

Use of Nonhuman Primates in TB vaccine research

Shelby O'Connor, Ph.D.

Department of Pathology and Laboratory Medicine

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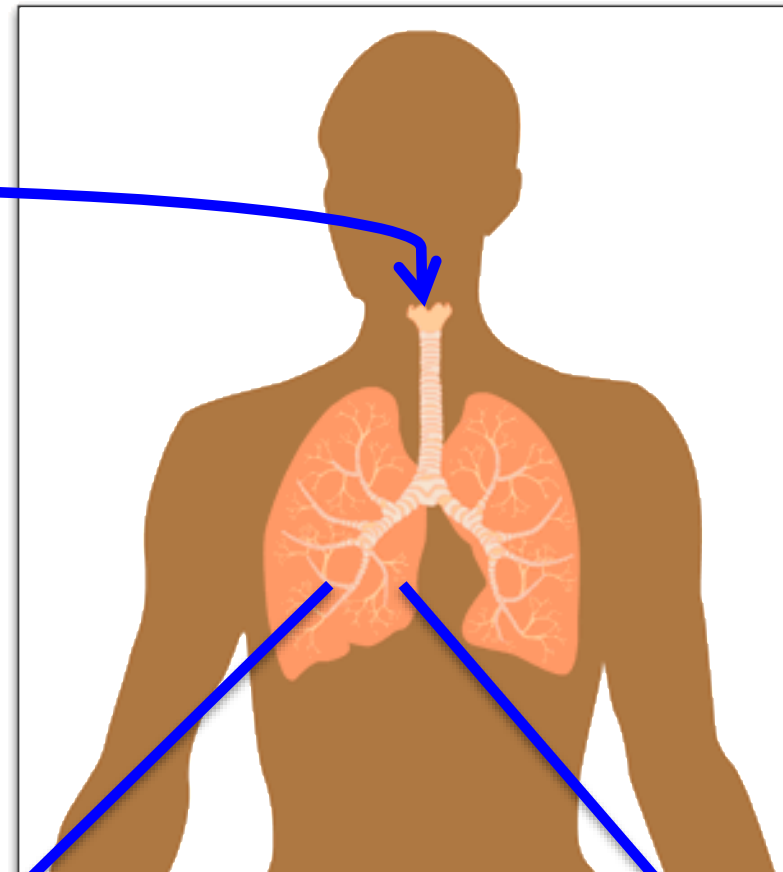
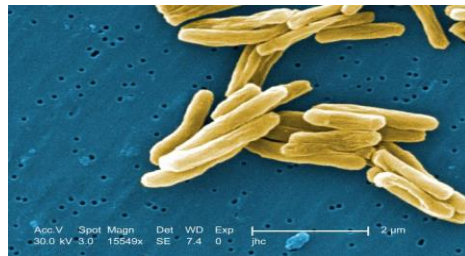
THE UNIVERSITY
of
WISCONSIN
MADISON

Mtb exposure leads to active or latent TB



**Active
Pulmonary TB**

Mtb, SEM, CDC., 2008



**Reactivation TB
(5-10% lifetime)**

← **Immunosuppression**
Age
??

**Latent
Infection
(90-95%)**

Asymptomatic

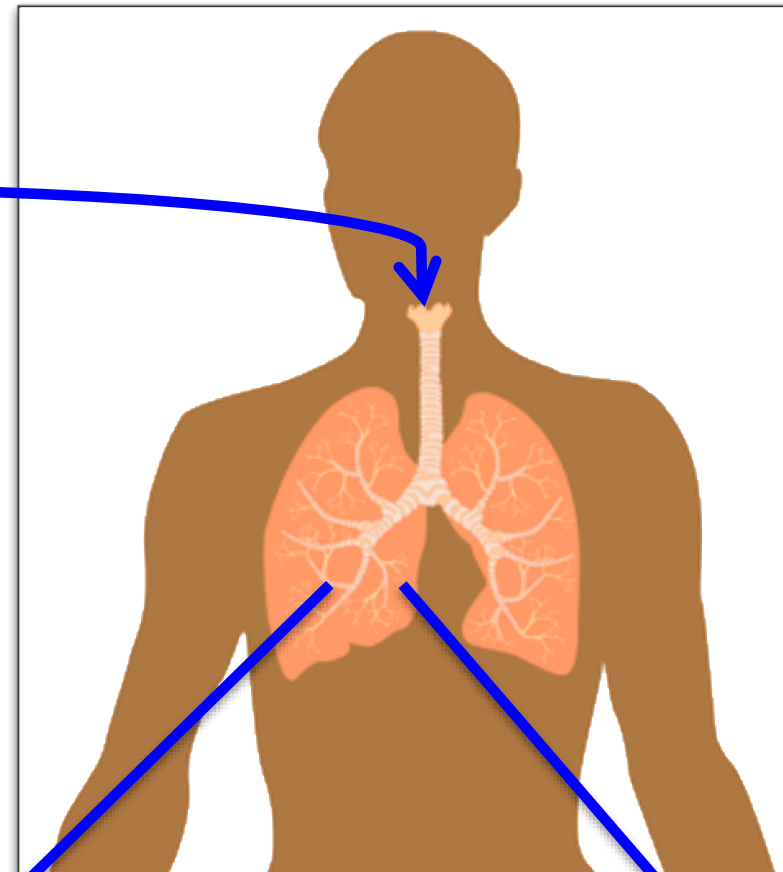
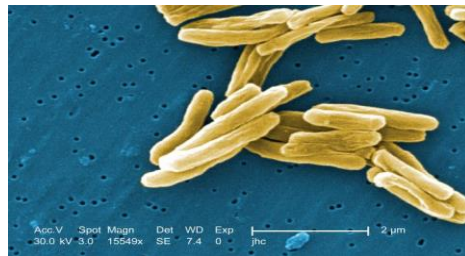
**Primary Active
Disease
(5-10%)**

Mtb exposure leads to active or latent TB



**Active
Pulmonary TB**

Mtb, SEM, CDC., 2008



**Reactivation TB
(5-10% lifetime)**

Immunosuppression

Age
??

**Latent
Infection
(90-95%)**



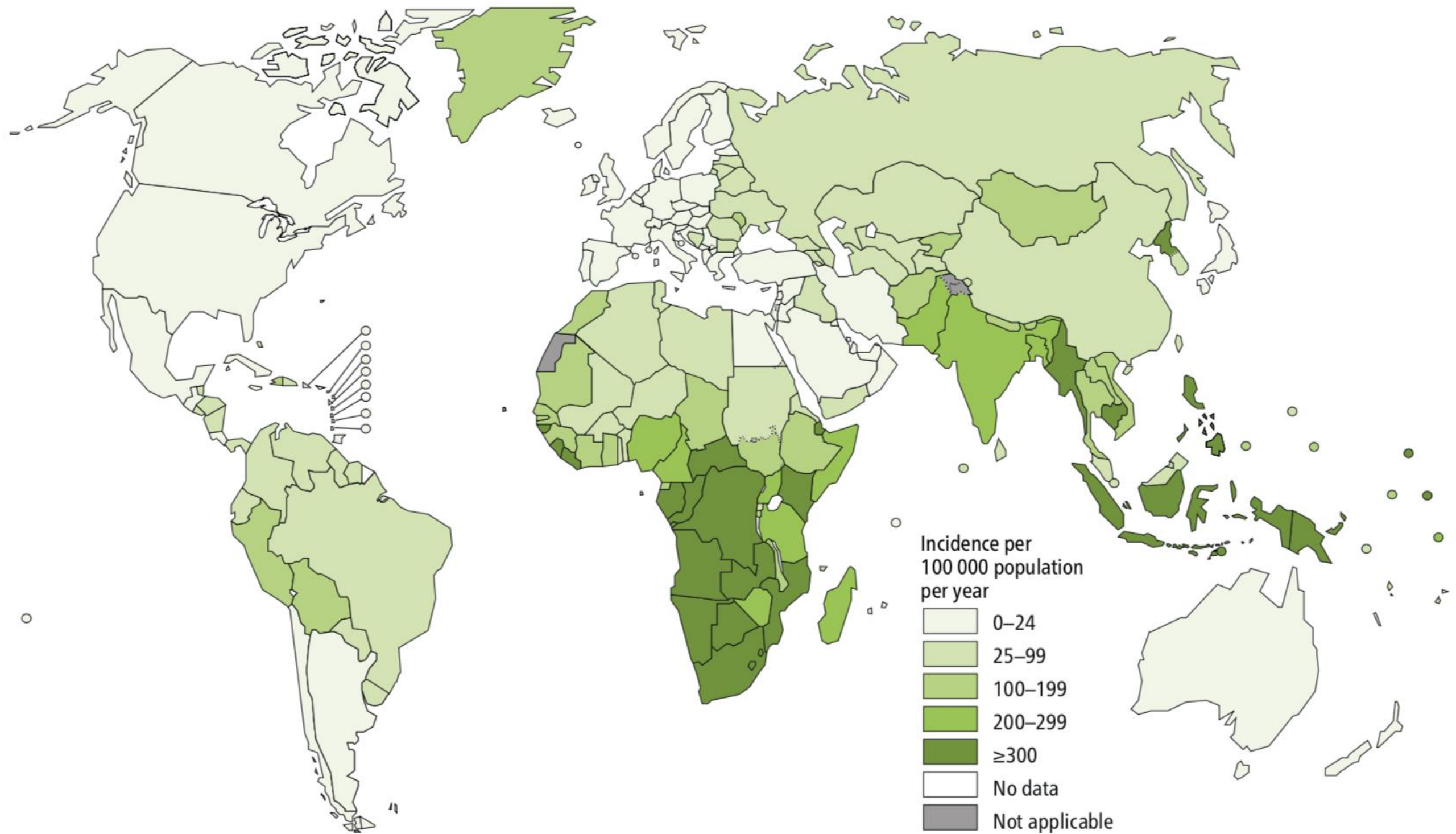
Asymptomatic

**Primary Active
Disease
(5-10%)**

To 10% per year!!

To 10% per year!!

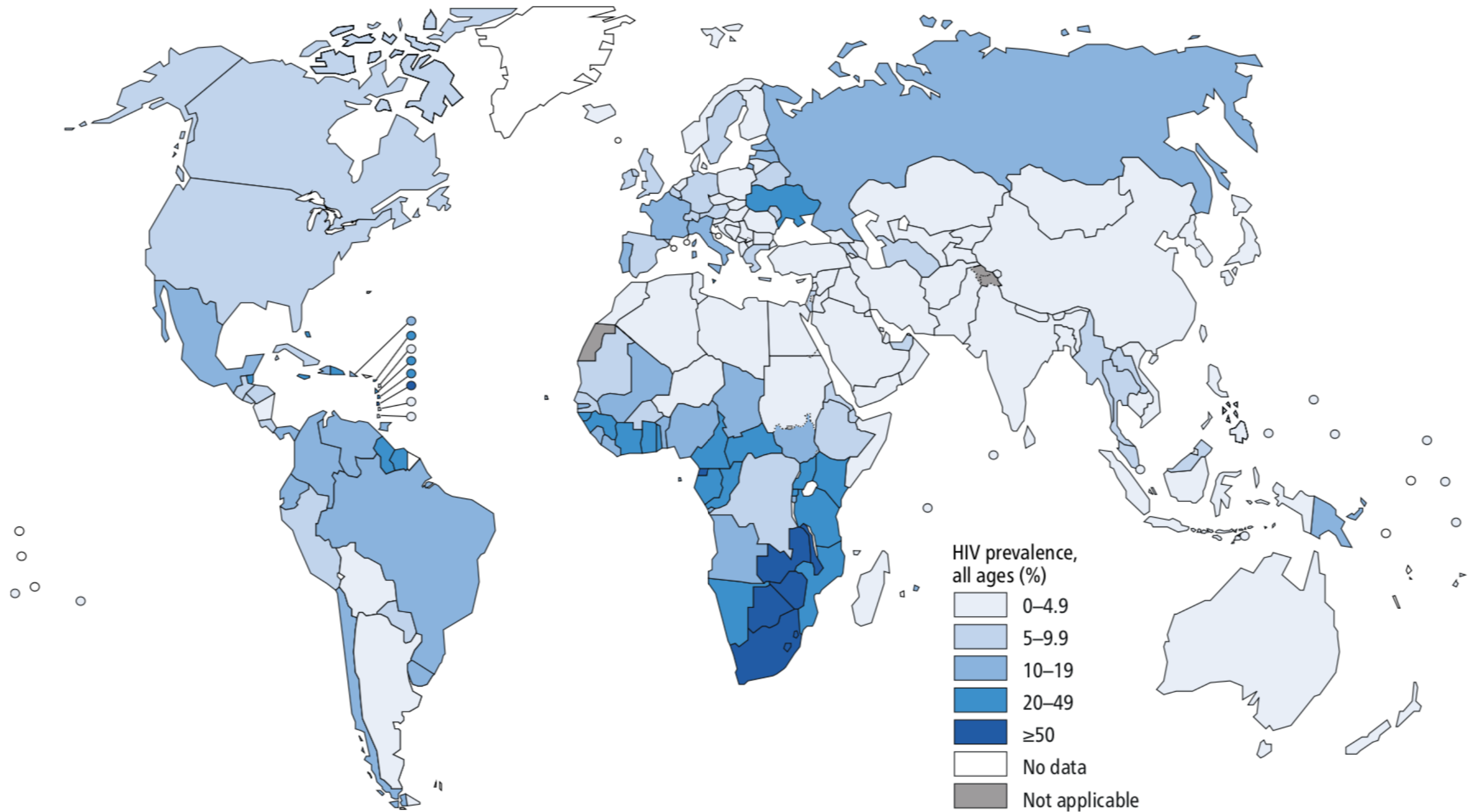
Tuberculosis is a global health crisis



WHO, Global Tuberculosis Report, 2017

Tuberculosis is the most common cause of morbidity and mortality in HIV+ individuals

Estimated HIV prevalence in new and relapse TB cases, 2016

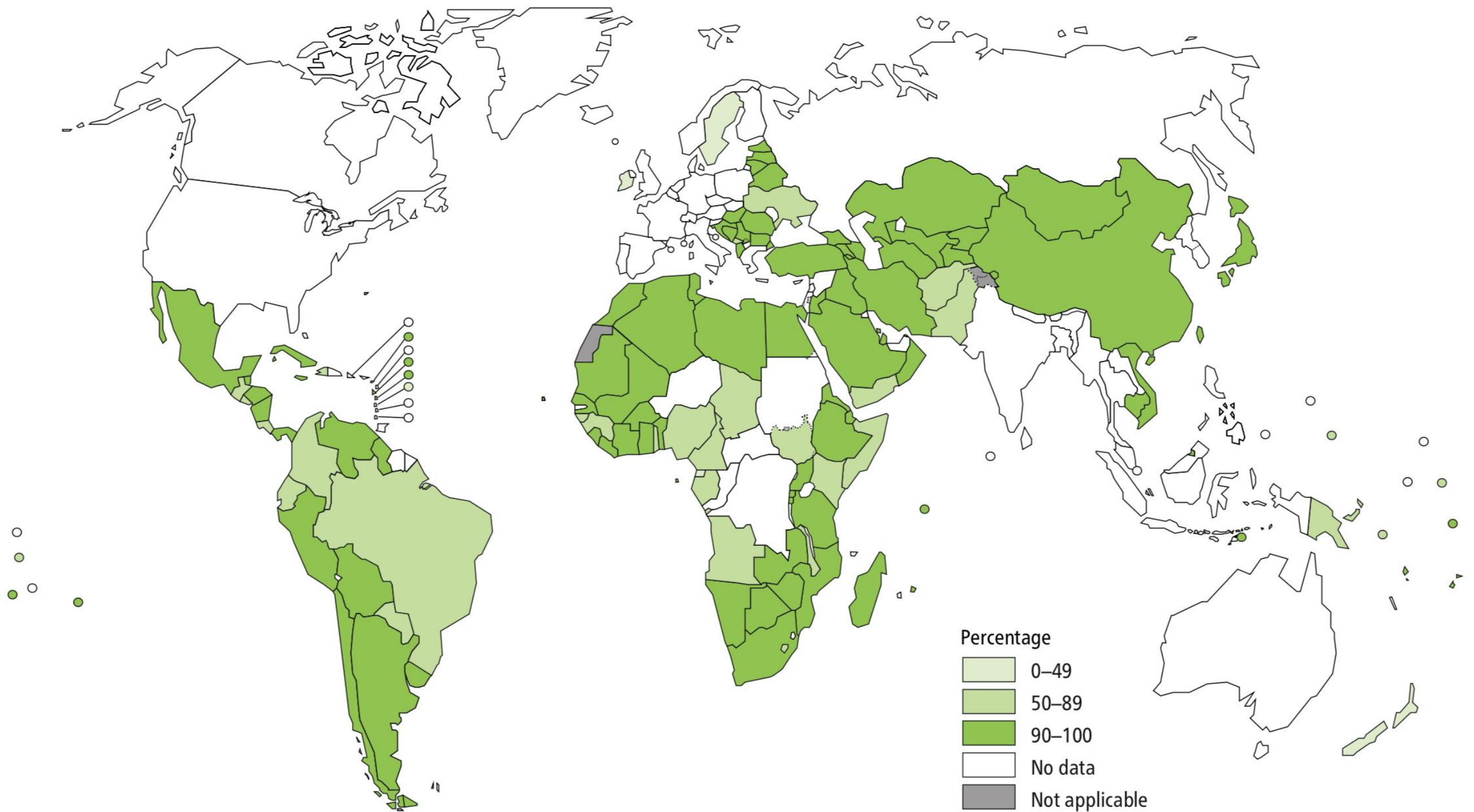


WHO, Global Tuberculosis Report, 2017

BCG: Bacille Calmette Guerin

- First TB vaccine
- A live attenuated strain of *Mycobacterium bovis* that was cultured every 14 days for 230 passages (nearly 9 years!)
- First human test was in a neonate who lived in a house with a TB patient
- Vaccinated 20,000 neonates between 1921 and 1924
- Protects newborns from life threatening extrapulmonary TB (miliary TB) and TB meningitis

Neonates are still being vaccinated with BCG in high burden TB settings



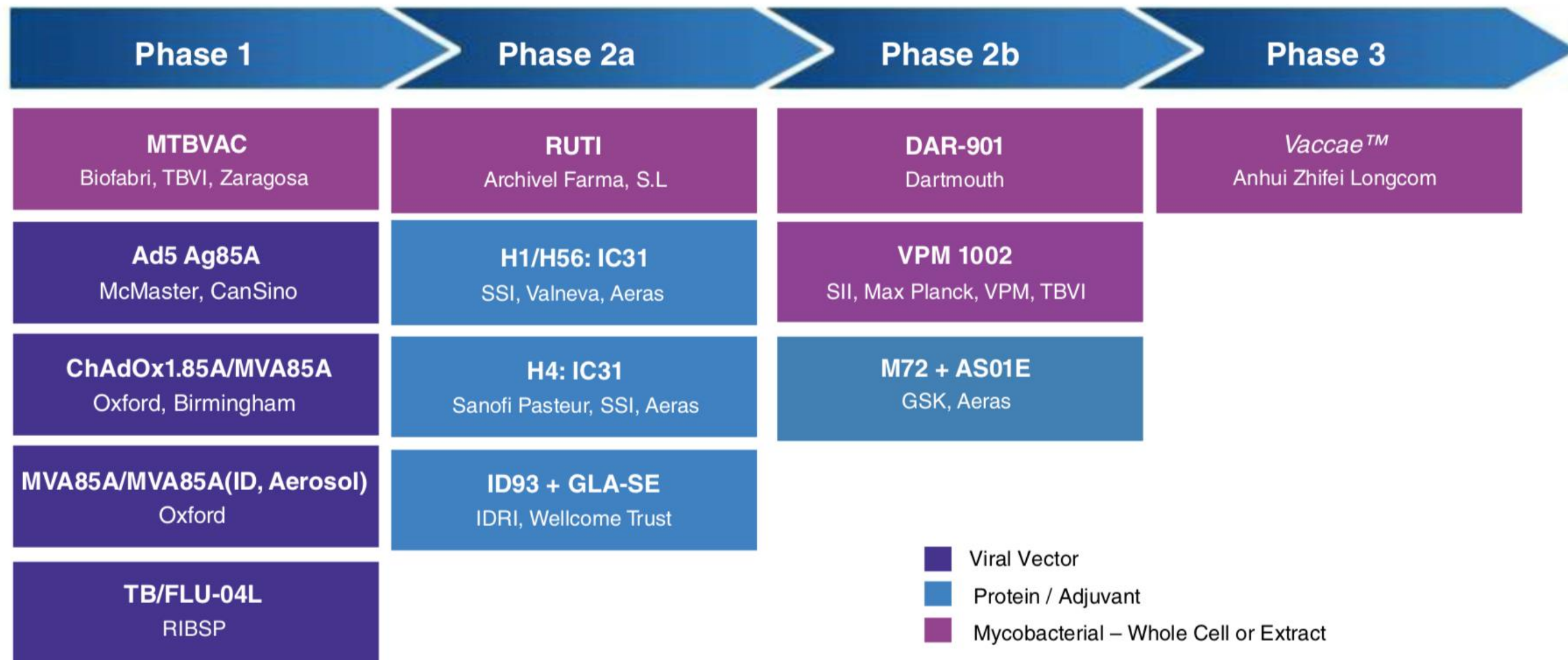
Why is BCG insufficient as a vaccine?

- Limited and variable effect on reducing pulmonary TB in adults
- BCG is a live vaccine and can cause a systemic infection in immunocompromised individuals
- HIV+ infants are at an increased risk of developing disseminated BCG, although they should still be vaccinated (*WHO, 2018*)

TB Vaccine Pipeline 2017

13 Candidates

5/4/3/1



Revised on February 2, 2017

Please note: Information is self-reported by vaccine sponsors

What are some animal models used to test TB vaccines?

Mice

Advantages:

1. Genetically defined
2. Rapid evaluation of interventions
3. Small size and low cost
4. Availability of immunological tools



Disadvantages:

1. TB granulomas and disease are different than humans
2. No disseminated disease

Guinea Pig

Advantages:

1. Susceptible to TB
2. Granulomas are more 'human-like'
3. Bigger than mice
4. Can still be used for vaccine efficacy and drug development

Disadvantages:

1. Lack of good immune reagents
2. No latent TB
3. More expensive than mice



Rabbit

Advantages:

1. Very susceptible to TB
2. Form 'human-like' necrotic granulomas
3. Can develop latent TB, depending on infecting strain
4. Can still be used for vaccine efficacy and drug development
5. Model for TB Meningitis

Disadvantages:

1. Lack of good immune reagents
2. More expensive than other small models



Image By Larry D. Moore
<https://commons.wikimedia.org>

Nonhuman primates (NHPs)

Advantages:

1. Susceptible to TB
2. Form a spectrum of granulomas similar to humans
3. Can still be used to test interventions
4. Longitudinal tracking
5. Immune reagents are available
6. *Gold standard*

Disadvantages:

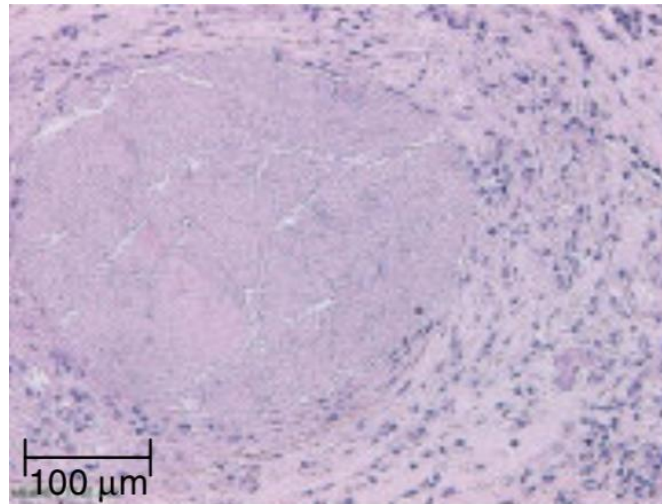
1. VERY expensive
2. Genetically outbred



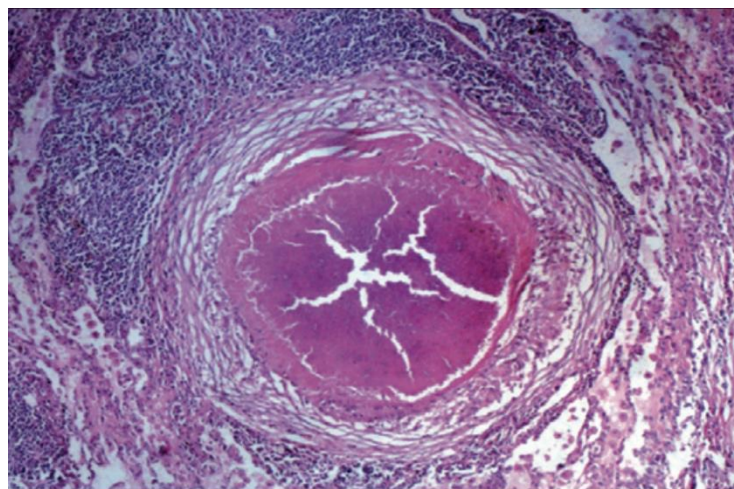
Is TB disease in NHPs
similar to humans?

Humans and NHPs develop similar granulomas

Human

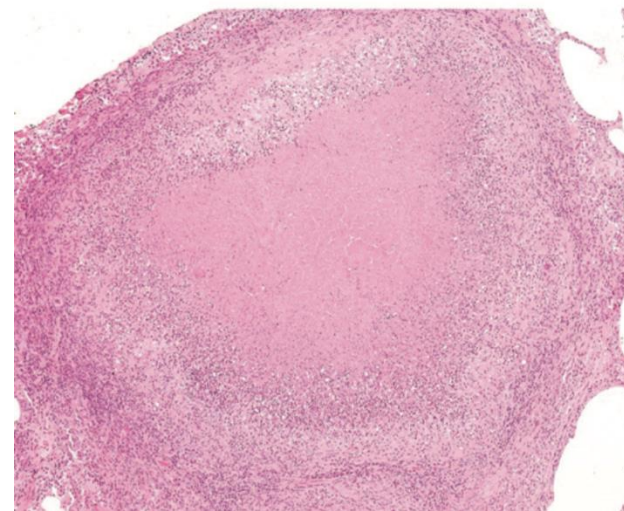


Caseous/necrotic

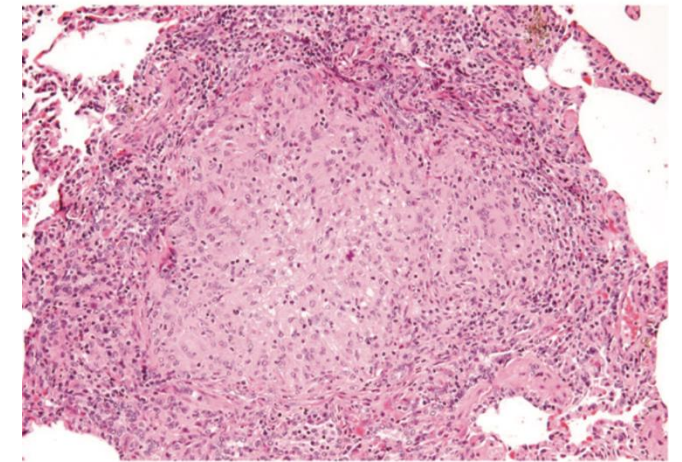


Caseous/necrotic

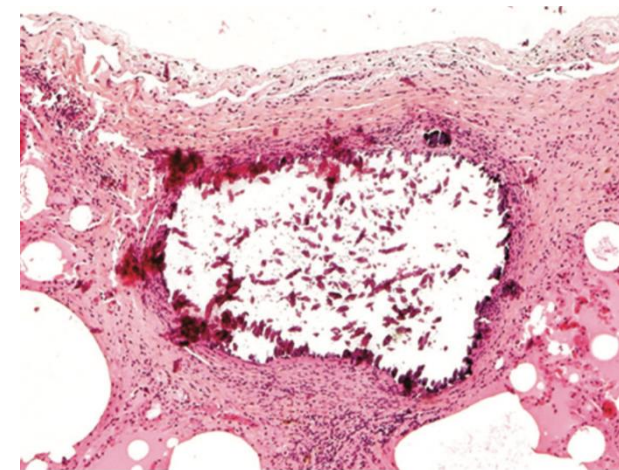
Cynomolgus macaque



Caseous/necrotic



nonnecrotic



mineralized

Ways to measure TB disease in NHPs

- Clinical markers: ESR, BAL culture, Gastric aspirates, Cough
- Serial PET/CT imaging
- Measuring bacterial growth at necropsy



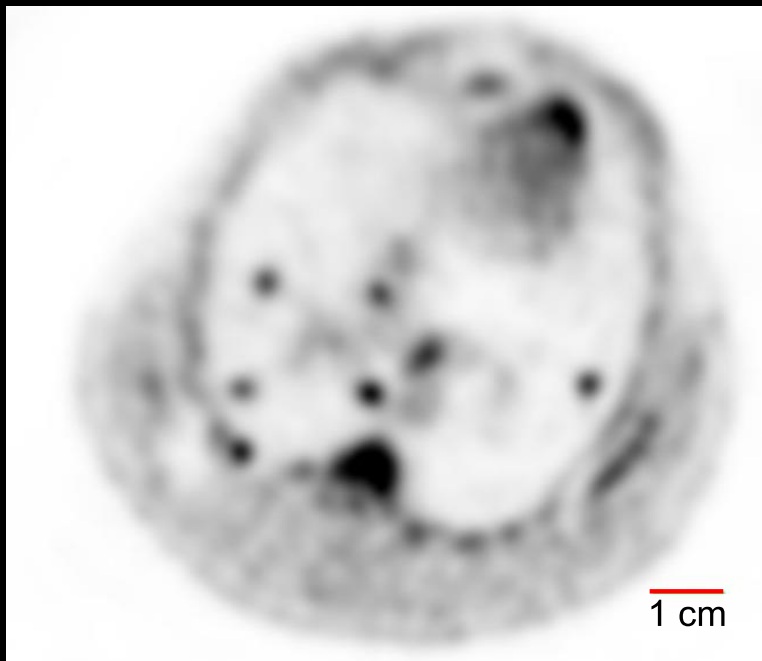
University of Pittsburgh Regional Biocontainment Laboratory **BSL-3 PET/CT Imaging Suite**



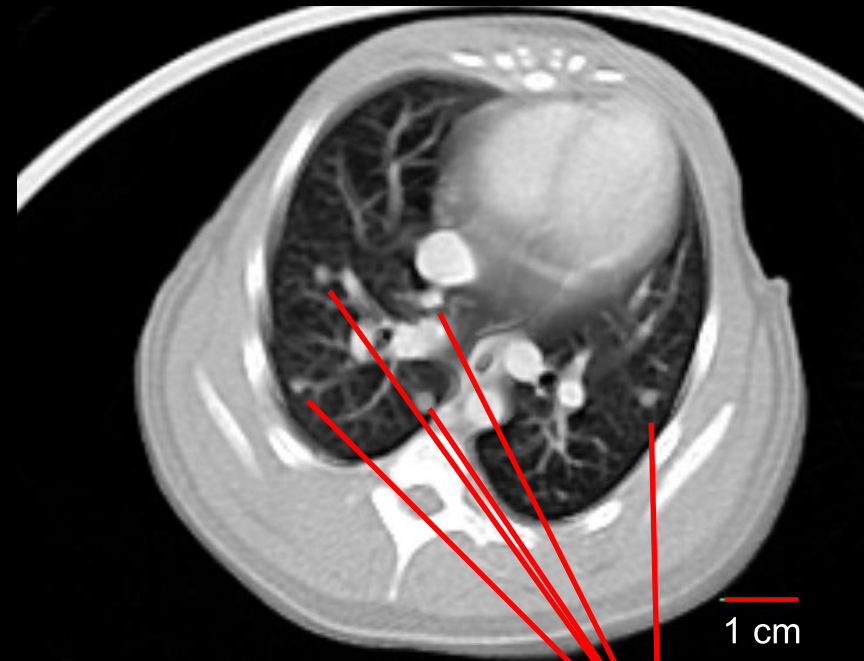
- **Siemens MicroPET Focus 220**
- **Neurologica CereTom CT scanner**
- **Integrated animal handling system**
- **eVent Inspiration LS critical care ventilator (not shown)**
- **Spectra AG5 vital signs monitor (not shown)**
- **Bear Hugger warming device (not shown)**
- **Isoflurane anesthesia machine (not shown)**

^{18}F -Fluorodeoxyglucose (FDG) PET/CT analyses to track lesions

PET

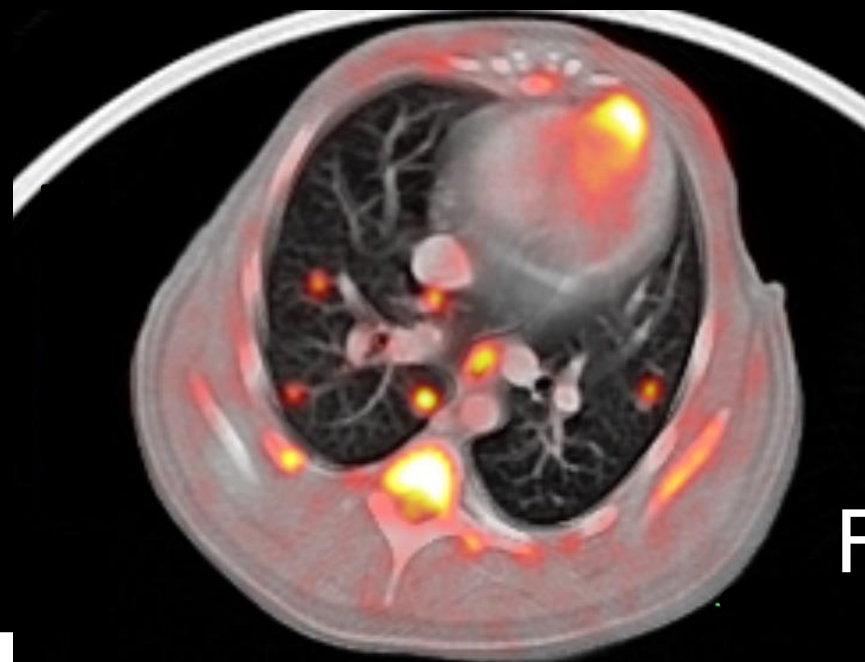


CT

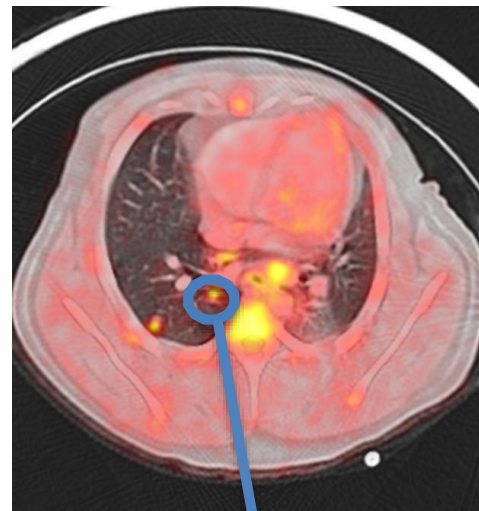


TB granulomas

Fusion

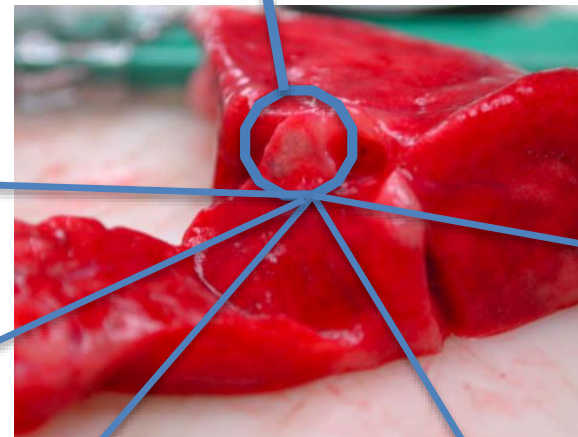


**Pre-necropsy PET/CT
provides map of lesions
(lesion size and SUV)**



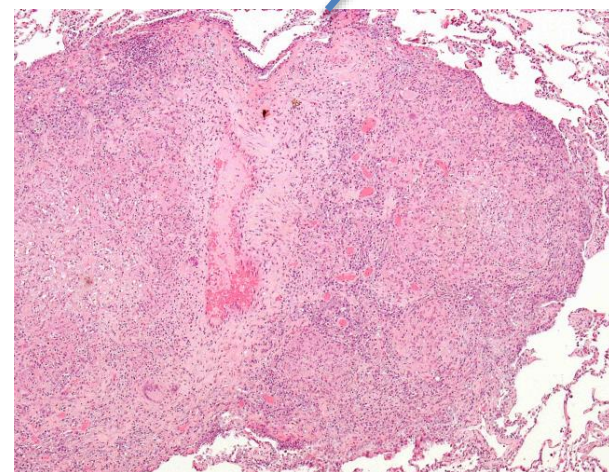
**Study individual
granulomas**

**Image-guided necropsy
yields individual lesions**

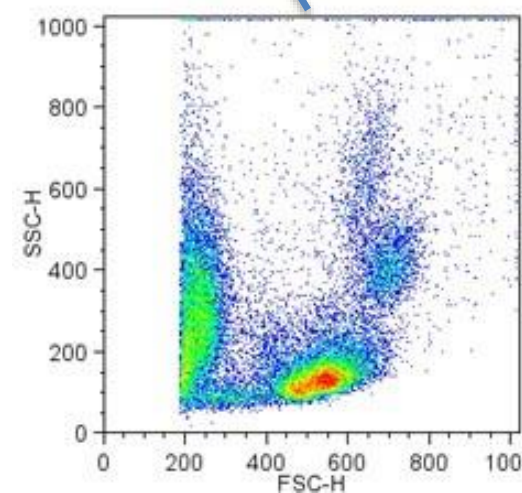


CFU

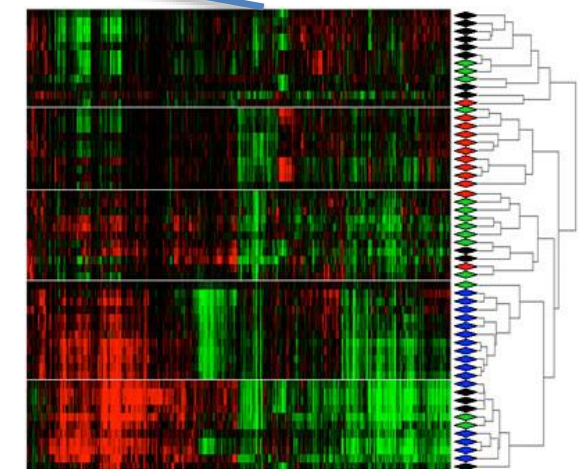
**[Rx] and/or
imaging mass
spectrometry**



Histopathology



Immunology



**Gene
expression**

Adapted from:
Scanga & Flynn. In:
Tuberculosis. ed. S.H.E.
Kaufmann. Cold Springs
Harb. Perspect. Med. 2014.

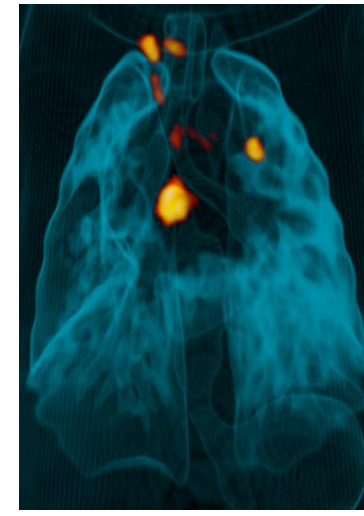
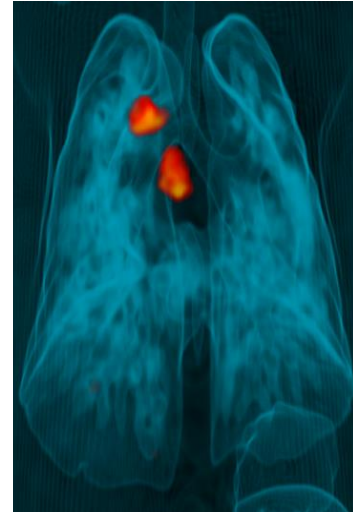
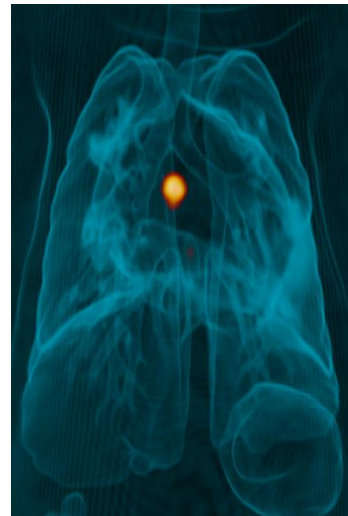
Spectrum of TB progression in NHPs by PET/CT

Chinese
cynomolgus
macaques

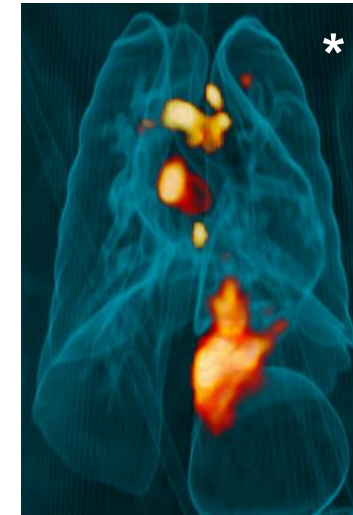
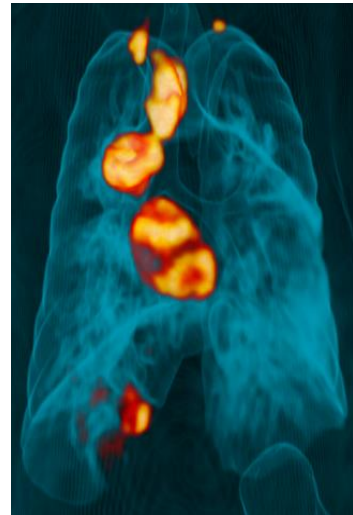
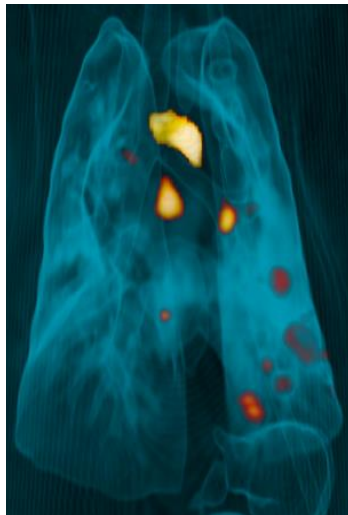
rhesus
macaques

Mauritian
cynomolgus
macaques

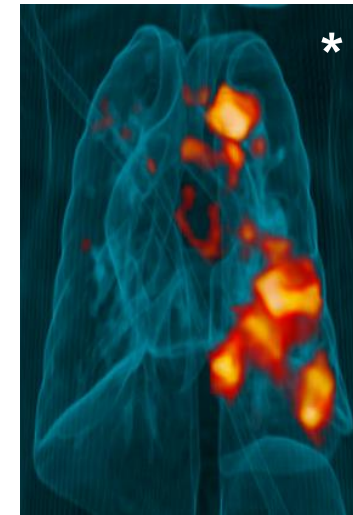
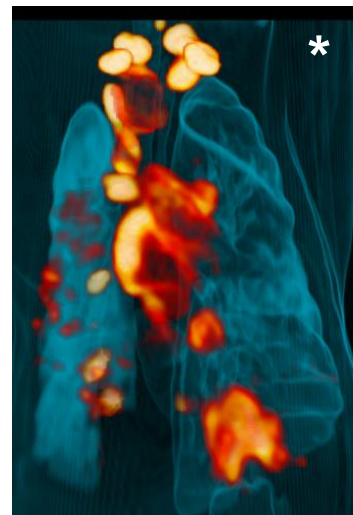
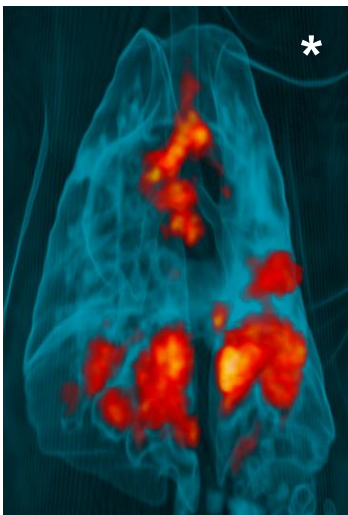
Good



“Typical”

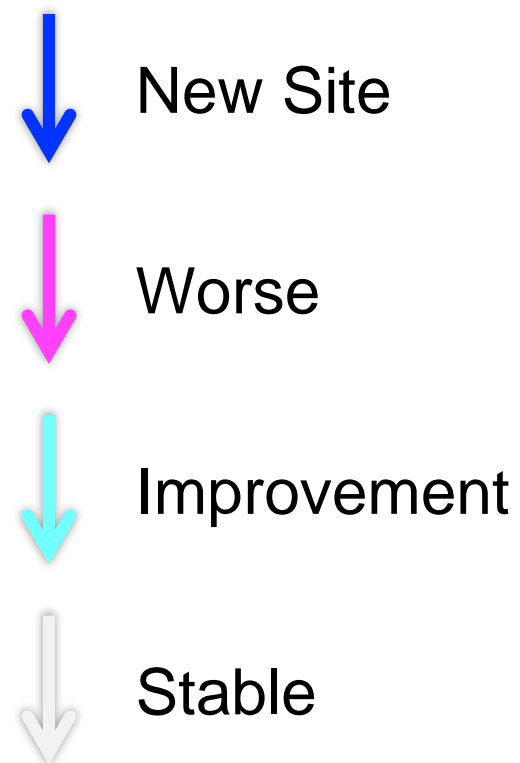


Bad

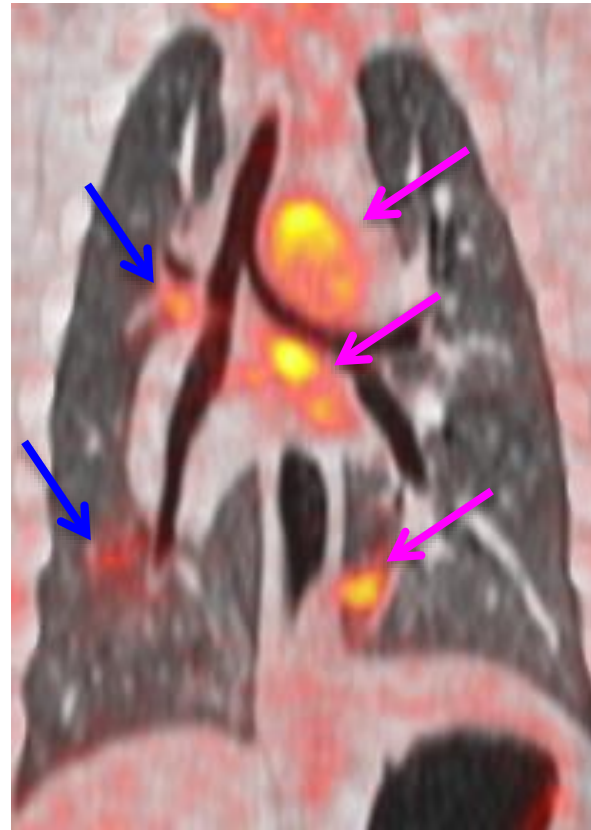


Pre-necropsy
images are
taken at 5-6
months or at
humane
endpoint*

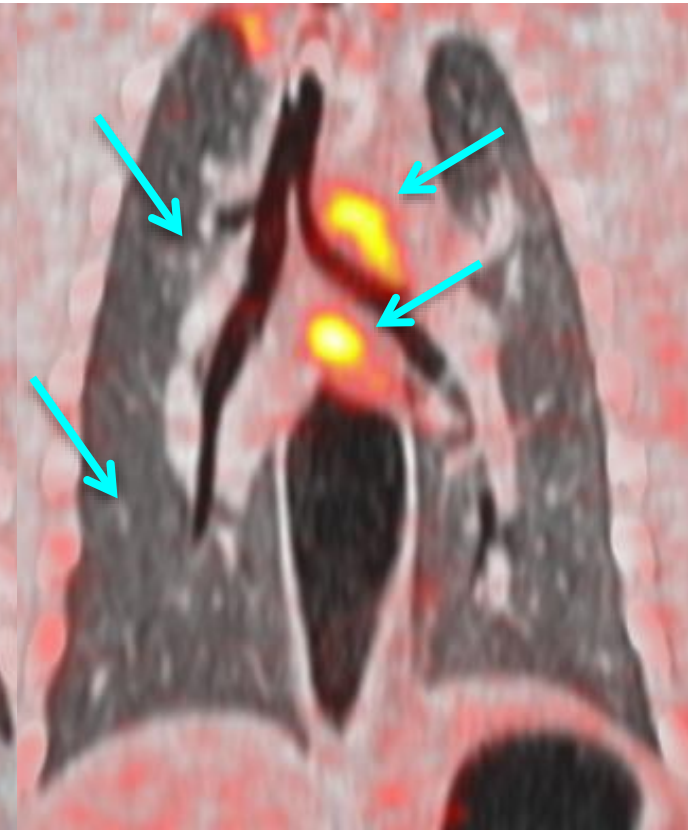
Individual granulomas can be tracked (CONTROL)



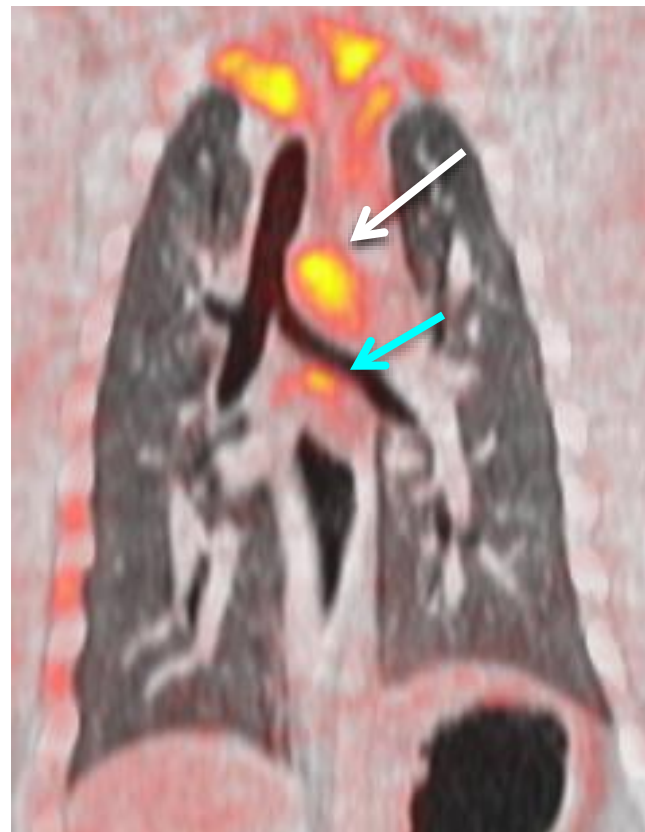
8 weeks



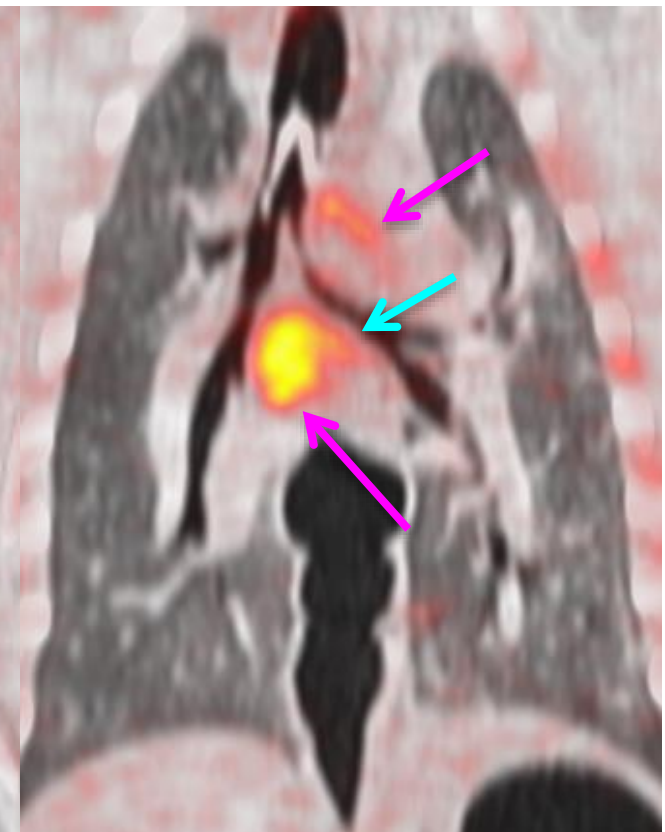
12 weeks



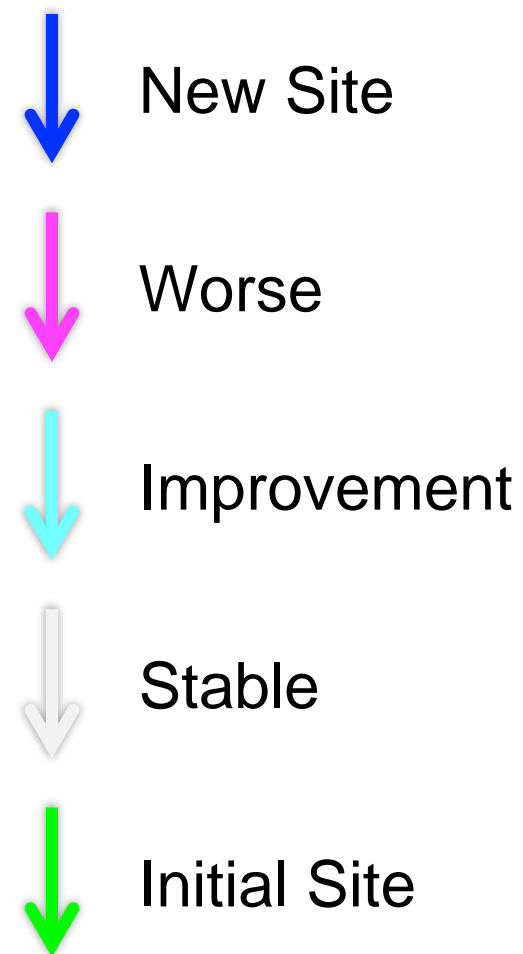
16 weeks



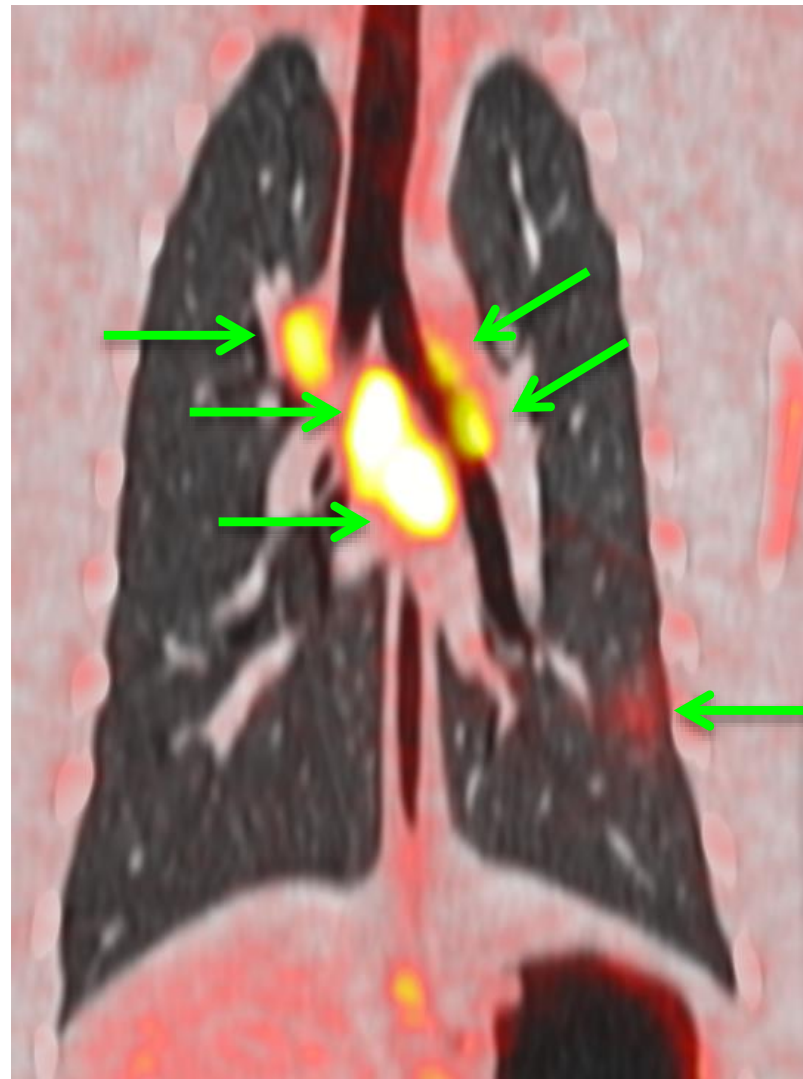
20 weeks



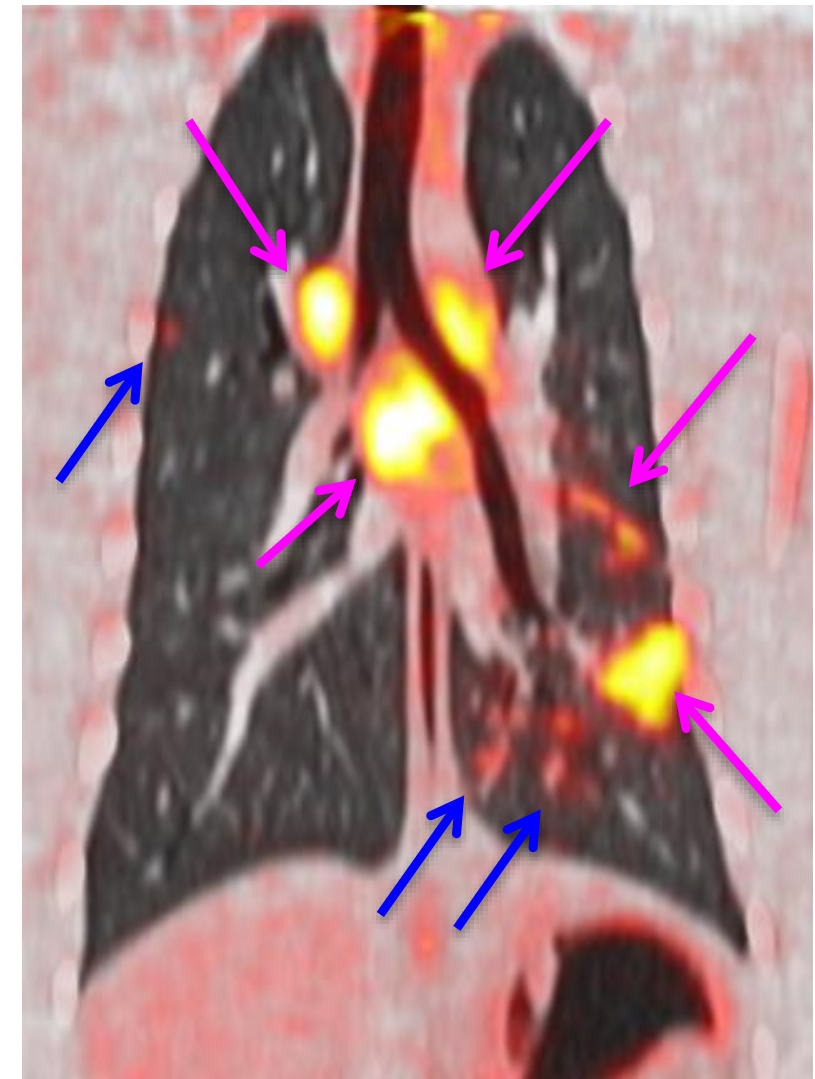
Individual granulomas can be tracked (NO control)



4 weeks



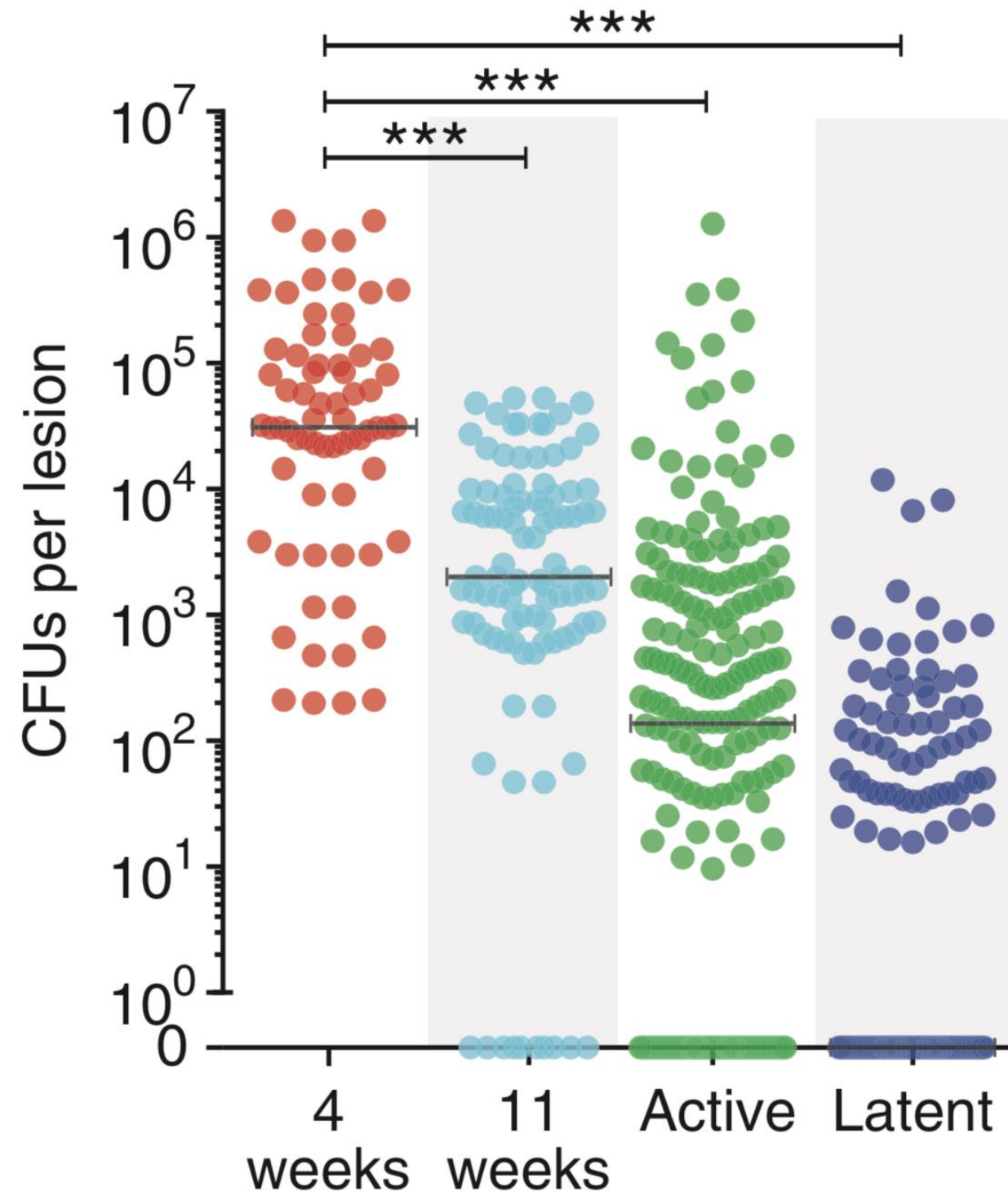
8 weeks



Bacterial Colony Forming Units (CFUs) decline as adaptive immunity develops

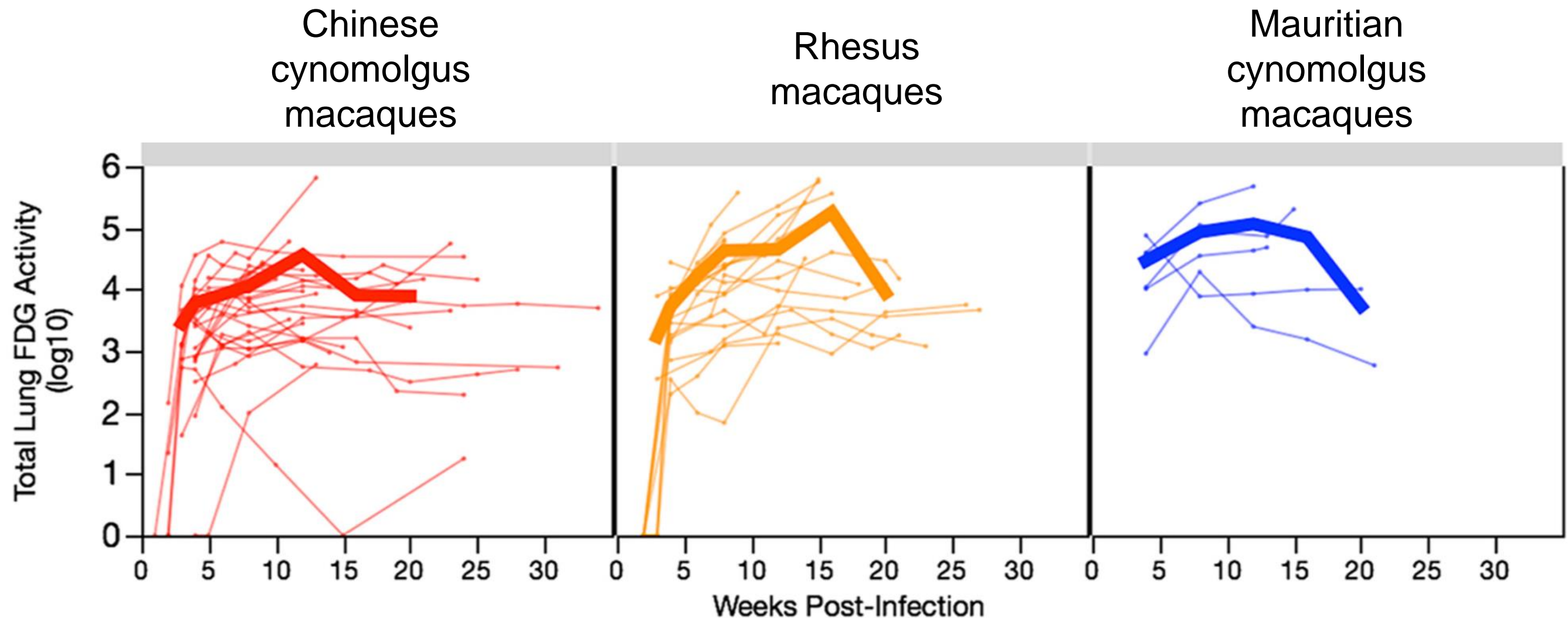


CFU

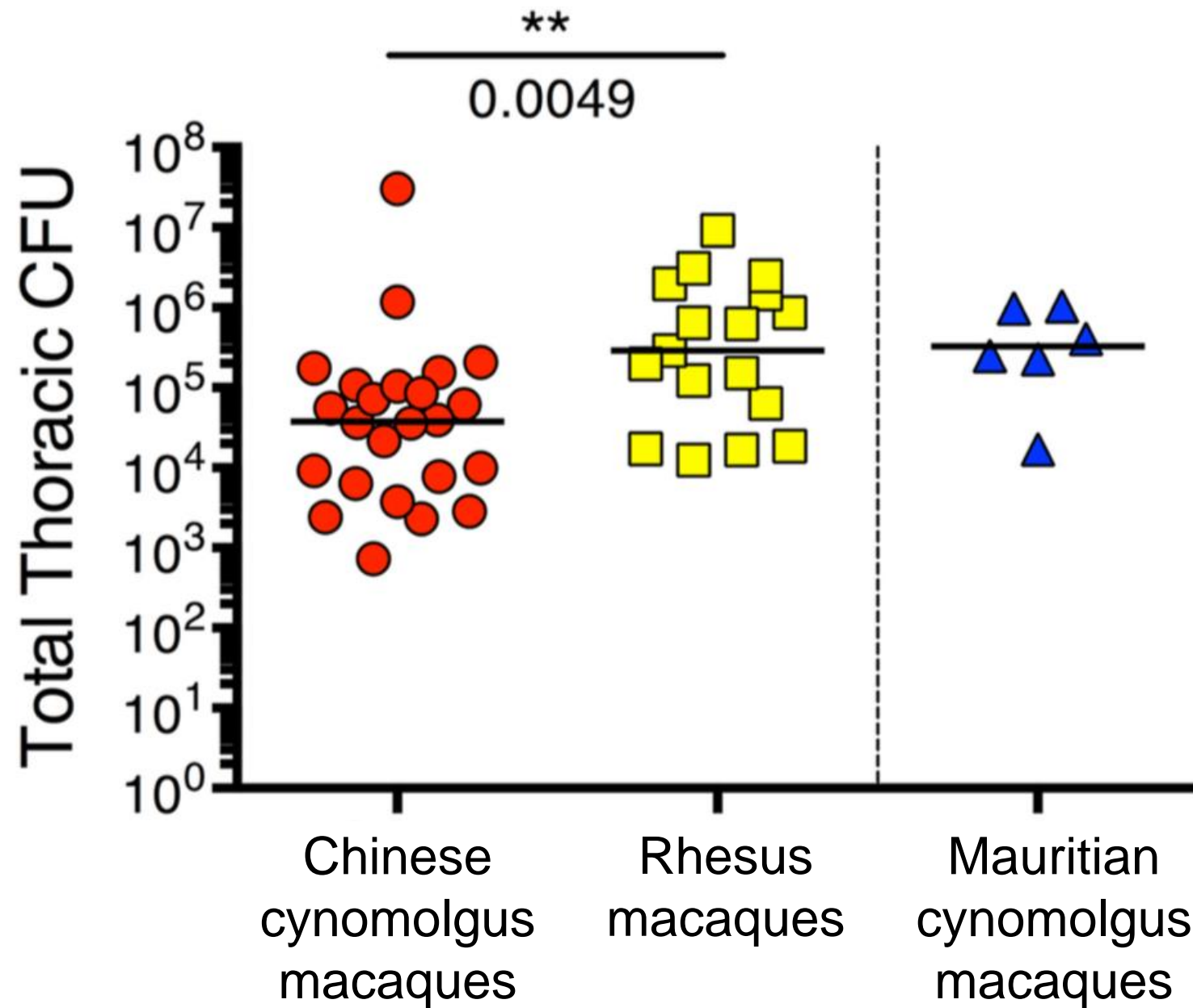


TB disease is not the
same in all NHP
populations

FDG avidity increases during infection and is different across NHP populations



Bacterial CFU at necropsy is higher in different NHP populations

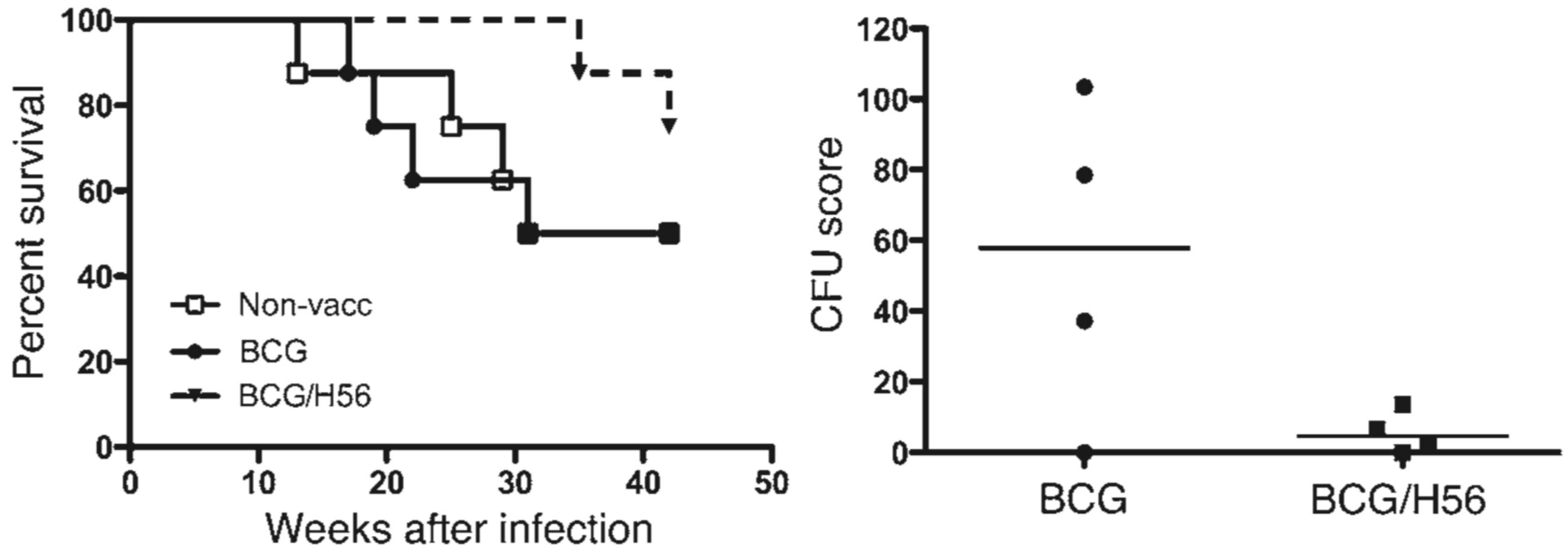


Each symbol = one animal

TB vaccines can be
tested in NHPs

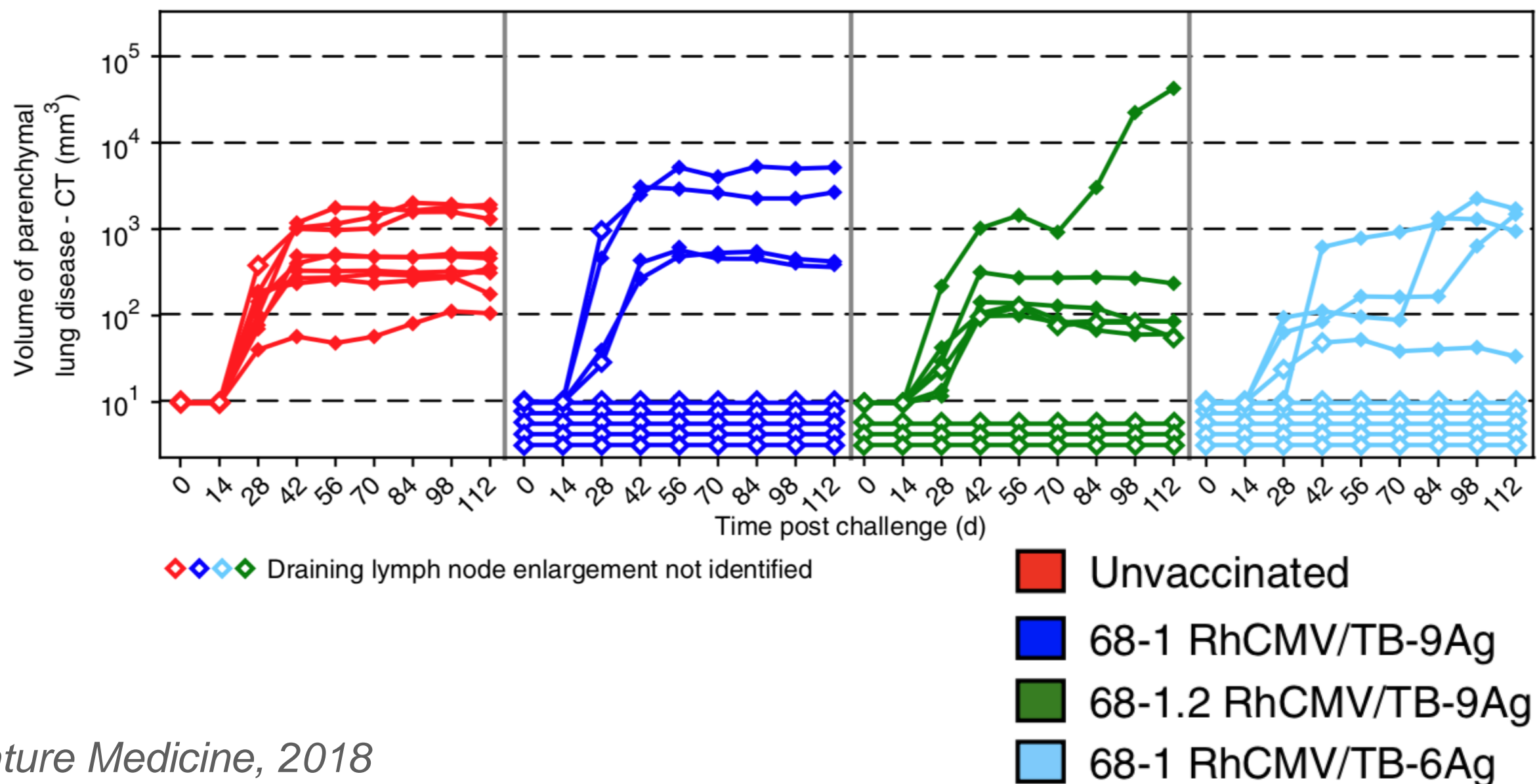
The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection

Philana Ling Lin,¹ Jes Dietrich,² Esterlina Tan,³ Rodolfo M. Abalos,³ Jasmin Burgos,³ Carolyn Bigbee,⁴ Matthew Bigbee,⁴ Leslie Milk,⁴ Hannah P. Gideon,⁴ Mark Rodgers,⁴ Catherine Cochran,⁴ Kristi M. Guinn,⁵ David R. Sherman,⁵ Edwin Klein,⁶ Christopher Janssen,⁶ JoAnne L. Flynn,^{4,7} and Peter Andersen²



Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine

Scott G Hansen^{1,8}, Daniel E Zak^{2,8}, Guangwu Xu^{1,8}, Julia C Ford¹, Emily E Marshall¹, Daniel Malouli¹, Roxanne M Gilbride¹, Colette M Hughes¹, Abigail B Ventura¹, Emily Ainslie¹, Kurt T Randall¹, Andrea N Selseth¹, Parker Rundstrom¹, Lauren Herlache¹, Matthew S Lewis¹, Haesun Park¹, Shannon L Planer¹, John M Turner¹, Miranda Fischer¹, Christina Armstrong¹, Robert C Zweig¹, Joseph Valvo², Jackie M Braun², Smitha Shankar², Lenette Lu³, Andrew W Sylwester¹, Alfred W Legasse¹, Martin Messerle⁴, Michael A Jarvis⁵, Lynn M Amon², Alan Aderem², Galit Alter³, Dominick J Laddy⁶, Michele Stone⁶, Aurelio Bonavia⁶, [†]Thomas G Evans⁶, Michael K Axthelm¹, Klaus Früh¹, Paul T Edlefsen⁷ & Louis J Picker¹



But, we really need a
TB vaccine for HIV+
individuals!

NHP co-infection models :

1. **Mtb** infection first; **SIV** infection second

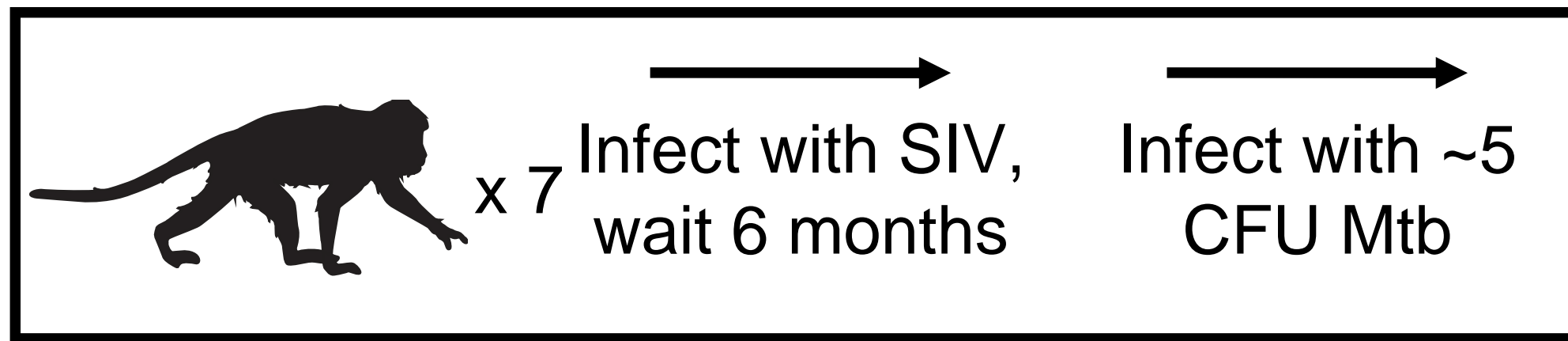
- Cynomolgus macaque – Latent Mtb Erdman followed by SIVmac239
(*Diedrich et. Al., PLoS ONE, 2010*)
- Rhesus macaque – Latent Mtb CDC1551 followed by SIVmac239
(*Foreman et. al., PNAS, 2016*)

2. **SIV** infection first; **Mtb** infection second

Hypothesis: SIV infection disrupts the development of immune responses to an Mtb infection, which leads to rapid TB progression



- Serial PET/CT imaging
- Defining T cell populations
- Characterize T cell function in PBMC and granulomas



Mtb infection
ONLY



SIV (6 months)
followed by Mtb

Mtb infection
ONLY

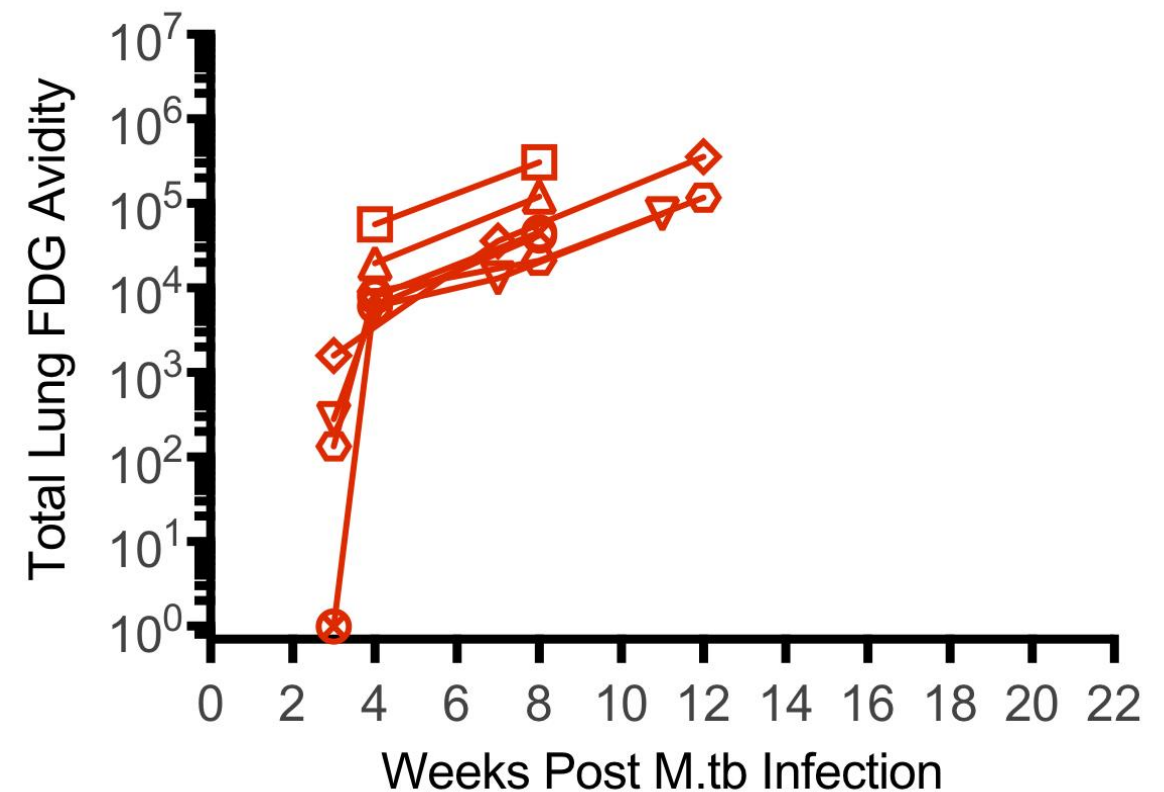
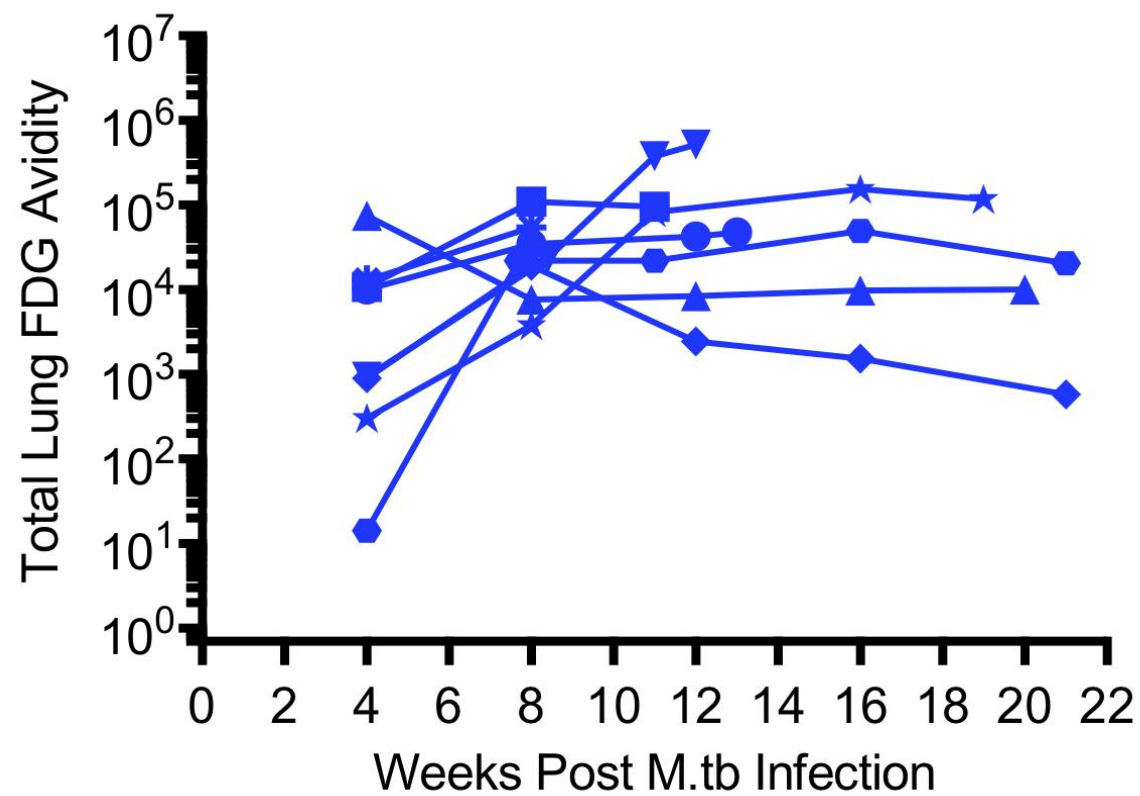


SIV (6 months)
followed by Mtb

Longitudinal PET/CT scanning does not differentiate between groups

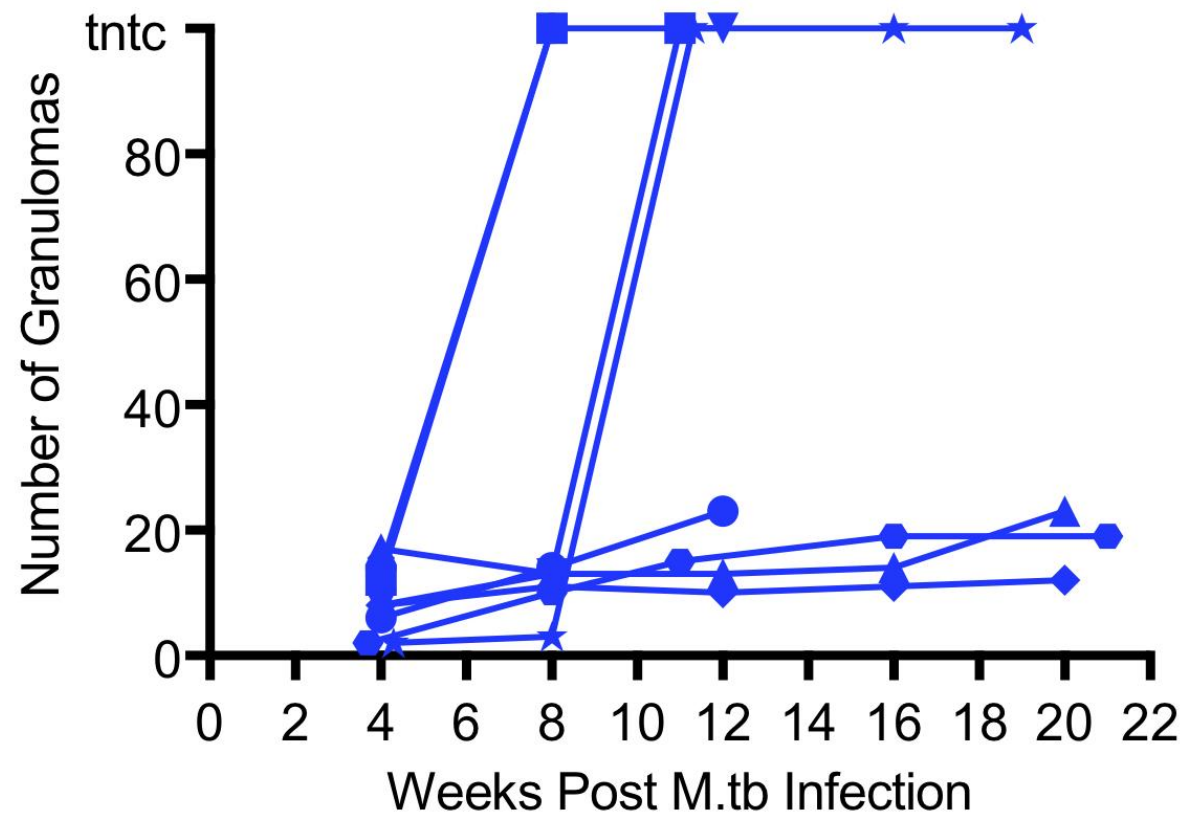
— Mtb only

— SIV/Mtb

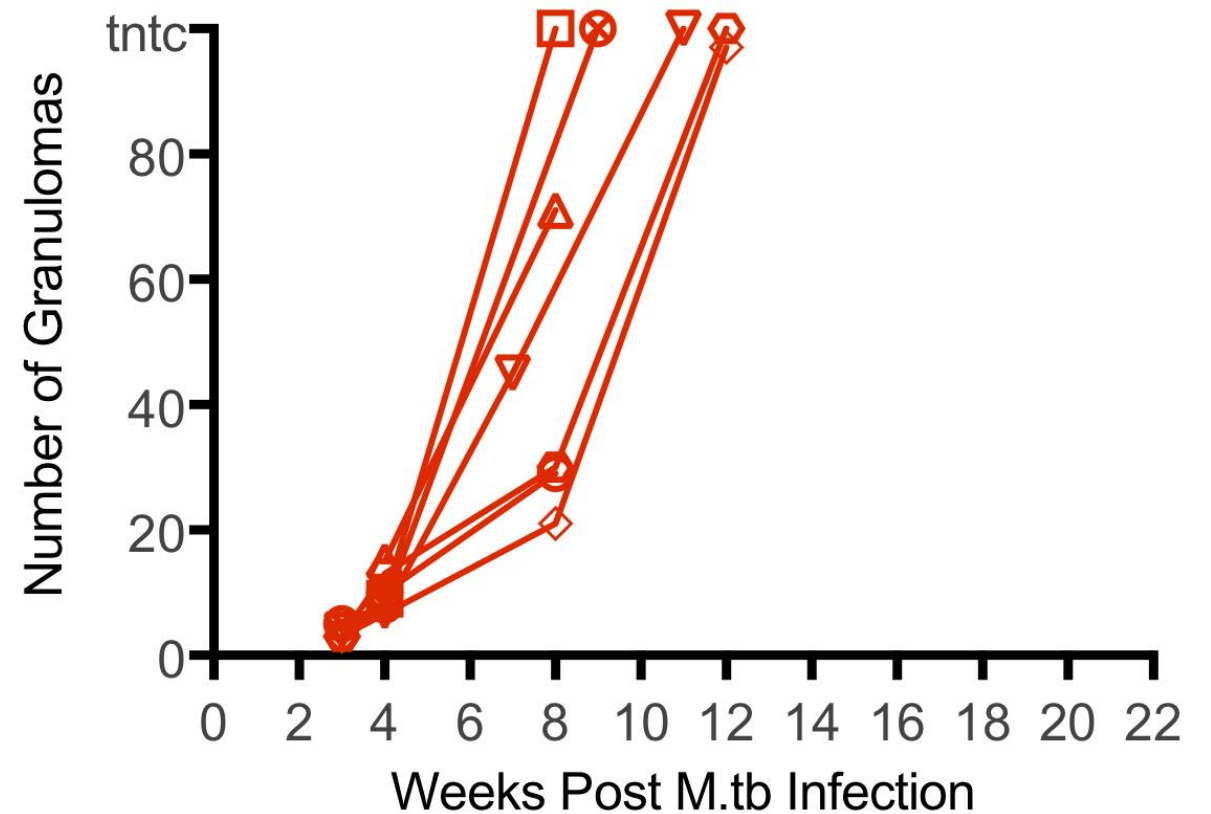


Granuloma counts reveal rapid dissemination in SIV/Mtb animals

— Mtb only

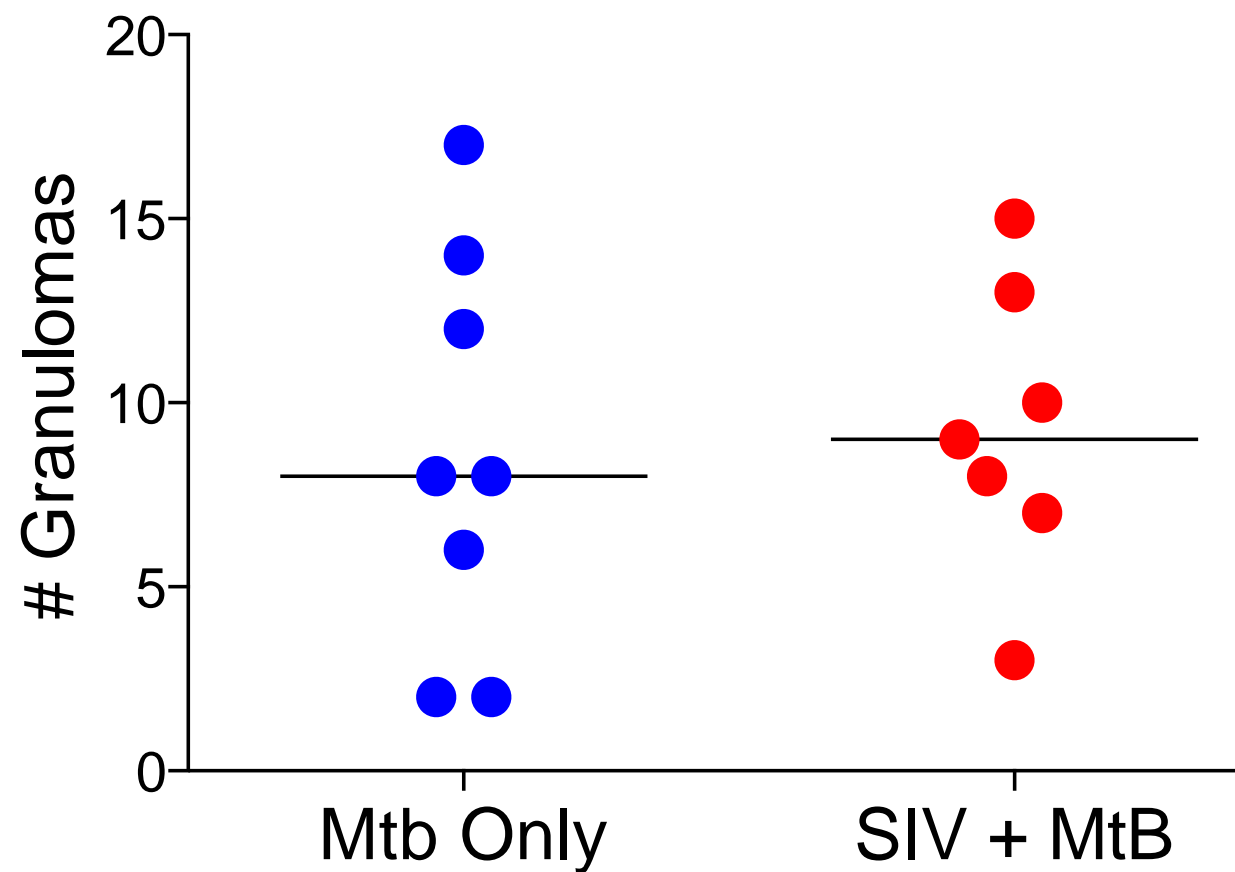


— SIV/Mtb



Granuloma counts reveal rapid dissemination in SIV/Mtb animals

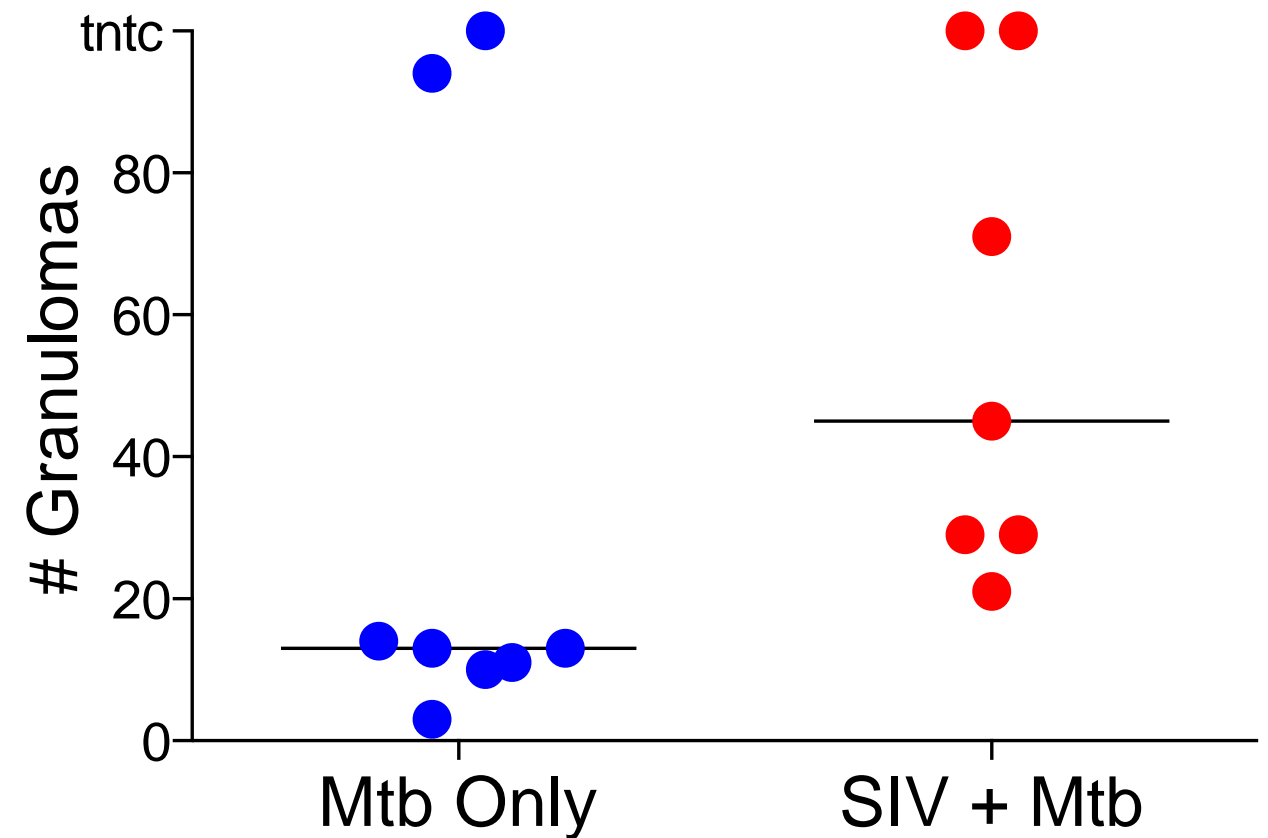
4 weeks



p=0.6668

— Mtb only

8 weeks



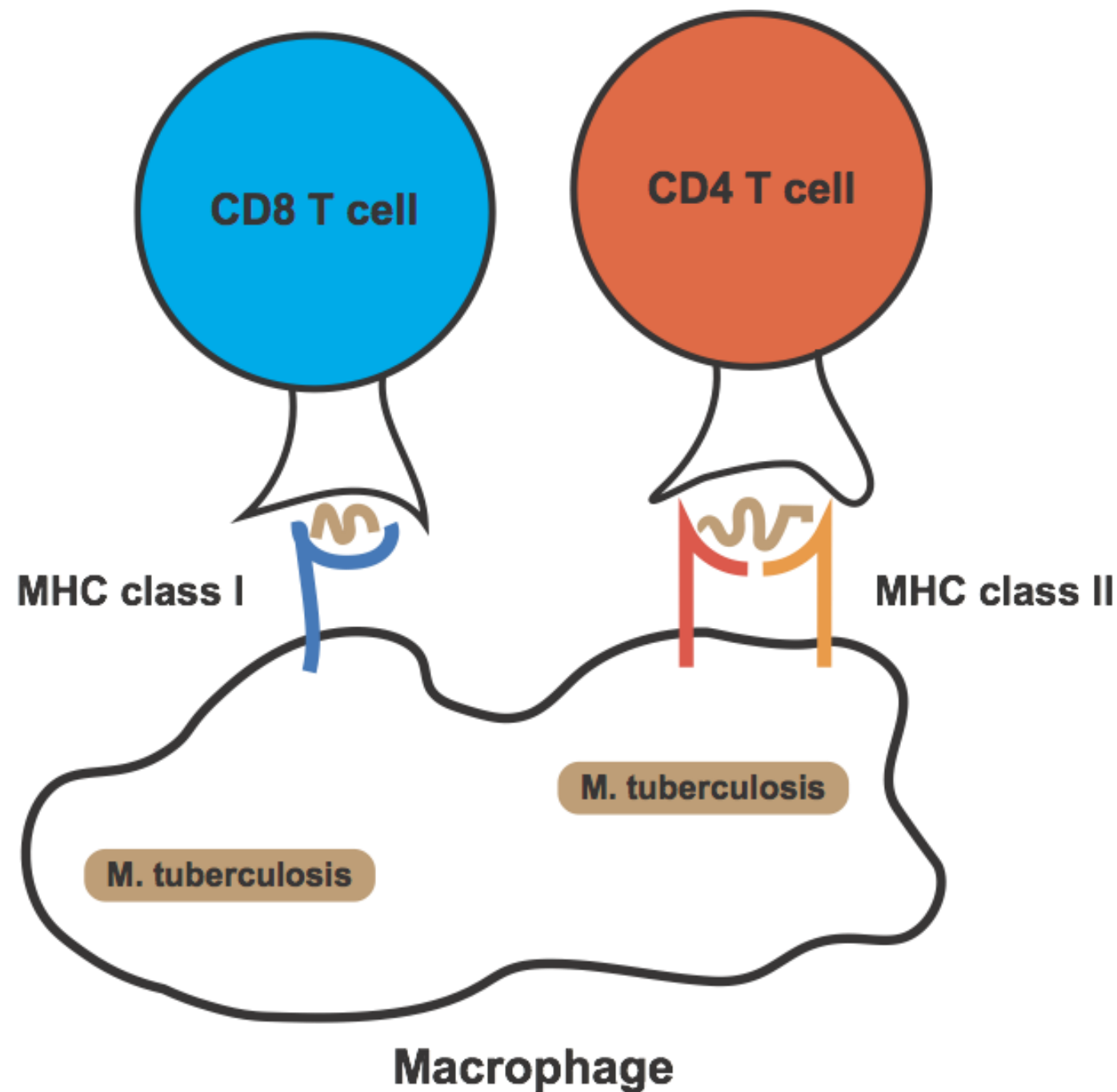
p=0.0491

— SIV/Mtb

Balance between too much and too little of an immune response!

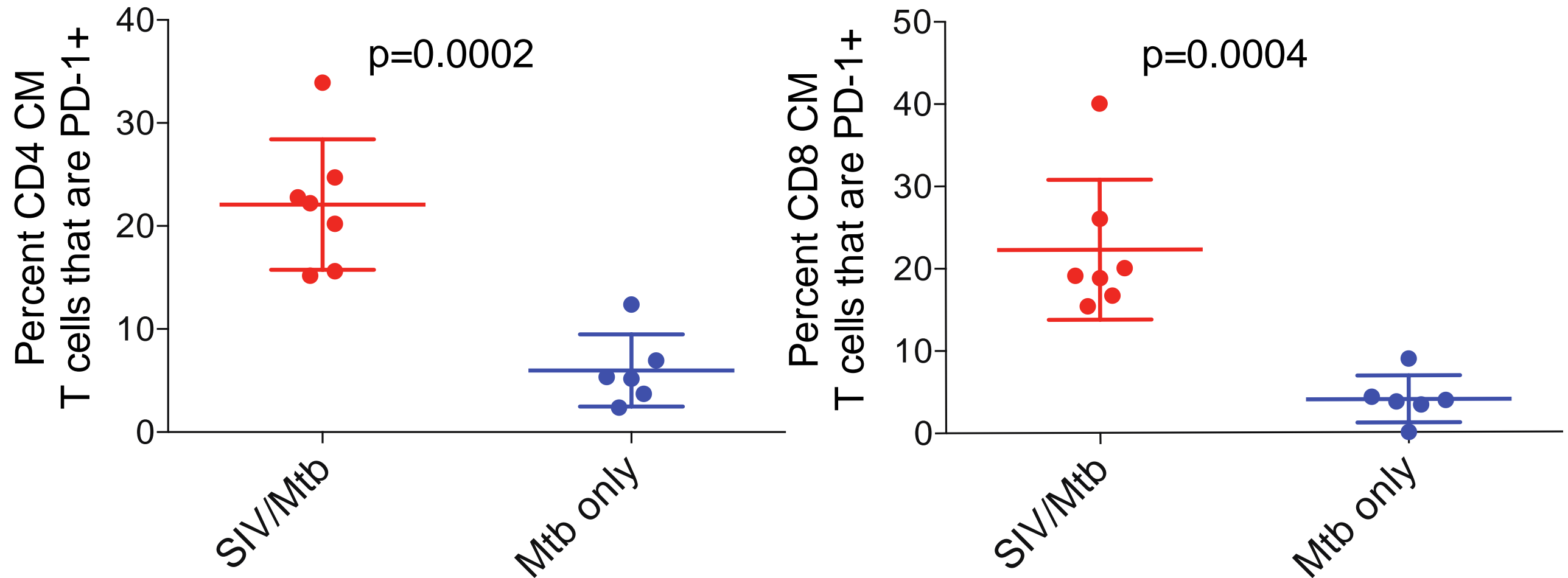


Conventional T cell responses to Mtb



SIV infection is known to lead to hyperactivation

Central memory CD4 and CD8 T cells have higher PD-1 expression in co-infected animals



Final Thoughts

- TB disease can be modeled in macaques infected with a low dose of *M. tuberculosis*
- Macaques develop a spectrum of TB disease that is similar to what is observed in humans
- SIV co-infection exacerbates TB disease
- Future studies in SIV+ and SIV-naïve macaques can be used as a platform for testing TB vaccines

Thank you!

SLO Lab (Current)

Alexis Balgeman

Amy Ellis

Matt Sutton

Ryan Moriarty

Anna Batchenkova

Nadean Kannal



U of Pitt

Charles Scanga

JoAnne Flynn

Mark Rodgers

Erica Larson

Cassy Ameal

Tonilynn Baranowski

Pauline Maiello

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NIH R21AI127127

T32 GM081061

P51 Genetics Services

UW-Madison

