T2016-101

Host-directed drug targeting against tuberculosis

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Identification of host genes hijacked by \textit{M. tuberculosis} (Mtb)

Goals: Host-directed drug development against Mtb

Supplementing pathogen-directed targeting by antibiotic treatments, would prolong the life span of antibiotics, thereby reducing the frequency of treatment failures.

TB is ninth leading causes of death worldwide

- WHO report shows 10.4 million new cases of TB in 2016
- 1.3 million died from TB in 2016 (+ 0.4 million by TB + HIV).
- 0.5 million people a year become infected with MDR-TB.

[Map showing Estimated TB incidence rate, 2016]
Partnership

- Professor Frank Brombacher (ICGEB, Cape Town)
  Pathogen Immunology: Mtb & NTD
  - Mtb pathology
  - Tg/KO mice technology
  - BSL3 laboratory

  \[\text{Interdisciplinary collaboration}\]

- Collaboration starting: 2010
- Focus on Macrophage cells
  - Macrophages are primary infected cells in TB.
  - Two activation states:
    - classical activation: M1 (IFNg) – killing effectors
    - alternative activation: M2 (IL4/IL13) – Regulation of immune response
  - Protective and subversive mechanisms of mph genes in TB
Identification of validation candidates

Host-protective and subversive gene expression must be affected by Mtb. Macrophage-specific genes are suitable for drug targets.
Establishment of validation experiments

**Activities and Results -- 2**

Lentivirus

- Target KD (shRNA)
- TB infection
- Colony Formation Assay

**Category:** Priority targets with no drugs/inhibitors

**Category:** Priority targets with specific inhibitors

**Category:** Priority targets with repurposed drugs

BSL3: Cape Town
Priority targets with no drugs/inhibitors

RSAD2

Knockdown efficiency

Mtb growth in MDM, 2 days

Mtb growth in MDM, 5 days
Priority targets with no drugs/inhibitors

**BCL2A**

**Knockdown efficiency**

**Mtb growth in MDM, 2 days**

**Mtb growth in MDM, 5 days**

Human MDM

TB patients
Activities and Results -- 5

**Priority targets with no drugs/inhibitors**

**CAMPK2**

**Knockdown efficiency**

- Human MDM
- TB patients

**Mtb growth in MDM, 2 days**

**Mtb growth in MDM, 5 days**
### Priority targets with specific inhibitors

**Berberine, inhibitor of Daxx gene**

*Human MDM*

![Graph showing CFU/1x10^5 MDM for Berberine compared to Medium at 3 and 6 dpi.](image)

- **3 dpi**: Medium > Berberine
- **6 dpi**: Medium > Berberine

### Priority targets with repurposed drugs

**IGF1 shRNA & Tyrophostin**

**shRNA**

- Mock
- IGF1

**Inhibitor**

- DMSO
- Tyrophostin

![Graph showing CFU/1.5x10^6 BMDM for different treatments at 0, 2, and 5 dpi.](image)

- 0 dpi: DMSO > Tyrophostin
- 2 dpi: Tyrophostin > DMSO
- 5 dpi: Tyrophostin > DMSO

Tyrophostin: IGFR1TK inhibitor
Summary

- Creation of Mtb infection time course data and selection of validation candidates
- Establishment of validation experiment
- Preliminary experiment revealed promising results (although it needs reproducibility check)
- Validation of additional candidates (Priority targets with no drugs/inhibitors) is going on

Lessons learned:
Equal partnership is successful in our project, with distinct role in each party.
We can focus on the achievement.
This happens due to long and fruitful collaboration between parties.

Request of advice:
Mtb growth inhibitory assay is not enough. Perhaps we need more analysis to identify best target(s).
How much validation we need for the next step?
How can we propose our next activity to the GHIT?