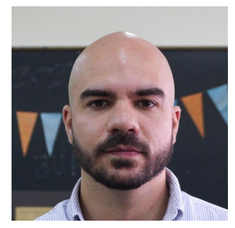
Mycobacterium abscessus double reporter strain as a readout of therapeutic efficacy – high-throughput screening development and validation

Gabriel S. Oliveira[1,2], Clara M. Bento[1,3,4], António Pombinho[1,3], Rita Reis[1,3], Kevin Van Calster[5], Linda De Vooght[5], André Maia[1,3], Paul Cos[5], Maria Salomé Gomes[1,2], Tânia Silva[1,2]

[1] i3S – Instituto de Investigação e Inovação e Saúde, Universidade do Porto, Porto, Portugal; [2] ICBAS – Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, Portugal; [3] IBMC – Instituto de Biologia Celular e Molecular, Universidade do Porto, Porto, Portugal ; [4] Programa Doutoral em Biologia Molecular e Celular (MCBiology), Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Porto, Portugal; [5] Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Wilrijk, Belgium

Corresponding authors: jorge.oliveira@i3s.up.pt; tania.silva@i3s.up.pt



Gabriel S. Oliveira

Tânia Silva

Overview

With our developed 3-step high throughput screening assay, we tested a ChemBridge compound library.

- We use a double reporter strain of *Mycobacterium abscessus,* liquid handling robotics, and automated microscopy and analysis to reduce variability.
- With an increase in assay complexity, fewer compounds progress from screening through hit validation to intracellular activity testing.
- Discovered 3 hits with potential to treat these infections.

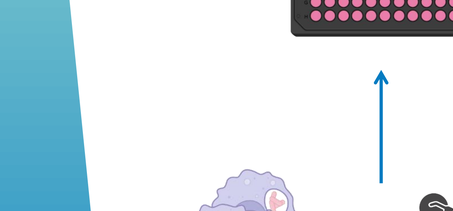
Introduction

Infections caused by *Mycobacterium abscessus* pose significant challenges due to high antibiotic resistance and limited treatment options. Current therapies are toxic and ineffective. Traditional methods for screening new drugs against *M. abscessus* are time-consuming, impeding researchers from reaching the necessary throughput to treat this “incurable nightmare” as testing thousands of compounds becomes impractical.

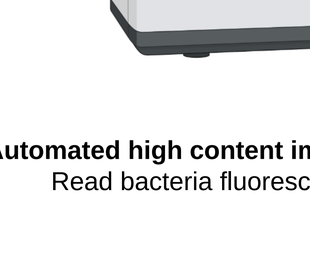
Thus, we developed a high-throughput screening assay using a double-reporter strain of *M. abscessus* capable of emitting both luminescence and fluorescence, enabling rapid evaluation of candidate compounds.

Methods

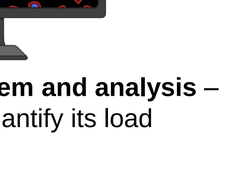
Task 1. Screening phase



Reagent dispenser – Add media and planktonic bacteria 2.5x10^5 CFUs/mL

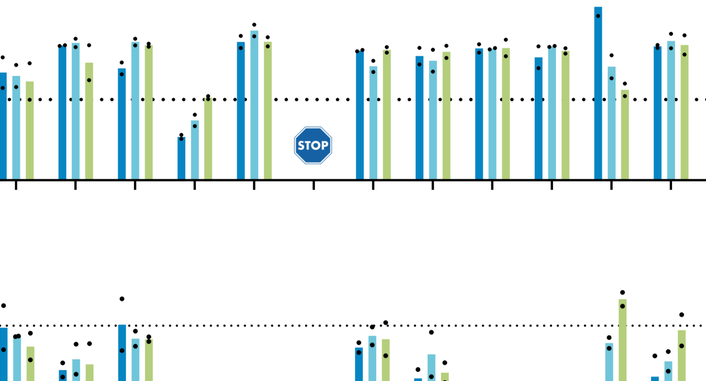


Liquid handler – Add compounds at 3.33 µM



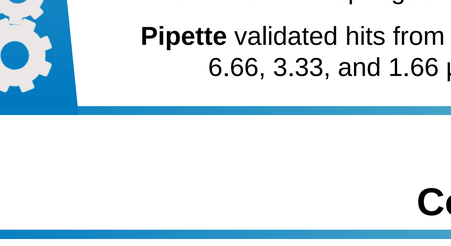
Luminescence

Normalised data

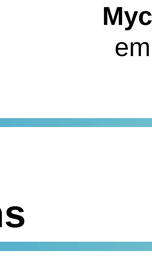


Results of 1 plate out of 29. **80 hits** were identified.

Task 2. Hit validation

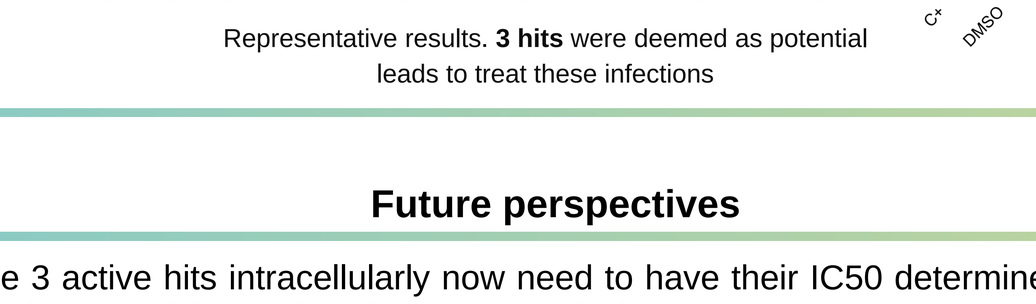


Reagent dispenser – Add media and planktonic bacteria 2.5x10^5 CFUs/mL



Pipette – Add hits from task 1 at 6.66, 3.33, 1.66 µM

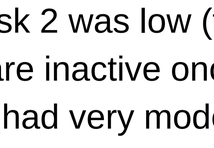
Luminescence

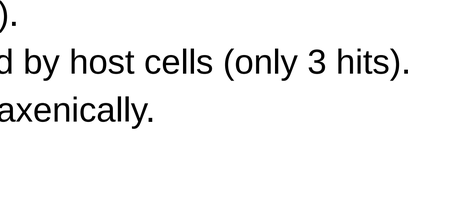


Representative results. **28 hits** were validated.

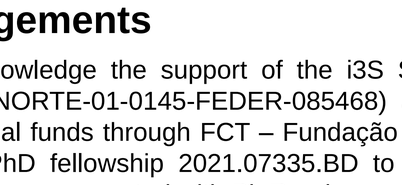
Task 3. Intracellular activity

Fluorescence





Automated high content imaging system and analysis – Read bacteria fluorescence and quantify its load

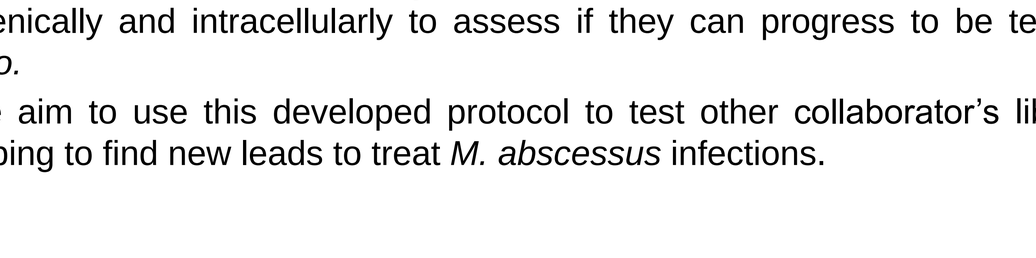


Infect 4x10^4 macrophages with MOI 1

Pipette validated hits from task 2 at 6.66, 3.33, and 1.66 µM

$$\frac{\text{Bacteria Intensity} * \text{Bacteria Area}}{\text{Nº Bacteria regions}}$$
$$\text{Nº cells}$$

Mycoload formula that better emulates counting of CFUs



Representative results. **3 hits** were deemed as potential leads to treat these infections

Assay complexity

Hits

Conclusions

- The data analysis of the initial screening was lenient, as the number of validated hits in Task 2 was low (from 80 to 28).
- Most compounds are inactive once internalized by host cells (only 3 hits).
- 2 of the 3 final hits had very moderate activity axenically.

Future perspectives

- The 3 active hits intracellularly now need to have their IC50 determined both axenically and intracellularly to assess if they can progress to be tested *in vivo.*
- We aim to use this developed protocol to test other collaborator’s libraries, hoping to find new leads to treat *M. abscessus* infections.

Acknowledgements

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