Increased efficacy of chemotherapy against *Mycobacterium tuberculosis* by additive immunotherapy using a multistage MVA vaccine
Vaccine Candidates for Tuberculosis

1: Pediatric vaccine (prophylactic)

2: Adult vaccines (prophylactic and post-infection)

3: Immunotherapy (DR-TB)
- Combination with antibiotics to shorten treatment/improve lung pathology and/or increase success rate
- Vaccination post-treatment to prevent rebound or re-infection

INFECTION PHASES AND DISEASE OCCURRENCE

Acute infection

Latent infection

Acute disease (5%)

Reactivation of infection (5–10%)

Latency

Infectious load
Vaccine Platform

MVA - a safe and strong T cell-based inducer

Vector:
- Recombinant non-replicative Poxvirus
- Modified Vaccinia Ankara virus strain (MVA)
  (safety largely established eg vaccination campaign against smallpox)

MVA-TB

Antigens:
- Multiple TB antigens (active, latency, resuscitation)

High plasticity of the MVA has allowed to generate highly complexed candidates
- MVA-TB candidates express multiple Ag covering the three phases of the disease
The MVATG18598 lead candidate
Antigenic design and *in vitro* detection of antigen expression

<table>
<thead>
<tr>
<th>Phases</th>
<th>Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>Ag85B, CFP10 / ESAT6, TB10.4 / Rv0288</td>
</tr>
<tr>
<td>Latency</td>
<td>Rv2626, Rv3407, Rv1813</td>
</tr>
<tr>
<td>Resuscitation</td>
<td>RpfB, RpfD</td>
</tr>
</tbody>
</table>

MVATG18598 expresses all 10 Ag spanning the 3 phases of the disease and is genetically stable, *i.e.* fit for manufacturing.

**Notes:**
- MVATG18598 lead candidate
- Antigenic design and *in vitro* detection of antigen expression
- MVATG18598 expresses all 10 Ag spanning the 3 phases of the disease and is genetically stable, *i.e.* fit for manufacturing
- Chicken Embryo Fibroblasts
- Western blot
- **Legend:**
  - Rv2626
  - Ag85B
  - CFP10/ESAT6
  - TB10.4/Rv0287
  - RpfB-RpfD
  - Rv3407/Rv1813
  - Mock
  - MVATGN33.1
  - MVATG18598

**Manuscript in revision**
Immunogenicity of MVATG18598 in naïve mice

Example of IFNγ response in BALB/c mice

MVATG18598
10^7 pfu, s.c.

BALB/c mouse

IFNγ ELISpot assay

MVATG18598 induces IFNγ-producing T cells specific of 6/10 antigens belonging to all phases of the disease in naïve BALB/c mice
MVATG18598 induces cytotoxic activity specific of antigens belonging to all phases of the disease in mice (total 8: 10 Ags)
Efficacy testing of MVATG18598: The TB post-exposure mouse model

- Well-validated murine TB post-exposure models to evaluate the efficacy of promising new antimicrobials and immunotherapeutic products.
  
  *The model developed by Dr Nuermberger’s lab (Johns Hopkins University, Baltimore) has been shown to be predictive of clinical efficacy for novel antibiotics (Williams et al., 2012; Tasneen et al., 2015)*

- **Endpoints:** prevention of reactivation and/or decrease of Mtb infection

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**Objective is to test capacity of MVATG18598, when combined with an existing suboptimal drug regimen, to prevent and/or control *Mycobacterium tuberculosis* regrowth**
Vaccination schedule study

Investigating positioning and route of immunization (Dr Nuermberger, JHU) (Williams et al., 2012; Tasneen et al., 2015; Nuermberger E, 2017)

1. Exploration of vaccine positioning
   • Combination with or post-antibiotic treatment

2. Exploration of route of immunization
   • Comparison between SC and IN routes

→ Vaccine: 10 Ag MVATG18598 in a 3x injections schedule

- H37Rv challenge
  2.5 log_{10} cfu

- BALB/c mice

- RHZ

- Bacterial load

- Immunology

- Weeks

- MVATG18598 or MVATGN33.1
  (SC or IN)

- MVA: 10^7 pfu/injection
- RHZ: 5 days/week, oral gavage
- R: Rifampicin (10 mg/kg/day), H: Isoniazid (10 mg/kg/day), Z: Pyrazinamide (150 mg/kg/day)
Vaccination schedule study

Bacterial loads in lungs

Significant reduction of bacterial load in group of mice vaccinated SC when the MVATG18598 vaccination is combined with chemotherapy.
## Vaccination schedule study
### Prevention of relapse

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vaccination Route</th>
<th>Positioning</th>
<th>Reactivation</th>
<th>Bacterial load (CFU (log_{10}) (mean ± SEM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHZ</td>
<td>-</td>
<td>-</td>
<td>15 / 15</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>RHZ + MVATGN33.1</td>
<td>SC</td>
<td>During</td>
<td>10 / 15</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>RHZ + MVATG18598</td>
<td>SC</td>
<td>During</td>
<td>9 / 15</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>RHZ + MVATGN33.1</td>
<td>IN</td>
<td>After</td>
<td>13 / 15</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>RHZ + MVATG18598</td>
<td>IN</td>
<td>After</td>
<td>10 / 14^a</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

R, Rifampicin; H, Isoniazid; Z, Pyrazinamide.
SC, Subcutaneous; IN, Intranasal

^aOne mouse died during the experiment.

- **RHZ only**: Reactivation observed in 100% of mice
- Best effects seen when vaccination is combined with antibiotic treatment regardless of the route, the **SC** route slightly better
- Likely importance of **innate immunity** *i.e.* empty vector displays some effect
Antigen-specific immune response in mice at the relapse evaluation

Antibody response at Week 27

MVATG18598 induces a **significant sustained memory B cell response** specific of active and Rv2626 antigens regardless of route and positioning of the vaccine. No correlation with lung bacterial burden demonstrated at **Week 27**.
Efficacy of MVATG18598 in a post-exposure mouse model
Investigating number of injections - Dr Nuermberger’s lab/TBA

**Objective**: Evaluate accelerated schedules of administration of the MVATG18598 and their impact on Reactivation and/or Bacterial load.

*Transgene has used multiple, closely spaced (1 week) injections of MVA-therapeutic vaccines in oncology/HPV/HCV with positive results (Quoix et al., 2011, Di Bisceglie et al., 2014).*

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**Protocol 2**

**Number of injections**

- **H37Rv challenge**: BALB/c mice at 0 weeks
- **RHZ**: Weeks 4, 10, 13, 15
- **MVATG18598**: (10^7 pfu/injection, sc) at Weeks 7, 10, 13, 15
- **Bacterial load Immunology**: Weeks 27
Comparative study of the number of MVATG18598 injections

Results

- **RHZ only**: Rebound observed in 83% of mice
- Increasing number of injections does not increase efficacy observed
- **3x injections schedule 3 weeks apart** results in best effect both on the “Reactivation” and “Bacterial load” read-outs (confirmation of previous protocol)

### Table: Treatment vs. Efficacy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nb inj.</th>
<th>Reactivation</th>
<th>Bacterial load</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>10 / 12</td>
<td>83</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>RHZ + MVATG18598</td>
<td>6 / 12</td>
<td>50</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>RHZ + MVATG18598</td>
<td>8 / 12</td>
<td>67</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>RHZ + MVATG18598</td>
<td>7 / 12</td>
<td>58</td>
<td>1.9 ± 0.5</td>
</tr>
</tbody>
</table>

R, Rifampicin; H, Isoniazid; Z, Pyrazinamide.
IFN\(\gamma\) response at the end of the study

IFN\(\gamma\) production by splenocytes stimulated with antigen peptides at the end of the study

- MVATG18598 vaccine induces detectable Ag-specific IFN\(\gamma\) production at **Week 27** in contrast to antibiotic-treated only mice
- Highest production in response to stimulation with TB10.4 peptides
- No correlation with lung bacterial burden demonstrated

Mann-Whitney test:
* \(p<0.05\); ** \(p<0.01\)
§ \(p<0.05\); §§ \(p<0.01\) as comp. with RHZ
Antigen-specific immune response in mice at the relapse evaluation

Antibody response at Week 27

- Confirmation of results from 1st protocol: same antigens induce significant antibody response following 3x injections of MVATG18598 that is not detected in RHZ treated mice.
  - 6/10 Ags principally targeted
- No correlation with lung bacterial burden demonstrated at Week 27

Mann-Whitney test:

§, p<0.05; §§, p<0.01 comp. ‘RHZ’
*, p<0.05; **, p<0.01
Evaluation of MVATG18598 in a C57BL/6 mouse-based post-exposure model of TB infection (Cardona et al., 2005; Aagaard et al., 2011)
TBVAC2020 consortium

MVA vaccines injected
- **MVATG18598** (10 Ag): Resulted in significant reduction of bacterial load in lungs (Protocol 1) and some decrease (trends) on % of relapsers (Protocols 1 and 2)
- **MVATGN33.1** as empty MVA negative control

Two independent runs launched
- 12 mice/group per run
- Data from both runs pooled to reach statistical relevance (technical pbs encountered)

**H37Rv**: Pasteur strain, aerosol, $10^2 \text{ cfu}$
**MVA**: $10^7 \text{ pfu/injection}, \text{ SC}$
**P**: *Rifapentine* (10 mg/kg/day), **H**: *Isoniazid* (25 mg/kg/day), **oral gavage**
Evaluation of MVATG18598 in a C57BL/6 mouse-based post-exposure model of TB infection (TBVAC2020 Consortium)

Bacterial loads in lungs

**Treatment**  | **Reactivation** | **Bacterial load**
---|---|---
| **Number** | **%** | **CFU (log_{10}) (mean ± SEM)**
---|---|---
PH | 16 / 16 | 100 | 4.3 ± 0.1
PH + MVATGN33.1 | 7 / 7 | 100 | 3.9 ± 0.3
PH + MVATG18598 | 10 / 10 | 100 | 3.6 ± 0.3

P, Rifapentine; H, Isoniazid.

- MVATG18598 controlled significantly bacterial regrowth but in this model does not impact Relapse
- Empty MVATGN33.1 (negative control) did not display efficacy

Mann-Whitney test:
*, p<0.05 compared with PH group

Data not available for all mice. Number of exploitable samples per group are indicated.
IFN$\gamma$ response at the end of the study
Cumulative results of IFN$\gamma$ ELISpot assay from both runs

- MVATG18598 vaccine induces detectable responses at Week 23 in contrast to antibiotic-treated only mice or MVATGN33.1-vaccinated mice
- This observation is in alignment with that observed in Protocol 2
- No correlation with lung bacterial burden demonstrated at Week23

Mann-Whitney test:
- * p<0.05
- ** p<0.01
- *** p<0.001
Antigen-specific immune response in mice at the relapse valuation

Antibody response at Week 23

- Antibody response was significantly induced in MVATG18598-vaccinated mice while only sporadically detected in the antibiotic only-treated mice. 6/10 principally targeted antigens (similar to BALB/c mice)
- Antibody response was triggered against the same Ag as in BALB/c mice studies
- No correlation with lung bacterial burden demonstrated at Week 23

Mann-Whitney test: §§, p<0.01; §§§, p<0.001 comp. ‘RHZ’
***, p<0.001
**Global analysis**

MVATG18598 displays significant activity on prevention of reactivation and lung bacterial load

Compilation of mouse protocols with MVATG18598 injected 3x during antibiotherapy through SC route

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>Reactivation</th>
<th>Bacterial load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactivation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$p$-value</td>
<td>Odds Ratio [95% CI]</td>
</tr>
<tr>
<td>‘ATB’ vs. ‘ATB+MVATGN33.1’</td>
<td>0.051</td>
<td>9.2 [1.3;65.4]</td>
</tr>
<tr>
<td>‘ATB’ vs. ‘ATB+MVATG18598’</td>
<td>0.007</td>
<td>9.9 [1.9;50.2]</td>
</tr>
<tr>
<td>‘ATB+MVATGN33.1’ vs. ‘ATB+MVATG18598’</td>
<td>0.167</td>
<td>1.1 [0.3;4.6]</td>
</tr>
</tbody>
</table>

Stratified logistic regression test

Generalized Linear Model (GLM) test

**Significant impact of MVATG18598 on percentage of Reactivation**

- Results are unique in the field in murine models: -30/40% prevention of relapse
- Empty MVA may have inherent efficacy effect ($p$-value=0.051) through activation of innate response. Balance innate/adaptive immunity to be clarified (mechanism of action)

**MVATG18598 immunization results in significant reduction of CFU load in lungs at the relapse evaluation timepoint**

- Exact role of adaptive immunity (B and/or T) and specific role of each of the vaccine-encoded Ags need to be clarified, clearly adaptive immunity is not observed in the ATB-only treated group in our experimental conditions
Hypothesis: Dual effect of MVATG18598 on controlling Mtb growth

MVATG18598

- MVA backbone
  - Innate immune response
    - % relapse
- Antigens
  - Adaptive immune response
    - % of Relapse and CFU load

Mycobacterium tuberculosis growth
Global conclusion and Next steps

• Transgene has developed a unique multi-antigenic therapeutic MVA-based vaccine
• The lead MVATG18598 is genetically stable and fit for manufacturing
• The lead MVATG18598 is immunogenic
  • Production of IFNγ
  • Cytotoxic activity
  • Polyfunctional T cells (not shown)
  • Production of antibodies (not shown)
• Preclinical Proof-Of-Concept achieved with the lead MVATG18598
  • Impact on bacterial load post-relapse (CFU in lungs)
  • Prevention of reactivation of the disease (percentage of relapsers)
• Manuscript in revision
• Clinical development plan
  • Exchange with KOLs: MVATG18598 should be moved to the clinic to reach PoA/PoC
  • Clinical development plan in progress
  • Transgene is seeking partnership (out-licensing, co-development, etc.)
Acknowledgements

Lyon, France
• Geneviève Inchauspé
• Stéphane Leung-Theung-Long
• Marie Gouanvic
• Charles-Antoine Coupet
• Aurélie Ray
• Valentina Ivanova-Segura

Illkirch, France
• Martine Marigliano
• Jean-Baptiste Marchand
• Nathalie Silvestre
• Doris Schmitt
• Chantal Hoffmann
• Huguette Schultz
• Murielle Klein
• Sophie Steinbach
• Patricia Kleinpeter
• Benoît Sansas
• Dominique Villeval
• Julie Heymann
• Sophie Jallat
• Annick Hoh

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NIH support through grant awarded to Emergent BioSolutions/Transgene subcontractor

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Heena Soni

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Jelle Thole
Béatrice De Vos
Danielle Roordink

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Benoît Sansas
Dominique Villeval
Julie Heymann
Sophie Jallat
Annick Hoh