Development and Characterization of a Robust Foamy Macrophage Assay for use in TB Drug Discovery

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Background

The tuberculosis lesion environment is highly complex. Mycobacterium tuberculosis (M.*tb*) reside in various niches and their metabolism and other characteristics, including drug tolerance depend on the specific environmental characteristics of those niches.
So-called persister bacteria refer to those that are difficult to eradicate with drug treatment and that may contribute to the long durations of TB treatment required for cure.
Many *in vitro* models have been developed in attempts to mimic these niches with the objective of determining potential anti-TB compound activity in models that reflect M.*tb*'s characteristics *in vivo* (Table 1)

In TB pathology, M.*tb* may exist as extracellular bacteria (breath, sputum, tissues) or as an intracellular organism (Figure 1). Upon M.*tb* infection, macrophages are the first-line antimicrobial defence and they play a key role by triggering immune responses. However, M.*tb* is able to circumvent the macrophages' defences by activating an inappropriate inflammatory response and promoting dysregulation of lipid metabolism, needed for the long-term intramacrophage survival of the bacilli[1,2]. Indeed, "foamy" macrophages are observed in TB pathology, and also in some rodent models of TB - but no high throughput and robust *in vitro* screening model has been described for this particular niche.

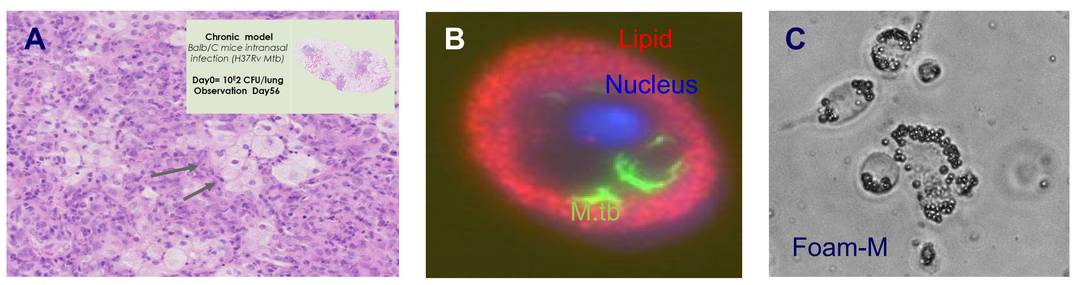

Figure 2: Foam-M cells are Lipid loaded

A - Histopathological characterization of Foamy macrophages in Lung lesions in preclinical Mycobacterium tuberculosis chronic model, Haematoxylin and Eosin (HE) staining
B - Human blood donor derived macrophages (hMDM), Fluorescent staining (nile red, dapi (blue), M.tb (green))
C - Lipid loaded THP1 Foam-M model, light microscopy

- **Aim**: based on Daniel et al., 2011[3], a foamy macrophage (Foam-M) model was developed adapting differentiation of THP1 cells to generate lipid-loaded macrophages where M.*tb* is slowly or non-replicating (not shown).

Compound ID	Intra-Macrophage Foam-M IC80 (µM)	Intra- Macrophage[14] IC80 (µM)	Extra-cellular R IC80 (µM)	Extra-cellular NR 4-stress test [13] IC80 (µM)
Rifampicin	0.26	0.15	0.09	0.6
Bedaquiline	0.12	0.07	0.05	2,8
Clofazimine	1,2	0.06	0.16	ND
Ethambutol	>30	11	4	>30
Isoniazid	4	0.22	0.35	>30
Linezolid	>30	1,6E-06	1,6E-06	>30
Moxifloxacin	4	0.56	0.29	1,9
Delanamid	>30	0.04	0.003	ND
Pretonamid	8,4	0.29	0.3	1,1

Table 2: TB compounds are less active in the Foam-M assay compared to THP1

- Nine TB reference drugs were tested in the Foam-M THP1 assay compared to standard IC80 in THP-1 infected as well as replicating and non-replicating extracellular M.*tb* assays (Table 2)

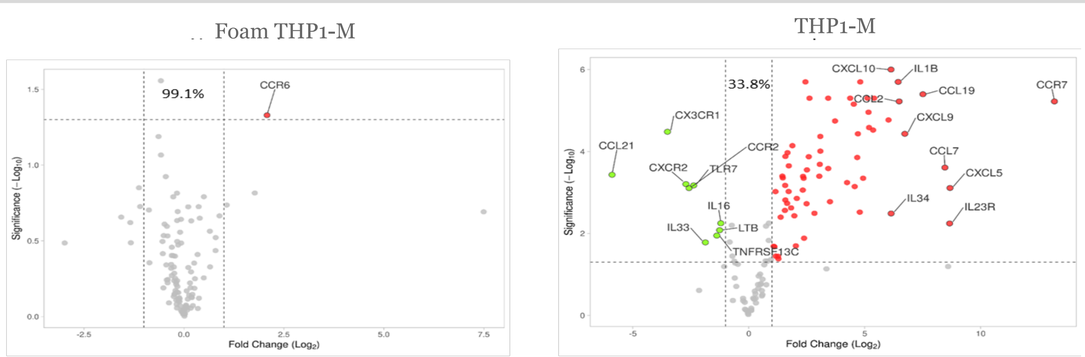

Figure 5: Infection of Foam-M does not trigger an anti-inflammatory response in host cells

Volcano plots showing significantly upregulated (red) or downregulated (green) and not significant (grey) genes in Foam-M THP-1 (A),THP-1 (B). Student's t-tests were used on ΔCt to compare treated samples to their non infected controls (FC ≥ 2 ; p- value ≤ 0.05 were used as a cut off to identify significantly differentially expressed genes (DEGs). Genes with Ct ≥ 32 were excluded from the analysis.

- Transcriptomic approach was used to quantify 184 human related genes in two different THP1 infected macrophage models compared to their non infected controls[14]. The top 10 genes DEG are labelled.
- Macrophage response in Foam-M THP-1 does not trigger an inflammatory response upon M.*tb* infection as compared to *in vitro* intracellular infection models (THP1 and human primary macrophages[14]).

Suspected Niches	In vitro mimetic assay
A Sputum	Synthetic Sputum assay[12]
B Biofilm	Biofilm assay[5, 6,7]
C Intramacrophage	Cholesterol as carbon source[16,17] / Intra-Macrophage assays[18]
D Granuloma / Caseum	*In vitro* granuloma assays[9,10] / Caseum assay[11]
A-E Phenotypic tolerant bacteria	Non replicative assays (NR)[19] Eg: nutient depletion, 4-stress assay, Stationnary phase, oxygen depletion)
E Foamy macrophage	Foamy macrophages[3]

Table 1: *In vitro* "mimetic" niche assays

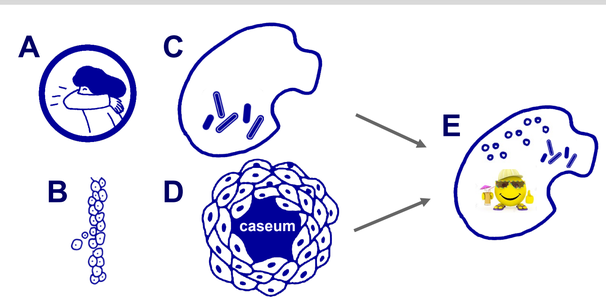

Figure 1: Mycobacterium tuberculosis (M.*tb*) resides in various niches

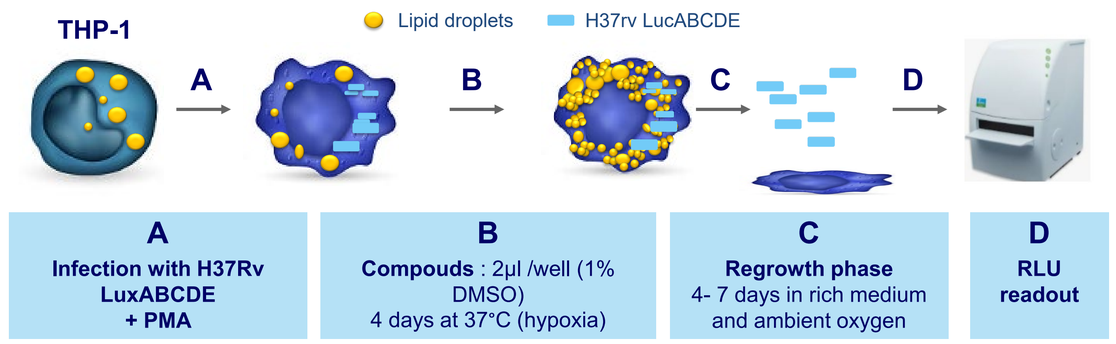

Figure 3: Foamy Macrophage assay (Foam-M)

- Parameters were optimized and validated to result in a robust assay in a 96 well plate format

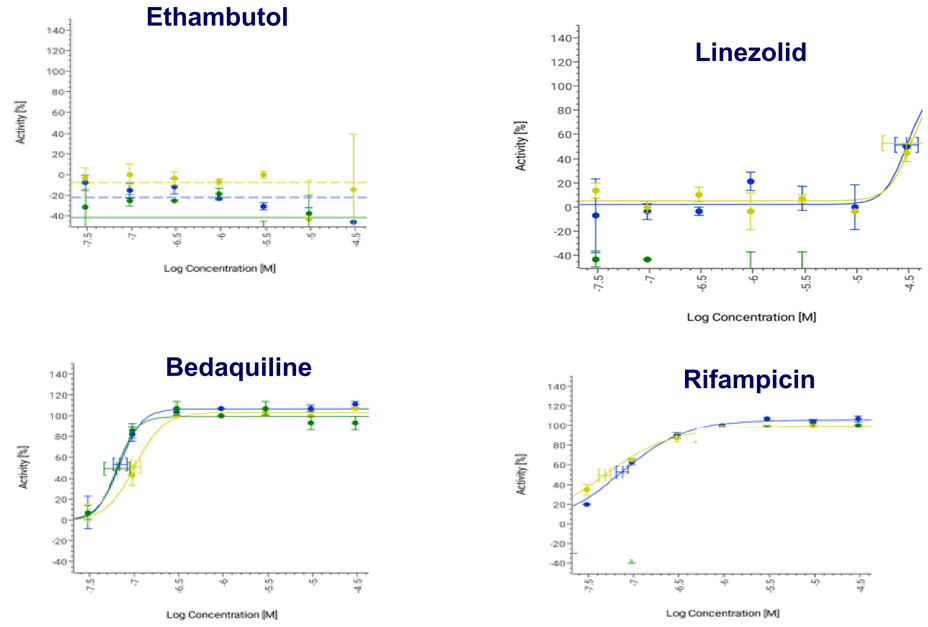

Figure 4: Ethambutol and Linezolid are inactive in the Foam-M assay whereas Bedaquiline and Rifampicin are the most active

Concentration–Response Curve (CRC) are shown. Data is normalized to the mean of 1 µM Rifampicin (killing) and DMSO control wells (no killing)

- Typical CRC for Ethambutol, Rifampicin, Bedaquiline and Linezolid are shown (Figure 4).

Conclusion : a new "niche assay"?

Foamy macrophages may be an important niche for M.tuberculosis during infection. We describe the development of a robust 96 well plate Foam-M assay designed to mimic this niche, and suitable for medium-high throughput evaluation of compound activity during drug discovery.

IC80 comparisons for TB drugs tested in the Foam-M assay, as well as Evotec's standard 384 well intramacrophage assay, indicate lower potency for all tested drugs except for Rifampicin and Bedaquiline in the Foam-M compared to the standard THP-1 assay. No activity could be detected for Linezolid or Ethambutol in the Foam-M assay, at the tested concentrations. Notably, potencies in Foam-M cells did not follow those in extracellular NR tests although M.tb growth was not detected in Foam-M cells during the hypoxia phase of the assay.
Further work is ongoing to (a) fully characterize this model and its utility in drug discovery, including relevance to TB animal models, and to clinical infection (b) understand the behavior of TB drugs in the Foam-M model.

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