

# BCG-induced immune correlates of protection against tuberculosis

EDCTP meeting, Burkina Faso

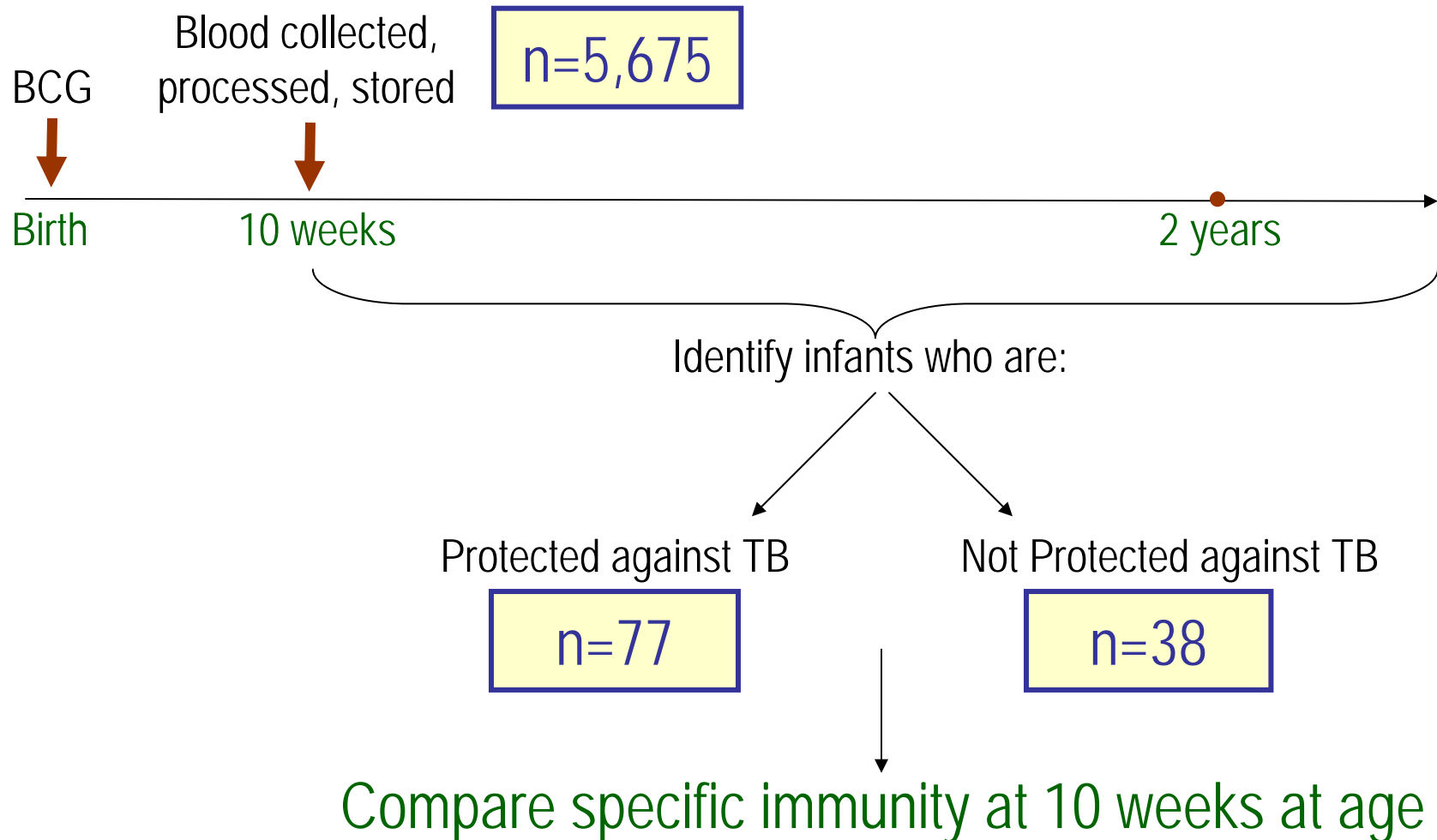


# South African TB Vaccine Initiative



Hassan Mahomed, Tony Hawkrige, Greg Hussey

# To determine BCG-induced immune correlates of protection against TB



# Immune correlates study approach

1. Soluble cytokines

2. T cell-associated cytokines

3. T cell proliferation, etc.

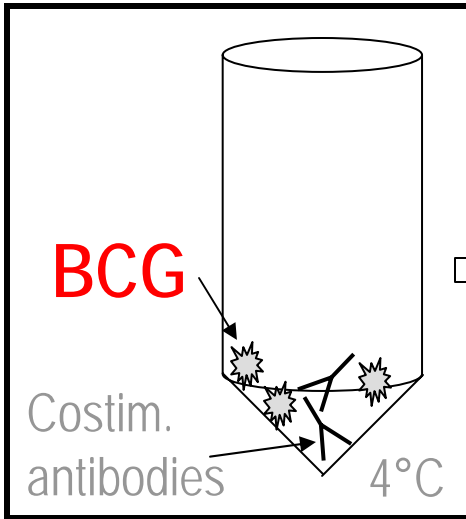
4. T cell antigenic repertoire

5. Antibody responses

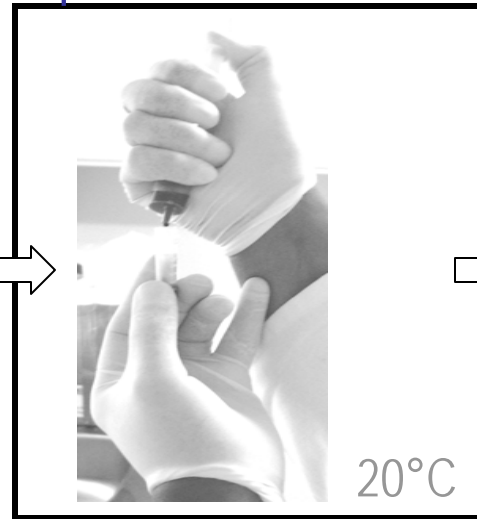
6. mRNA expression

7. Innate immune cells

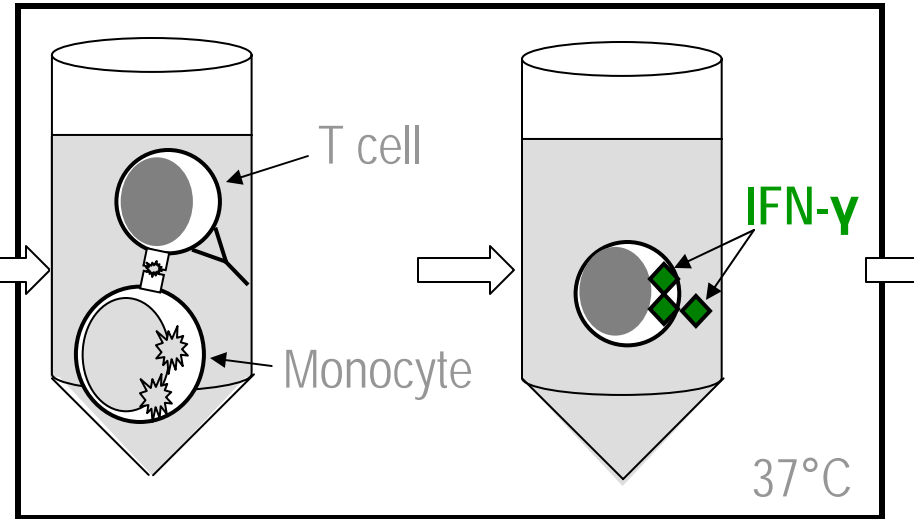
Preparation,  
Transport to  
Clinic



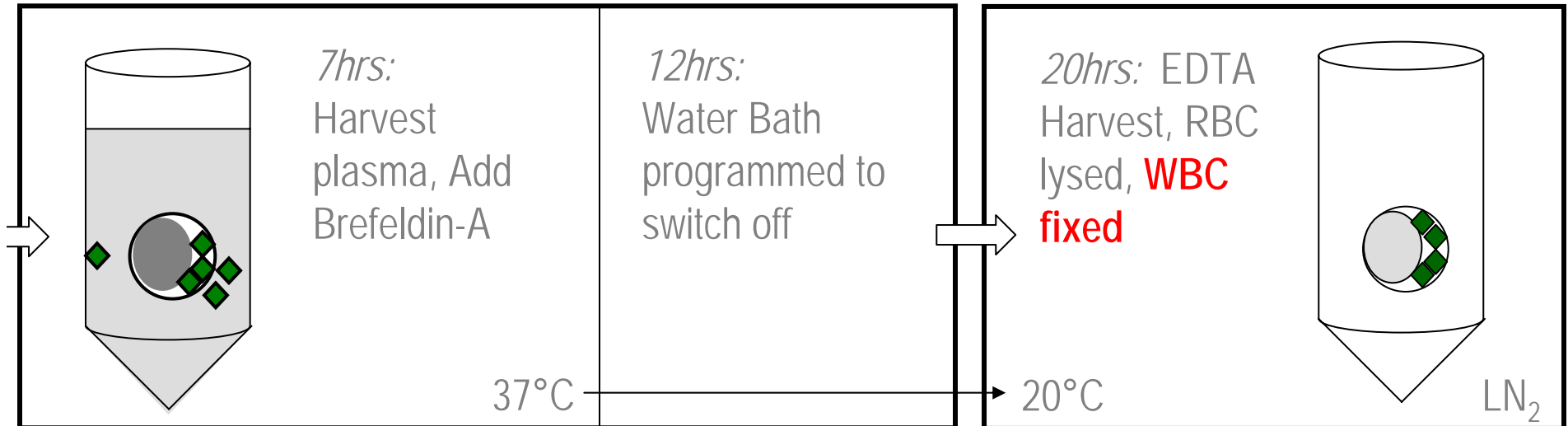
Clinic: Blood  
Added to  
Opened Tube



Transport to Lab in  
Portable Incubator

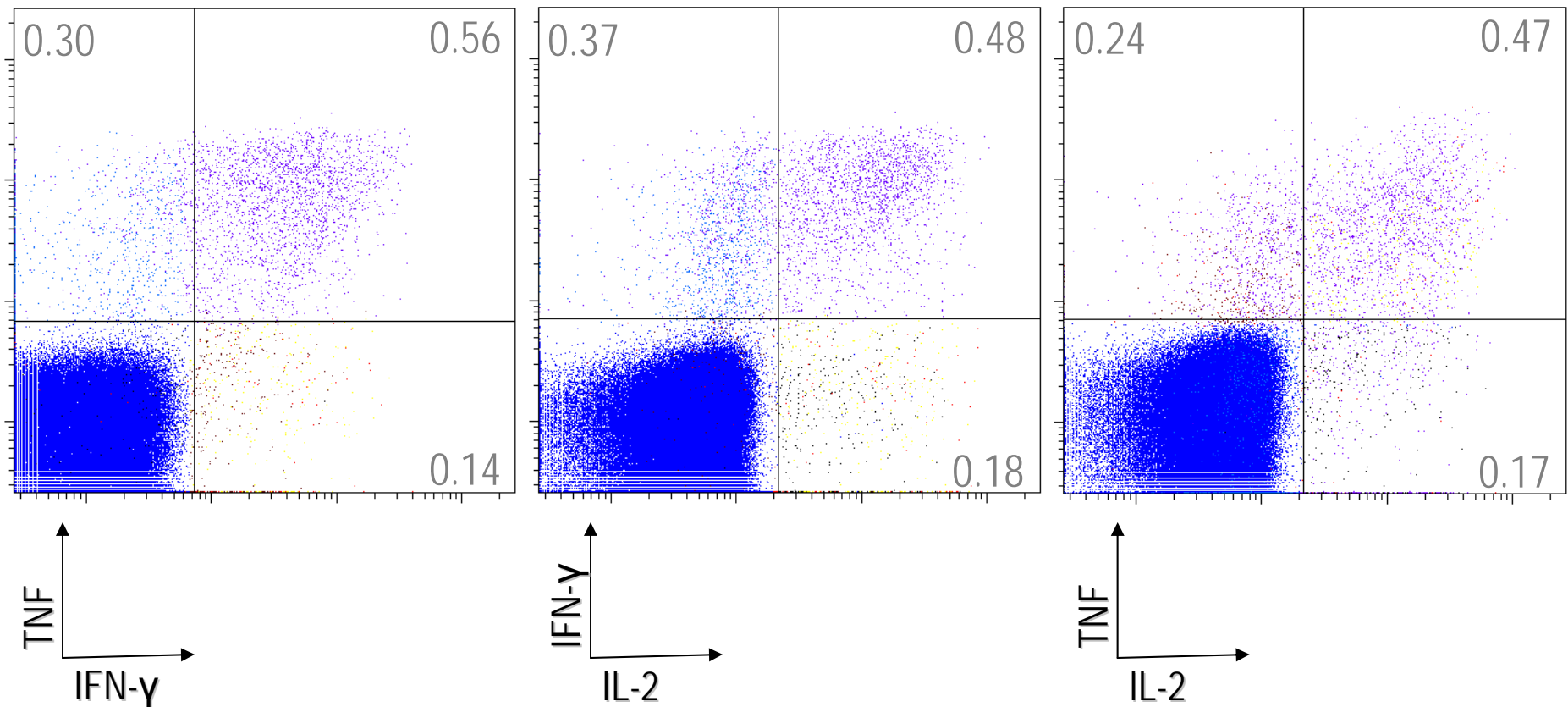


Lab: Transfer to Water Bath



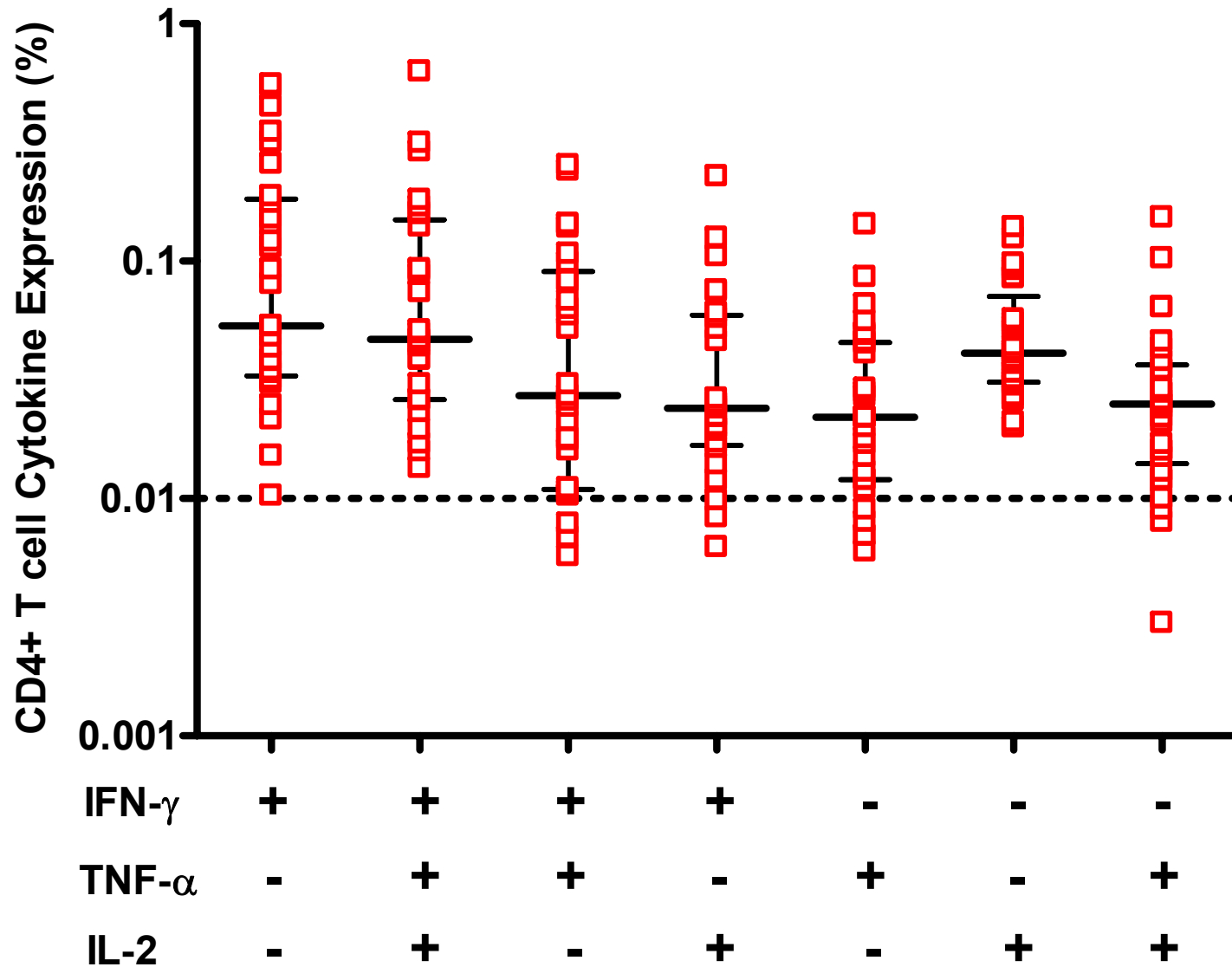
# BCG-induced T cell cytokine expression

Unstimulated levels < 0.05%



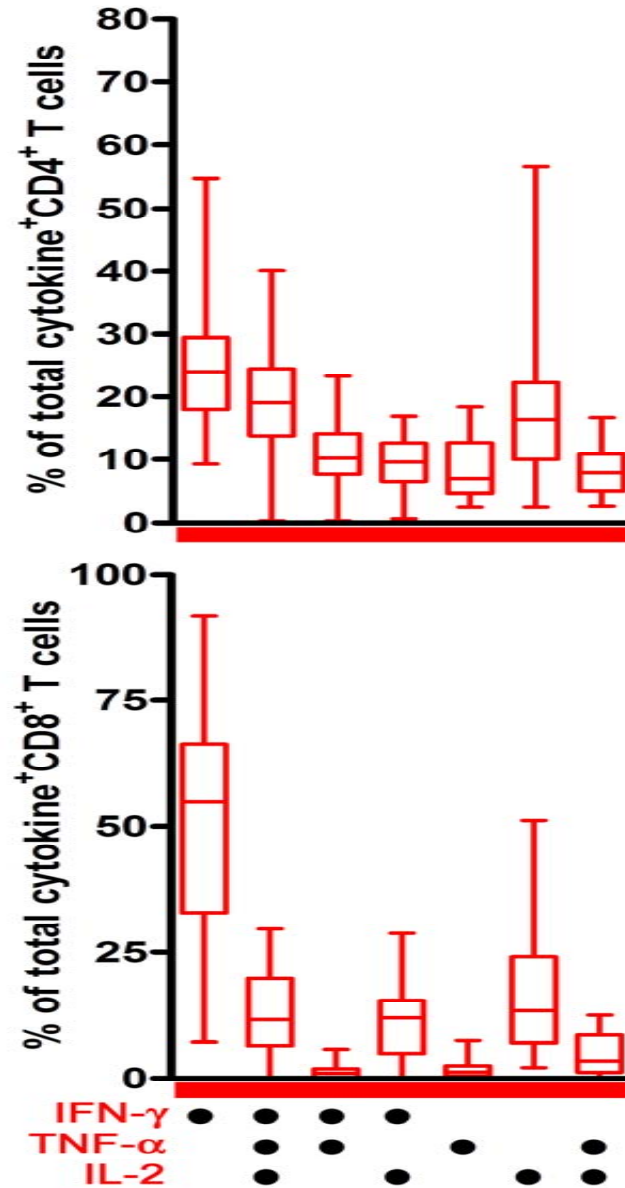
\*Gated on CD4<sup>+</sup> T cells. 12 hours incubation of whole blood with BCG, from a 10-week old infant, vaccinated with BCG at birth.

# BCG-induced T cell cytokine expression



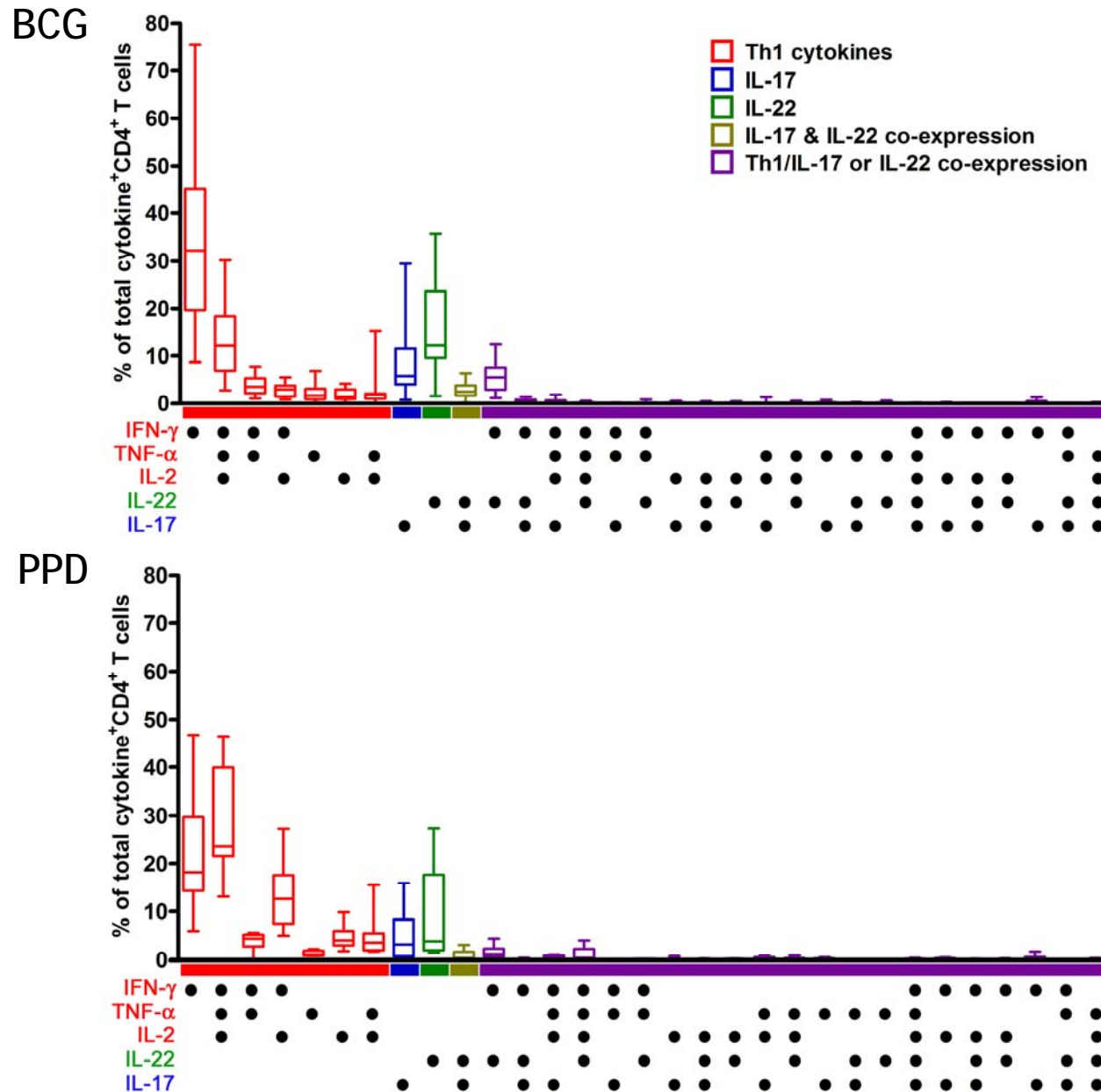
\*12 hours incubation of whole blood with BCG, in 29 10-week old infants, vaccinated with BCG at birth. Medians and interquartile ranges shown.

# BCG-induced T cell cytokine expression



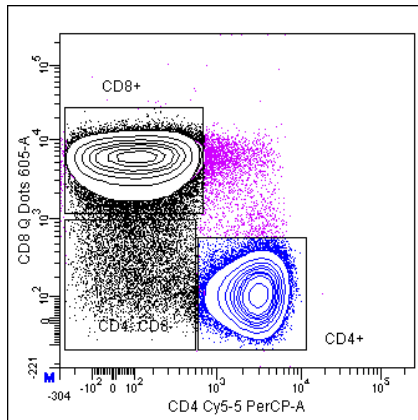
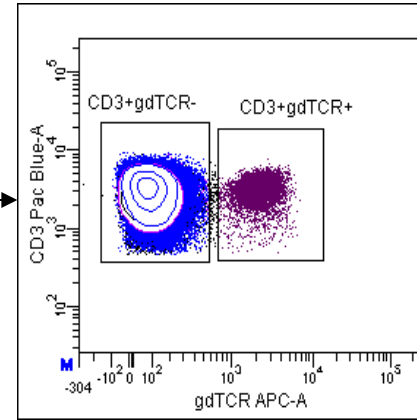
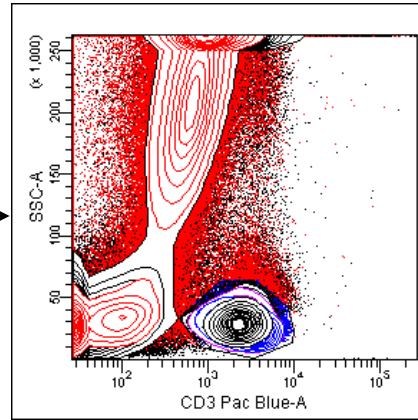
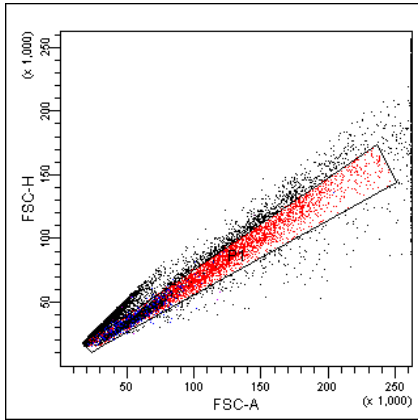
\*12 hours incubation of whole blood with BCG, in 29 10-week old infants, vaccinated with BCG at birth.

# Specific T cell cytokines in adults

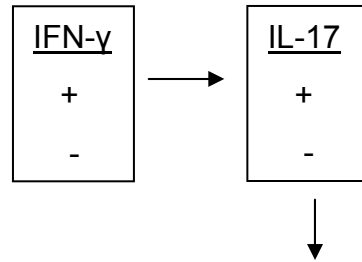


\*12 hours incubation of whole blood from mycobacteria-exposed adults with antigen.

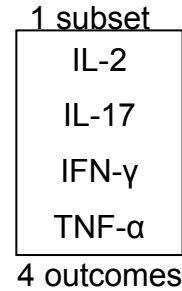
# Immune correlates study analysis



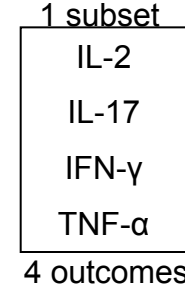
**CD4+ or CD8+  $\gamma\delta$ - T cells**



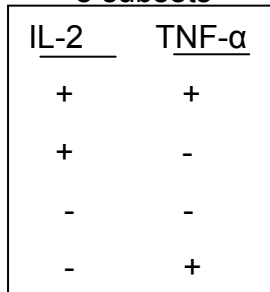
**$\gamma\delta$ + T cells**



**CD4-CD8-  $\gamma\delta$ - T cells**



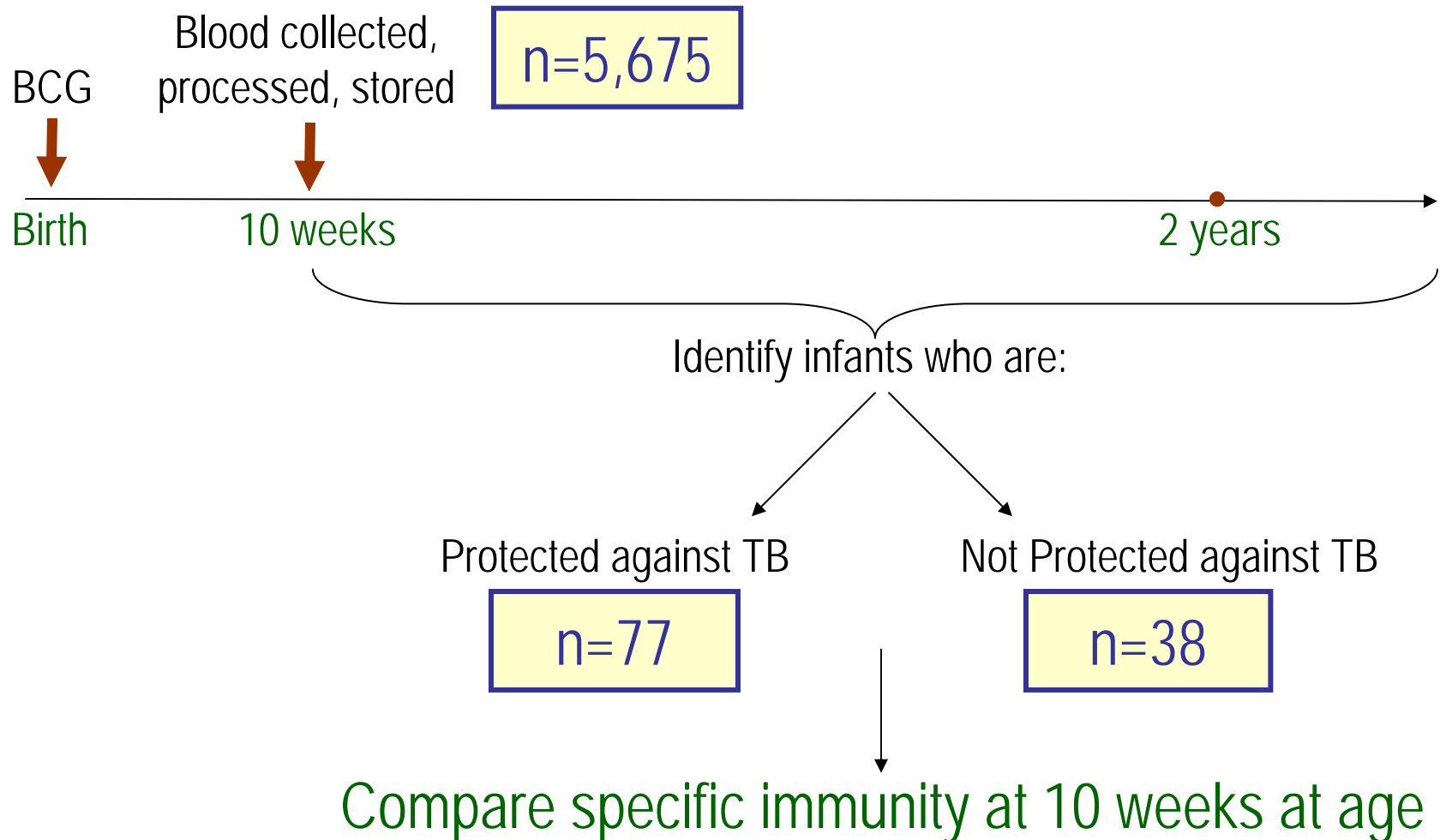
8 subsets



64 outcomes for each

136 outcomes

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# Immune correlates study approach

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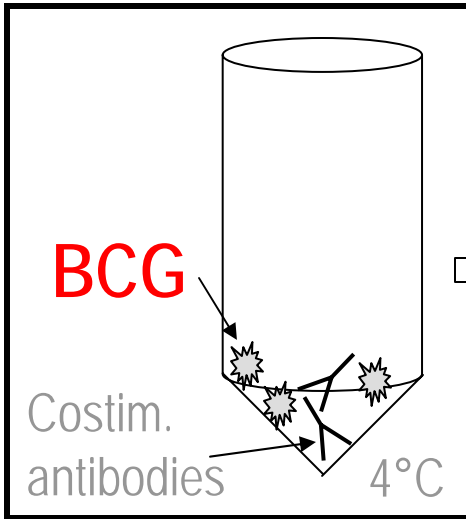
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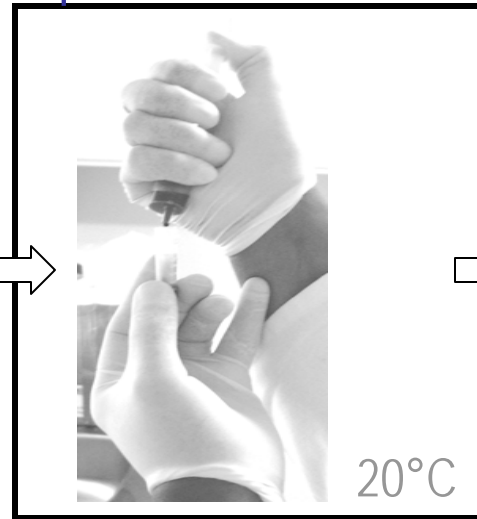
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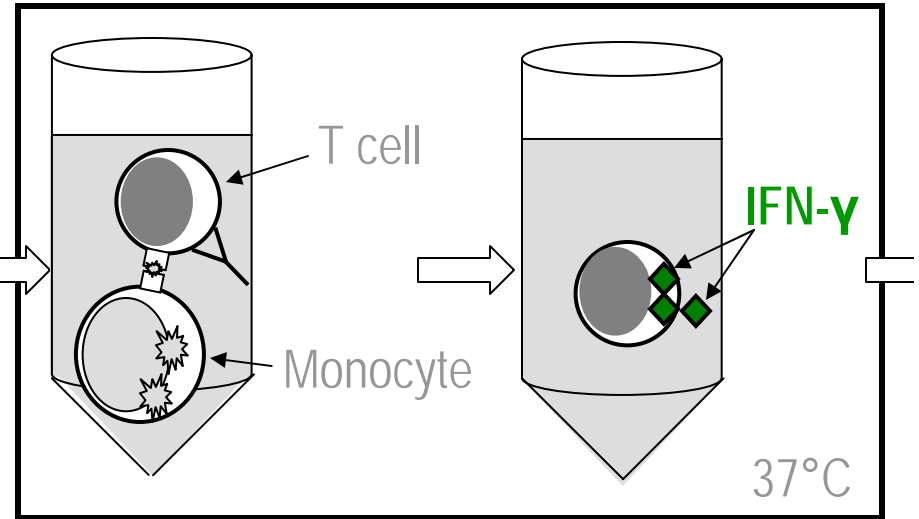
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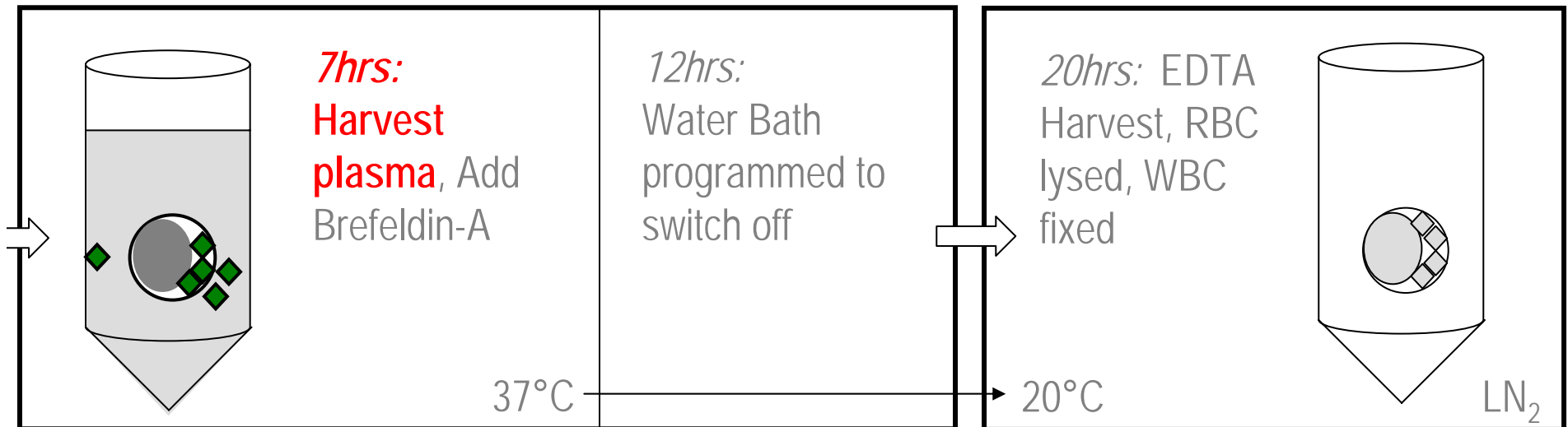
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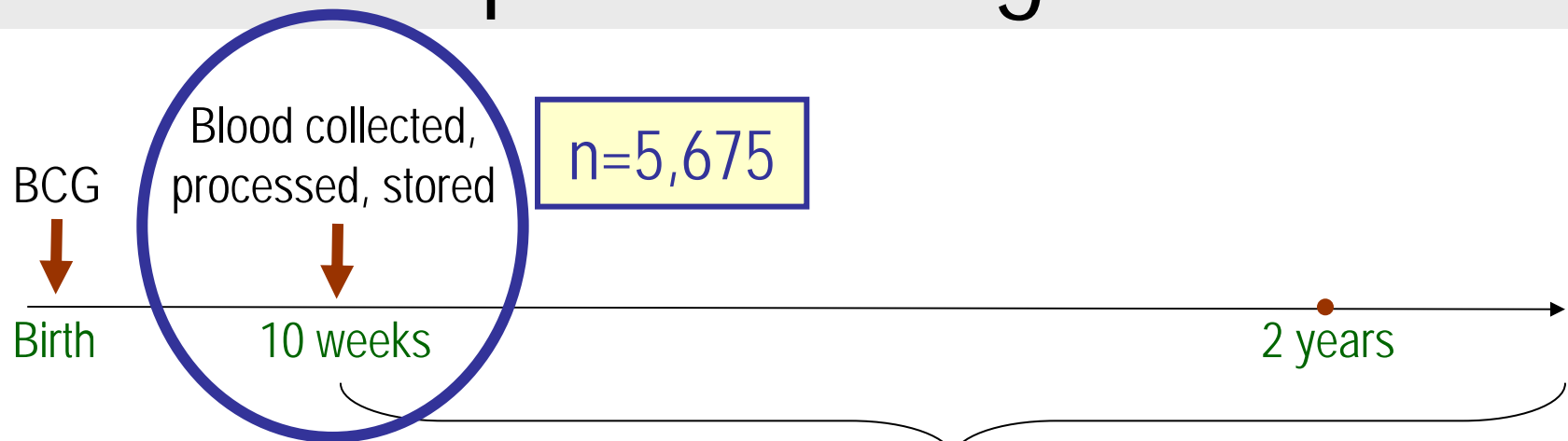
Transport to Lab in  
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Lab: Transfer to Water Bath



# To determine BCG-induced immune correlates of protection against TB



Identify infants who are:

Protected against TB

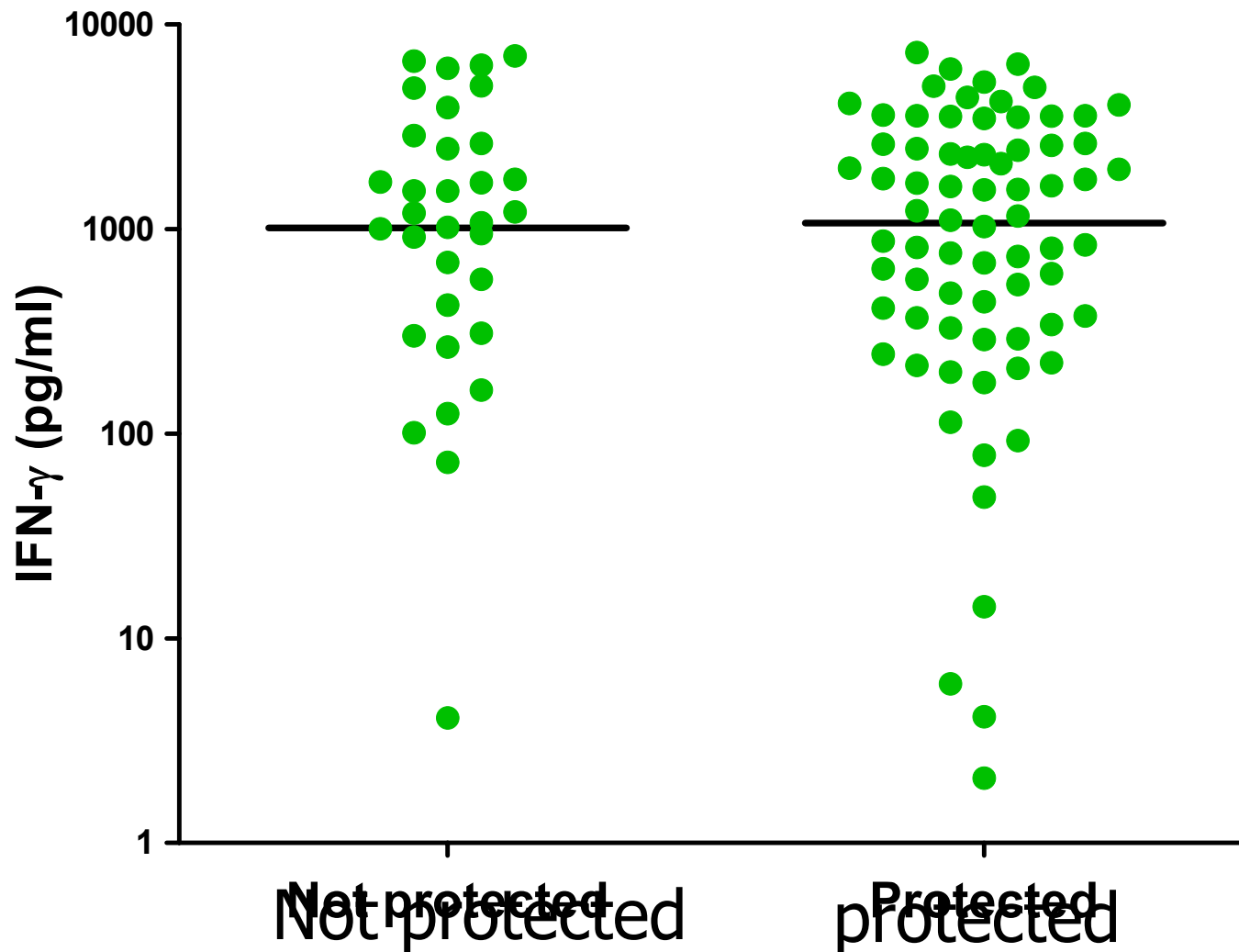
n=77

Not Protected against TB

n=38

Compare specific immunity at 10 weeks at age

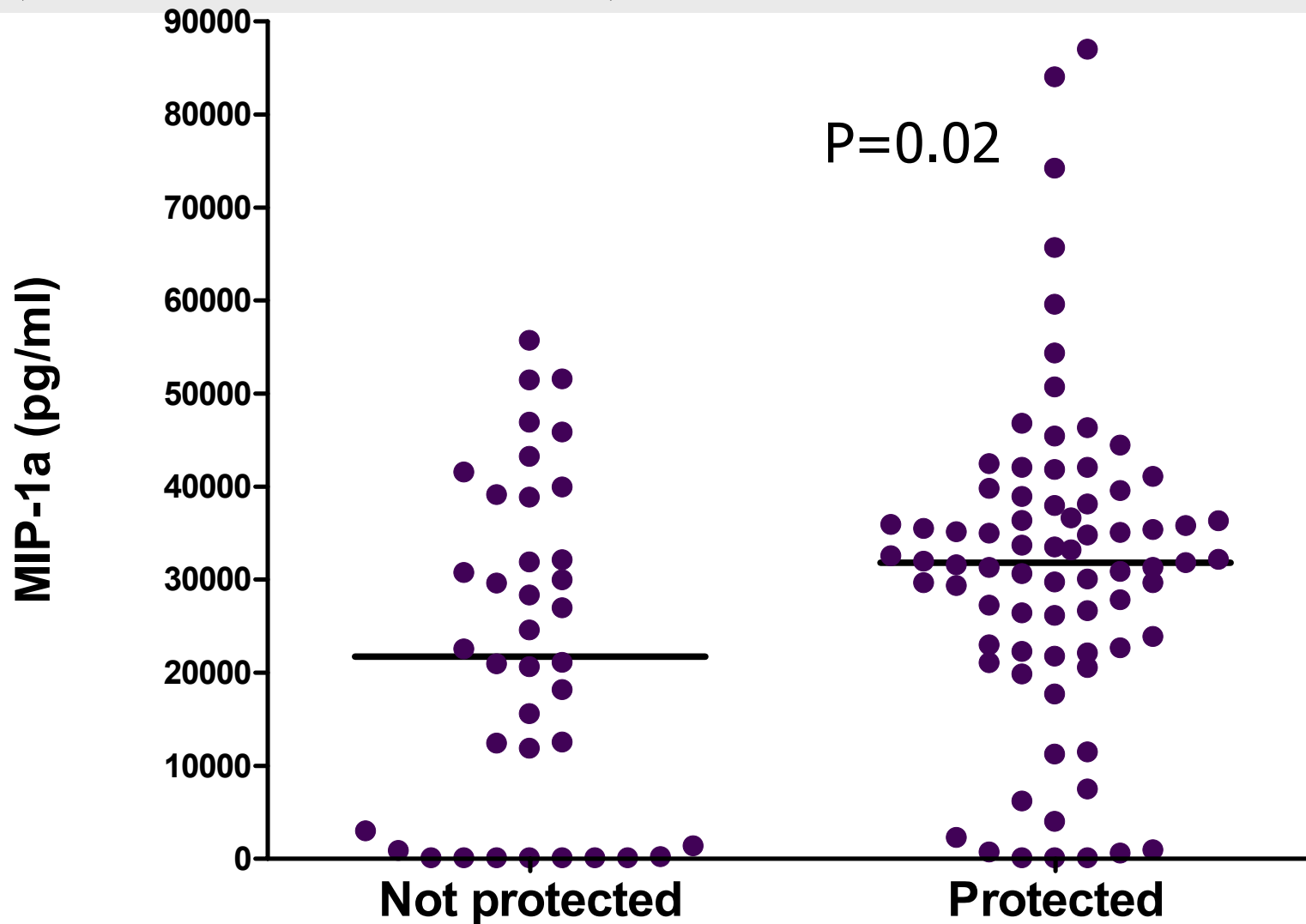
# IFN- $\gamma$ is not a BCG-induced immune correlate of protection against TB



**IL-1 $\beta$ , IL-2, IL1RA, IL-4, IL-5, EGF, IL-6, IL-7, TGF- $\alpha$ , Fraktalkine, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-1 $\alpha$ , G-CSF, GM-CSF, TNF- $\alpha$ , Eotaxin, IL-12p40, MIP-1 $\beta$ , IP-10, VEGF, sCD40**

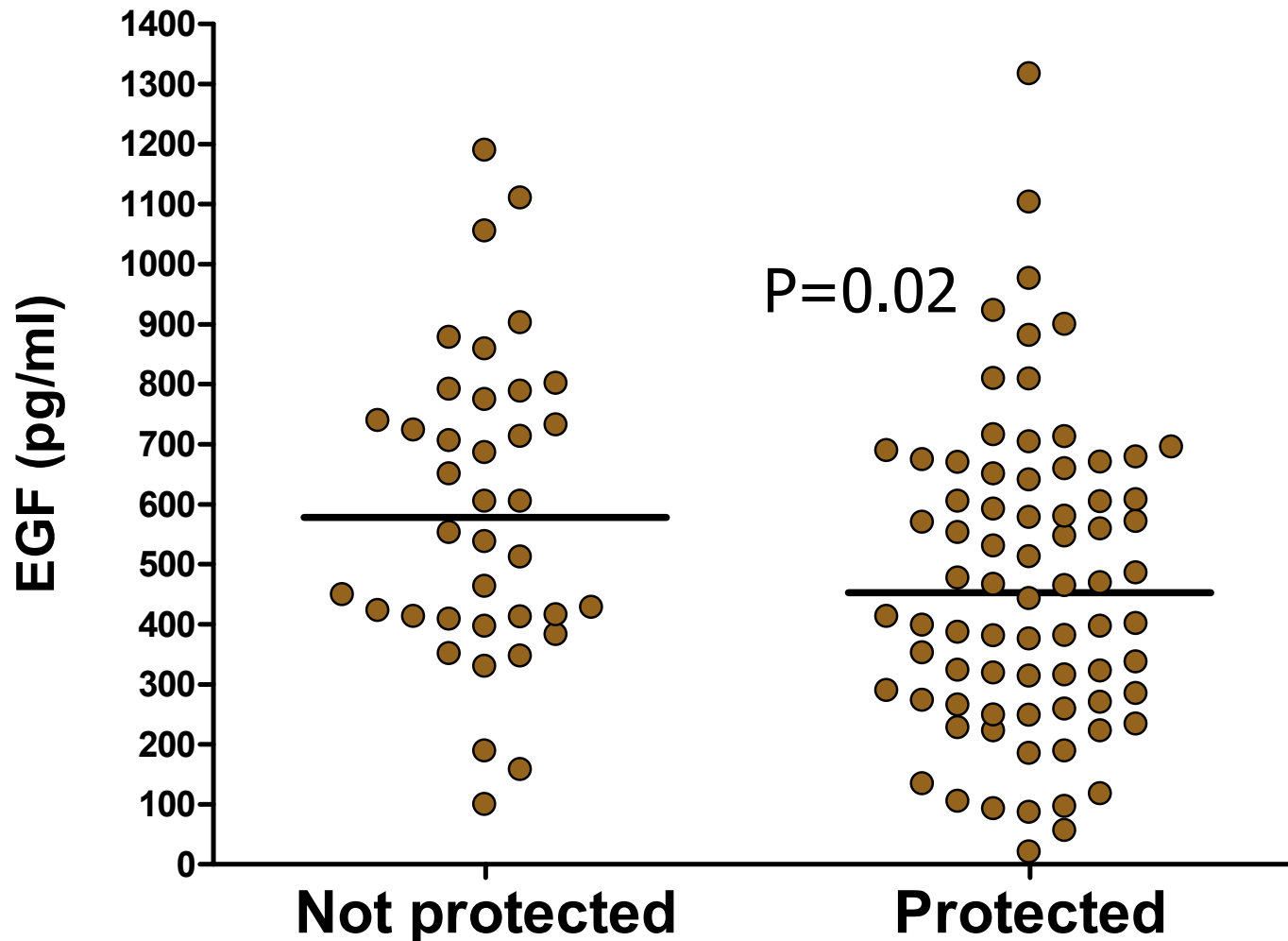
\*Measured in plasma collected 7 hours after incubation of whole blood with BCG; Linco beads, Luminex.

# MIP-1 $\alpha$ levels were higher when protected (BCG stimulated)



\*Measured in plasma collected 7 hours after incubation of whole blood with BCG; Linco beads, Luminex.

# Plasma from UNstimulated blood: EGF levels were lower when protected



\*Measured in plasma collected 7 hours after incubation of whole blood with nothing; Linco beads, Luminex.

# UNstimulated plasma: Differences detected by univariate analysis

	<i>When protected</i>	<i>p value</i>
IL-13	Higher	0.03
EGF	Lower	0.02
Fraktalkine	Lower	0.04

\*Measured in plasma collected 7 hours after incubation of whole blood with nothing; Linco beads, Luminex.

# Multivariate logistic regression analysis of data from UNstimulated blood

**MIP1- $\beta$**

**TNF- $\alpha$**

**IL-4**

**IL-17**

**EGF**

**IL-13**

**Eotaxin**

**TGF- $\alpha$**

**Fraktalkine**

**GM-CSF**

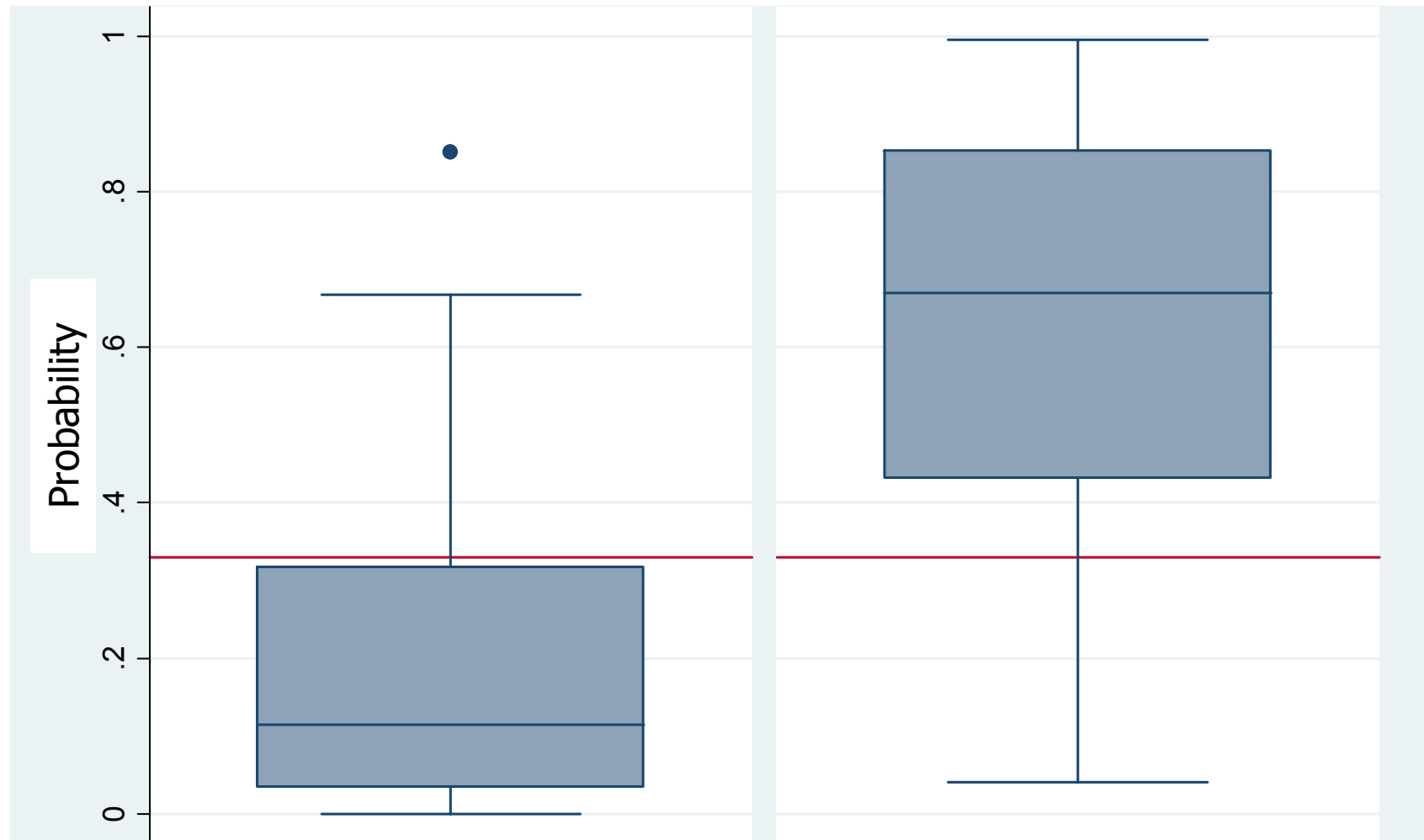
$$\eta = -1.31 + 1.70 * \text{MIP1beta} - 1.97 * \text{TNFa} - 0.76 * \text{IL4} + 0.89 * \text{IL17} + 1.02 * \text{EGF} - 1.53 * \text{IL13} - 1.24 * \text{Eotaxin} + 1.05 * \text{TGFa} + 2.19 * \text{Fractalkine} - 1.16 * \text{GMCSF}$$

$$\hat{p} = \exp\left(\frac{\eta}{1 + \eta}\right)$$

# Model: data from UNstimulated blood

Not protected

Protected



# Model: UNstimulated blood

MIP1- $\beta$

TNF- $\alpha$

IL-4

IL-17

EGF

IL-13

Eotaxin

TGF- $\alpha$

Fraktalkine

GM-CSF

---

Sensitivity 84.21%

Specificity 78.95%

Positive predictive value 66.67%

Negative predictive value 90.91%

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False + rate for classified+ 33.33%

False - rate for classified- 9.09%

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Correctly classified 80.70%

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# Model: BCG-stimulated blood

MIP1- $\alpha$   
Fraktalkine  
IL-12p70

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Sensitivity	65.79%
Specificity	71.05%
Positive predictive value	53.19%
Negative predictive value	80.60%

---

False + rate for classified +46.81%  
False - rate for classified - 19.40%

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Correctly classified 69.30%

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# Conclusions

1. IFN- $\gamma$  alone measured at 7hrs in a WBA is not a BCG-induced immune correlate of protection against TB
2. Combined analysis of multiple measures may allow differentiation
3. Analysis of unstimulated blood may yield answers
4. Critical determinants of protection  $\neq$  Immune correlates of protection?



# Other acknowledgements

Greg Hussey, Willem Hanekom, Tony Hawkrige, Hassan Mahomed,  
many members of the SATVI clinical team (n=85!)

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Gilla Kaplan, Gregory Harbacheuski (UMDNJ)

Sarah Joseph

Holden Maecker

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