EMI-TB: Mucosal TB Vaccine Development
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www.emi-tb.org

Funded by the EU
Our goal:
1. Develop a mucosal vaccine candidate as a boost to BCG
2. Better understanding of mucosal immunity in TB and better linking to vaccine induced protection
**Antigens:**

**Fusion proteins (FPs) with HBHA**
- Fusion Protein 1 (FP1): Ag85B-ACR-HBHA
- Fusion Protein 2 (FP2): MPT64-ACR-HBHA
- Fusion Protein 3 (FP3): Ag85B-TB10.4-HBHA
- Fusion Protein 4 (FP4): MPT64-TB10.4-HBHA

**Fusion proteins (FPs-b) without HBHA**
- Fusion Protein 1 (FP1b): Ag85B-ACR
- Fusion Protein 2 (FP2b): MPT64-ACR
- Fusion Protein 3 (FP3b): Ag85B-TB10.4
- Fusion Protein 4 (FP4b): MPT64-TB10.4
- Fusion protein 5 (FP5b): ESAT6-CFP10

**Individual antigens:**
- Ag85B, Acr, MPT64, TB10.4, ESAT-6

**Delivery systems:**
- Nanoparticles
- *Bacillus subtilis* bacterial spores
- Asymmetric apoptotic-like liposomes
- Protein + adjuvants
- Streptavidin multi-antigen complexes
How can we mimic infection with a vaccine?

Nanoparticles, spores etc coated with FPs-HBHA
0.5 million BCG Pasteur
Base of tail

BCG (s.c.)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

weeks

Vaccine (i.n.)

MTB (aerosol)

Cull

H37Rv 200 CFU

• C57BL6 mice
• 10/group (3 for immunogenicity and 7 for MTB)
• Immune responses:
  - Antibodies
  - T cell proliferation
  - Cytokines
  - Polyfunctional T cells
  - Lung lavage

• Lung and spleen CFU
• Lung immunopathology
EMI-TB mucosal vaccine candidates for boosting BCG

<table>
<thead>
<tr>
<th>Delivery system:</th>
<th>Yc-NaMA NANO</th>
<th>B. subtilis spore</th>
<th>PS/PS Lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen:</td>
<td>Ag85B-Acr-HBHA</td>
<td>Ag85B-Acr-HBHA</td>
<td>Ag85B + ESAT-6</td>
</tr>
<tr>
<td>Location:</td>
<td>Surface</td>
<td>Surface</td>
<td>Encapsulated</td>
</tr>
<tr>
<td>Adjuvant:</td>
<td>Poly I:C</td>
<td>Poly I:C</td>
<td>Poly I:C</td>
</tr>
<tr>
<td>Boost BCG:</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Antibody Response:</td>
<td>Strong</td>
<td>Moderate</td>
<td>Weak</td>
</tr>
<tr>
<td>T cell response:</td>
<td>Good</td>
<td>Good</td>
<td>Strong</td>
</tr>
<tr>
<td>Resident Lung Tm:</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mouse CFU reduction:</td>
<td>0.5-1 Log</td>
<td>0.5-1 Log</td>
<td>0.5-1 Log</td>
</tr>
<tr>
<td>Suitable for aerosol</td>
<td>Moderate</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Current Status:</td>
<td>Guinea Pig/NHP</td>
<td>Guinea Pig/NHP</td>
<td>Guinea Pig/NHP</td>
</tr>
<tr>
<td>Next:</td>
<td>NHP</td>
<td>NHP</td>
<td>NHP</td>
</tr>
</tbody>
</table>
Spores can adsorb antigens on their surface.

Antigen (FPs) + Bacterial spores

Immune responses
Protection against MTB challenge

Bacillus subtilis spores (heat inactivated)
Adjuvanticity of Spore-FP1

Dendritic Cells

- **CD80**
- **CD86**
- **CCR7**
- **PD-L2**
- **PD-L1**
- **MHC I**
- **MHC II**

Macrophages

- **CD80**
- **CD86**
- **CCR7**
- **PD-L2**
- **PD-L1**
- **MHC I**
- **MHC II**

**Graphs:**

- **IL-1β**
- **IL-6**
- **TNF-α**

- **Unstimulated**
- **LPS**
- **Spores (1, 10, 100 MOI)**
Spore-FP1 Protection in mice

A

Lungs

Spleen

B

Lungs

Spleen

MGIA
Lung immune response to Spore-FP1

BCG Prime

6 weeks

Intranasal immunisation

3 weeks

Intranasal immunisation

3 weeks

Cull

CD44<sup>hi</sup>CD62L<sup>b</sup>

T<sub>RM</sub> cells

PBS

BCG

FP

Spores

Spore-FP

CD4+

3.87%

3.56%

5.85%

3.47%

14.9%

CD8+

1.31%

1.87%

5.63%

2.63%

12.5%

CD103

CD69
T Cell Responses

CD4^+ T-cells

Ag85B

ACR

FP1

% Ki67^+

Naive T CM TEM

IFN-γ

pg/mL

PBS BCG Spore-FP

IL-17A

pg/mL

PBS BCG Spore-FP

CD44^loCD62L^hi

CD44^hiCD62L^hi

CD44^hiCD62L^lo

PBS

BCG

Spore-FP

Unstimulated FP Ag85B
Spore-FP1 protection in Guinea pigs

1\textsuperscript{st} Study (joint):

and

(No BCG prime)

2\textsuperscript{nd} Study:

(With BCG prime)

May 2018
Carriage of protein by spores facilitates transportation through mesh
Aerosolised Spore-FP1 NHP studies

1st study (completed): Safety: 3 EMI-TB candidates are well tolerated and safe in Macaques

2nd study: Immunogenicity

3rd study: Protection

Study week post BCG vaccination: Collection of clinical data & blood samples: Weight, temperature, ESR, chest X-ray. Immunology: PBMC cryopreservation, ELISPOT, serum storage, etc...

6 NHP per group
Where are we now...

- Standard mouse model
  - Screening multiple candidates, immune responses, protection

- Modified mouse models

- Guinea pig models

- Safety
  - NHP

- Protection
  - Immune responses

- Candidate for human phase I clinical trial

- Validation of protection

- 36 vaccine Candidates regimens
  - Nano-FP1
  - Spore-FP1
  - Lipo-AE

- Spore-FP1
Summary

• 36 mucosal vaccine formulations tested – 3 selected

• Spore-FP1, Nano-FP1, Lipo-AE protective in standard mouse model

• Spore-FP1 protective in Guinea Pig model

• All 3 formulations safe in NHP

• Currently, spore-FP1 starting MTB challenge trial

• Expected conclusion of experimental studies: early 2019

• Expected output: mucosal vaccine candidate for human clinical trials
Passive vaccination with human IgA protects against MDR-TB infection in mice

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Background

Despite the decreasing incidence of TB, rates of MDR-TB are increasing rapidly. Therefore, novel and effective treatments for MDR-TB are urgently required.

Immunotherapy using the human 2E9IgA antibody has the potential to provide prophylactic immunity for combating MDR-TB. 2E9IgA specifically targets ACR, a protein found on Mtb associated with dormant infection.

Previous work has demonstrated promising results for the antibody when given together with the cytokine interleukin-2 (IL-2) in humanised genetically modified mice.

Aims of the study

To test if 2E9IgA and IL-2 immunotherapy could be used for treatment of MDR-TB.

Specific research objectives were:

1. Investigate the mechanism of action of 2E9IgA.
2. Test whether combined IgA/IL-2 therapy could suppress aerosol MDR-TB infection in transgenic mice expressing human IgA receptor.

Methods

Production of 2E9IgA. 2E9IgA was produced using a Chinese hamster ovary cell line and had different conditions to facilitate the production of IgA and IgG antibodies.

Functional characterisation of 2E9IgA. ELISAs were used to determine whether 2E9IgA binds to the ACR antigen.

PCR screen for human IgA receptor transgenic mice. BALB/c mice were screened for CSF3R expression prior to MDC-TB challenge experiments.

Infection of human cells with BCG in vitro. Human monocytes and THP-1 cells were infected with fluorescent BCG-GFP and treated with 2E9IgA. Levels of infection were measured.

Immunotherapy of MDR-TB in human IgA receptor transgenic mice. Transgenic mice expressing the human IgA receptor were used to investigate the effects of 2E9IgA given with IL-2. Levels of infection were determined by plating lung homogenates and counting mycobacterial colonies.

MDR-TB immunotherapy in mice

Conclusions

- 2E9IgA binds to ACR, BCG and the human IgA receptor CD90.
- 2E9IgA reduced BCG-GFP infection of THP-1 cells and human monocytes in vitro.
- Using aerosol MDR-TB mice challenge model, immunotherapy with 2E9IgA significantly reduced MDR-TB infection compared to no treatment control.
- These results suggest that ACR is a promising target for vaccine induced mucosal antibodies.
- Combined immunotherapy has the potential for adjunct treatment of MDR-TB.

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Andy C. Tran
Thank you!

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