TB has killed more people than any other infectious disease the past 200 years
Mycobacterium tuberculosis (Mtb) infection:
- > 1/3 of the world population is infected
- > 9.6 million people develop TB disease every year, ~1.5 million die
- Deadly interaction with HIV infection
- Multidrug (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) strains emerging rapidly

Infection can be latent for decades

Reactivation disease develops in 3-10% of latently infected persons
Tuberculosis anno 2016

- **Mycobacterium tuberculosis**
  - > 1/3 of the world
  - > 9.6 million people
  - Deadly interaction
  - Multidrug (MDR), extensively drug resistant (XDR), totally drug resistant (TDR) strains emerging rapidly

- Infection can be latent

- Reactivation disease develops in 3-10% of latently infected persons
**The increasing threat of MDR and XDR TB at the borders of the EU**

- *Mycobacterium tuberculosis* (Mtb) infection:
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  - > 9.6 million people develop TB disease every year, ~1.5 million die
  - Deadly interaction with HIV infection
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- Infection can be latent for decades
- Reactivation disease develops in 3-10% of latently infected persons

![WHO, 2015](image.png)
We have BCG so why worry?

- most used global vaccine (> 4 billion doses)
- protects against severe TB in childhood

but:

- it fails to protect adults consistently against pulmonary TB
- it does not protect against reactivation of latent TB
- it does not induce immunity against several relevant antigens of Mtb, including latency antigens

-> BCG vaccination has limited impact on TB pandemic
High global health benefit from more effective TB vaccination

**TB incidence**

**TB mortality**

---

Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics

Laith J. Abu-Raddad\(^{a,1}\), Lorenzo Sabatelli\(^{b}\), Jerusha T. Achterberg\(^{a,b,3}\), Jonathan D. Sugimoto\(^{a,5}\), Ira M. Longini, Jr.\(^{a,d}\), Christopher Dye\(^{c}\), and M. Elizabeth Halloran\(^{a,d,2}\)
# The Current Global Clinical Portfolio of TB Vaccine Candidates

<table>
<thead>
<tr>
<th>Phase 1</th>
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</table>

- **Viral Vector**
- **Protein / Adjuvant**
- **Mycobacterial – Whole Cell or Extract**
A recent phase IIb trial showed no efficacy of a new TB vaccine

Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

Michele D Tameri*, Mark Hatherill*, Bernard S Landry, Thomas J Scriba, Margaret Ann Snowden, Stephen Lockhart, Jacqueline E Shea, J Bruce McClain, Gregory D Hussey, Willem A Hanekom, Hasson Mohamed†, Helen McShane, and the MVA85A 020 Trial Study Team

Summary
Background BCG vaccination provides incomplete protection against tuberculosis in infants. A new vaccine, modified Vaccinia Ankara virus expressing antigen 85A (MVA85A), was designed to enhance the protective efficacy of BCG. We aimed to assess safety, immunogenicity, and efficacy of MVA85A against tuberculosis and Mycobacterium tuberculosis infection in infants.
• Ag85A may not be the “right” antigen: gene expression analysis shows its expression is regulated (e.g. Commandeur et al, JI 2013)

• Cells other than Th1 may be needed for full protection

• BCG may have masked possible protective MVA85A effect

• MVA85A might have shown protection against severe forms of TB (endpoint was mild TB)

• Possible protective MVA85A effect may depend on population setting
Antigenic phase variation during *Mtb* infection

*in vitro and in vivo*
Antigenic phase variation during *Mtb* infection

*in vitro* and *in vivo*

MTB exposed to:
- low O$_2$, NO
- lack of nutrients
- low pH

- macrophages
- epithelioid cells
- lymphocytes

→ physical containment of bacilli
Antigenic phase variation during *Mtb* infection

*in vitro and in vivo*

**MTB exposed to:**
- low O$_2$, NO
- lack of nutrients
- low pH

**Hypoxia induced**

<table>
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<tr>
<th>O$_2$</th>
<th>20%</th>
<th>0.2%</th>
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**Schoolnik et al. RNA-microarray: dormancy regulon (DosR)**

**Granuloma in lung tissue**

**Droplets either sneezed or exhaled**

**Physical containment of bacilli**

### Table: Hypoxia Induced Genes

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**Sensor histidine kinase**

**2-comp. response reg.**

**20%**

**0.2%**
Mtb DosR regulated latency Ags tested all across Africa.
Strongly recognized across different African populations

Black et al., B4TB GC6 consortium, CVI 16: 1203, 2009

Rv1733 as candidate
Amino acid sequences of Rv1733c synthetic long peptides (SLPs).

The addition of a single peptide based vaccine significantly improves BCG efficacy

Mtb latency antigens Rv3407 + Rv1733c expressed in rBCG induce superior protection in mice

Reece et al, JID 2011
**Model**

**Phase of infection**
- Primo infection
- Latent infection
- Reactivation of infection

**Phase specific Ag expression by MTB**
- Ag set #1: secreted Ag, ..
- Ag set #2: latency / starvation Ag
- Ag set #3: reactivation-Ag

**BCG priming**
- ++
- -
- ?

**New vaccines (prime/boost)**
- +
- +
- +
This first example demonstrates:

based on a fundamental understanding of host pathogen interactions in TB, it is possible to identify candidate vaccine antigens that can improve BCG

A second example, applicable to many bacterial infectious diseases.

Starting with a very simple hypothesis:

Mtbc antigens targeted by vaccination must be expressed *during infection* in the primary target organ, which is the *lungs* of *susceptible* individuals.

---


*J. Immunology* 190:1659-1671 (2013)
Super TB susceptible (sst1) mice

C57BL/6J (B6)
Resistant to TB

C3H-sst1 locus generates caseous necrosis in *Mtb* lung granulomas

A genome wide unbiased antigen discovery approach

1. Mtb RNA isolated directly from the lungs of infected mice
2. Four congenic mouse strains, ranging from hyper susceptible (sst1) to genetically resistant
3. qRT-PCR performed on all first genes of all predicted Mtb operons (n=2170)
4. Data sets -> analysis:
5. Selection of genes that were *persistently and highly expressed* (in multiple different genetic backgrounds)
6. Recombinant antigen / peptide production and testing in human T cell assays
Classification of newly identified IVE TB antigens

2170 Mtb genes

selected genes

19 genes selected

Previously identified (in vitro)

* Dos Regulon genes \( n = 2 \)

* Enduring hypoxic response (EHR) \( n = 6 \)

/ Starvation

Newly identified \( n = 11 \)
Immunogenicity of IVE-TB antigens (human)

IFN-γ responses to TB disease associated antigens in mantoux positive individuals

Exposed to TB index case → positive mantoux

Follow up 2 years (0, 3, 6, 12 and 24 months)

Whole blood diluted 1:10 incubated with recombinant IVE-TB proteins for 6 days
Immunogenicity of IVE-TB antigens (human)

E/C positive, Mtb positive donors

E/C negative, Mtb positive donors

Mtb negative donors

WBA assay
Vaccine evaluation in guinea pigs
Ag85B-esat6-Rv2034/Caf09 equivalence to BCG

Bacterial load

Commandeur et al, Vaccine 2015
Summary of *Mtb* T-cell antigen discovery in humans

1. **MtB Latency antigens**
   - *MtB* DosR regulon encoded antigens:
     - Leyten et al 2006; Lin et al 2007; Commandeur et al 2011a; Schuck et al 2009; Goletti et al 2009; Black et al, 2009; Arroyo et al, 2015; other papers

2. **MtB Rpf antigens**
   - Commandeur et al 2011b; Schuck et al 2009; ...

3. **MtB secreted antigens**
   - *MtB* esx antigens (SSI group’s papers)

4. **MtB HLA-class Ia presented CD8 T-cell epitopes**
   - *MtB* genome wide algorithm-based inquiry for HLA class Ia ligands
     - Tang et al, J. Immunol. 2010; library approach Lewinsohns lab

5. **MtB HLA-class Ib presented CD8 T-cell epitopes**
   - First identified *MtB* specific HLA-E presented peptides for human CD8 T-cells
   - Unorthodox specificity, phenotype, function, disease correlation

6. **MtB HLA-class II presented CD4 T-cell epitopes**
   - *MtB* genome wide algorithm-based inquiry for HLA class II ligands

7. **IVE-TB antigens expressed during *in vivo* lung infection**
   - *MtB* genes highly expressed during in vivo infection
   - Strong protective efficacy in mice and guinea pigs
     - S Commandeur et al, J Immunol 2013; PLoS ONE 2014; Vaccine 2015
Summary of Mtb T-cell antigen discovery in humans

1. Mtb Latency antigens
   - Mtb dosR Ag are potently recognized by human T-cells across different TB exposed populations
     - Multiple epitopes, presented by diverse HLA class I and II molecules
     - Mono- as well as multi-functional CD4 and CD8 T-cell responses
   - Recognition is invariably associated with latent infection in humans and mice
     - -> Vaccine antigens
     - -> Biomarkers of immune associated infection control
   - No response to latency antigens induced by BCG (humans, mice)
     - Partial explanation for BCG’s inefficiency? -> possibilities for repair by boosting
   - Leyten et al 2006; Lin et al 2007; Commandeur et al 2011a; Schuck et al 2009; Goletti et al 2009; Black et al, 2009; Coppola et al, CVI 2015
Summary of *Mtb* T-cell antigen discovery in humans

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Unconventional immunity – donor unrestricted T-cells

αβ T-cells

DI Godfrey, AP Uldrich, J McCluskey, J Rossjohn & DB Moody
Nature Immunology 16, 1114–1123 (2015) doi:10.1038/ni.3298
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7. IVE-TB antigens expressed during *in vivo* lung infection
   - Analysis of in vivo *Mtb* gene expression patterns uncovers new *Mtb* antigens with protective potential
     - S Commandeur et al, J Immunol 2013; PLoS ONE 2014; Vaccine 2015

---

1. Mtb Latency antigens
2. Mtb Rpf antigens
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Multi-phase, multi-component TB vaccines

Disease incidence

age

infection / early disease

reactivation / late disease
Multi-phase, multi-component TB vaccines

Disease incidence over age

- Prevent infection / early disease
- Reactivation / late disease

BCG
- Secreted antigen1
- Secreted antigen2
Prevent reactivation / late disease. Clearance possible?

Multi-phase, multi-component TB vaccines
Conclusions

There are no effective vaccines against TB yet

The biology of TB infection is complex and involves extensive immune evasion and inflammation, both compromising host immunity

New antigen discovery approaches are yielding novel classes of Mtb antigens, with promising vaccine potential. This is much needed after the MVA85A failure

Latency as well as IVE-TB antigens are promising. Can induce protection in humanized mice and guinea pigs

IVE-TB approaches would have predicted MVA85A vaccine failure

IVE antigen discovery approaches can be applied to other infectious diseases

HLA-E restricted CD8+ T cells can inhibit Mtb in macrophages, despite being Th2 like and helping B cells through IL4
Collaborators

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Susanna Commandeur
Mariateresa Coppola
Susan van den Eeden
Kees Franken
Annemieke Geluk
Simone Joosten
Marielle Haks
Krista van Meijgaarden
Corine Prins
Kimberley Walburg
Louis Wilson

Stanford University, Palo Alto
Greg Dolganov
Gary Schoolnik

Oslo
Fredrik Oftung

Harvard School Public Health
Igor Kramnik

PHE, UK
Simon Clark
Ann Rawkins