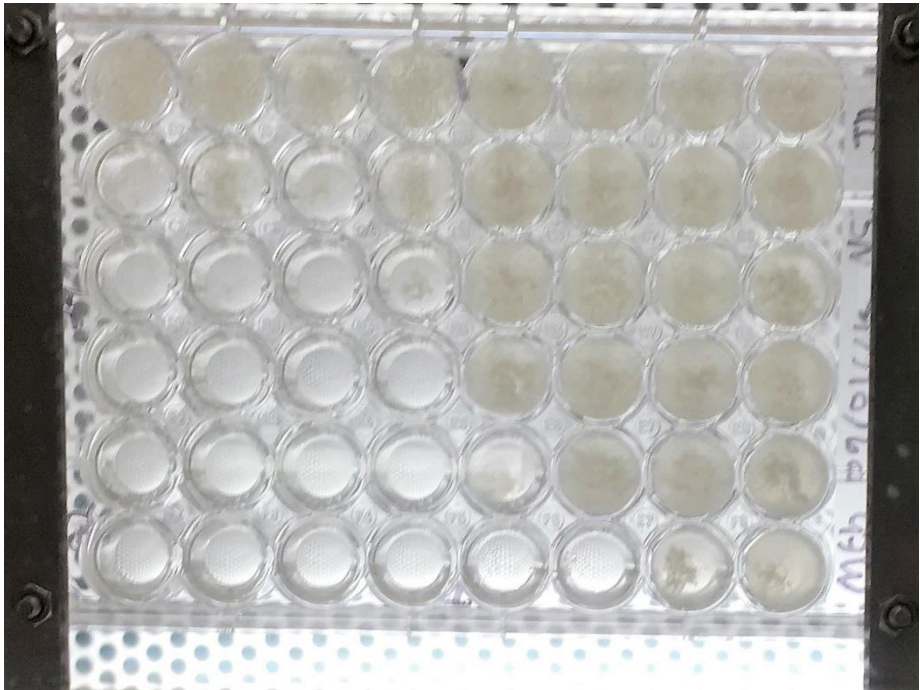


FIGURE 2. Hypothesis of special populations of the bacterial population in lesions killed by different drugs.



Differentially Culturable Bacteria

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Colony Forming Units (CFU)	Most Probable Number (MPN)	Most Probable Number + Culture Supernatant (MPN_CSN)
Solid Media	Liquid Media	Liquid Media
Middlebrook 7H10, 7H11, Löwenstein–Jensen	Middlebrook 7H9 (MGIT) , Youmans, Sautons	Middlebrook 7H9 + Culture Supernatant

Culture Supernatant Dependant (CSN_D) Mycobacteria

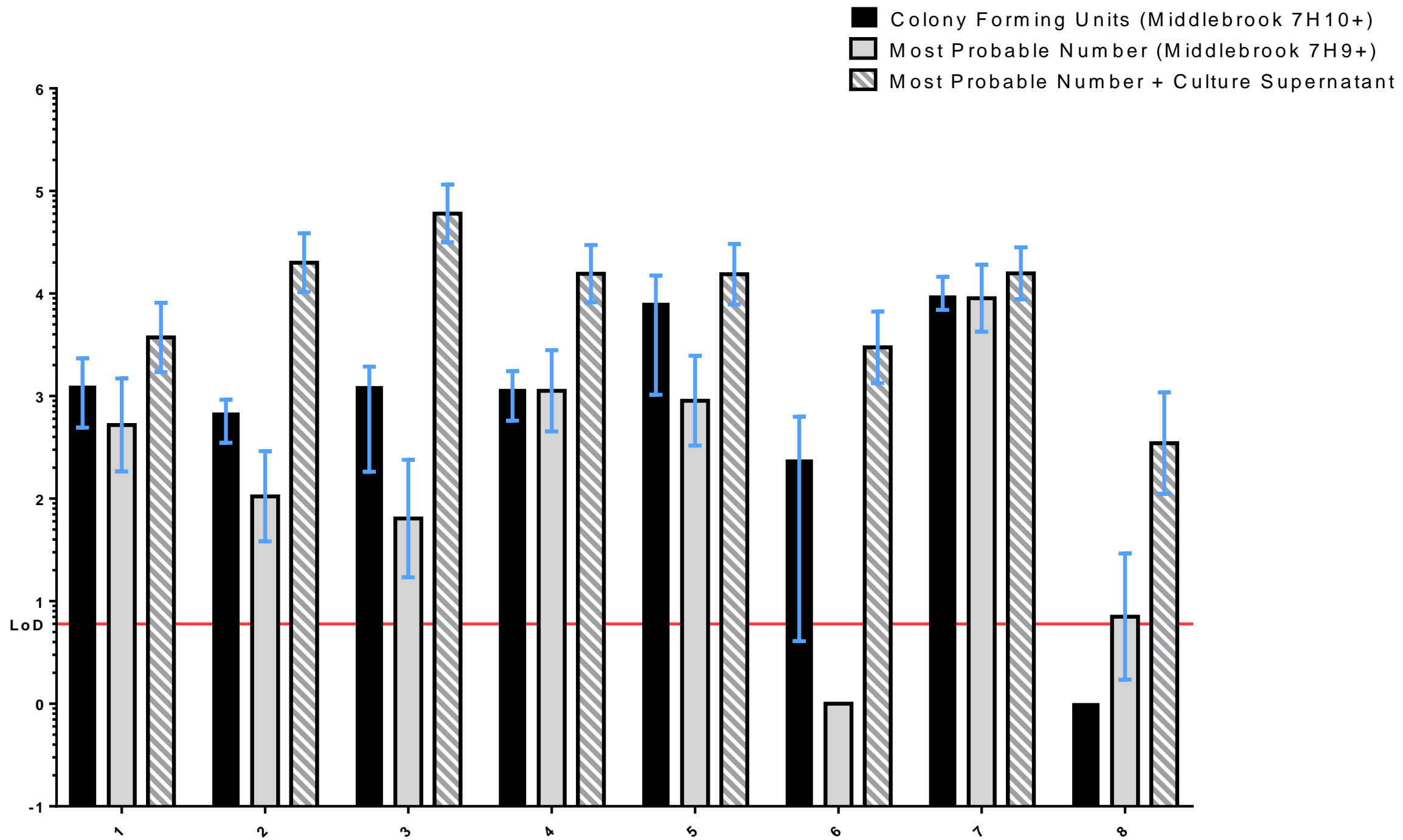
[A.K.A Resuscitation Promoting Factor Dependant (RPF_D) and Culture Filtrate Dependant (CF_D) Mycobacteria]

- 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.

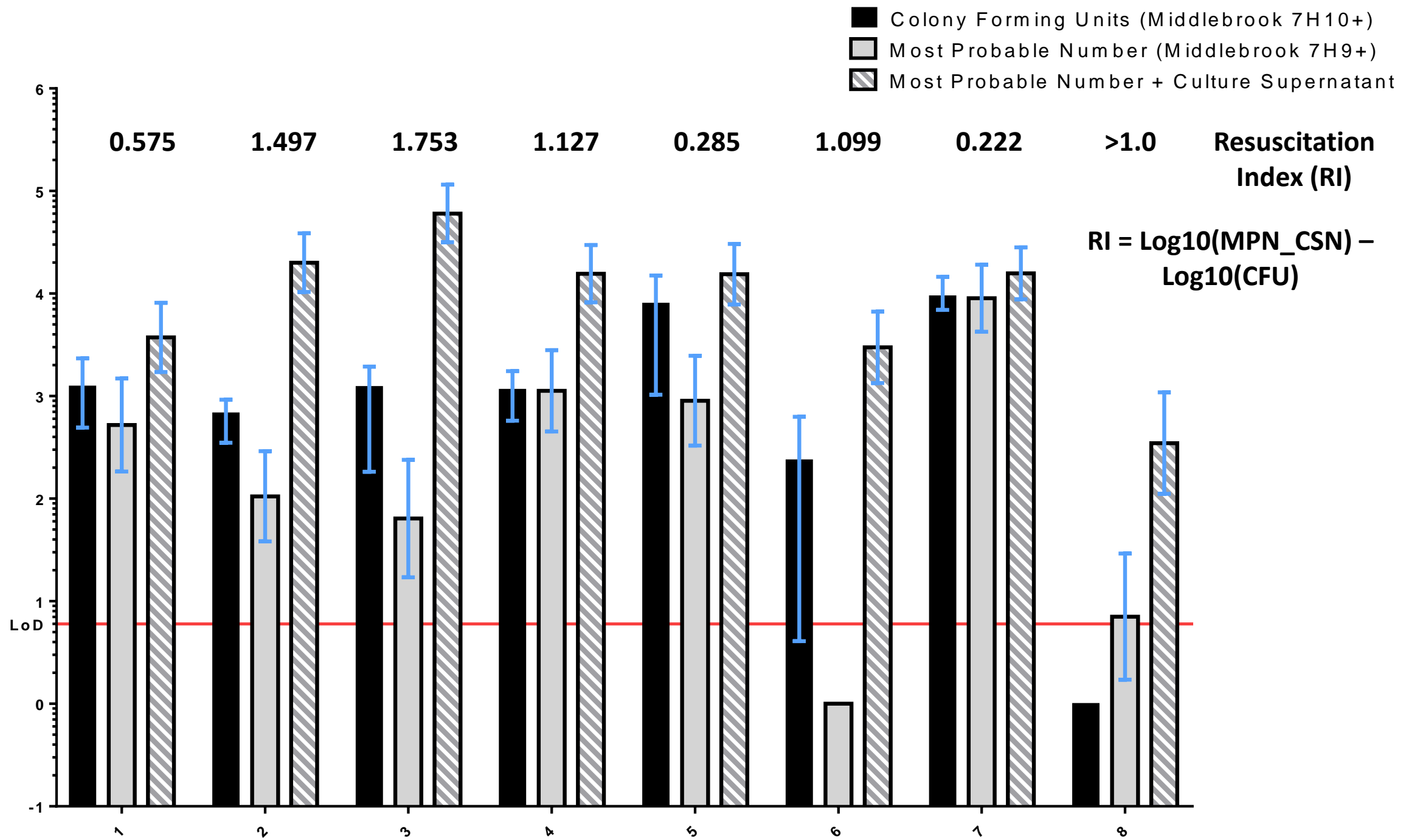
References :

Turapov O, O'Connor BD, Sarybaeva AA, Williams C, Patel H, Kadyrov AS, Sarybaev AS, Woltmann G, Barer MR, Mukamolova GV. Phenotypically Adapted Mycobacterium tuberculosis Populations from Sputum Are Tolerant to First-Line Drugs. Antimicrob Agents Chemother. 2016 Mar 25;60(4):2476-83. doi: 10.1128/AAC.01380-15. Print 2016 Apr. PubMed PMID

Bacteria per ml of sputum (Log10)



Bacteria per ml of sputum (Log10)



Lipid Body (LB) containing Mycobacteria

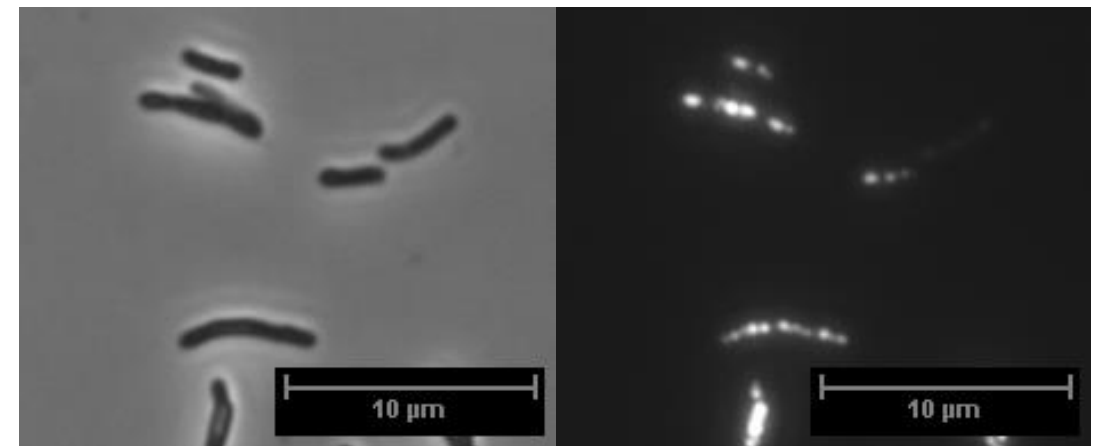
- In response to stresses such as NO, nutrient limitation and hypoxia Mtb form large intracellular lipid incisions.
- Lipid bodies formation can induce antimicrobial tolerance.
- High LB levels 3-4 weeks into treatment has been correlated with unfavourable outcome.

Daniel, J. 2011. *Mycobacterium tuberculosis* Uses Host Triacylglycerol to Accumulate Lipid Droplets and Acquires a Dormancy-Like Phenotype in Lipid-Loaded Macrophages. *PLoS Pathog.* E1002093

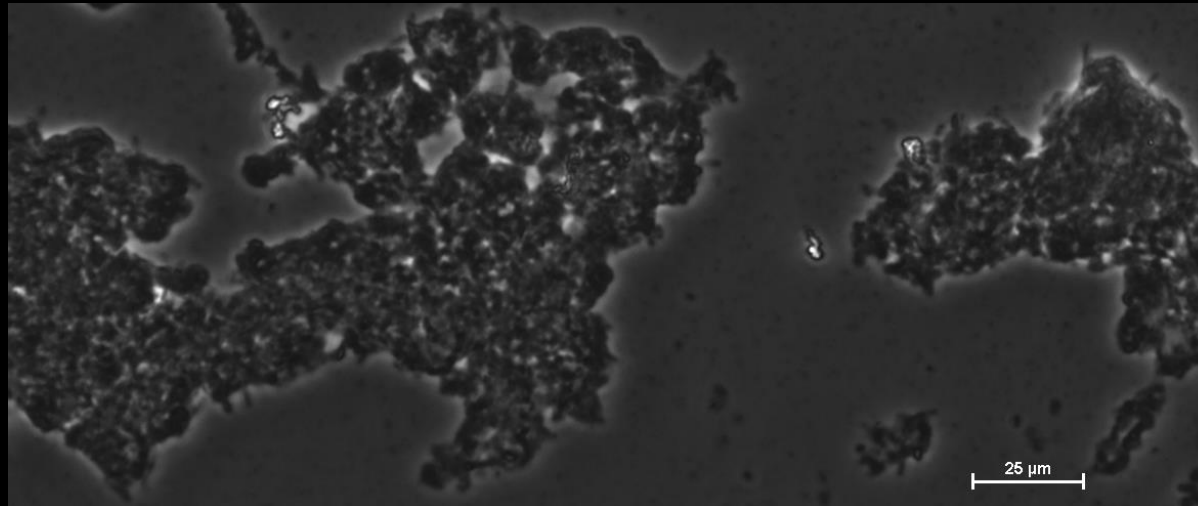
Garton, N. Cytological and Transcript Analyses Reveal Fat and Lazy Persister-Like Bacilli in Tuberculous Sputum. - *PLoS Med.* - e75.

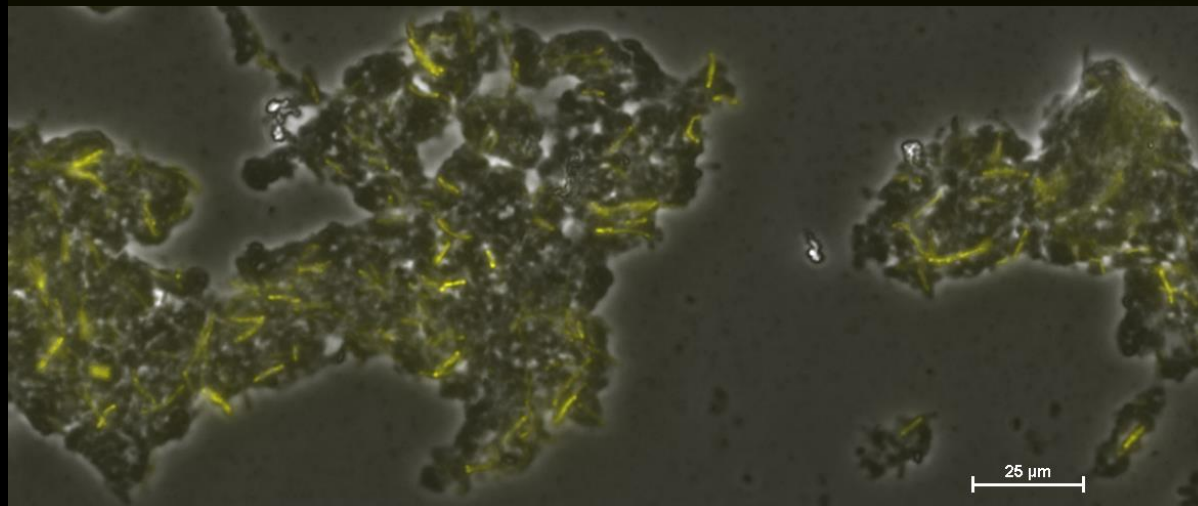
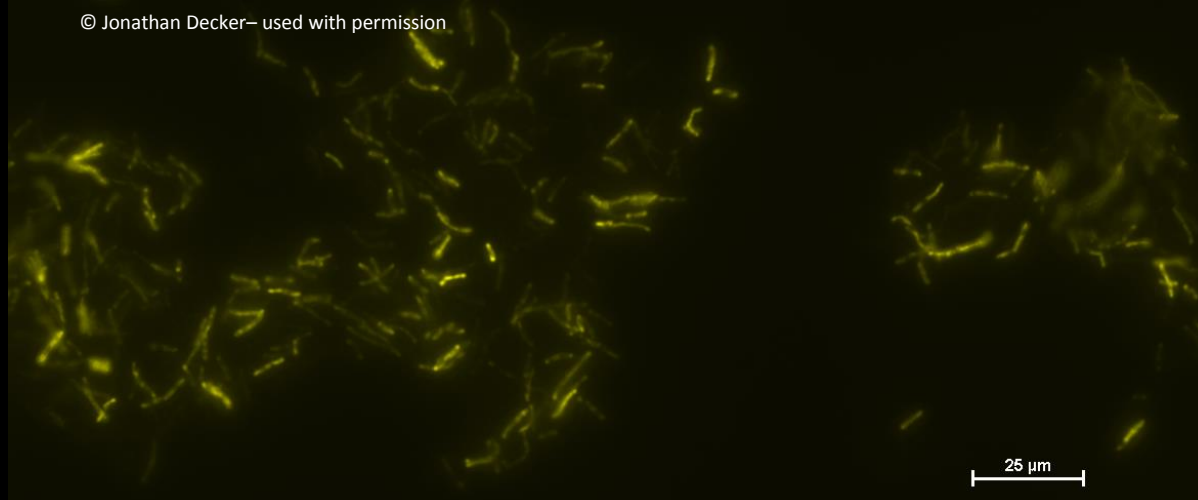
Sloan, D. 2015 Pharmacodynamic Modeling of Bacillary Elimination Rates and Detection of Bacterial Lipid Bodies in Sputum to Predict and Understand Outcomes in Treatment of Pulmonary Tuberculosis. *Clin Inf Dis* v61

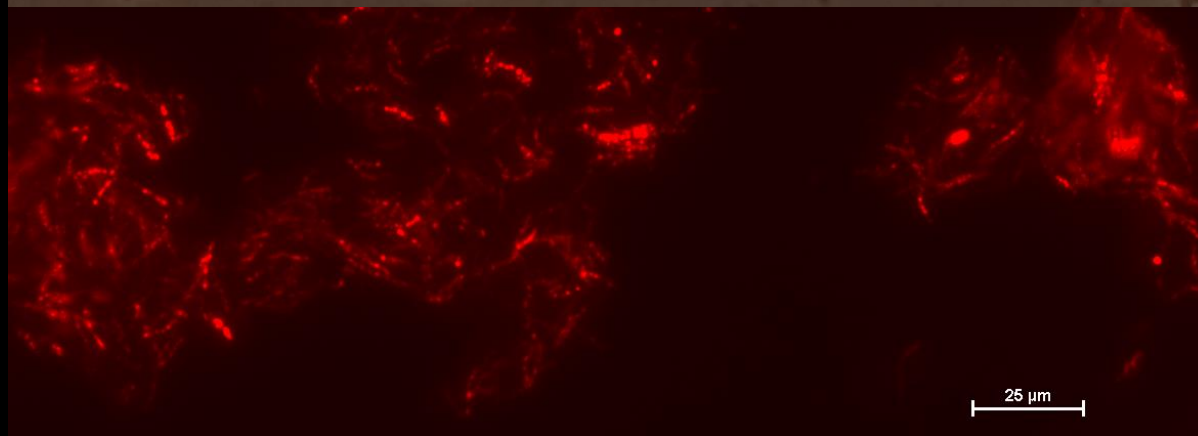
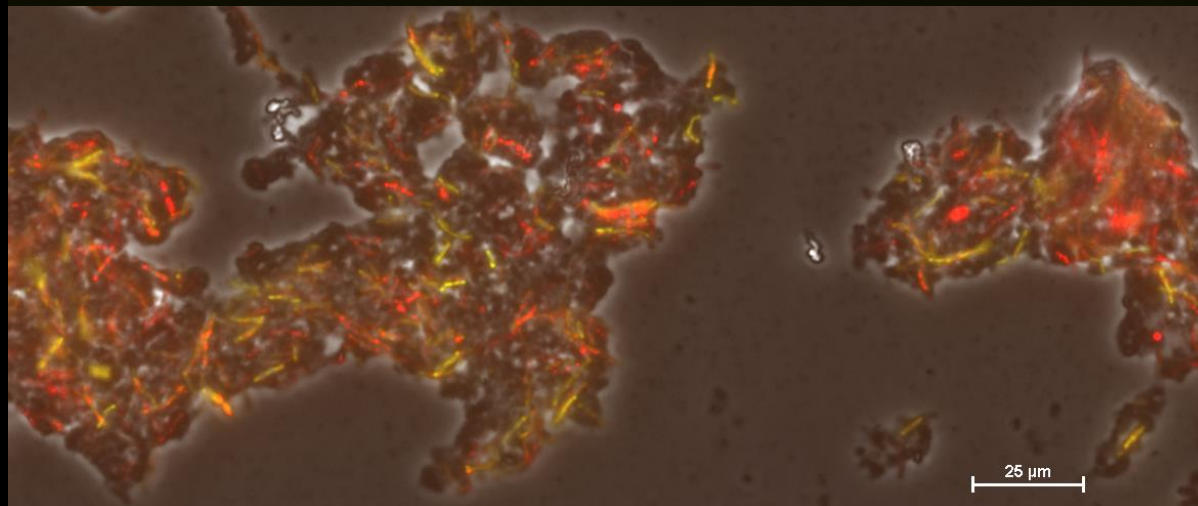
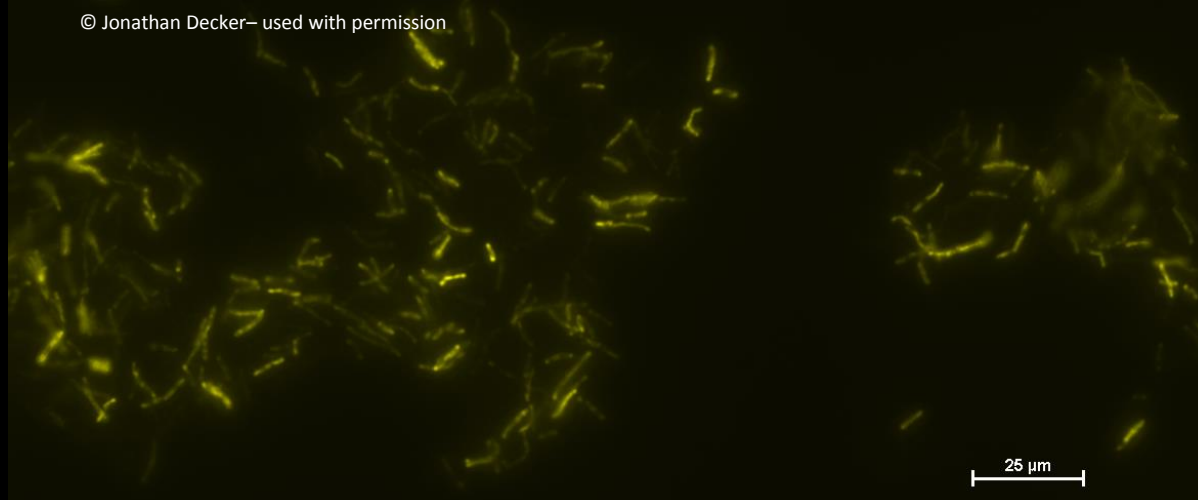
Deb C, Lee CM, Dubey VS, Daniel J, Abomoelak B, Sirakova TD, Pawar S, Rogers L, Kolattukudy PE. A novel in vitro multiple-stress dormancy model for *Mycobacterium tuberculosis* generates a lipid-loaded, drug-tolerant, dormant pathogen. *PLoS One.* 2009 Jun 29;4(6):e6077. doi: 10.1371/journal.pone.0006077. PubMed PMID: 19562030; PubMed Central PMCID: PMC2698117

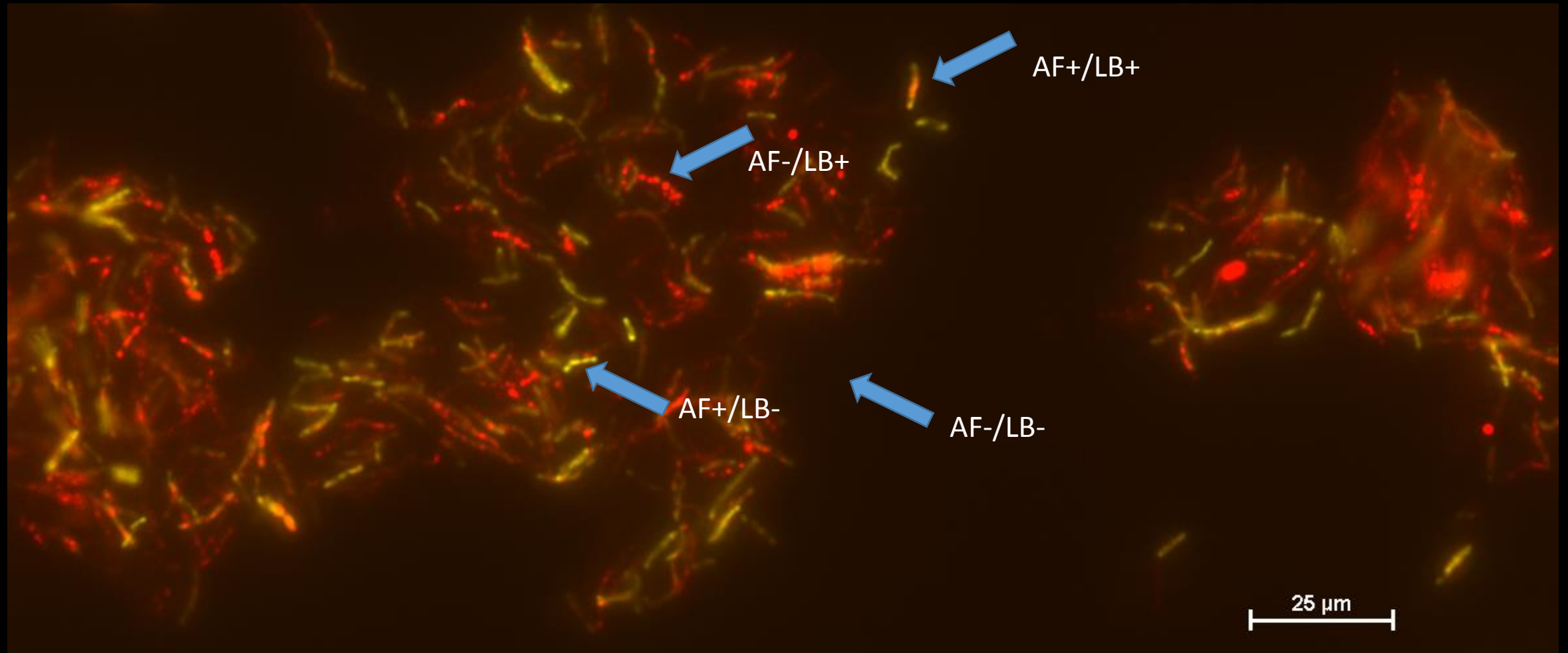


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Persister Study

Validation Study

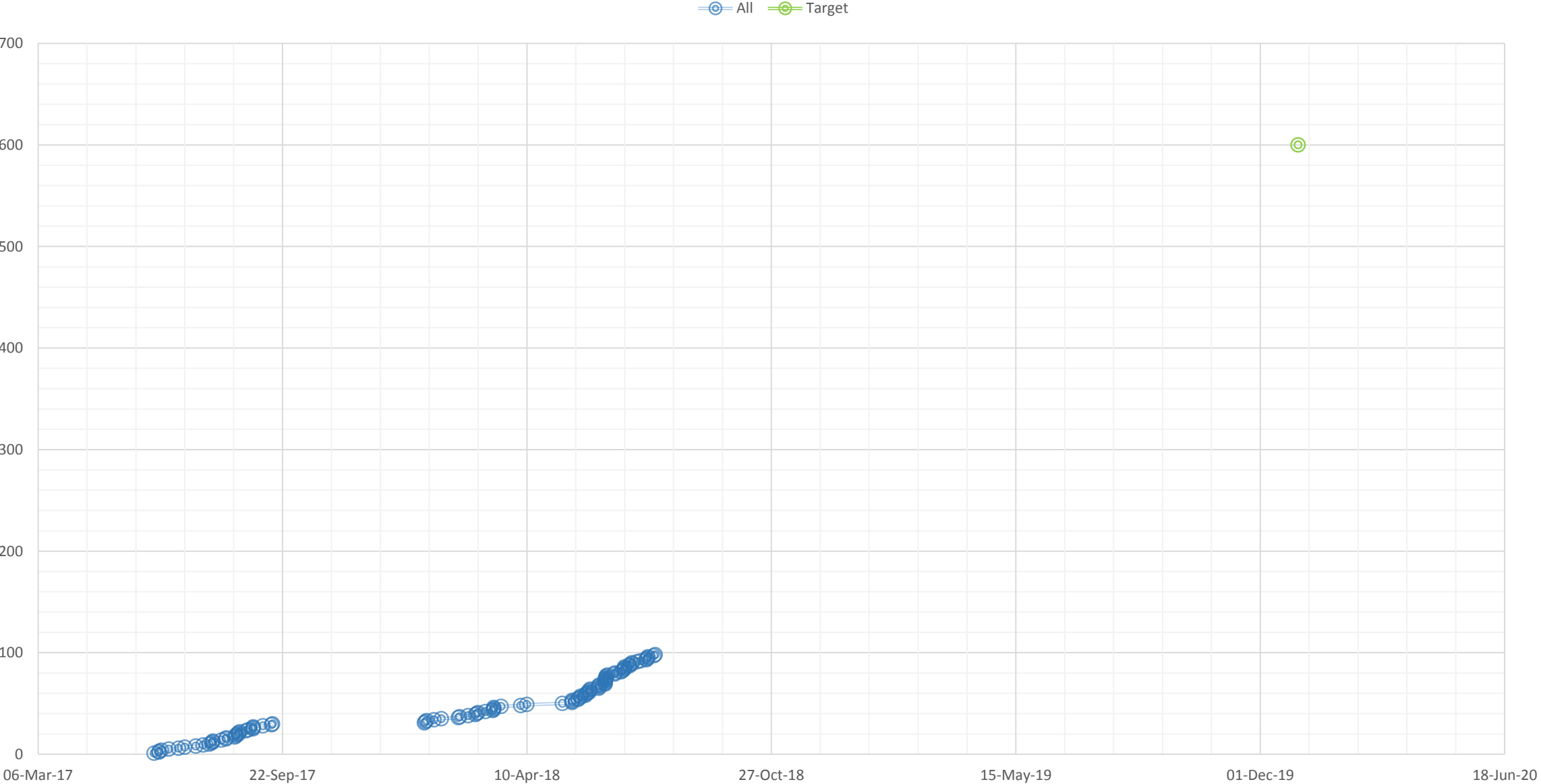
Validations that LB levels 4 weeks into treatment is predictive of unfavourable outcome

Case-Control Study

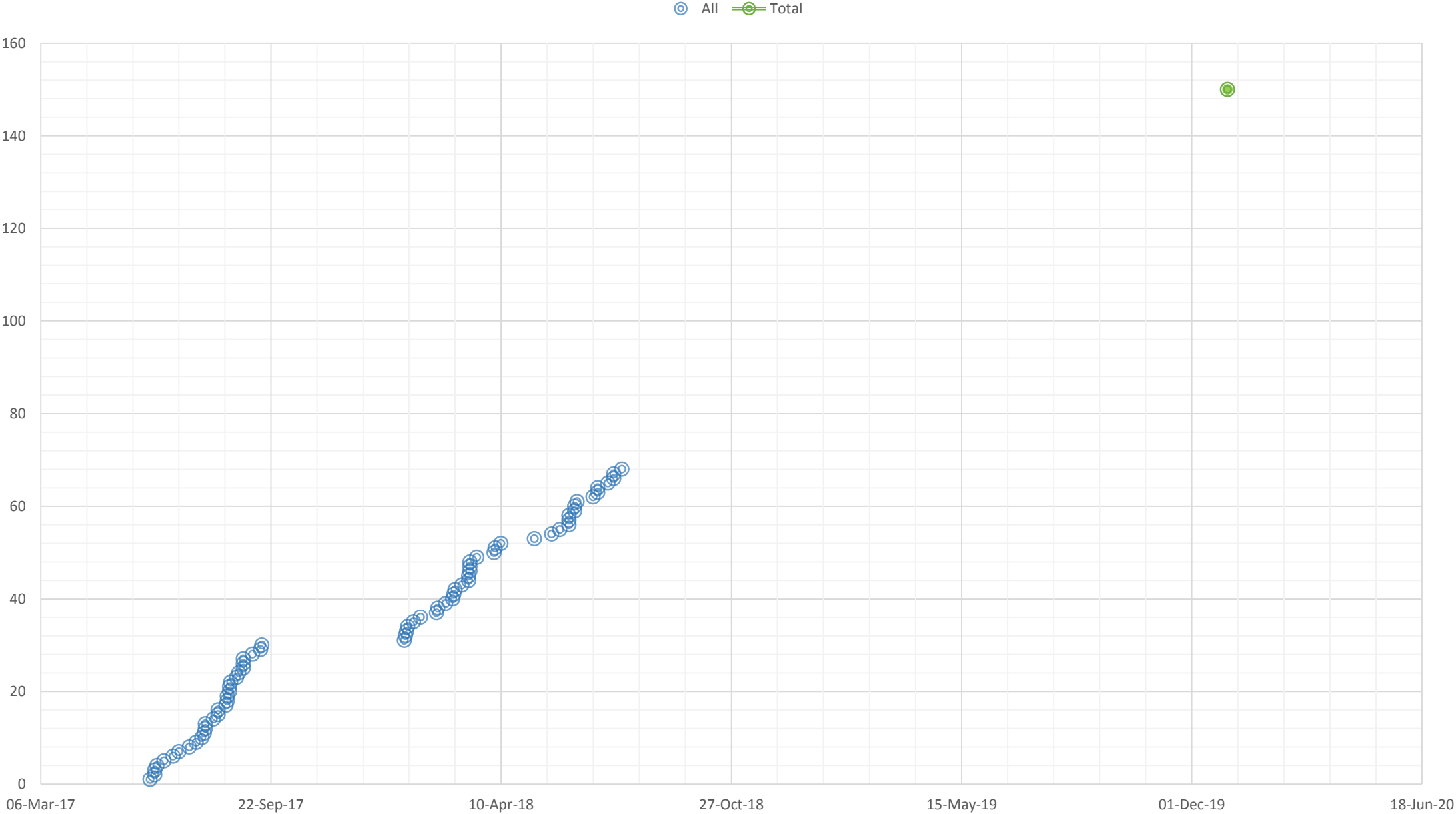
To determine if persister determinants can predict treatment failure and/or relapse.

Correlation Study

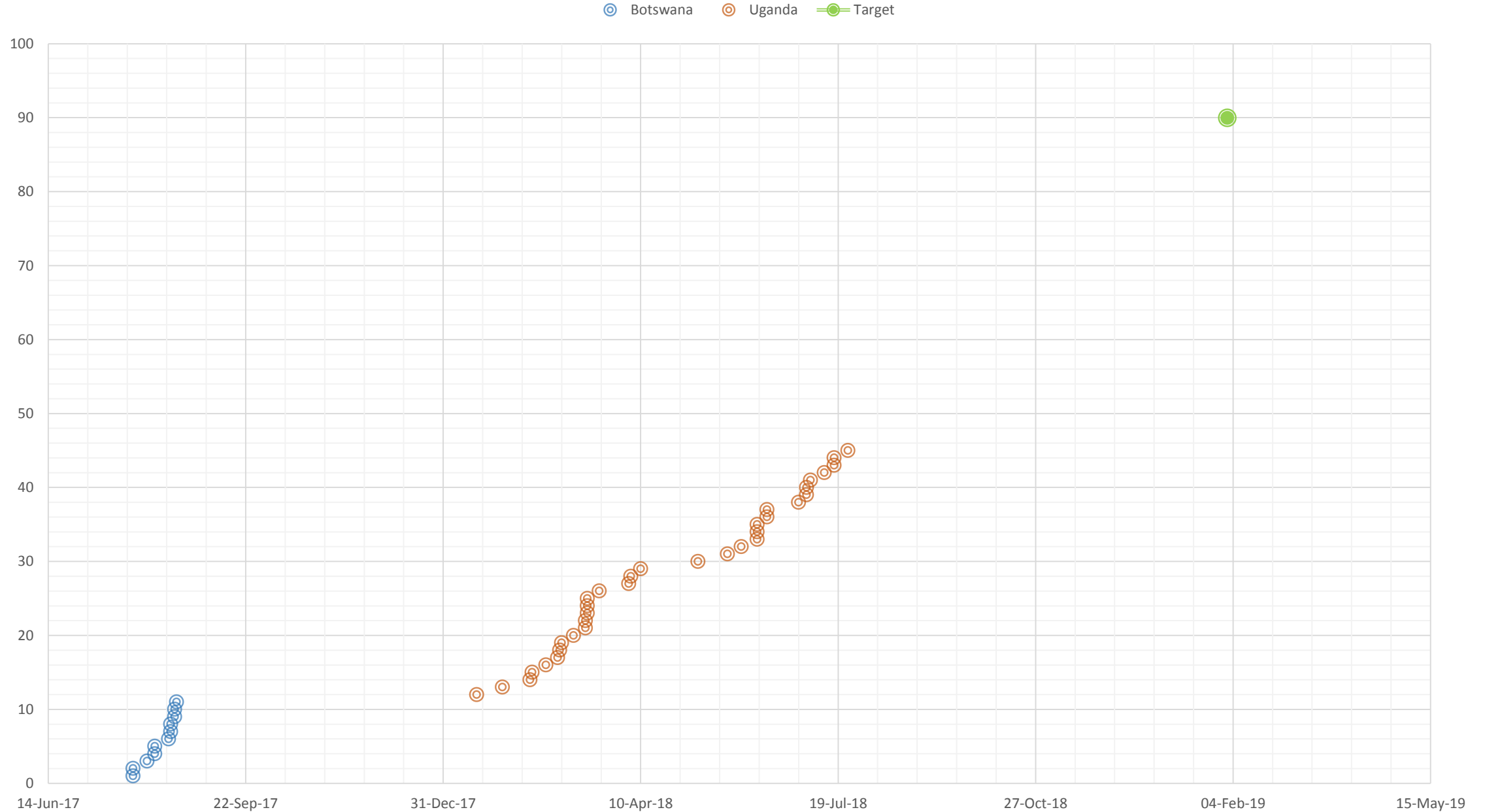
Validation Study Recruitment



Case - Control Study Recruitment



Correlation Study Recruitment



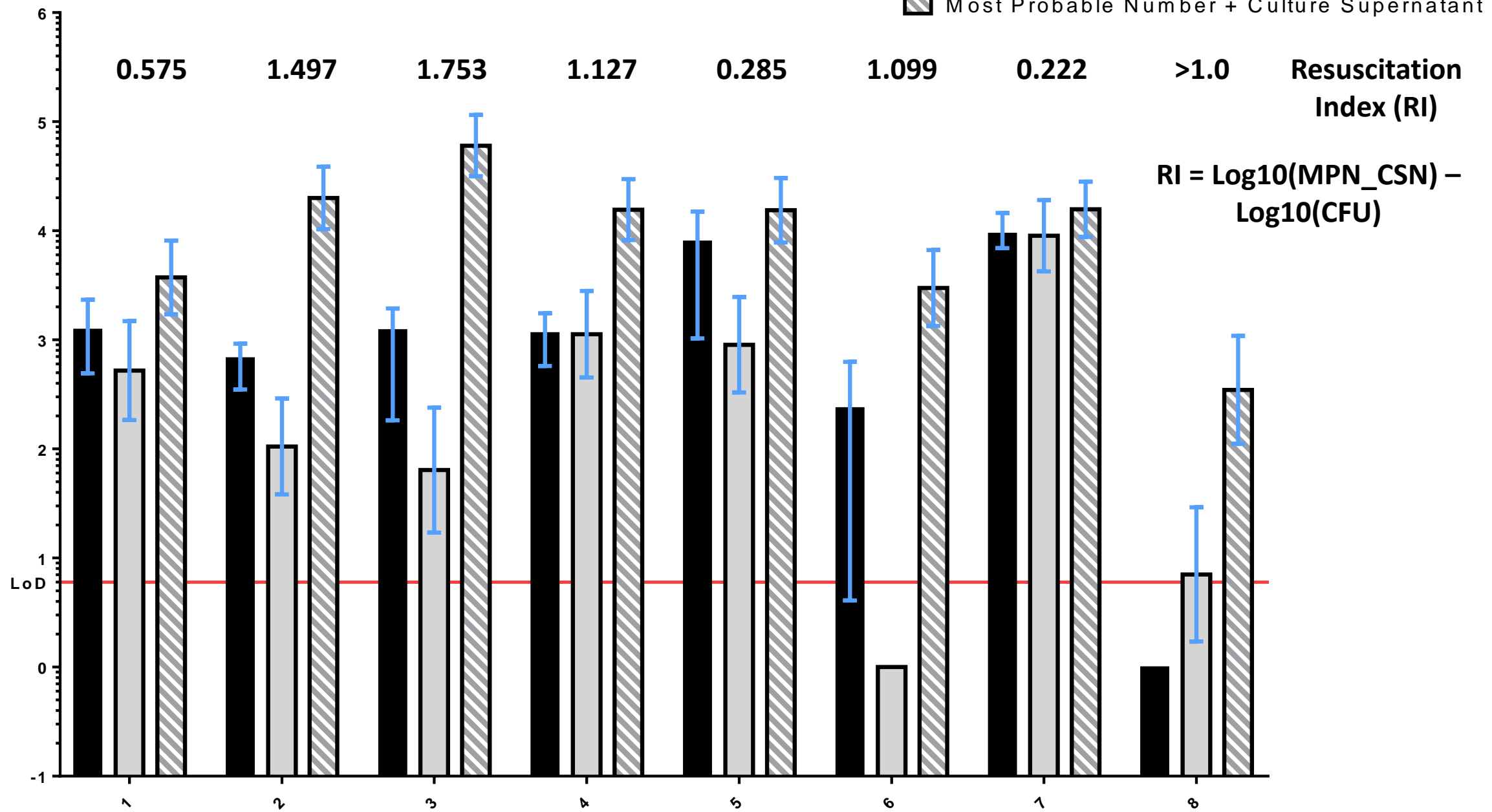
Additional Persister Studies

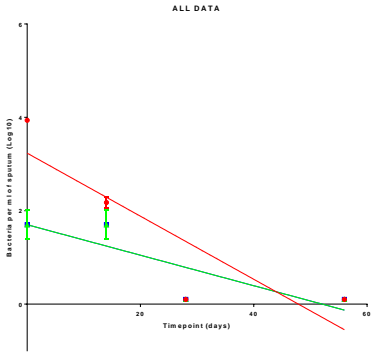
The Consortium for TB Biomarkers (CTB2) Persister Study

Aim:

To determine if persister determinants can predict treatment failure and/or relapse.

Bacteria per ml of sputum (Log10)

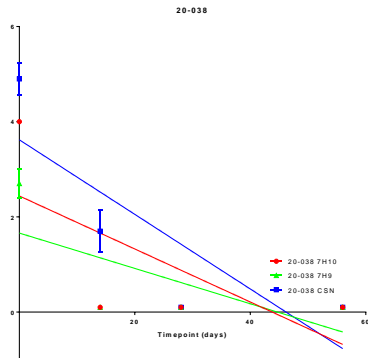
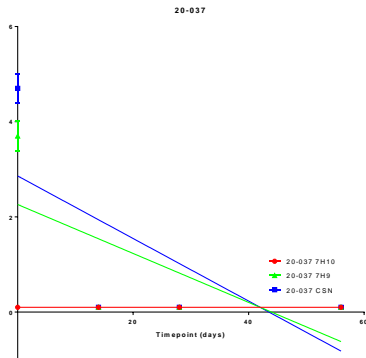
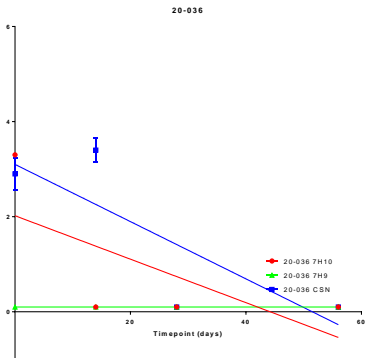
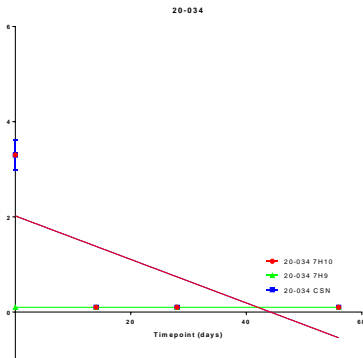
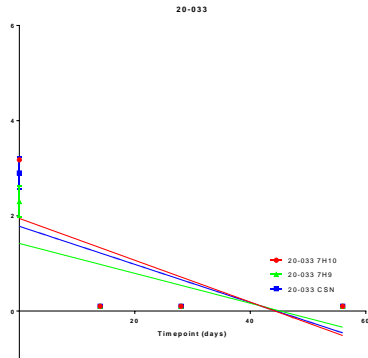
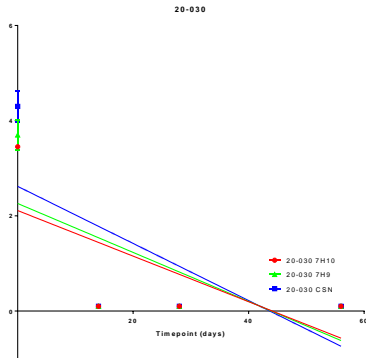
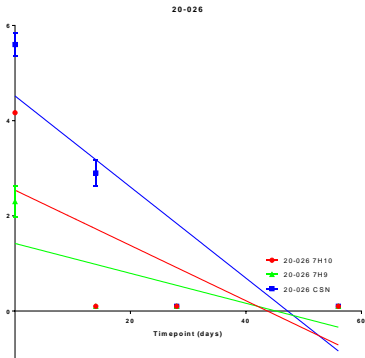
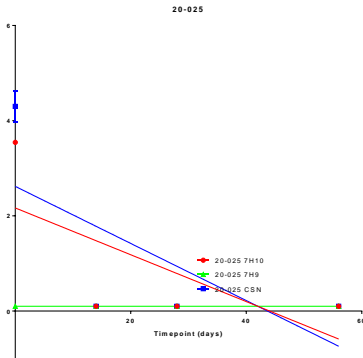
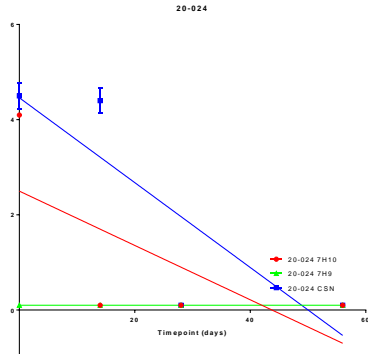
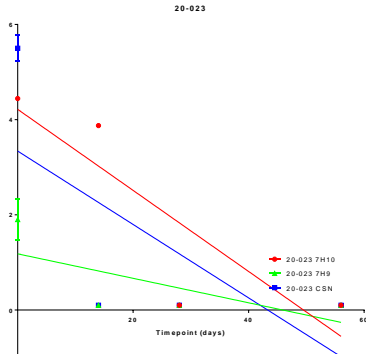
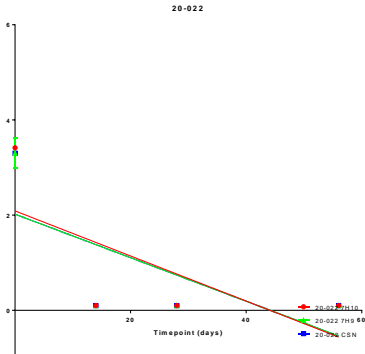




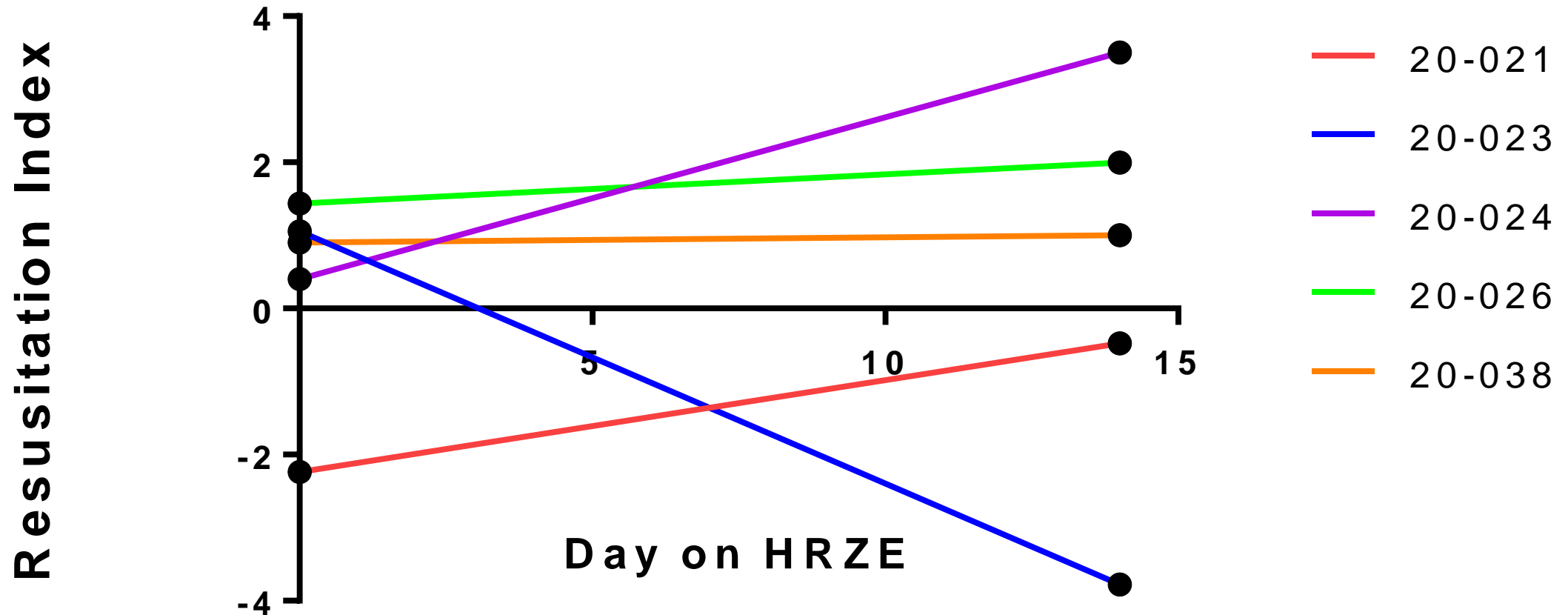
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Resusitation Index increases over time



Summary

- The RIFASHORT: Persister Study is up and running !

Acknowledgments

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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 months post treatment is a strong predictor of unfavourable outcome.