TB Human Challenge Consortium

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Including work and slides from:

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Why do we need a TB Human Challenge model?

- Urgent need for better TB vaccine
- No validated immunological correlate
- Common TB animal models do not adequately represent human TB, for vaccine testing purposes
  - Mouse, Guinea pig, Rabbit, Bovine, NHP
- Capacity to perform and fund efficacy trials is limited

- A TB human challenge model would facilitate vaccine development
  - Optimisation of dose, route of vaccination etc.
  - Selection of which candidates progress to efficacy trials
  - Potential to identify immune correlates using challenge model
Other Human Challenge models

- **Controlled Human Malaria Infection (CHMI)**
  - Test efficacy of vaccines and drugs against *Plasmodium falciparum*
    - Sporozoite challenge (bites of infectious mosquitoes)
    - Inoculum of blood-stage parasites
    - Efficient drug treatment at end of study

- Gonorrhea
- Cholera
- Respiratory syncytial virus
- Influenza
- Zika
Controlled Human *Mycobacteria* Infection?

- Issue of using *M. tuberculosis*
  - Safety
    - BCG, attenuated *Mtb*
  - Route of delivery?
    - Skin, pulmonary
  - What output measurement?
    - Simple, accessible detection: reporters?
Controlled Human *Mycobacteria* Infection?

- Issue of using *M. tuberculosis*
  - Safety
    - **BCG**, attenuated *Mtb*
  - Route of delivery?
    - **Skin**, Pulmonary
  - What output measurement?
    - Simple, accessible detection: **reporters**?
A TB Human Challenge Skin model

Human intradermal challenge model

Intradermal injection of mycobacterial reporter in vaccinees

Measure signal loss over time, through skin

Assess vaccine efficacy

- Evaluate fluorescence and bioluminescence reporters for use in an intradermal challenge model for TB vaccine evaluation
  - Detect reporters expressed in BCG in pig skin and mice
Choice of reporters?

- **Bioluminescence**: requires metabolically active organisms
  - decay in bioluminescence indicates vaccine-induced killing
  - Alternative: unstable fluorescent reporters.

- **Fluorescence**: does not require live organisms
  - fluorescent proteins stable
  - provides long-term indicator of bacterial presence
Property of some molecules to absorb light at one wavelength and emit light of longer wavelength

- **GFP** (395/509nm): extensively used in Mycobacteria
  - Yellow Fluorescent Protein (525/538nm)
- **Far-Red shifted proteins**: Turbo-635 (588/635nm)
  - ‘Katushka’ from Sea anemone *Entacmaea quadricolor*
  - Fast maturation and a high pH-stability and photostability
- Unstable reporters improved measure of cell death
  - Dual stable-unstable format
**GFPasv-Turbo635** *M. bovis* BCG in pig skin

**Turbo635 fluorescence**

**Time 0 h**

**Time 24 h**
GFPasv - Turbo635 *M. bovis* BCG in pig skin

**GFPasv fluorescence**

**Time 0 h**

**Time 24 h**
BCG-Turbo635: stability and detection \textit{in vivo}

- C57BL/6 mice injected ID with $10^6$, $10^4$, $10^3$ bacteria
  - Imaged daily for 7 days
  - Fluorescent signal detected $10^6$
    - Persisted for 5 days

IVIS® Spectrum-CT
A TB human challenge model

Human intradermal challenge model

Intradermal injection of BCG dual reporter in vaccinees

Measure signal loss over time, through skin

Assess vaccine efficacy

IVIS® Spectrum-CT
Prototype imaging device

Human Challenge Imaging System
(Aeras, Dr. T. Baer)
Photonics, Stanford University

- Nikon D-SLR
- Solid state lasers
  - Single or Dual detection
- Wi-Fi or cable for data transfer
Next steps:

- Turbo635 + YFP operons
- Stable/unstable combinations
  - Testing of LOD and stability
  - Minipig vaccination model
- **BCG limitations as reporter strain...**
Controlled Human *Mycobacteria* Infection?

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    - BCG, *attenuated Mtb*
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  - What output measurement?
    - Simple, accessible detection: *reporters*?
Yes..

- BCG
- Auxotrophs
- Regulatory mutants

But

... the safest strains fail to grow in animal models and humans

Sabanadamurthy I&I 73:1196 (2005)
The problem

How do you measure vaccine efficacy if your challenge strain doesn’t grow?

Timed kill switch

- Regulated Expression of a toxin
  - Phage lysins, Rnases etc
- OFF \textit{in vitro} (Tet-dependent)
- ON during infection (Tet absent)

+ Tetracycline
Toxin OFF \textit{(in vitro)}

–Tetracycline
Toxin ON \textit{(in vivo)}

Eric Rubin
Failsafe mechanism

Slow starvation

- Non-natural amino acids not found in the host
  - This could allow us to create “auxotrophs” in any essential gene
  - Growth \textit{in vitro} with NN-AA
  - Growth fails \textit{in vivo} without NN-AA

\begin{verbatim}
AUG UUU GCU UGG UGG UAG AUG CUG GUG GGG GAG UAA
\end{verbatim}
**Slow starvation**

**Alanine racemase**

- No additive
- + Non-natural AA

**Biotin synthetase**

- No additive
- + Non-natural AA
Pulmonary detection

Engineer a challenge strain to produce a reporter molecule that can be sensitively and quantitatively detected

**Volatile molecules?**
- Grape – methyl anthranilate
- Banana – isoamyl acetate
- **Wintergreen** – methyl salicylate
- Cinnamon – cinnamaldehyde
- Lemon – limonene
- Garlic – sulphur compounds
**Mycobacterium smegmatis**

Chorismate $\rightarrow$ Isochorismate $\rightarrow$ Salicylic Acid

Detection limit $\sim 10^4$ bacteria

Eric Rubin
TB041: A clinical challenge trial to evaluate controlled human infection with BCG administered by the aerosol inhaled route compared with the intradermal route in healthy, BCG-naïve, UK adult volunteers

- **Dose escalation study:**
  - $10^3$, $10^4$, $10^5$ BCG SSI* or Saline inhalation

- **Outcome measures**
  - Safety
    - Day 14 bronchoscopy
      - No macroscopic abnormalities to date
      - No SAEs
  - BCG recovery from BALF
  - Immunogenicity

*No longer available.
Revised trial design with BCG Bulgaria (InterVax) including $10^4$, $10^5$, $10^6$
BALF PPD responses

Aerosol response at least as good as Intradermal

Helen McShane, University of Oxford
Summary – TB Human Challenge Model

• An **attenuated** *Mtb* reporter challenge strain to test vaccines
  • Timed expression of toxin as kill switch (ER)
  • Non-natural amino acids to make synthetic auxotroph (ER)

• An attenuated *Mtb* **reporter** strain
  • Fluorescent for non-invasive detection in the **skin** (BR)
  • Expressing volatile compounds for detection in **expired breath** from the **lungs** (ER)

• Pulmonary administration trial in progress
  • Safety and immunobiology of aerosolised BCG (HMcS)
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Some questions

- How safe does a strain need to be?
- How long does a strain have to live?
- How sensitive does a reporter need to be?
- What kind of vaccines would this work for?
  - Can we detect a vaccine effect?
- Would this be acceptable to the regulators?