

# **The Quest for TB Biomarkers Discovery: the Journey from the Bench to the Bush; Introducing the Validation process**

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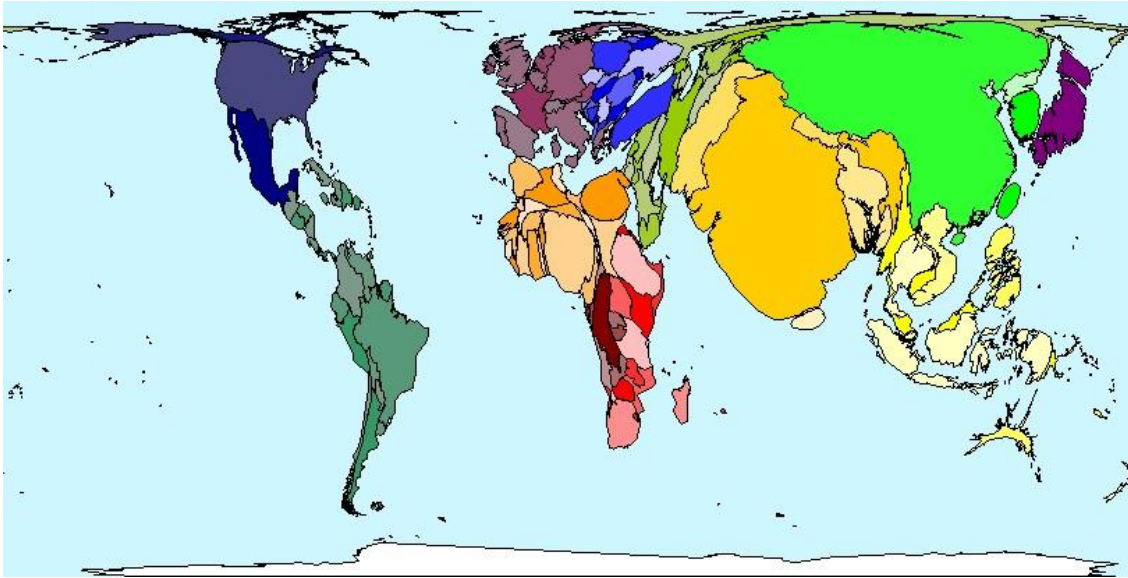
**TB Diagnostic Research: Beyond Basics**  
**TRC, Chennai, December 14, 2010**

# **Disclaimer: Shreemanta K Parida**

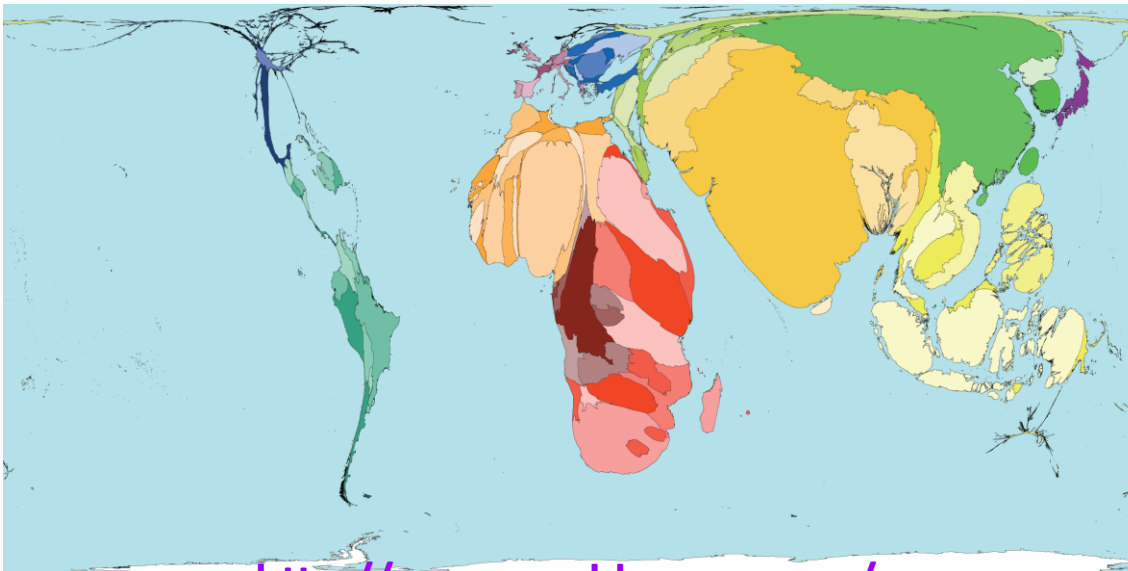
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# Worldmapper Cartographs

(Gastner and Newman, PNAS 2004 Diffusion-based method for producing density-equalizing maps)



**Total Global population:**  
The size of each territory shows the relative proportion of the world's population living there.

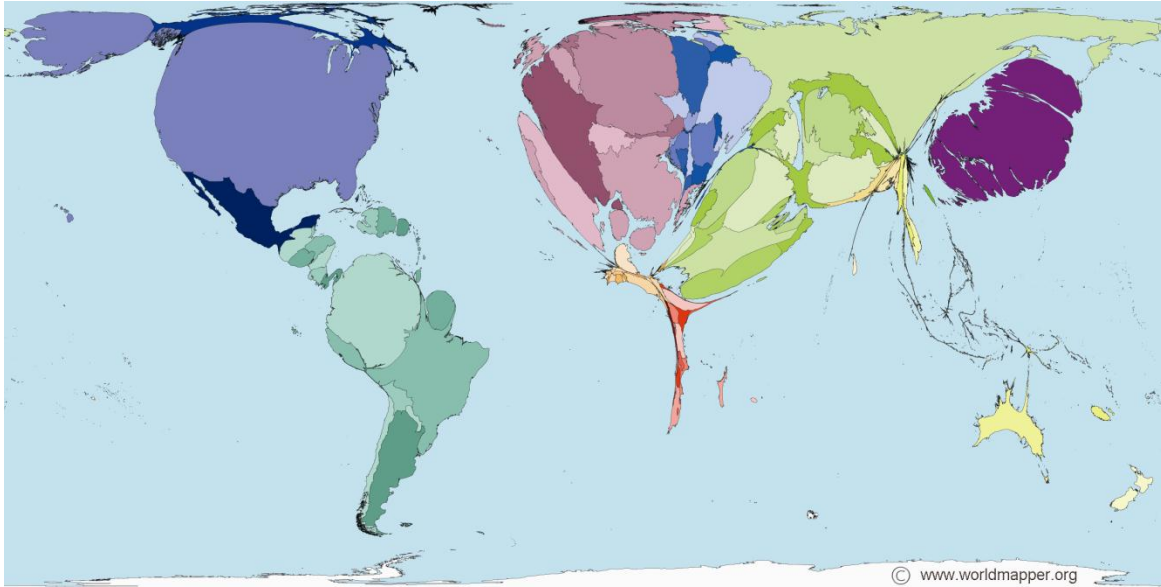


**Global TB cases:**  
Territory size shows the proportion of worldwide TB cases found there.

<http://www.worldmapper.org/>

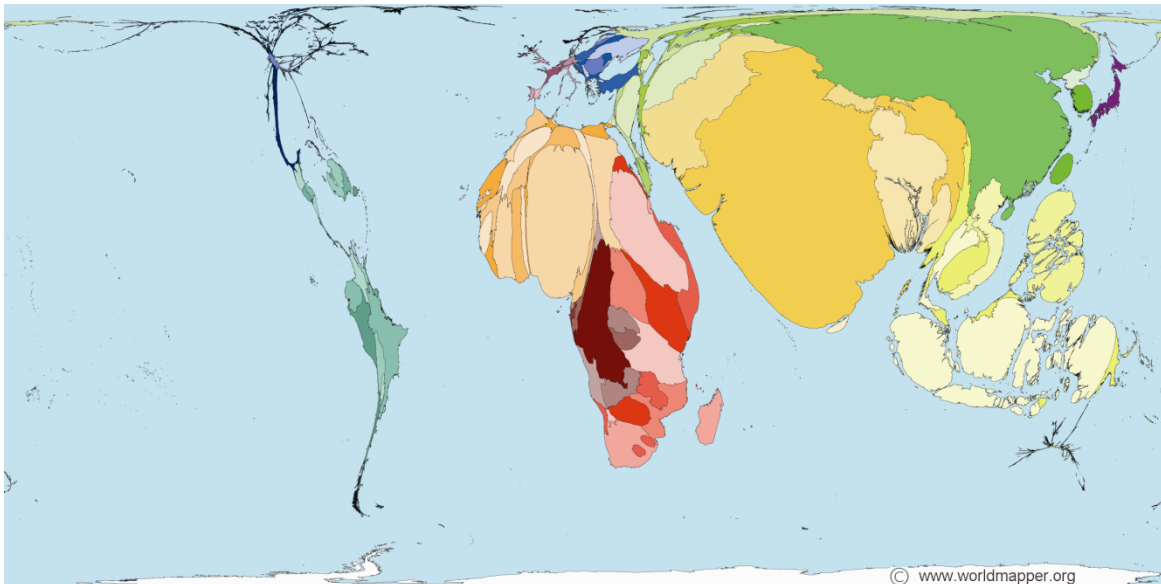
# Worldmapper Cartographs

(Gastner and Newman, PNAS 2004 Diffusion-based method for producing density-equalizing maps)



## Influenza Outbreaks:

Territory size shows the proportion of people worldwide living where there is an influenza outbreak, per week, between 2000 and 2005.

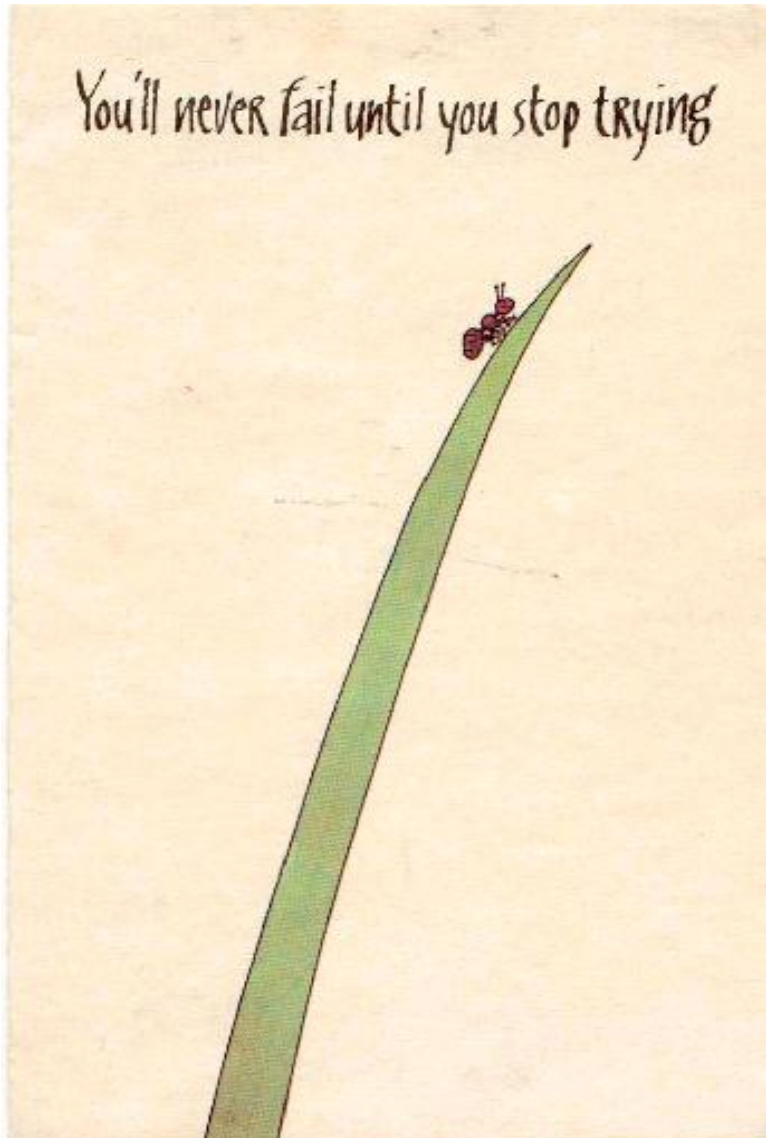


## Global TB deaths:

Territories are sized in proportion to the absolute number of people who died from tuberculosis in one year.

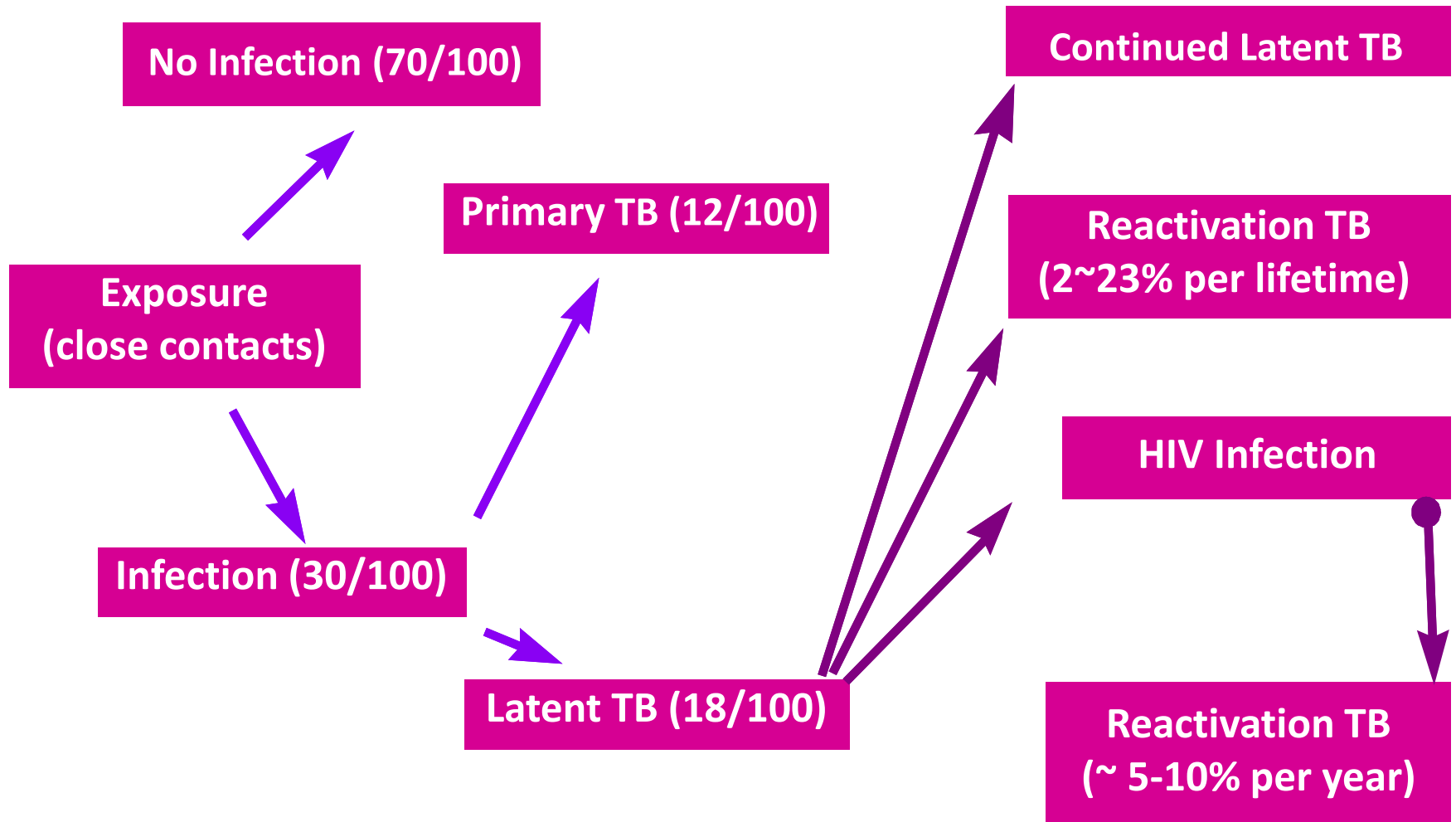
<http://www.worldmapper.org/>

# Tools to persist and conquer....!!!



All great undertakings  
are achieved through  
mighty obstacles.

# Outcome associated with exposure to *Mycobacterium tuberculosis*



Adapted from Parrish et al, 1998

# Grand Challenges in Global Health



Max-Planck-Institut  
für Infektionsbiologie



MAX-PLANCK-GESellschaft

**Biomarkers of protective  
immunity against TB in the  
context of HIV/AIDS in Africa**

**MPIIB (Coordinator)**  
**Berlin, Germany**

**LUMC**  
**Leiden, The Netherlands**

**SSI**  
**Copenhagen, Denmark**

**STANFORD UNIVERSITY**  
**Stanford, USA**

**MRC**  
**Banjul, The Gambia**

**EHNRI**  
**Addis Ababa, Ethiopia**

**UMCU**  
**Utrecht, The Netherlands**

**AHRI**  
**Addis Ababa, Ethiopia**

**MAK**  
**Kampala, Uganda**

**KPS**  
**Karonga, Malawi**

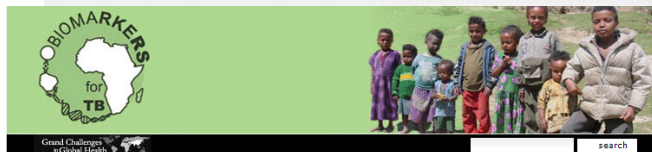
**CWRU/TBRU**  
**Cleveland, USA**

**AERAS**  
**Rockville, USA**

**LSHTM**  
**London, UK**

**SUN**  
**Tygerberg, South Africa**

**UCT/SATVI**  
**Cape Town, South Africa**



you are here: home



Home

## The Grand Challenges in Global Health

The Grand Challenges in Global Health initiative was launched in 2003 by the Bill & Melinda Gates Foundation, in partnership with the National Institutes of Health, to harness the power of science and technology to dramatically improve health in the world's poorest countries.

<http://www.biomarkers-for-TB.net>

<https://gc6.biomarkers-for-tb.net>

# Definitions

**BIOMARKER(S):** Characteristic(s) that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes or physiological/pharmacological responses to an intervention.

**CORRELATES OF PROTECTION:** Measurable sign(s) in a host in response to an infectious agent indicating whether the individual is being protected against becoming infected and/or developing disease.

**SURROGATES OF PROTECTION:** Validated markers of correlates of protection.

**CLINICAL ENDPOINT:** Characteristic or variable that reflects the final outcome of disease in terms of function, effect, progress, recovery, survival or death.

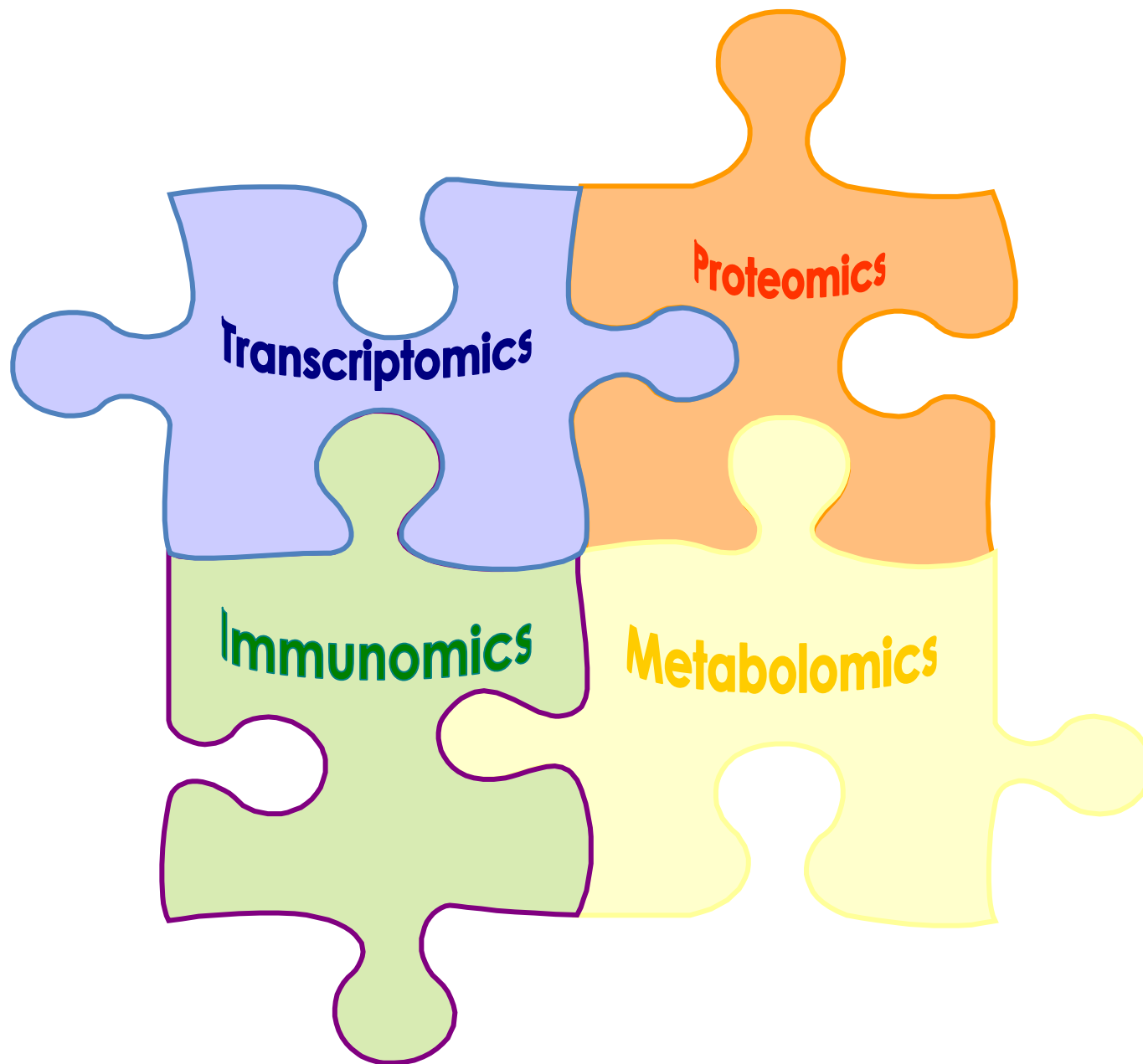
**SURROGATE ENDPOINT:** Biomarker that is intended to substitute for a clinical endpoint, predicting clinical outcome in terms of benefit, or harm or lack of benefit or harm.

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## Biomarkers in TB

- Differences in the immune response between individuals exposed to TB and protected from the disease to those who develops active disease.
- Particular attention on people coinfectd with both *Mtb* and HIV with or without ART.
- Harness design and testing of new TB vaccines, drugs and diagnostics, especially in areas with high HIV infection rates.

[www.biomarkers-for-TB.net](http://www.biomarkers-for-TB.net)



## Types of Markers



### ◆ Immunologic

Selection of antigens

### ◆ Transcriptomics

RNA

100,000 transcripts

Differentially expressed genes which distinguish latent infection from active TB

### ◆ Proteomics

Proteins

1,000,000 proteins

Differentially expressed proteins which distinguish latent infection from active TB

### ◆ Metabolomics

Biochemicals

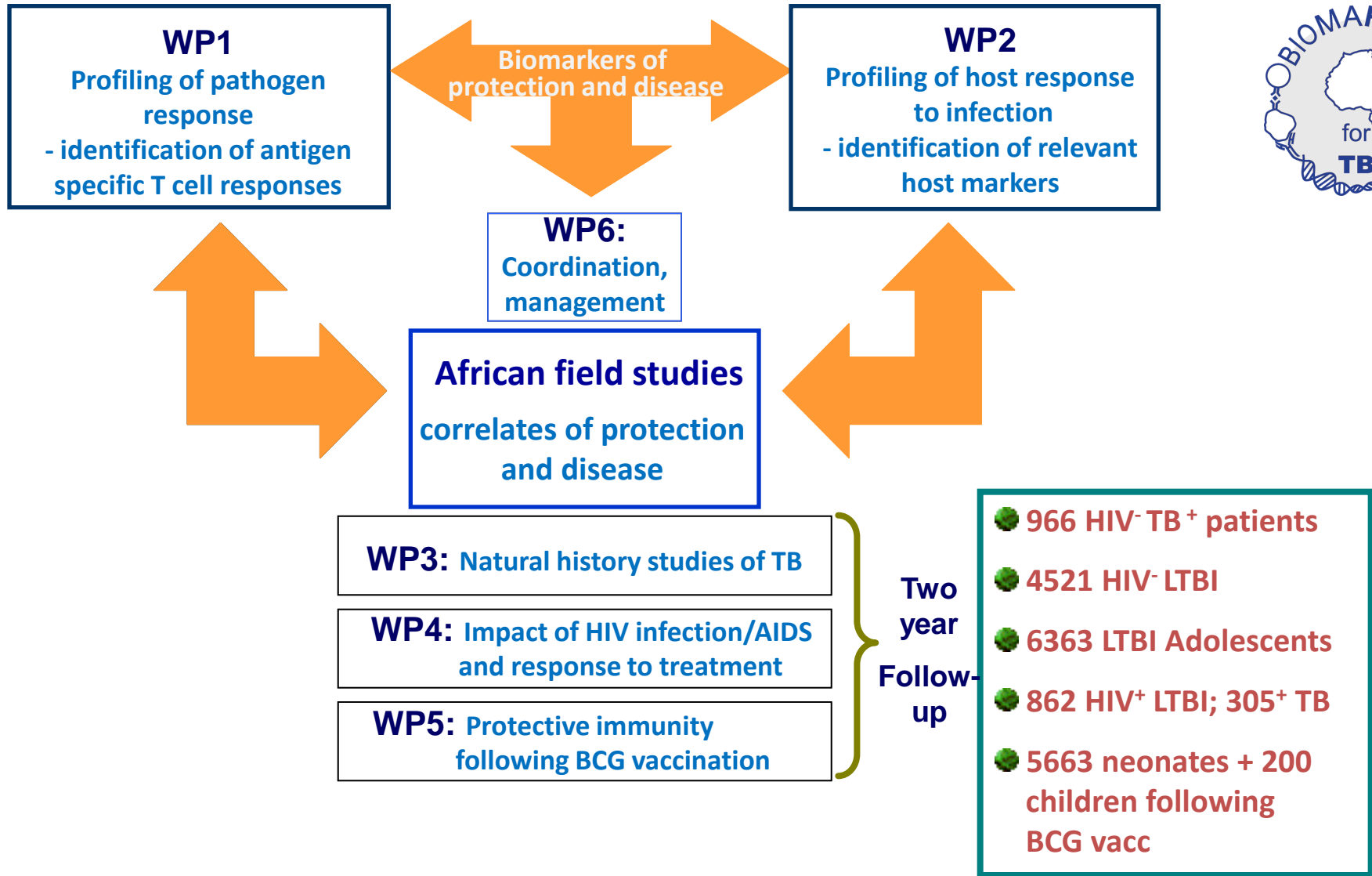
2,400 compounds

Metabolites which distinguish latent infection from active TB



**Combinations**

# Biomarkers of protective immunity and surrogate markers of TB disease in Africa



## WP 3: Natural protective immunity against TB

HIV -ve newly diagnosed Pulmonary TB patients

Household contacts

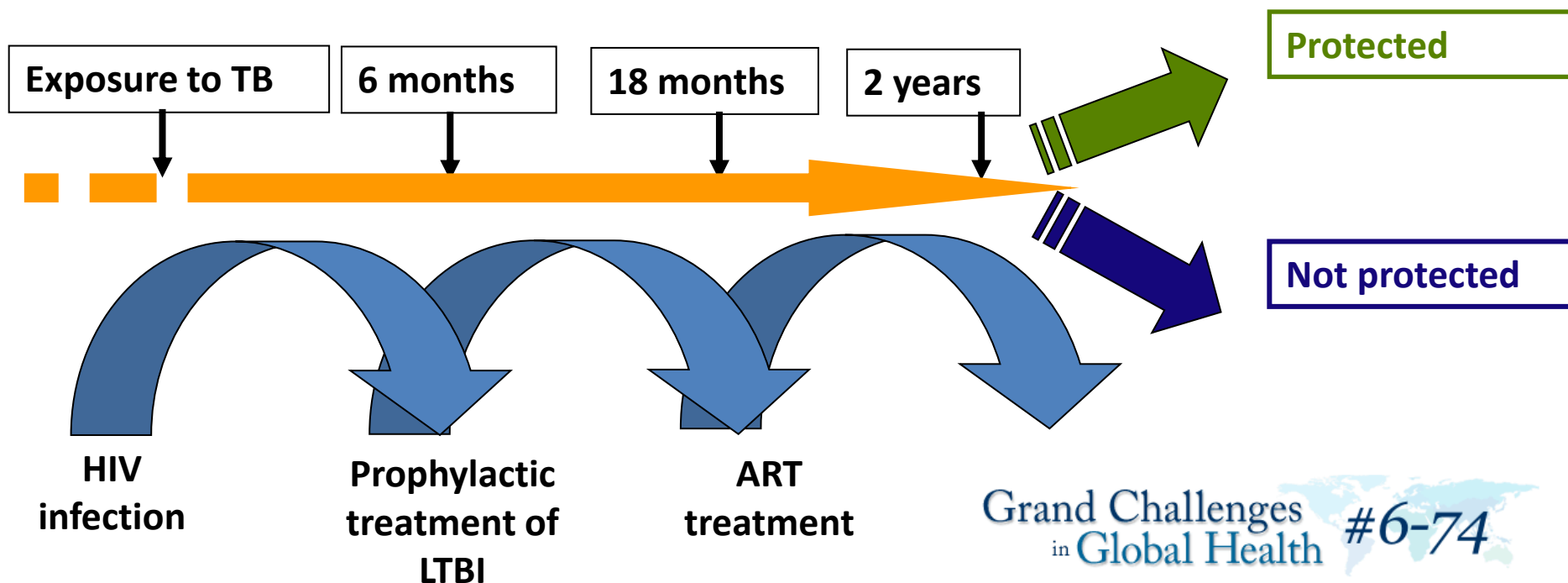


## WP4: Impact of HIV-1/AIDS and response to treatment on immunity against TB

HIV +ve individuals



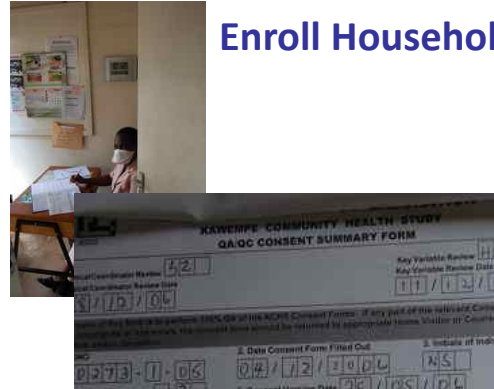
- Immunological markers
- Diagnosis
- Phenotyping



## Diagnose and Rx Index Case



## Counsel and Enroll Household



## Evaluate Household



## F/U Evaluations

### TST



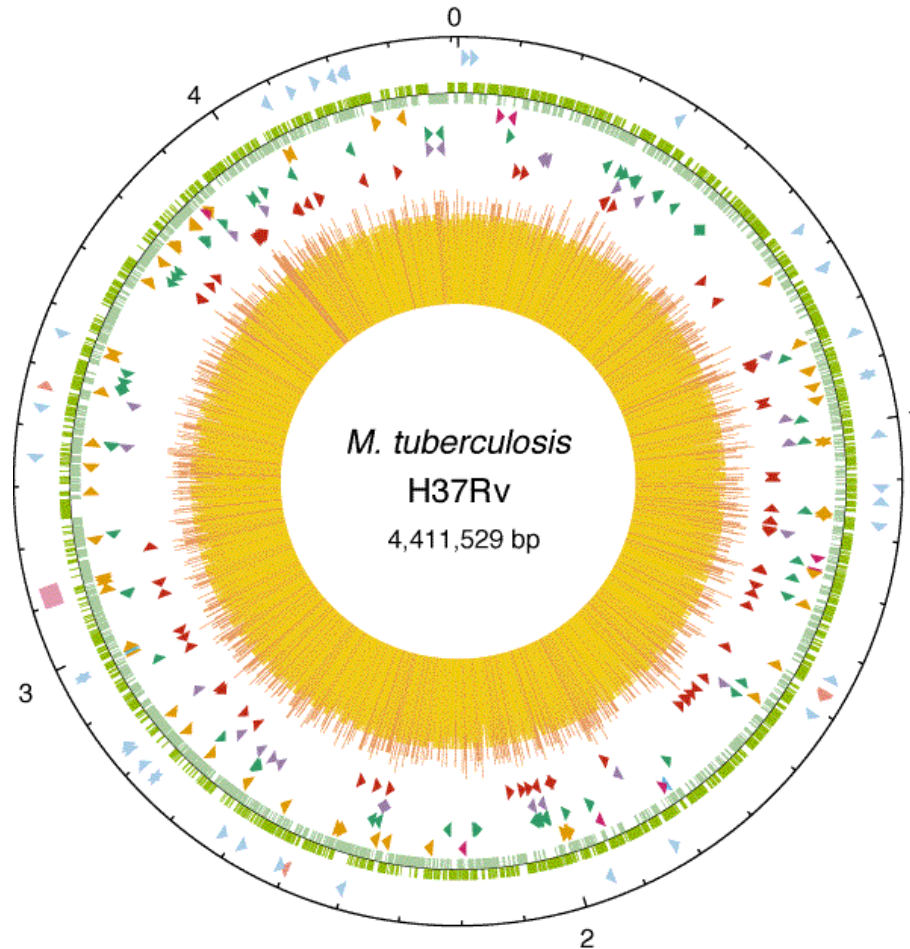
### Blood for Biomarkers



## Data Management



# *Mtb* genome



~4,000 ORFs

GC rich

Cole et al, Nature 1998

## Dormancy: Non-replicating Persistent (NRP) state

- hypoxic stress
- nitrosative stress
- pH stress
- nutrient shift
- Alpha crystallin(acr)hspX: Rv2031c
- Dormancy survival Regulator (DosR): Rv3133c
- Fused nitrate reductase (narX): Rv1736v
- Nitrate/nitrite transporter (narK2): Rv1737c
- Isocitrate lyase (icl): Rv 0467

Enduring Hypoxic Response (EHR)  
genes: 230

Rustad et al, Cell Micro 2009



## Production of recombinant proteins

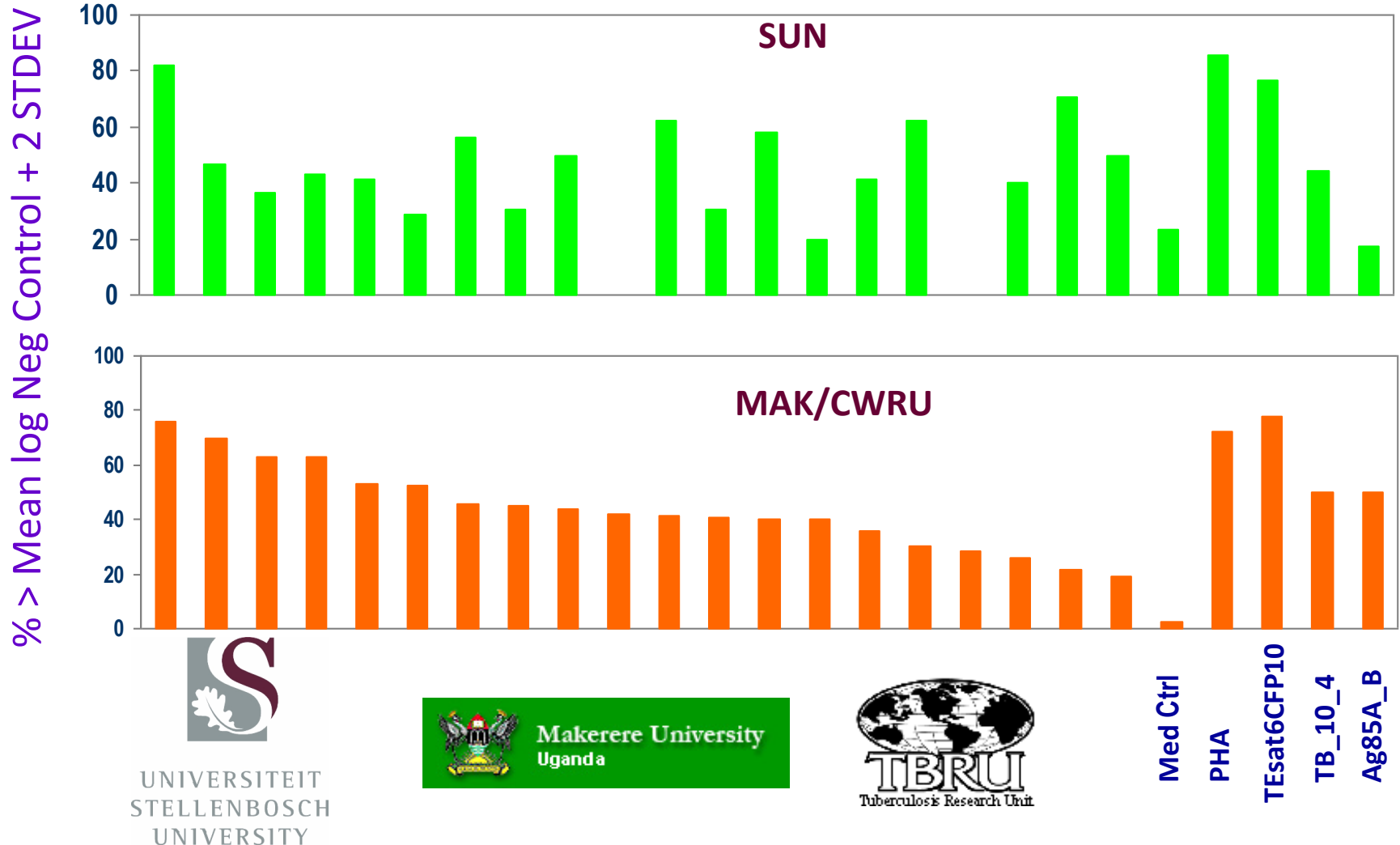
- panel of 86 TB antigens for screening  
(0.5-5 mg)

- set of ~20 TB antigens for cohort studies  
(~150mg+)

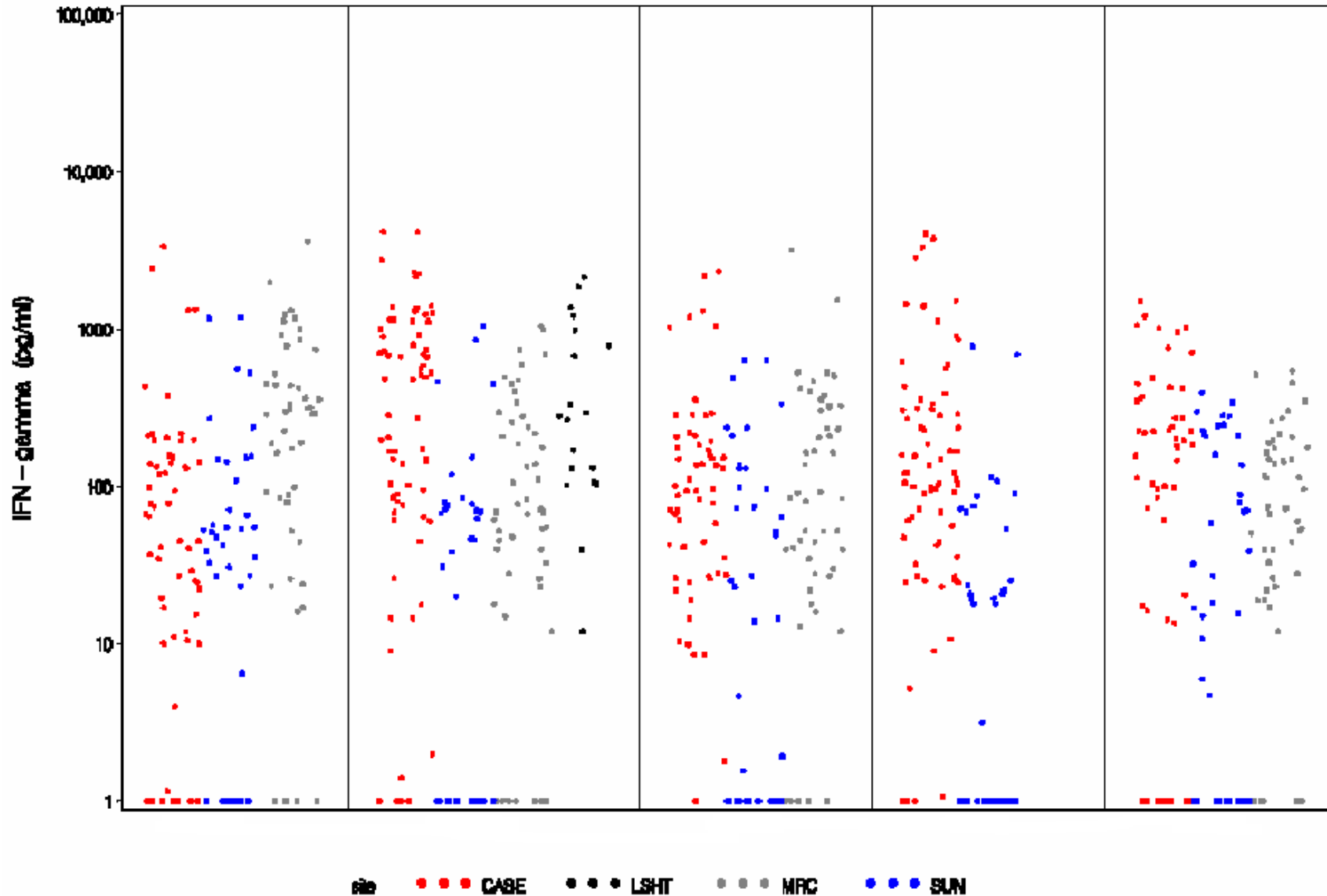
- QC of antigen batches

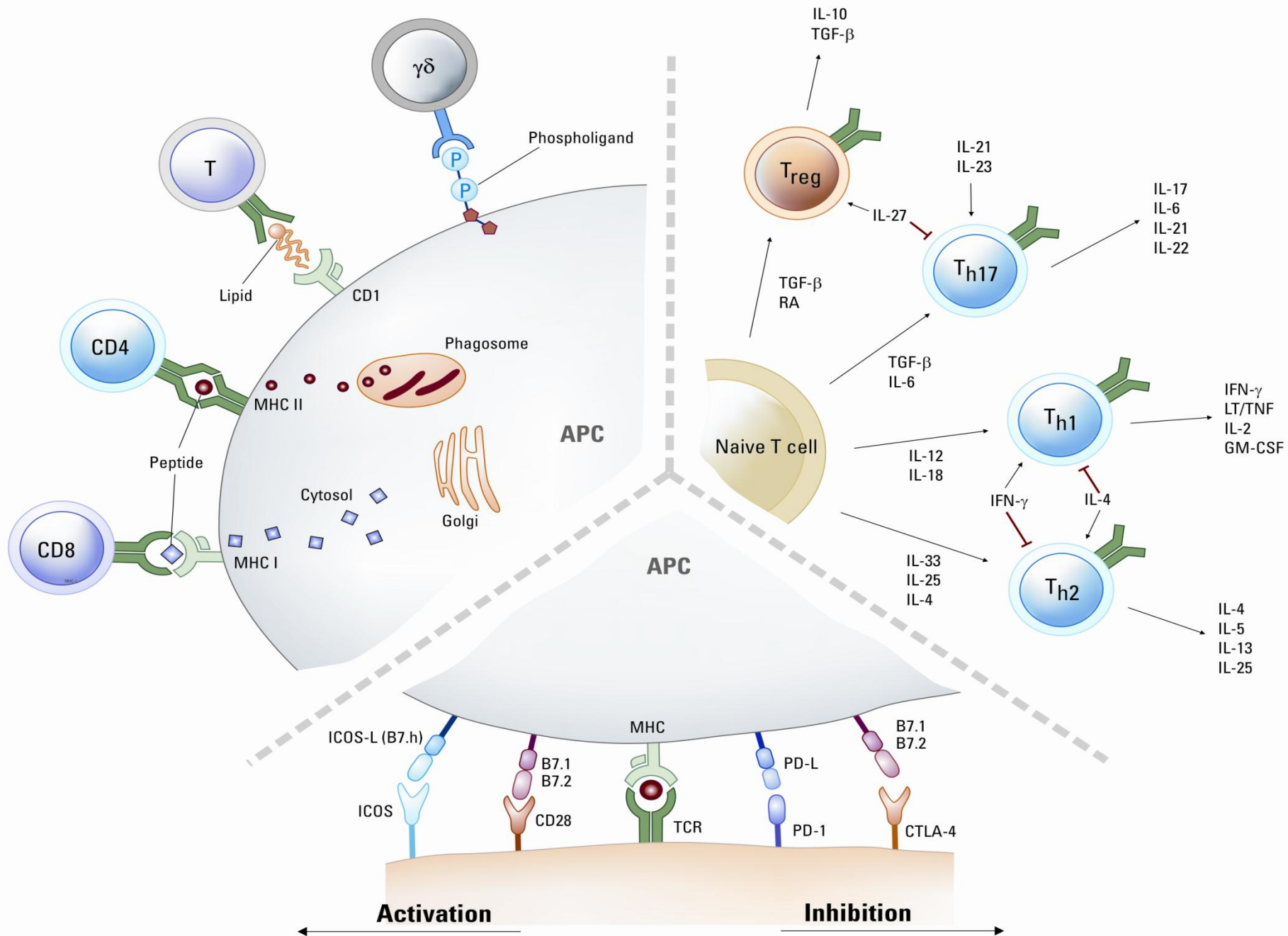


# Immunogenicity screening of 86 TB antigens by 6-day whole blood culture (IFN $\gamma$ )

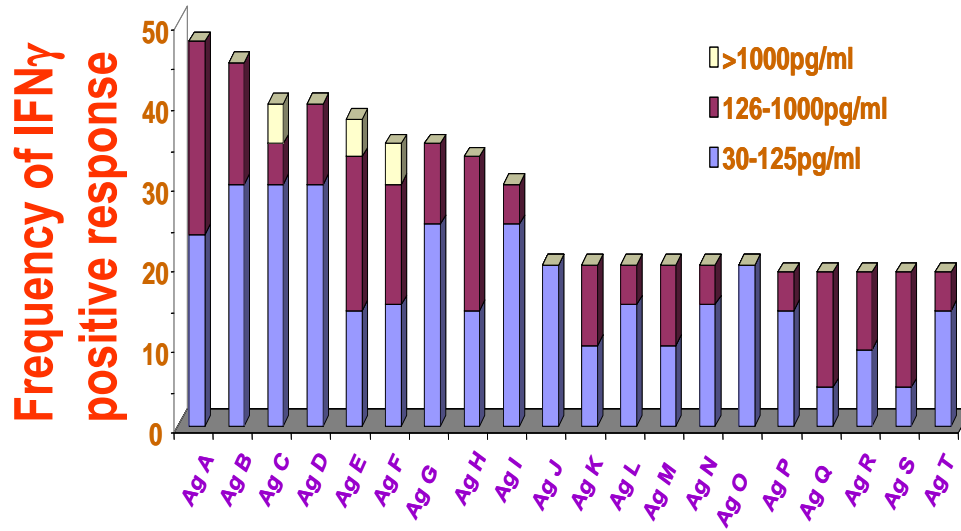


# Antigen Screening Results from Mining Exercise – Top 5



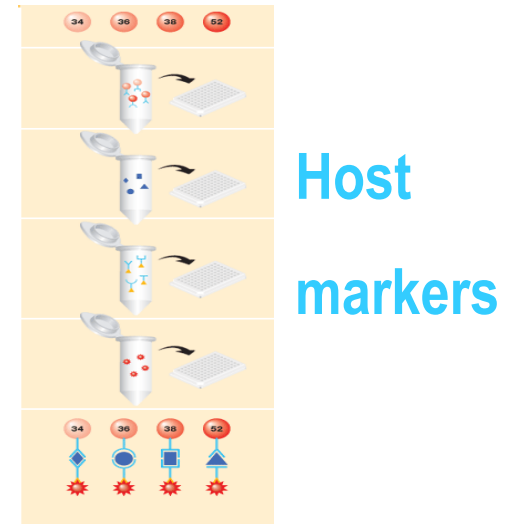


## Screening of 86 antigens



5 antigens

## Screening of 42 cytokines



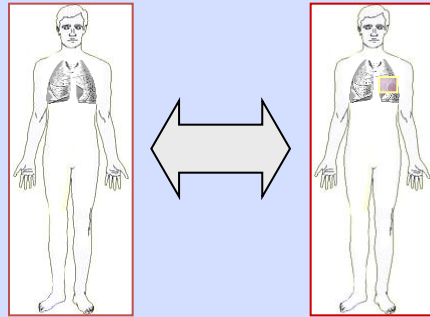
3 cytokines

Combinatorial approach:  $5 \times 3 = 15$  possible biomarkers

# Biomarkers to distinguish TB patients and healthy contacts



## Microarray comparison

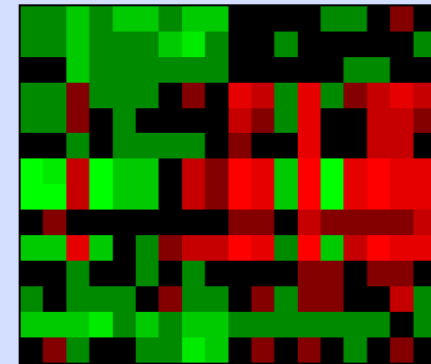


Healthy contacts

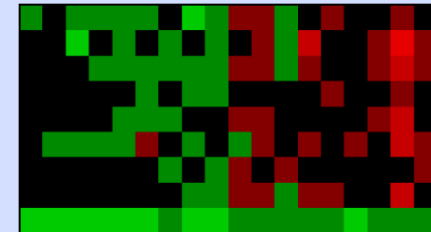
TB patients



## Antimicrobial



## Inflammation



## Chemokines



## Vesicle trafficking



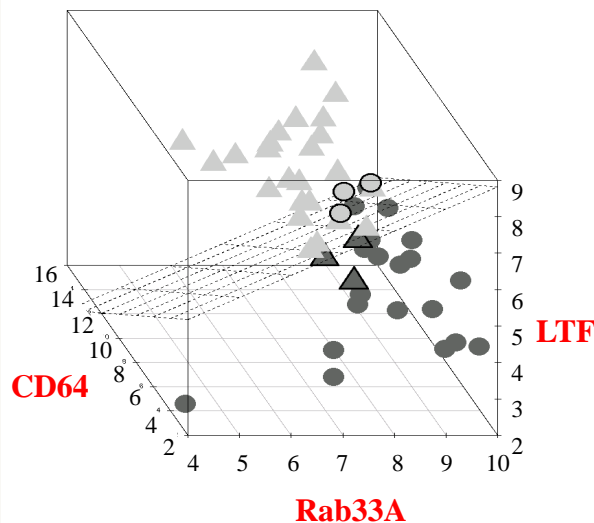
Healthy contacts

Tuberculosis patients

Genename	Rank	SAM	P-value
FPR1	1	-3.15	0.01*
SERPING1	4	-2.43	0.11
FCGR2A	13	-2.13	0.27
DEFA4	17	-2.09	0.29
BPI	22	-2.04	0.34
FCGR1A	27	-2.00	0.37
DEFA1	33	-1.94	0.42
DEFA3	36	-1.93	0.44
MPO	38	-1.91	0.46
LTF	69	-1.70	0.69
C2	73	-1.69	0.70
C5R1	90	-1.63	0.78
PTX3	99	-1.61	0.80
MARCO	100	-1.61	0.80
CEBPD	7	-2.27	0.18
AQP9	30	-1.99	0.38
S100A9	35	-1.93	0.43
NCF2	40	-1.89	0.48
S100A8	44	-1.86	0.52
CCR1	66	-1.73	0.65
PSTPIP1	78	-1.66	0.74
CD163	81	-1.64	0.76
CCRL2	92	-1.62	0.78

CXCL1	6	-2.35	0.14
CXCL2	52	-1.83	0.54
CXCL3	88	-1.63	0.77

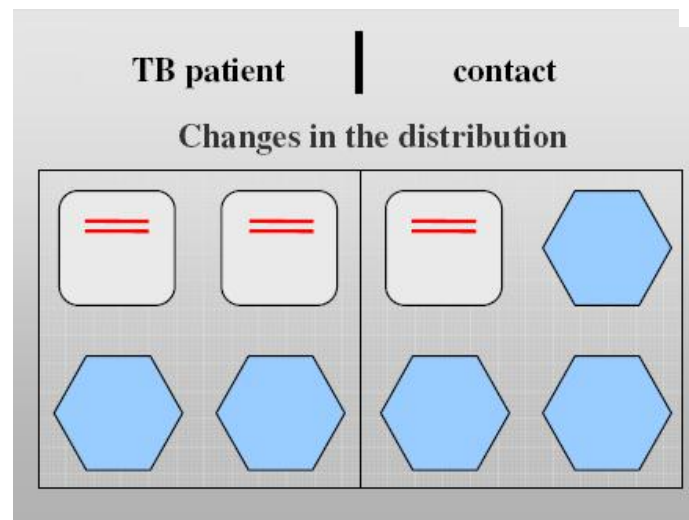
RAB13	3	-2.49	0.09
SEC14L1	8	-2.23	0.20
ASGR2	34	-1.94	0.42
ANXA3	48	-1.85	0.53
RAB32	82	-1.63	0.77



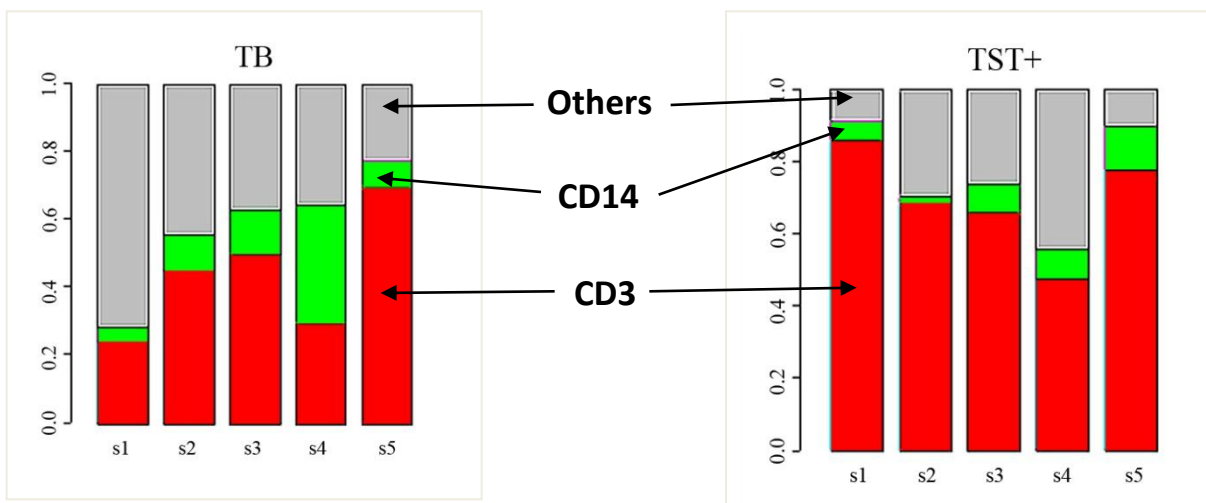
# Deconfounding of microarray data

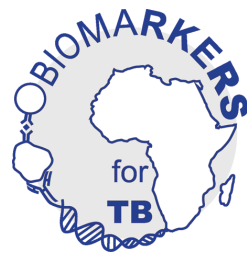
## Differential gene expression

- Differences in cell type proportions
- Cell type specific gene expression



Deconfounding: Mathematical dissection of heterogeneous tissue into its components (cell types)





# Deconfounding of microarray data

## Proof of principle \*

- ✦ Current deconfounding algorithm has been shown to be successful in mathematically dissecting heterogeneous tissue.
- ✦ Improved validity in detecting differentially expressed genes.

### Limitation of current algorithm:

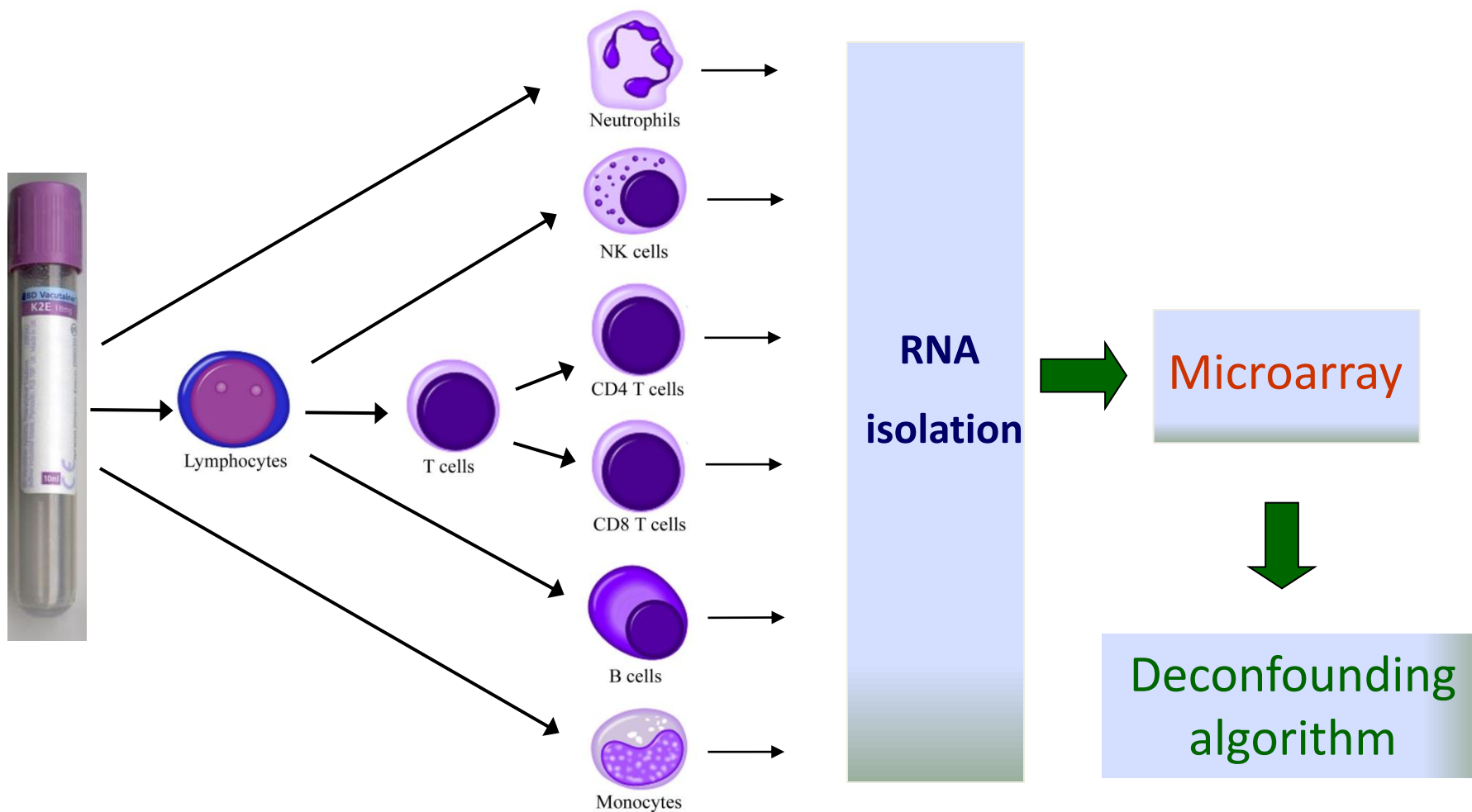
Current deconfounding algorithm limited to a single marker gene for T cells and monocytes/macrophages

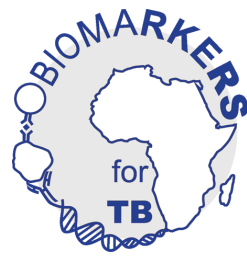
→ New experimental setup to identify cell type specific gene expression profiles

Jacobsen, M. et al. Methods Inf Med 2006, Jacobsen, M. et al. J Mol Med 2007

# Improving current deconfounding algorithm

## Expression profiling of all major cell types in blood



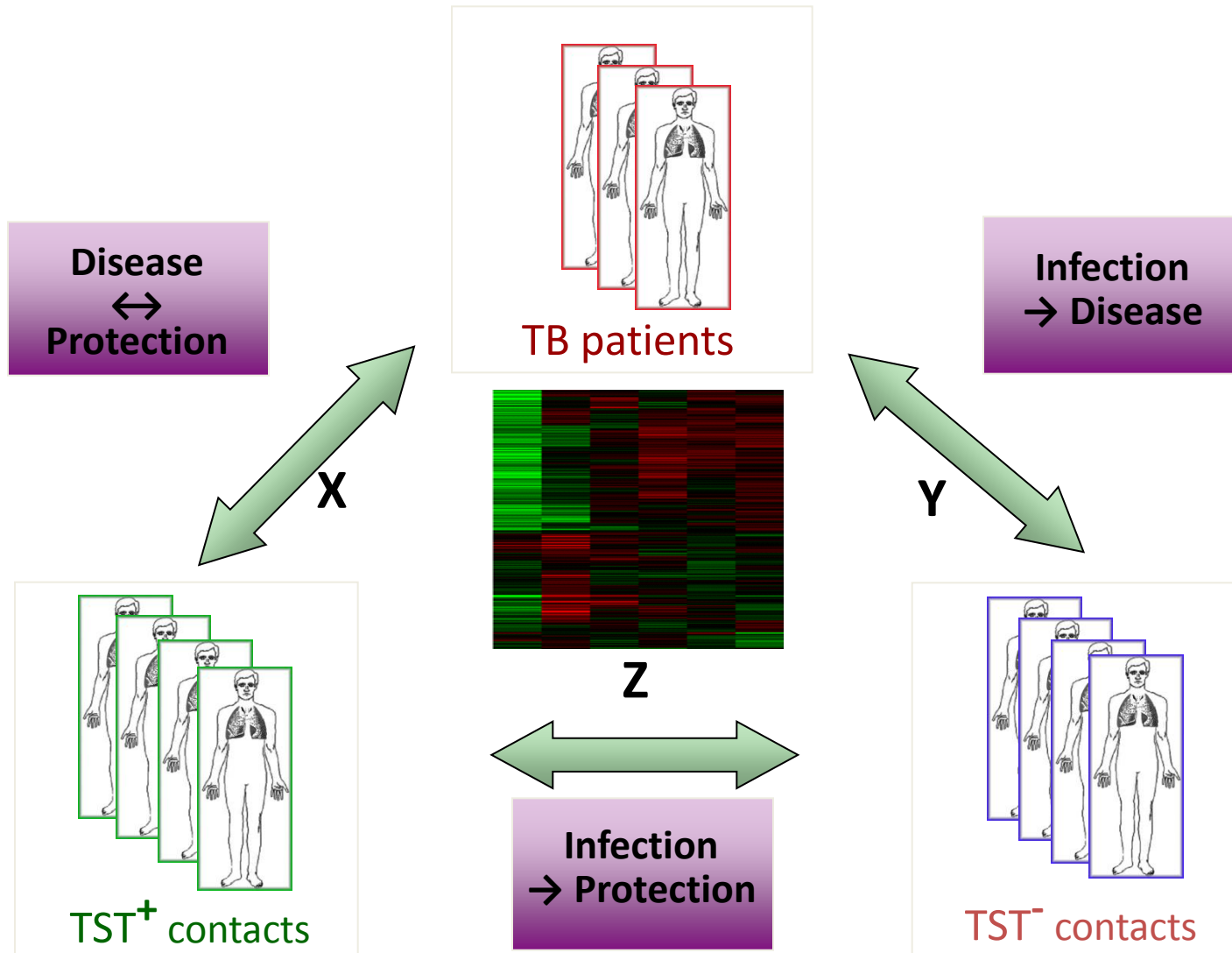


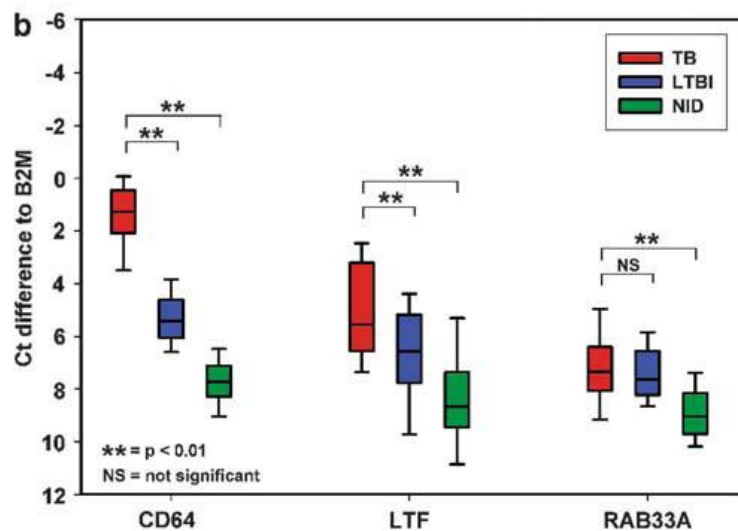
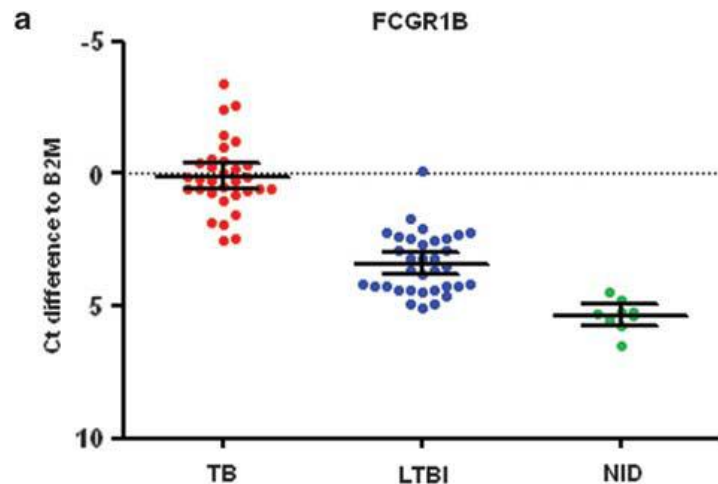
# Improving current deconfounding algorithm

## Expression profiling of all major cell types in blood

### Power of deconfounding can be greatly improved:

- ✦ Dissect tissue into all major components (cell types)
- ✦ Multiple markers per cell type
- ◇ Differentially expressed genes can be identified with greater validity (less false-positive and false-negative hits)
- ◇ Differentially expressed genes can be assigned to a specific cell type





Random forest analysis of gene subsets  
discriminating between TB and LTBI.

A		predicted	
		TB	LTBI
Gene subset	actual	CD64	28 (88%)
		LTF	4 (12%)
Gene subset	actual	LTBI	3 (9%)
		RAB33A	31 (91%)

B		predicted	
		TB	LTBI
Gene subset	actual	FCGR1B	30 (94%)
		CD64	2 (6%)
Gene subset	actual	LTF	1 (3%)
		GBP5	33 (97%)
		GZMA	

# Quest for Immune Correlates of Protection in TB



## Current Status:

- Recruitment completed – follow-up ongoing (would end in October 2010)
- Additional recruitment initiated and ongoing to obtain more Sec. Cases
- Assays
  - ELISA for IFN $\gamma$  – assay qualification at all site being completed
  - Luminex assay for Multicytokine studies
  - Transcriptomics – Microarray; MLPA
- Antigens - Large scale Production achieved

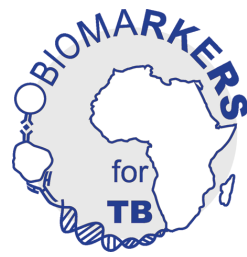
## Next Plans:

To do analysis centrally on all secondary cases and matched controls (4 x progressors) at the end of the follow-up period

Validation of the patterns showing association with protection:

- 1. Soluble cytokines
- 2. T cell cytotoxic molecule expression
- 3. Gene expression profiles
- Complementary analysis to delineate soluble cytokine expression patterns from longer term assays that associate with protection

# Thinking out of the box!



## Plausible paths:

- **Fine map the immune responses –**
  - T cell resp      Ag- specific frequency
  - Phenotypes – specific subsets
  - Cytokines, proliferative potential
  - Cytotoxic potential, functionality
  - Treg
- relevant host markers,
- pathogen markers
- different combinations of markers and platforms (biosignatures)
- **Unbiased global profiling** – differential gene expression / multiple cytokines
- **Integrated approach** of all composite data sets over time
- Further **longer term assay** comparisons
- **Robust bioinformatics** – systems biology approach
- **Validation**, validation, validation



AERAS  
Global TB Vaccine Foundation

AHRI  
ALERT  
Arman Hansen  
Research Institute

CASE WESTERN RESERVE  
UNIVERSITY EST. 1826

TBRI  
Tuberculosis Research Unit

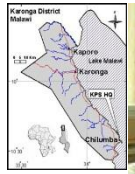
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Scientific Advisory Board (SAB)

# Biomarkers in TB



## Immunologic

- **Material**
  - Blood
  - Tissue
- **Application**
  - Diagnose disease 😊
  - Monitor vaccine trial 😊
  - Predict susceptibility 😐
  - Treatment outcome 😞
  - Monitor drug trial 😞



# Biomarker Needs in context of TB!

- **Surrogate markers of immune protection – need for assessing potential vaccine candidates**
- **Surrogate marker of bacterial clearance (clinical end-point)– need for assessing potential drug candidates**
- **Markers of relapse**
- **Markers of treatment failure (drug resistance)**
- **Diagnostic Markers**
- **Markers for infection**
- **Prognostic markers for reactivation/disease**

# The long and winding road from Bench to the Bush

**Bench**



... and  
back from  
Bush to  
Bench



**Bush**







**Baruj Benaceraf**  
**The Nobel Prize in Physiology or Medicine 1980**

As I address myself to the young immunologists, my message is still one of the encouragement. There are plenty of uncharted territories for you to explore, numerous mysteries to unravel and revelations to behold.

Stay away from the beaten track.

Don't be afraid **to question established dogma**, to dare the impossible, or to seek the unexpected.

Nature rewards and yields its magic secrets to the most daring imaginative.

**Hard work and dedication, while essential are not enough.**

-- *Kyoto*, August 1983