Monitoring tuberculosis progression using MRI and stereology
TB – the problem

- 2 million deaths; 9 million new cases p.a.
- TB kills someone every 15 secs,
- 9,153 cases in UK (2009),
- Latent infection (one third of world’s population)
- Co-infection with HIV, drug resistance (MDR, XDR)
Mycobacterium tuberculosis - antibiotic therapy

Drug sensitive strains:

6+ months therapy:
• 2 - 4 antibiotics
• £5k

Drug resistant strains:

18+ months therapy:
• 5+ antibiotics
• £50-70k
• UK = £70 million pa

Issues:
- non-compliance
- side-effects

Increasing numbers of MDR / XDR strains
Mycobacterium tuberculosis - vaccination

BCG vaccine

- *M. bovis* [bacille Calmette Guerin]: live, attenuated
- inexpensive, safe, 3 billion doses
- effective in children - not adults
- variable efficacy globally [0 - 80%]
- live vaccine – not recommended for babies with HIV
- does not prevent infection [latency]
Background

The global needs:

• **Therapy**
  - new drug targets
  - shorter treatment times

• **Vaccines**
  - improve BCG / boost BCG (sub-unit vaccine)
  - **NO CURRENT CORRELATE OF PROTECTION**

• **In vivo Models**
  - Well characterised
  - Predictive
  - Clear readouts
Animal models of *M. tuberculosis* infection
Rational approach to model development and use

- Three species predominantly used for efficacy studies
- Vaccines move through progressively more complex models prior to clinical trial

- Immunogenicity, mechanisms of action
- Protection against *Mtb* challenge
- Detailed and relevant immunogenicity
- Safety
- Protection against *Mtb* challenge
Macaque models for TB vaccine evaluation

- 2 species are commonly used to model human tuberculosis

Rhesus macaque

*Macaca mulatta mulatta*: Indian and Chinese subspecies

Cynomolgus macaque

*Macaca fascicularis*: Native: e.g. S.E. Asia, Philippines. Introduced alien species: e.g. Mauritius.

History
- 1968 – 1975: Rhesus macaque; aerosol / intra-bronchial challenge; BCG effective
- 1996 – to date: Cynomolgus macaque: intra-tracheal challenge

- Lack of clear criteria for species selection for the evaluation of therapeutic interventions against tuberculosis.
- Need for further studies to:
  - Characterise the species and the model for different uses.
  - Define and improve study endpoints and readouts.
Aims

• To establish aerosol challenge models of TB infection in NHP

• To characterise and compare rhesus and cynomolgus macaque species as models of human tuberculosis for vaccine assessment.
  - Susceptibility to infection with *M. tuberculosis*
  - Similarity to humans

• To evaluate readouts of vaccine immunogenicity and efficacy
  – including the evaluation of advanced imaging approaches to enhance measurement of disease burden
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Aerosol Challenge Model
Clinical parameters

Humane endpoint indicators

- **Behavioural indicators**
  - Depressed or withdrawn behaviour,
  - Abnormal respiratory rates (dyspnoea).

- **Weight**
  - Loss of 20% of peak post-challenge weight.

- **Temperature**
  - Increased temperature

- **Measures of inflammation**
  - Erythrocyte Sedimentation Rate
    - ESR levels > normal

- **Chest X-ray**
  - Severely abnormal chest X-ray

- **Haematology:**
  - Full blood count (CBC)
  - Hb Haemoglobin level < normal limits
Aerosol Challenge Model Assessment of the Immune response

- Identification of correlates of protection, disease pathogenesis
  - Time course studies

- Immune responses investigated:
  - Cytokine
  - IFN-γ – model calibrator; ELISPOT, ELISA, ICS
  - Cellular responses: e.g. Polyfunctional T cells, proliferation
  - Mucosal responses: BAL, lung
  - Humoral responses
  - Omics’

- Aligned with human clinical trials

ELISPOT: Frequency IFN-γ secreting cells

ELISA: Quantity of IFN-γ secreted
Aerosol Challenge Model
Assessment of disease at necropsy

Pathology / Histology
• Scoring system for TB NHP disease pathology (qualitative)
• Pulmonary disease
  - Pathology score
  - Manual lesion count
• H&E for evaluation of lesion pathology

Bacteriology
• Organ-specific bacterial load

Evaluation of imaging for determination of pulmonary disease
• Visualisation of disease
• Quantification of disease
  (lesion counting; lesion : lung volume by stereology)
Aerosol Challenge Model development

VACCINE EVALUATION

- AEROSOL CHALLENGE DOSE ESTABLISHED
- READOUTS OF DISEASE BURDEN ESTABLISHED
- IMMUNE ASSAYS ESTABLISHED
  - ALIGNED HUMAN CLINICAL TRIALS
- HUMANE ENDPOINT CRITERIA ESTABLISHED
  - X-RAY CAPABILITY
  - HOUSING SYSTEM
    - CL3 CONTAINMENT
- AEROSOL DELIVERY SYSTEM: NHP
  - PLETHYSMOGRAPHY
Vaccine evaluation studies require readouts capable of demonstrating that vaccine candidates are able to protect against challenge with tuberculosis.

The ideal readout would be

- Discriminatory
- Sensitive
- Objective
- Quantitative
- Transferable
Readouts of disease burden for evaluation of TB vaccine efficacy

• **Current readouts**
  - **Survival:** Long term studies required, larger numbers.
  - **Clinical assessment: X-ray:** Qualitative, not easily transferable.
  - **Gross pathology:** Qualitative, not easily transferable.
  - **Bacterial load in tissues:** Technically challenging. Test groups with survivors and non survivors show a large variation in group bacterial load.
  - **Immune responses:** No correlate of protection.
**Improved readouts - Advanced Medical Imaging**

- **Imaging systems**
  - **Computed tomography (CT)** uses X-rays to construct good high resolution 3D images of tissue.
    - Shorter scan time, less detailed images, bone imaging possible
  - **Magnetic resonance imaging (MRI)** uses strong magnetic fields and non-ionising radiation to generate high contrast 3D images
    - Longer scan time, high resolution images, multiple scan types, not calcification

- Imaging of TB infected lungs performed ex vivo
  - Lungs inflated with formalin, embedded in agarose
  - Contained samples transported to Oxford for scanning (MR, CT)

- Visualisation of disease burden
- Quantification of disease burden
  - MR stereology
  - Total lung and lesion volumes determined from MR digital image stacks
  - Lesion : lung volume ratio determined as measure of disease burden

- Improved readouts
Imaging based readouts of disease burden
Visualisation of pulmonary disease: Pathology

Rhesus macaques

Dose *M. tuberculosis*
- High: 75 CFU
- Mid: 45 CFU
- Low: 30 CFU

- Pulmonary disease induced following aerosol exposure.
- Increased level of disease burden with increased exposure dose of *M. tuberculosis*.
Imaging based readouts of disease burden
Visualisation of pulmonary disease: MR Images

Rhesus macaques

Dose *M. tuberculosis*

- 75 CFU
- 40 CFU
- 30 CFU

• Even distribution of disease following aerosol exposure.
• Clearly shows the increased level of disease burden with increased exposure dose of *M. tuberculosis*. 
Imaging based readouts of disease burden
Visualisation of pulmonary disease: MR Images

- Increased level of disease burden in **Rhesus** and **Mauritian cynomolgus** macaques compared to **Chinese Cynomolgus** macaque
Imaging based readouts of disease burden
Quantification of pulmonary disease
Lesion counts

Rhesus macaques

<table>
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<th>Dose M. tuberculosis</th>
<th>500</th>
<th>30</th>
<th>30 CFU</th>
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<tr>
<td>X-ray Score</td>
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- Lesions enumerated from
  - Cross sectional CT or MR images
  - Sliced lung sections

- Excellent correlation of CT, MR, with pathology counts.

- Counting provides an over all view of disease burden

- Limitations
  - at high levels of disease
  - Lesion size not included
**Imaging based readouts of disease burden**

**Quantification of pulmonary disease**

Lesion volume : Lung volume [MR stereology]

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<th>Rhesus macaques</th>
<th>Dose M. tuberculosis</th>
<th>500</th>
<th>75</th>
<th>70</th>
<th>45</th>
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Lesion to lung volume ratio

Sharpe et al., 2009 - *Tuberculosis*

The lesion to lung ratio is an indicator of disease severity, the higher the ratio the greater the proportion of lung is taken up with lesions.
M. tuberculosis infection studies
Disease burden measures:

Quantitative measures
- Lesion: lung volume
- Lesion count

Qualitative measures
- Lung gross path score
- Total gross path score
- Chest X-ray score

Blue fill indicates animals which met humane endpoint before week 12. Lesion: lung volume ratio correlates with the other measures of pulmonary disease assessed.

Quantitative data was more sensitive than the Qualitative measures of pulmonary disease (gross pathology score, Chest X-ray score).
Lesion volume : Lung volume [MR stereology]

Vaccine efficacy readout

Sharpe et al., 2010
- Clinical & vaccine Immunology

A significant difference lesion volume to lung volume ratio was seen between the unvaccinated animals and animals vaccinated with BCG alone (p = 0.011)

Grey fills indicates animals which did not met humane endpoint before end of study (week 52)
‘In life’ imaging

Use of mobile scanner units

**MR:** ‘Abdomen ‘apex to base’

**CT:** ‘Head to base’
‘In life’ imaging at HPA Porton
CT imaging with software reconstruction

CT: voyage down the trachea

Bronchoscopy: Trachea
Summary

- Visualisation of pulmonary disease
  - MR imaging provided a clear indication of pulmonary disease burden in fixed tissues.

- Quantification of disease burden:
  Lesion counting
  - Provided an overall view of pulmonary disease burden, but didn’t take lesion size into account.
  - Agreement between lesion counts from CT or MR images with counts from pathological specimens validates the approach for use ‘in life’.

- MR stereology (lesion volume to lung volume ratio)
  - Provides a tool to quantify pulmonary disease induced by *M. tuberculosis* infection.
  - Superior to qualitative readouts of disease burden.
  - Provides a reliable measure against which to validate the Immunological correlates of vaccine-induced protective efficacy.
Future - Imaging

• Inclusion of imaging in future studies

• Application of stereology approach to CT image stacks

• ‘In life’ imaging
  • Development of a suitable containment strategy
  • Evaluation of MR and CT modalities
  • Evaluation of PET CT