ROLE OF CD8 IN PROGRESSION FROM LATENT TO ACTIVE TB

Disclosures

- David Lewinsohn:
  - OHSU inventor, CD8$^+$ T cell vaccines and diagnostics
  - Viti Inc., CEO, current
- Spouse Deborah Lewinsohn:
  - OHSU inventor, CD8$^+$ T cell vaccines and diagnostics
  - ViTi Inc., President, current
- OHSU and Drs. Deborah and David Lewinsohn have a financial interest in ViTi, a company that may have a commercial interest in the results of this research. This potential individual and institutional conflict of interest has been reviewed and managed by OHSU.
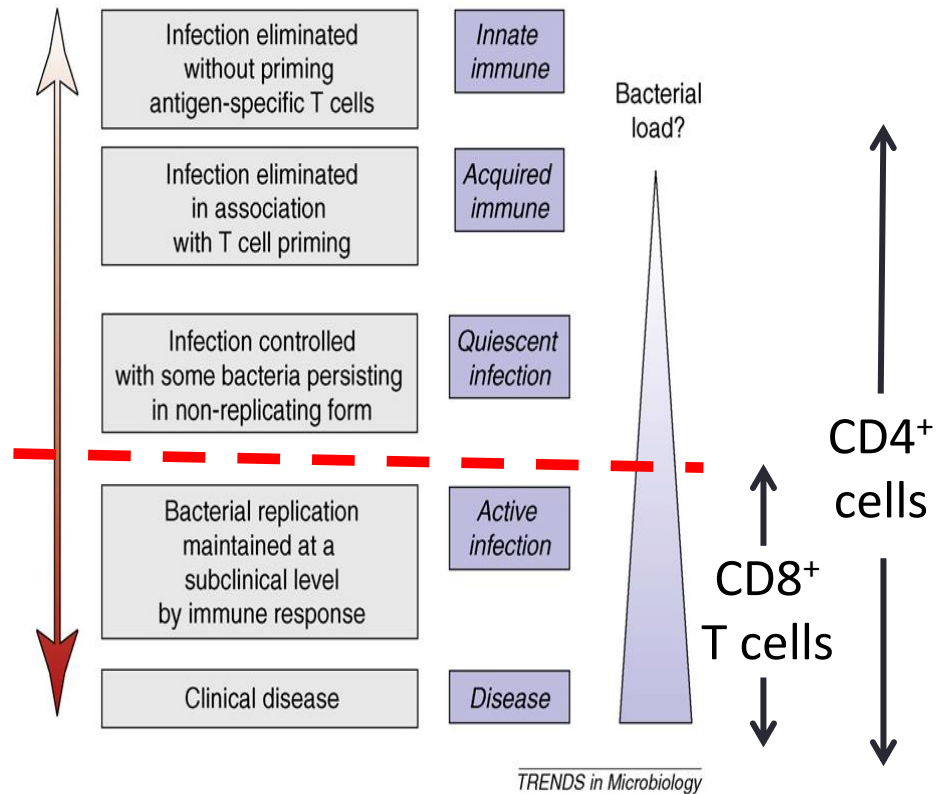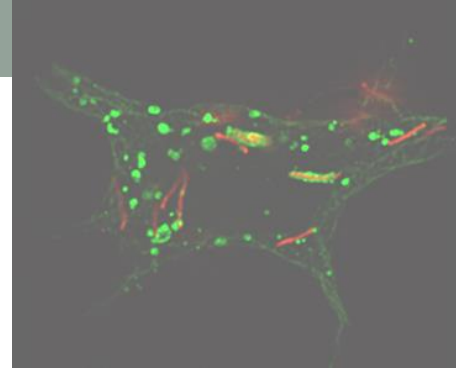
Using the Host Response to Discern Bacterial Burden

The Big Questions:

- Following exposure to Mtb who is at risk for disease?
- During the course of TB Treatment, can we predict who is likely to relapse?
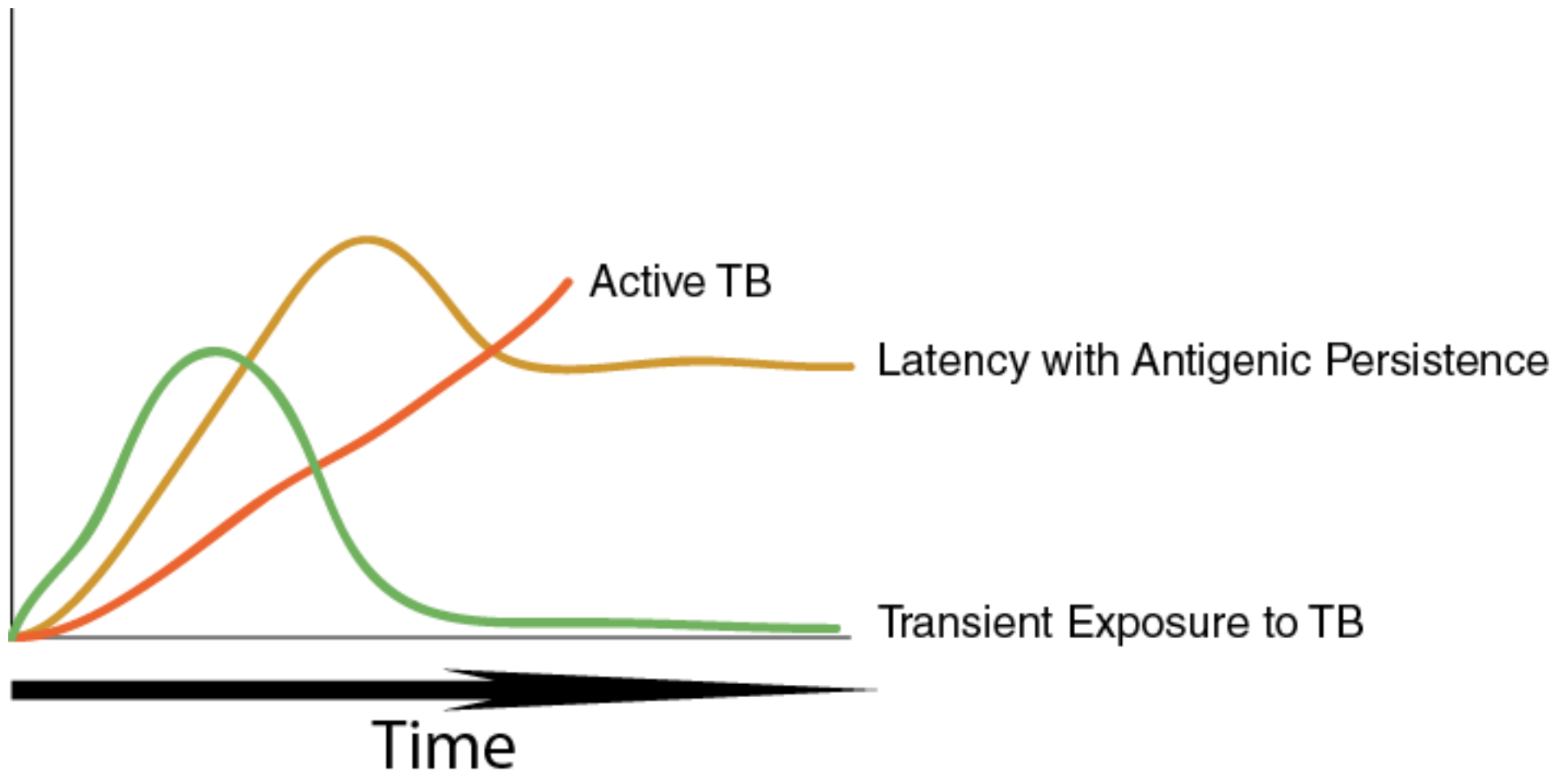- Can measuring the host response help us where bacterial burden is low?

Liklihood of Disease

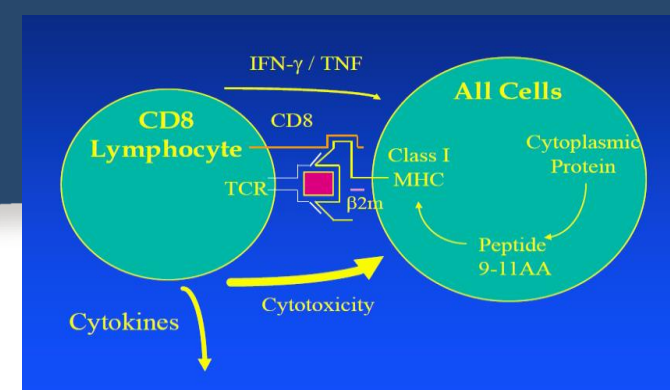Bacterial Burden
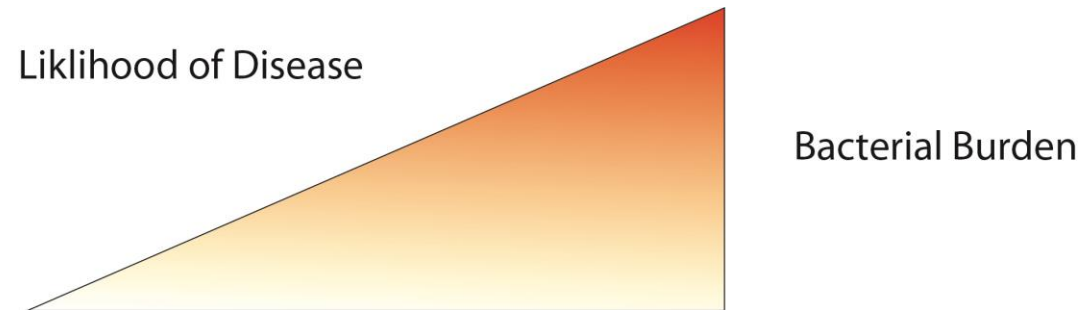
Spectrum of Infection with Mtb

Adapted from Young DB et al.,
Trends Microbiol 2009

Model of Memory: Possible Outcomes

Active TB

Latency with Antigenic Persistence

Transient Exposure to TB
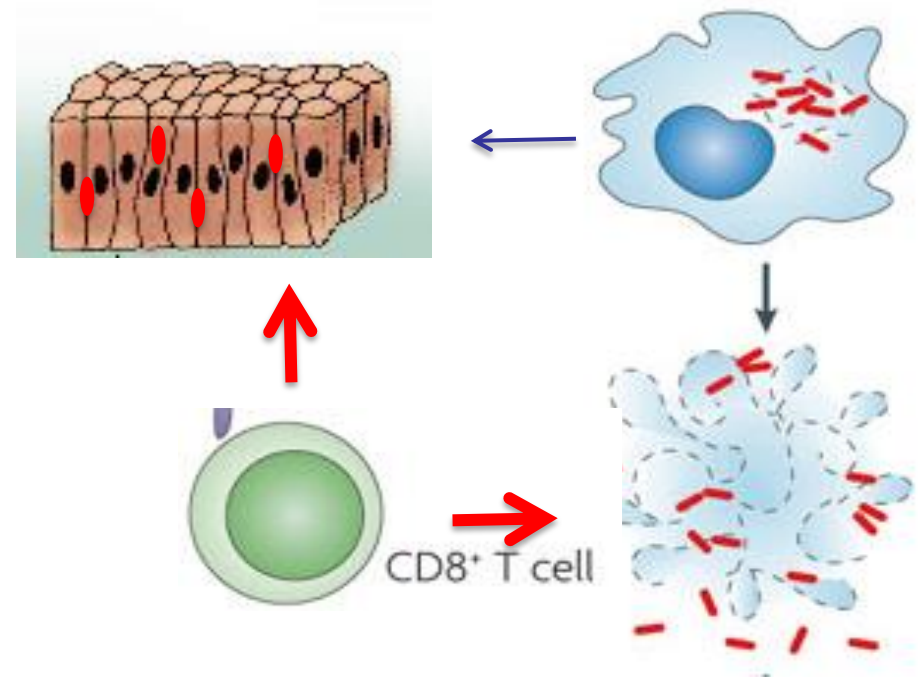
Time

Unique functions of CD8$^+$ T cells

- Recognition of MHC Class II negative cells
- Preferentially recognize heavily infected cells
- Discern bacterial burden

OREGON
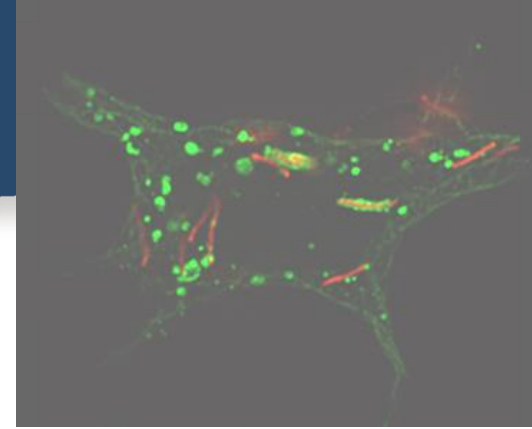HEALTH OHSU
&SCIENCE
UNIVERSITY

Unique functions of CD8⁺ T cells

- Recognition of MHC Class II negative cells
- **Preferentially recognize heavily infected cells**
- Discern bacterial burden

CD8 Clone 23

CD4 Clone 38.1-1

DC control   DC low   DC med   DC hi

Lewinsohn DA et al., AJRCCM, 2003
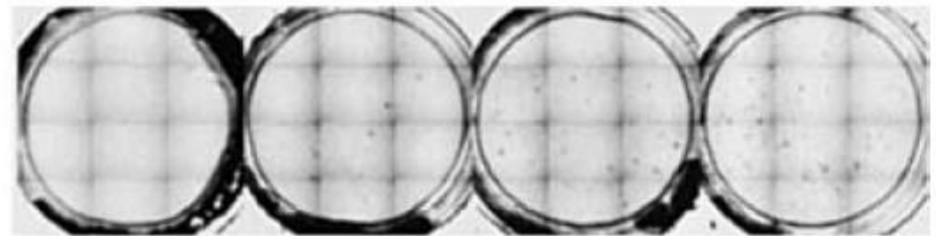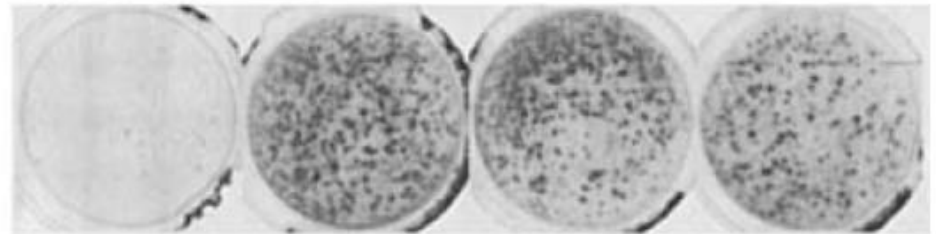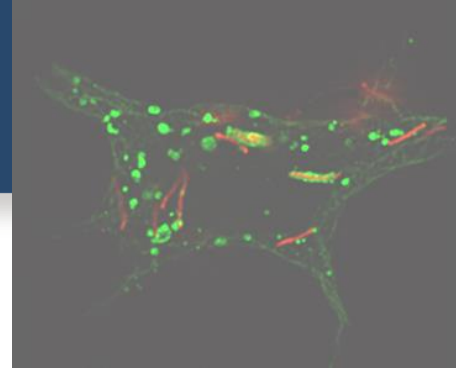
OREGON HEALTH &SCIENCE UNIVERSITY

Unique functions of CD8$^+$ T cells

- Recognition of MHC Class II negative cells
- Preferentially recognize heavily infected cells
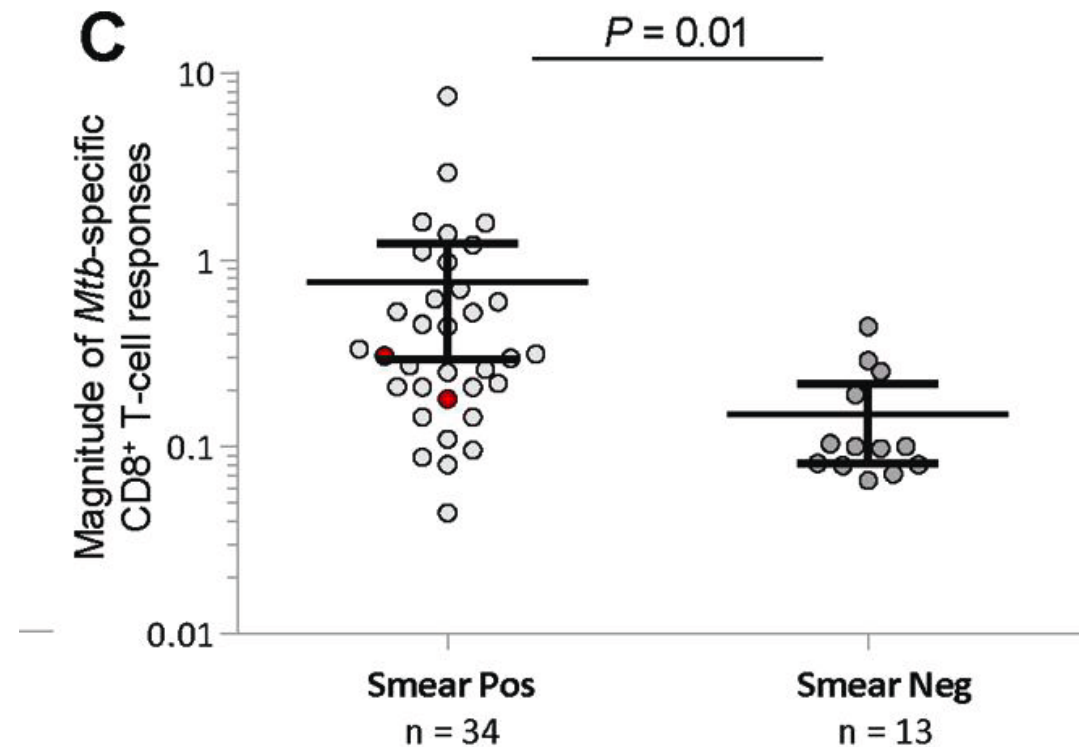- **Discern bacterial burden**
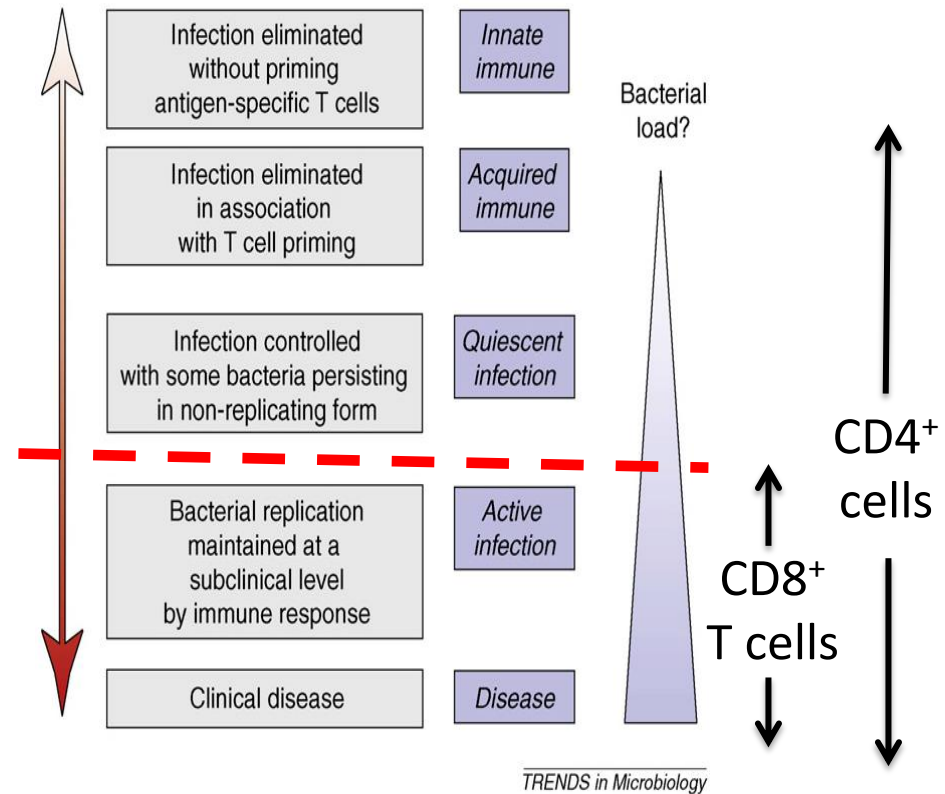
Adapted from Young DB et al., Trends Microbiol 2009

CD8+ T cells: A reflection of antigenic load?

- CD8+ T cells assessed in adults with LTBI or TB disease by ICS/FACS (Lausanne, Switzerland)

- CD8+ T cells found more frequently in PTB [67%] than ETB [37%] than LTBI [15%]

- CD8+ T cells at higher frequency in Smear-positive TB than Smear-negative TB


Rozat et al., EJI, 2013

CD8$^+$ T cells as surrogate for bacterial burden in adults

- NAA2/08-0023 CDC / TBRU Collaboration: Kathleen Eisenach & John Johnson

- Longitudinal study in smear positive, HIV negative adults in Kampala, Uganda, initiating treatment

- Blood drawn for ELISPOT analysis
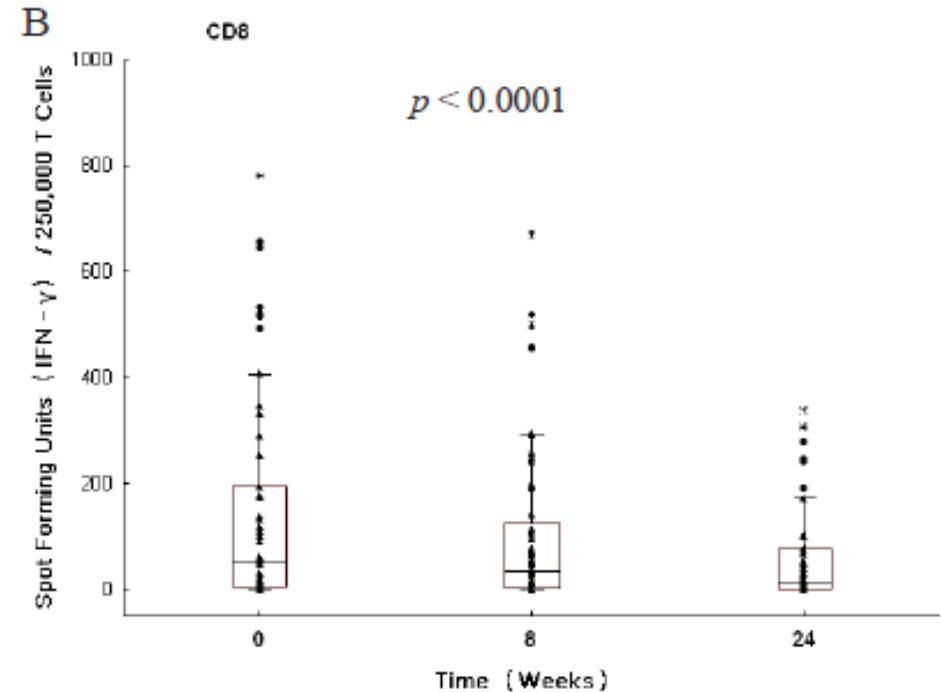
  - Baseline

  - Week 8

  - Week 24

Nyendak M et al, PLoS ONE, 2014

CD8+ T cells as surrogate for bacterial burden in adults

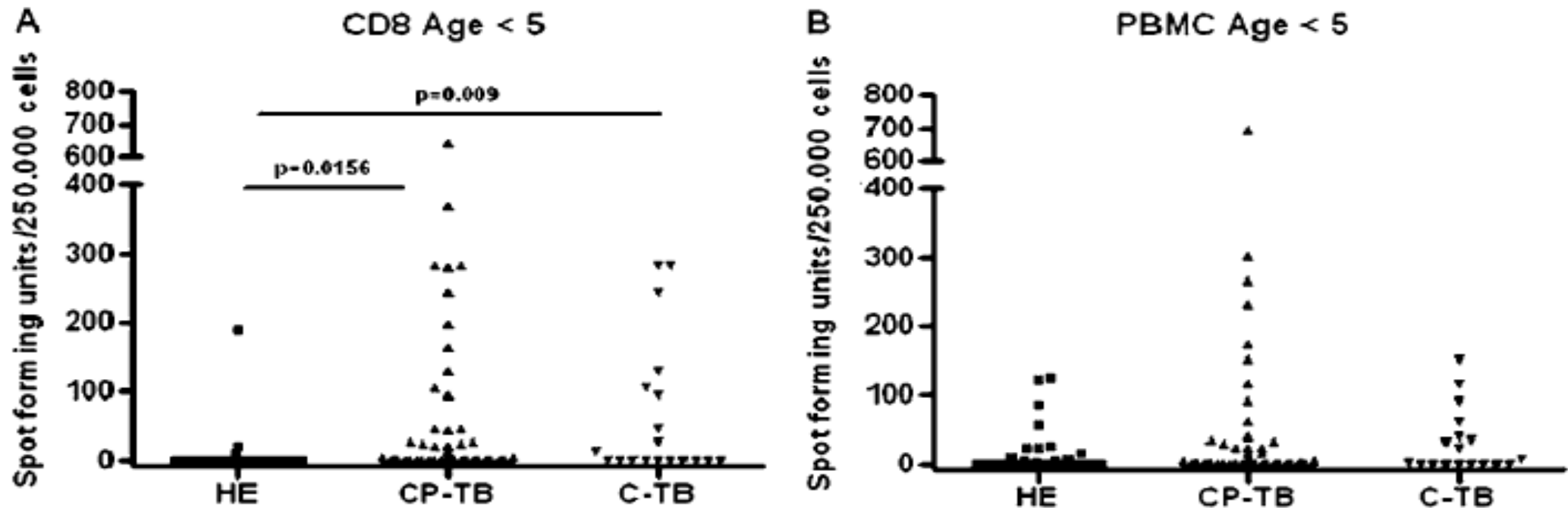**CD4 Response**
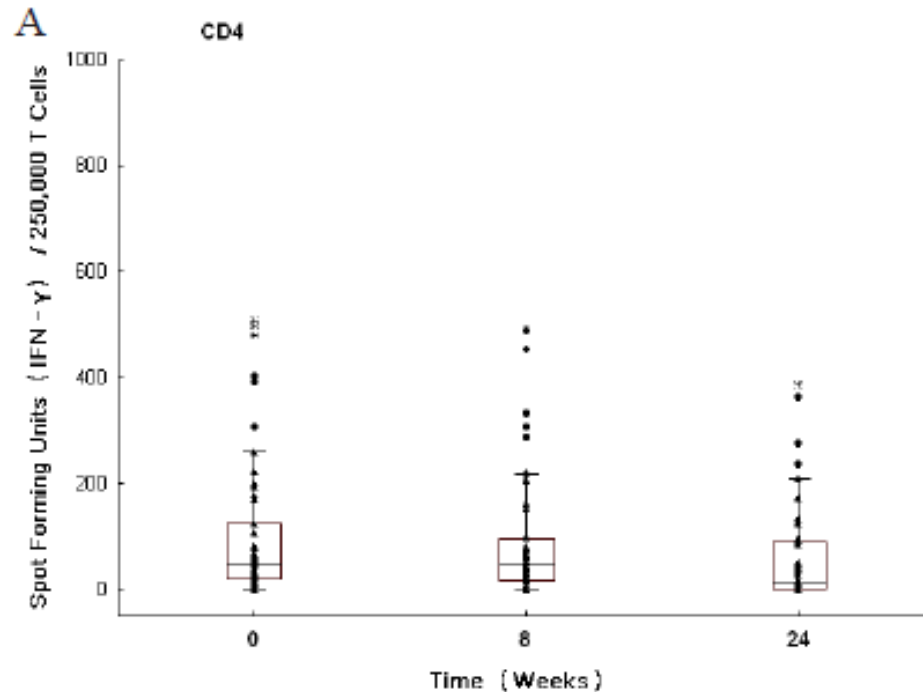
CD8 Response

Nyendak M et al, PLoS ONE, 2014

CD8⁺ T cells as surrogate for bacterial burden in children

- HIV negative Ugandan children <10 yrs hospitalized with pulmonary TB versus healthy household contacts.

- ESAT-6/CFP-10-specific T cells measured by IFN-γ ELISPOT.

- CD8⁺ T cells (PBMC depleted of CD4⁺ T cells) and whole PBMC measured.

Lancioni et al, AJRCCM, 2012

CD8⁺ T cells as surrogate for bacterial burden in children

Lancioni et al, AJRCCM, 2012

- Define Immunodominant HLA-Ia- and HLA-Ib- restricted Mtb Antigens
  – Lewinsohn Lab(s)
  – Karen Dobos, CSU
  – Dave Sherman, Seattle Biomed
  – William Hildebrand, UOHSC
  – Avigdor Shafferman, IIBR
- Define Clinical Utility
  – Henry Boom, TBRU, CWRU
  – Harriett Myanja & Sarah Kiguli, Makerere University, Kampala, Uganda
- Define Vaccine Utility
  – Helen McShane & Elena Sytlianou, Oxford University

OREGON
HEALTH
&SCIENCE
UNIVERSITY
OHSU

Mtb Genome
4011 genes
331,000 peptides

M. tuberculosis
H37Rv
4,411,529 bp

Known Antigens
"Esat-6 Like"
Composite Evidenced-based Score
"Secreted"
TubercuList Functional Score

H37Rv
411,529 bp
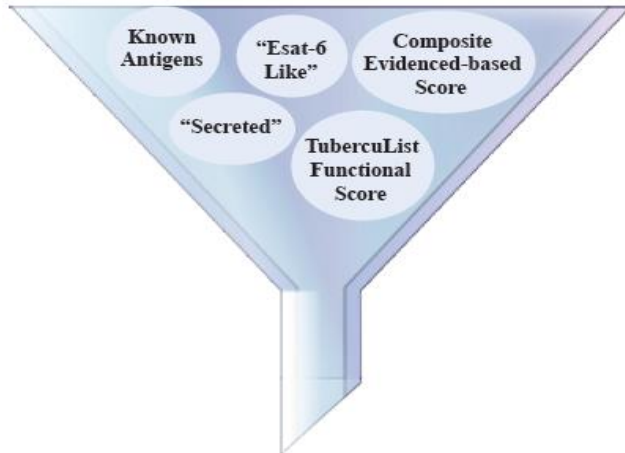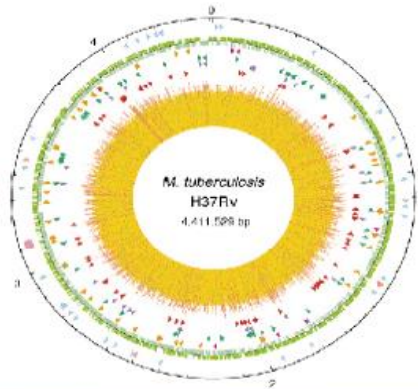
Genomic Peptide Library
389 Genes
38,989 peptides

Mtb HN878 genome (Beijing strain)
3922 ORFs

~42,000,000 potential 9, 10, 11-mer binders to 12 HLA supertypes
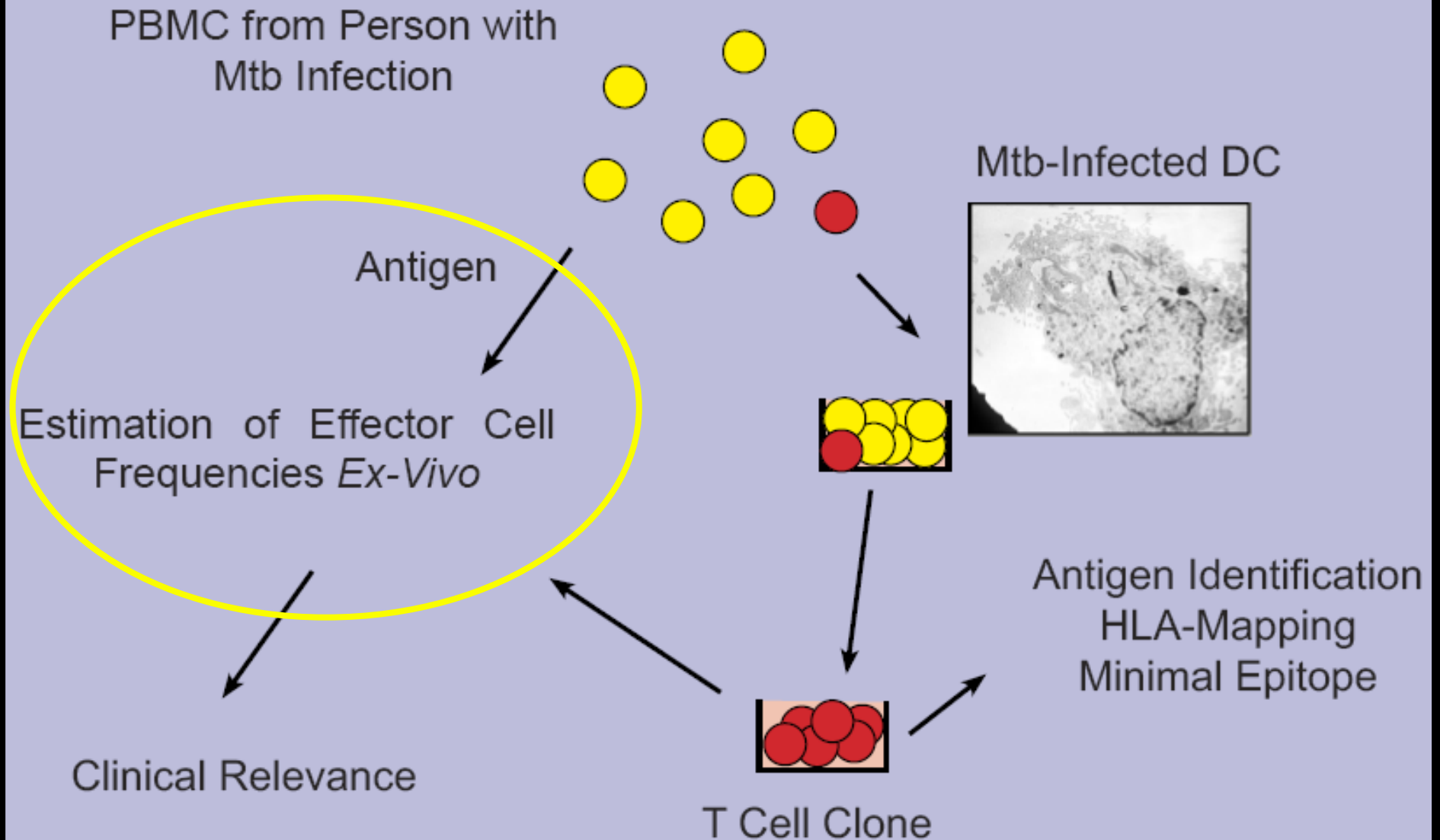
- *Median binding affinity* of predicted binders in the cluster
- *Density* of predicted binders in the cluster

CTL PEPTIDE LIBRARY

39,000 15-mer peptides
68,713 putative CTL binders

80% representation of the genome ORFs
> 95% high affinity epitopes

OREGON
HEALTH & SCIENCE
UNIVERSITY
OHSU

T Cell Based Identification of CD8 Antigens

PBMC from Person with
Mtb Infection

Antigen

Estimation of Effector Cell
Frequencies *Ex-Vivo*

Clinical Relevance

Mtb-Infected DC

Antigen Identification
HLA-Mapping
Minimal Epitope

T Cell Clone

Definition of CD8 Antigens Using Pooled Peptides

Subjects
 1/2 Pheresis / screen
 15 LTBI
 5 Active

CD8 T Cells

3×10^9 PBMC

4.5×10^8 CD8

Dendritic Cells

3×10^8 PBMC 3.6×10^7 DC

ELISPOT Assay

250,000 CD8 T cells / well
20,000 DC / well
Duplicate wells

Peptide Library
 50 peptides / well
 5 mcg / peptide
 Solid phase synthesis
  (Jerini)
Controls
 Media (7 wells)
 PHA
 HIV Gag

Ex-Vivo Determination of Frequency
• Commonly recognized
• Strongly recognized

Ex vivo T Cell Screens of Mtb-infected Donors (target n=20)

Caucasian (PPD+)

- ☑ D525
- ☑ D557
- ☑ D454
- ☑ D467
- ☑ D603
- ☑ D694

African American (PPD+)

- D584
- ☑ D627
- ☑ D632
- ☑ D634
- ☑

SE Asian (PPD+)

- ☑ D527
- ☑ D545
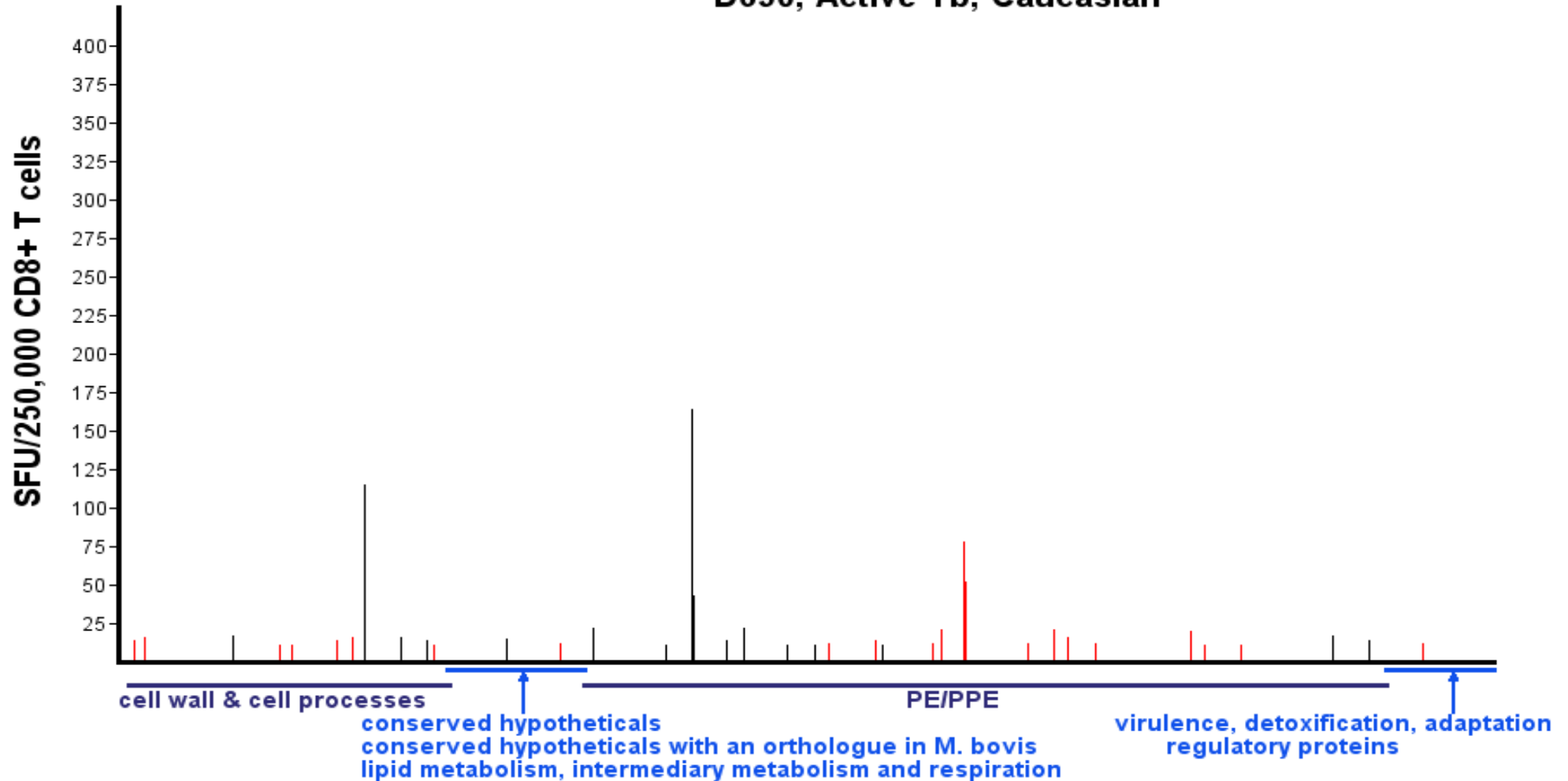- ☑ D564
- ☑ D608
- ☑ D610

ACTIVE TB

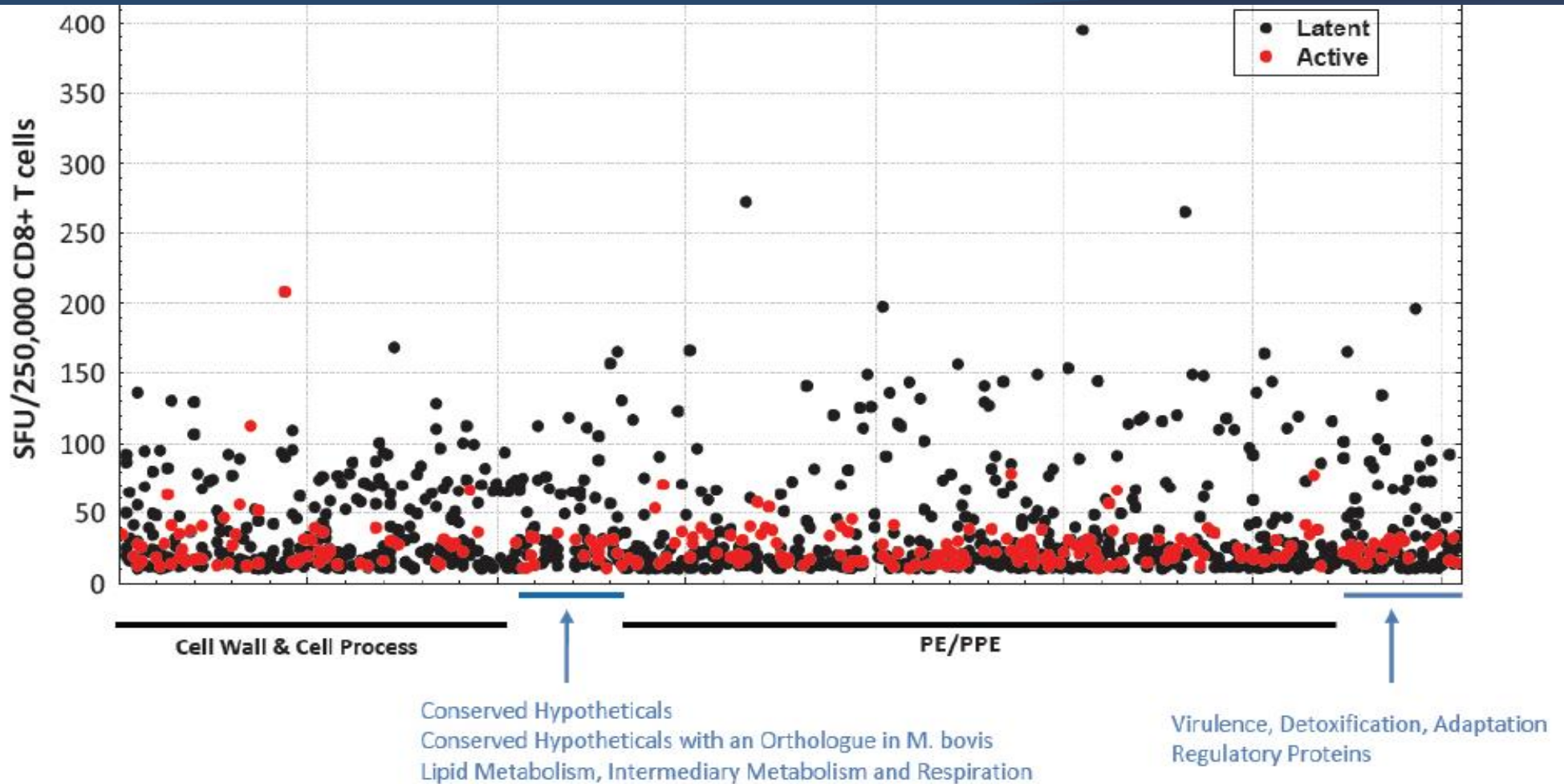- ☑ D560
- ☑ D635
- ☑ D690
- D691
- ☑ D695
- ☑

D690

D690, Active Tb, Caucasian

Summary of all ex vivo donor screens (Top 5%)

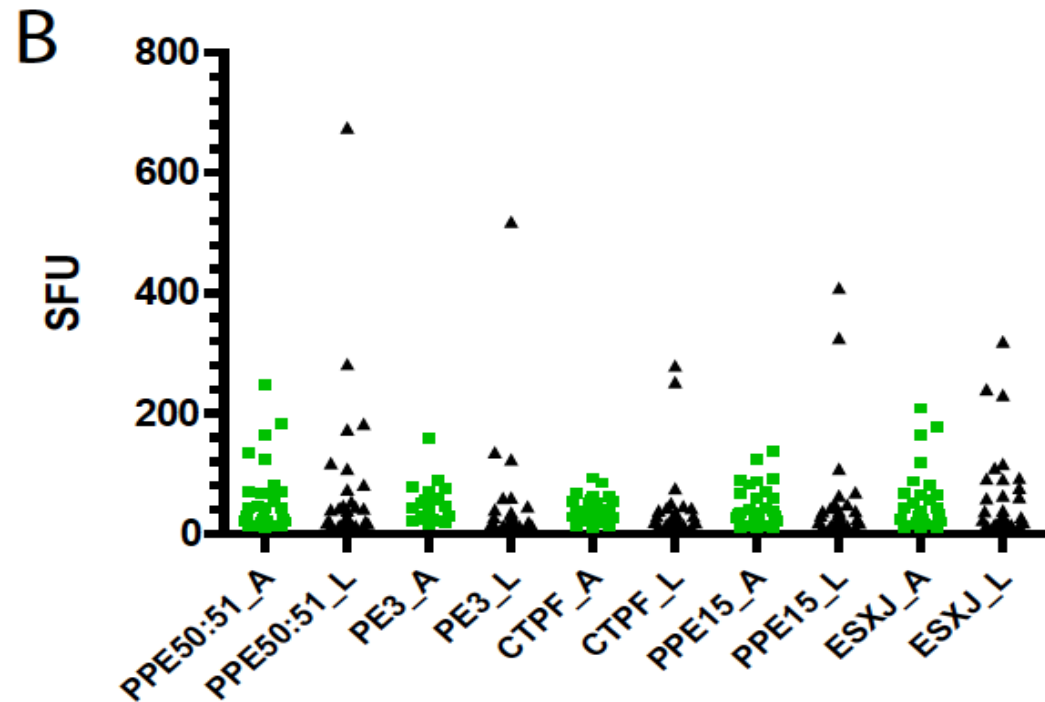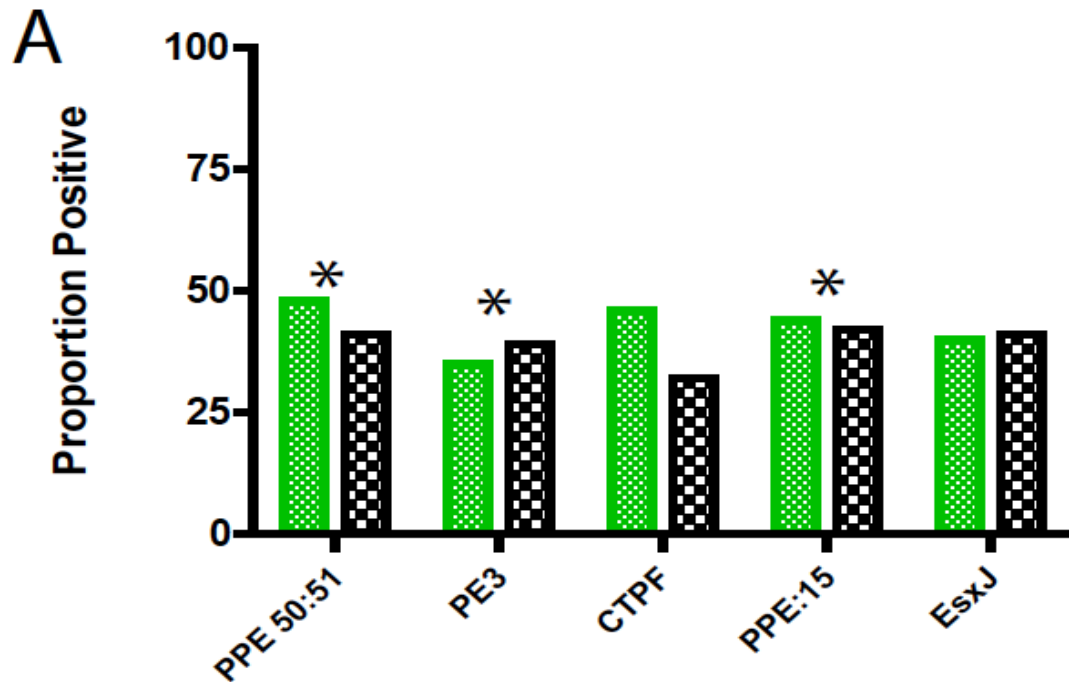# Clinical screening & validation
Kampala, Uganda

- ## TBRU Kawempe Community Health Study
  - Identification of commonly recognized antigens
    - 10 Active / 10 LTB I
  - Identification of disease specificity
    - 50 Active / 50 LTBI

Validation Summary
Immunodominant Peptide Pools
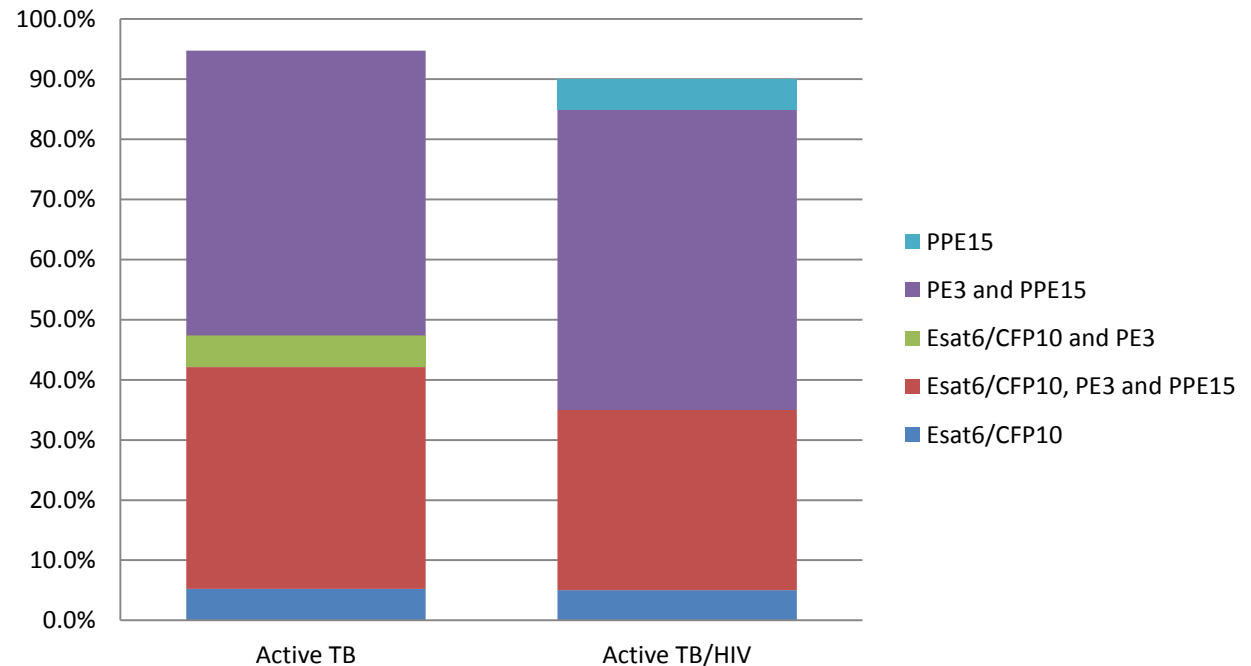### Top 5% for Any Donor
At Least Three donors

* Under evaluation per Elena Stylianou and Helen McShane

CD8 antigen combinations

- CD8^{+} T cell responses in adults with TB disease (Kampala, Uganda)
- HIV positive (*n*=20) & HIV negative (*n*=20)

% of Donors positive by antigen (CD8+ T cells, IFN-gamma)

Legend:
- PPE15
- PE3 and PPE15
- Esat6/CFP10 and PE3
- Esat6/CFP10, PE3 and PPE15
- Esat6/CFP10

Role of CD8⁺ T cells in the diagnosis of TB

- Recognition of MHC Class II negative cells
- Preferentially recognize heavily infected cells
- Discern bacterial burden
  - Specific to Mtb-infected individuals.
  - Positive correlation with degree of TB disease
  - Decrease with effective TB treatment
  - Additional sensitivity for TB disease with multiple TB antigens.
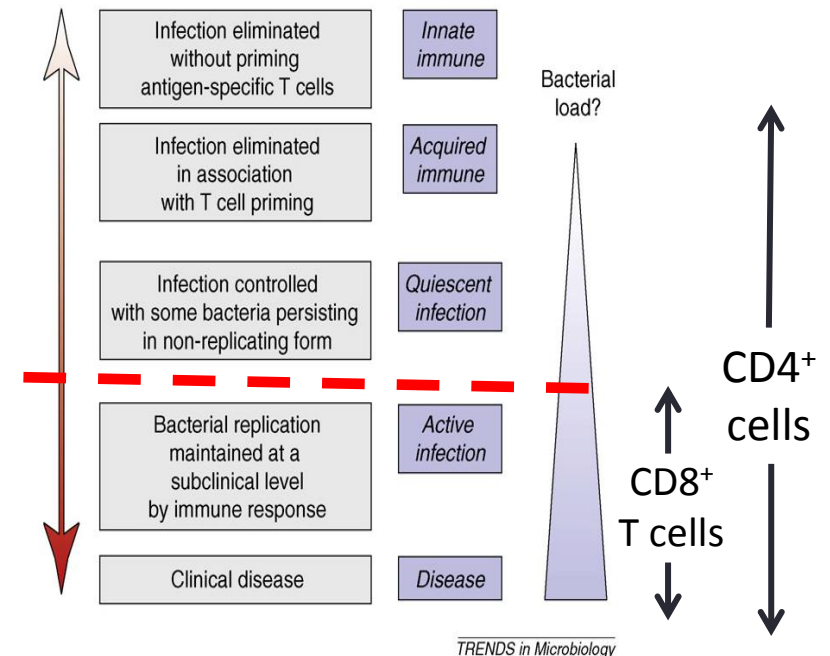
Adapted from Young DB et al.,
Trends Microbiol 2009

Acknowledgements