April 10, 2015

The non-human primate model in TB vaccine development

Tom Evans MD
Aeras
Agenda

• Issues
• Transmission studies
• Recent advancements
  – Outcomes
  – Endpoint analysis
  – Design
• Going forward
Development of the macaque model

Ho et al. I&I 2015
Primate Selection Issues

• Species
  – Indian-origin Rhesus Macaques
  – Chinese-origin Rhesus Macaques
    • Highly susceptible
    • Unclear if there are origin differences
    • Develop fairly severe hilar adenopathy (reminiscent of childhood TB)
  – Cynomolgus Macaques
    • Latency in 50% at low dose by intrabrochial administrations, but all infected, and disease mimics the heterogeneity seen in humans

• Age
  – Usually use adult (not adolescent)

• Almost always male

• Confounders- breeding colony, diet, microbiome, NTM exposure, stress

• Monkeys in almost all studies have all been infected!
Infection, vaccination and challenge

- **Route of challenge**
  - Intratracheal instillation
  - Intrabronchial instillation
  - Aerosol

- **Dose (15 CFU to 500 CFU)**
  - Aeras uses 15 CFU in rhesus, TBVI uses 500 CFU
  - 3-5 CFU comparison of cynomolgus vs rhesus and aerosol vs. intrabronchial under analysis

- **Strain**
  - Erdman used not the same at each center and attempts to standardize have not succeeded

- **Timing of vaccine boosts and challenge**
  - Natural boosts are likely to be in adolescents
  - Animal study boost of BCG tend to be at 8-16 weeks (at the peak of BCG)
  - Multiple sets of data suggest that at least one year is needed to boost BCG in order not to see “interference” with a vaccine effect
  - Group of monkeys immunized with BCG at birth, which will be challenged in mid-2016 to partially answer these issues
Inoculum - move to bar coded strains

Can measure each challenge separately and every strain in every challenge

Swarm model
- May be sufficient to reliably inoculate ~15 individual bacteria in unvaccinated animals (as established by barcode counting)
- Outputs at 4 weeks post infection:
  - number of bacteria that establish infection
  - progression of infection – max bacterial burden, killing (CFU/CEQ)
  - quantitative effect on dissemination (how many bacteria disseminate)

Repeated low dose infection model
- Goal would be to achieve limiting dose with barcoded Mtb (such that animals get infected ~half the time, presumably with one bacterium though this would be determined by barcode counting)
- Outputs at 4 weeks post infection:
  - How many doses were required to achieve infection? (determined at time of necropsy based on barcode tracking)
  - Progression of infection – max bacterial burden of doses that took and killing (at least of first dose that established infection)
  - Dissemination – only able to track barcodes that established infection
## Why choose one over the other?

<table>
<thead>
<tr>
<th></th>
<th>Low dose swarm</th>
<th>Low dose repeat</th>
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<tbody>
<tr>
<td><strong>PROS</strong></td>
<td>- Easier</td>
<td>- Thought to more accurately mimic human infection</td>
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<td>- Likely to require smaller group sizes than LDRC</td>
<td>- Provides more definitive evidence of protection against infection</td>
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<td>- More quantitative resolution around events post-infection if more bacteria establish infection</td>
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<td><strong>CONS</strong></td>
<td>- People are likely to be exposed to lower multiplicity of infection (MOI)</td>
<td>- More complex to track vaccine effects on disease progression given differences in dose timing</td>
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<td>- The low MOI in human infection may be sensed and responded to differently than a higher MOI infection</td>
<td>- May have somewhat less quantitative dynamic range for tracking vaccine effects on dissemination</td>
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Can we achieve natural transmission?
## Experimental Design

<table>
<thead>
<tr>
<th>Animal #</th>
<th>ID</th>
<th>Sex</th>
<th>Infection</th>
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<tbody>
<tr>
<td>WNP01 (35WK, P.I)</td>
<td>0602031 18</td>
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<td>Artificial</td>
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<tr>
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<tr>
<td>WNP03 (~18WK, P.E)</td>
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<td>WNP06 (~18WK, P.E)</td>
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<td>Natural</td>
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</table>
WNP04 (~ 18 week post-exposure)
Natural Transmission at Wuhan - Immunologic readout of infection by ELISPOT

PPD-induced IFN-γ (Elispot)

Exposure period

<table>
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<tr>
<th>Animal ID</th>
<th>Week 8</th>
<th>Week 24</th>
<th>Week 27</th>
<th>Week 42</th>
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<tbody>
<tr>
<td>WNP1</td>
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<td></td>
<td></td>
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<td>WNP2</td>
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<td>WNP3</td>
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<td>WNP5</td>
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<tr>
<td>WNP6</td>
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Courtesy of Wenzhe Ho, Wuhan
Low Dose Cynomolgous Macaque Model of TB Infection
JoAnne Flynn, Philana Lin, U. of Pittsburgh

+ 25 CFU M. tb (Erdman) via bronchoscope

\[ \rightarrow 100\% \]

2-6 weeks

Cynomolgus macaque

\[ \rightarrow 6-8 \text{ months} \]

ACTIVE TB

- Positive Chest x-ray
- Mycobacterial culture
  - repeated + GA or BAL culture
- Clinical signs
  - weight loss
  - appetite loss
  - cough

LATENT TB

- No signs of disease
- CXR negative between 2-6 months
- Mycobacterial culture negative after 2 months
- Clinical signs--none

Capuano, et al IAI 2003
Lin, et al IAI 2009
PET/CT imaging for serial tracking of disease

Visualization of very small lesions
Animals scanned weekly after TB challenge
Serial images reveal disease progression over time

Micro-PET/CT in BSL3 imaging suite at U. Pitt.

Tuberculosis granulomas (~1 mm)
Cyno Low Dose Challenge: Unvaccinated Controls

2051 2
4 Wks PI

2071 2
4 Wks PI

8 Wks PI

21 wks PI: Pre necropsy
Cyno BCG + H56 vaccinated animals

4 Wks PI

8 Wks PI

21 wks PI: Pre necropsy
CMV Vaccine Induces Reduction in Dissemination in both Pulmonary and Extra-Pulmonary Tissue in Rhesus Macaques

Wilcoxon Rank-Sum Test

RhCMV: RhCMV68-1
Study Endpoints- not addressed at all

No studies have had predefined Go-No go end points and appropriate powering analysis or even reliable positive controls! (BCG is variable)

• Survival vs. Fixed
  – Survival not feasible for cost reasons for most studies
  – 16 week observation likely sufficient for rhesus

• CFU
  – Stereology, granuloma specific?
  – Extra-pulmonary: HLNs, others?

• Serum chemistries
  – ESR, CRP, hemoglobin

• Gross Pathology and “scoring systems”
Categories of Protection

- **Lung Protection**
  - **Total PET Hot** (4, 8, 12 weeks, and pre-necropsy)
  - Granuloma Increase from 4 to 12 weeks
  - Percent Sterile Granulomas
  - CFU per granuloma (median)

- **Lymph Node Protection**
  - Lymph Node Count Lymph (4, 8, 12 weeks, and pre-necropsy)
  - Percent Sterile Lymph Nodes
  - CFU per lymph node (median)

- **Extra-Pulmonary Protection**
  - Extra-Pulmonary Score at Necropsy

- **Overall Protection**
  - CFU Score
  - Necropsy Score
What does the future hold: in two years

• Repeat positive control (CMV) in rhesus macaques
• Data on use of low dose swarm versus low does repeat challenge using bar coded libraries, and ability to track dissemination
• Data on how long to wait after BCG priming to boost in order to mimic
• Further data on protection afforded by aerosolized vaccines, and intensive work on lung immunology
• Better assessment of the primate humoral response to TB vaccine candidates