

Stop TB Task Force: Immunoassays in TB Vaccine Development

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Immunoassays in TB Vaccine Development

- What assays should be used to monitor immunogenicity of new vaccines?
- Should short-term (ie overnight) or longer-term (5-7 days) assays be used?
- Can biomarkers of protection be developed?
- Can assays “fit for purpose” be developed, that are suitable for large scale use?

Immunological Outcomes of New Tuberculosis Vaccine Trials: WHO Panel Recommendations

Wilens A. Hasakoni¹, Hazel M. Dockrell², Tom H. M. Ottenhoff³, T. Mark Doherty⁴, Helen Fletcher⁵, Helen McShane⁶, Frank F. Weichold⁷, Dan F. Hoft⁸, Shreemanta K. Parida⁹, Ulf J. Freth¹⁰

Summary Points

- The ability to compare clinical immunogenicity between different new tuberculosis vaccine candidates would be an important asset
- The WHO Initiative for Vaccine Research sponsored three meetings of experts to discuss assay harmonisation for new tuberculosis vaccine field trials
- Advantages and disadvantages of multiple T cell assay approaches were described, and specific recommendations for phase I/IIa trials made, including introducing a single and simple harmonised assay for all trials

Working Group Recommendations Regarding Assay Selection

- Time from blood collection to incubation should be standardised
- PBMCs should be stored for later analysis
- Harmonisation and standardisation of assays should be promoted
- A combination of shorter-term and longer-term assays would be the optimal way to measure vaccine-induced immunity
- A single, simple harmonised assay should be introduced in all trials

Hanekom et al, 2008

Assays for vaccine immunogenicity

- Good progress
- IFN γ is a robust stable cytokine to measure
- Detection using ELISA, ELISPOT, multiplex bead array, intracellular cytokine staining
- IFN γ detected in both short-term and longer-term assays

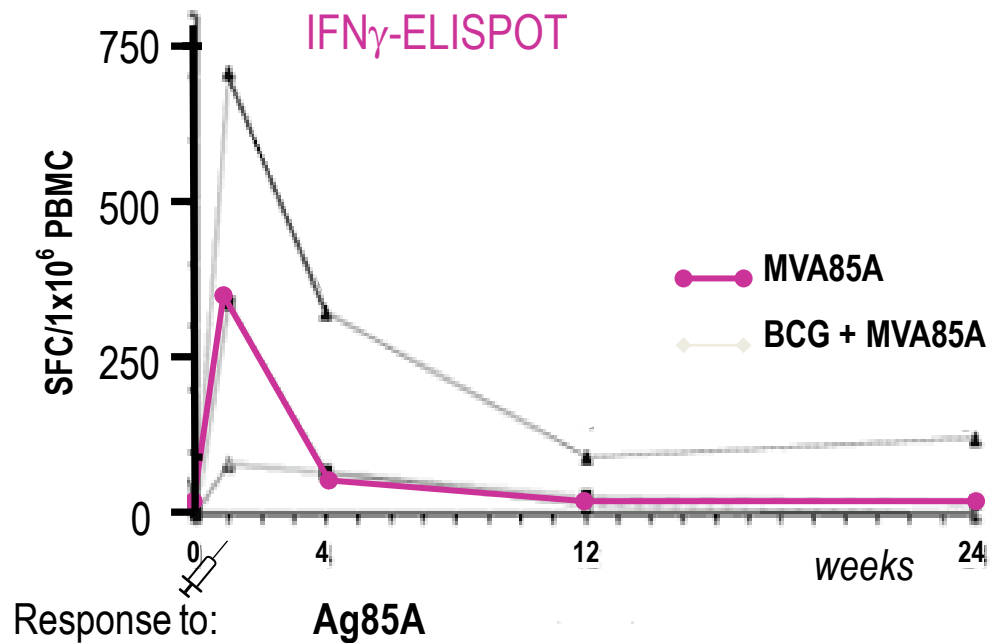
TBVAC-associated sub-unit TB vaccines: MVA85A

Phase 1 trial in
PPD skin test
negatives

MVA85A alone or
BCG + MVA85A

Immunogenicity:

- Excellent primary response
- Boosting post BCG



from H McShane & al., *Nature Medicine* 10, 1240 (2004)

7 day diluted whole blood assay

- Simple, easy to perform in field conditions; time from collection to assay less critical; IFN γ in sups stable with long-term storage
- Candidate assay to be used in all vaccine trials; IFN γ responses to PPD measure immunogenicity of BCG, revealing differences in different populations, maintenance of immunological memory over time....
- Assay reagents for IFN γ ELISA need to be standardised; standard protocols available; need for same ELISA readers/equipment with same curve fitting programmes

Can more complex biosignatures be used to assess protective efficacy of vaccines?

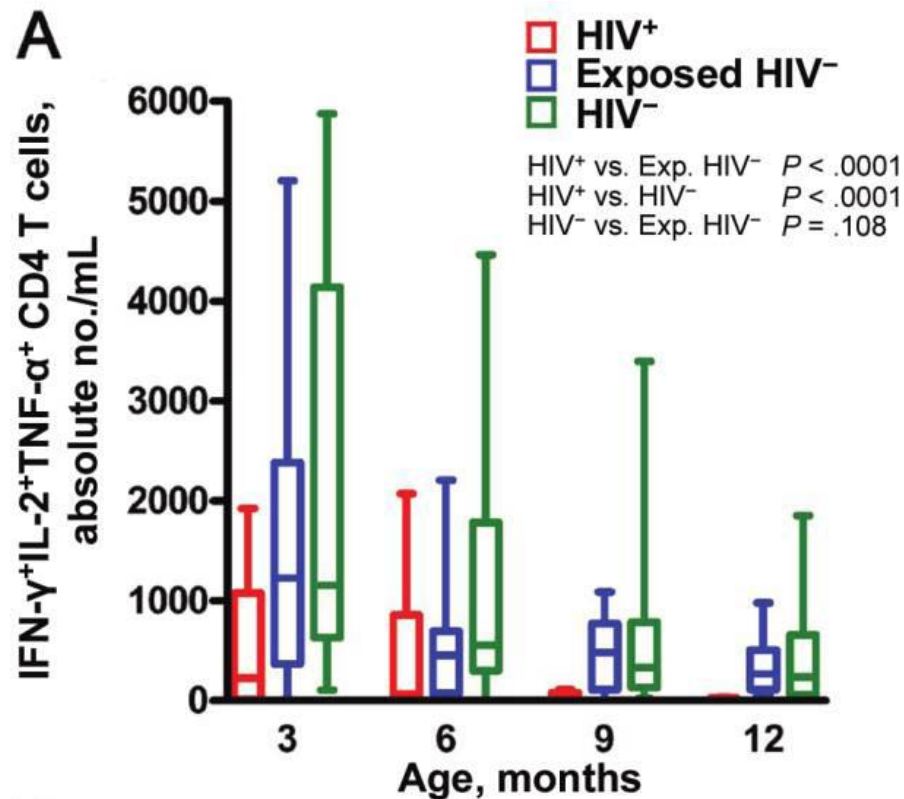
- Bead array –“Luminex”
- Intracellular cytokine staining
- Multiplex gene expression assays - MLPA
- Gene array
- Proteomics and metabolomics
- New generation assays

Extra information from multiplex assays:

- Multiplex bead array assays reveal broad cytokine/chemokine profiles in immune response to vaccination
- Such assays have shown unrecognised differences, in how different populations respond to BCG vaccination, for example how Malawian and UK infants respond to BCG vaccination

Intracellular cytokine staining quantitates and identifies cells making cytokines:

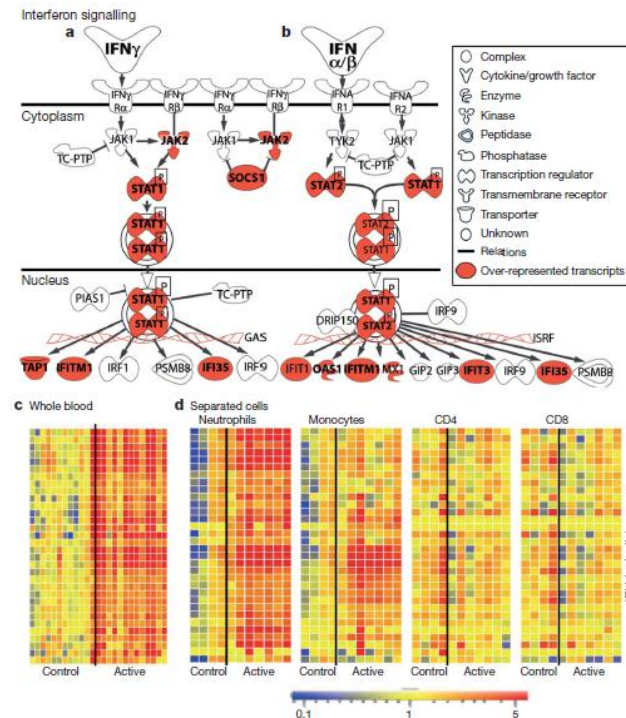
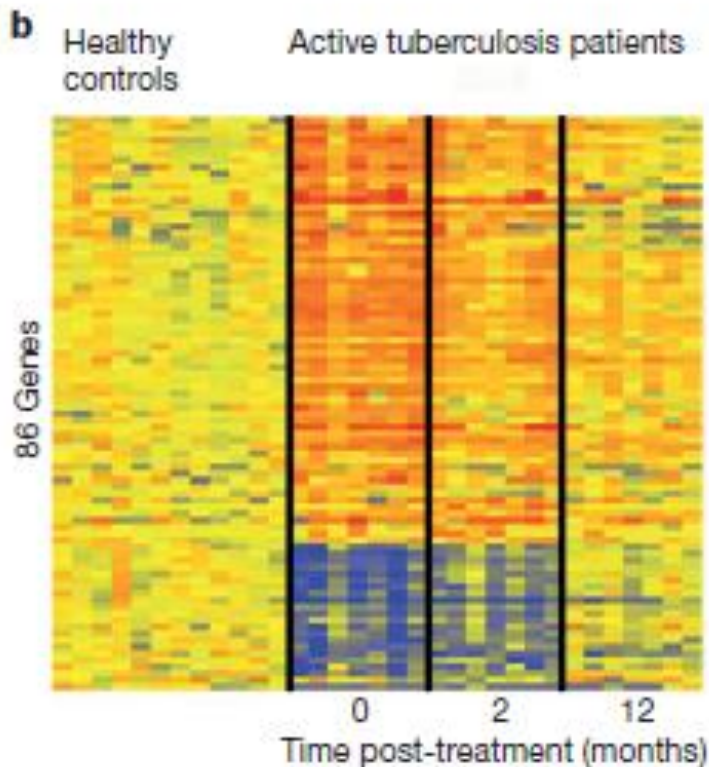
HIV infection reduces T cells making IFN γ , IL-2, TNF α in response to BCG vaccination






•HIV infected infants had fewer polyfunctional T cells

Mansoor et al J Infect Dis (2009) 199:982

Gene expression profiles are helping identify new candidates (including confirming the overlooked importance of innate immunity)



Standardised RT-MLPA assay developed at Leiden University Medical Centre (Haqs, Ottenhoff) for measurement of gene expression levels in TB studies

Microarray	RT - Multiplex Ligation Dependent Probe Amplification (MLPA)	Quantitative PCR (Taqman)
thousands of genes	± 60 genes	single genes
no selection of genes	selection of genes of interest	selection of gene of interest
± 2 µg RNA required	± 0.2 µg RNA required	± 0.05 µg RNA required
more expensive	less expensive	more expensive
enormous amount of data	genes can be changed easily	genes can be selected easily
not quantitative	(semi) quantitative	quantitative
single sample	96 well format	96 well format
		
Broad screen for new markers on few samples	Testing multiple candidates in larger groups	Testing single candidates in larger groups

The best test of ability to control mycobacterial growth might be an assay of mycobacterial growth inhibition

Mycobacterial Assay for Growth Inhibition : BCG Study (MAGI-BCG)

Helen Fletcher & Helen McShane

The Jenner Institute

University of Oxford

Objectives

Primary Objectives

- 1) Assessment of the variability and reproducibility of 4 different Mycobacterial Growth Inhibition Assays (MGIAs)
 - Whole blood + BCG/*lux* (Kampmann et al, JID 2000)
 - Whole blood + BCG (Wallis et al, JID 2003)
 - PBMC + BCG-infected monocytes (Li et al, I&I, 2002)
 - Antigen-expanded PBMC + BCG-infected monocytes (Worku et al, CID 2000)

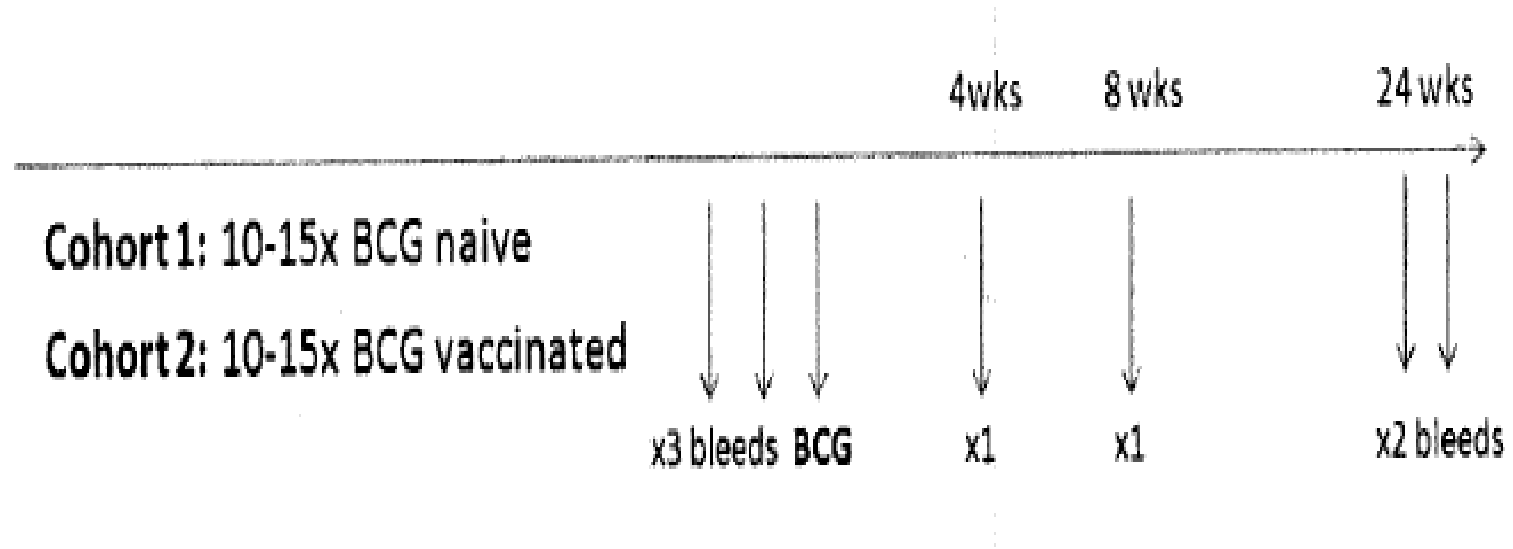
- 2) Comparison between these 4 different MGIAs in the ability to detect BCG induced immune responses.

- 3) Assessment of the transferability of 4 different MGIAs to an independent laboratory

Secondary Objective

- 1) Correlation of MGIAs with T cell immunology

Clinical study design



Screening commenced September 2010

Recruitment September – December 2010

Follow up January – June 2011

Funded by Aeras

Pipeline for assays to move towards use in vaccine trials

- Harmonisation: same protocols, reagents, (equipment)
- Qualification
- Validation
- “Fit-for-purpose”

Assays as well as vaccines must go down a challenging development pipeline.....

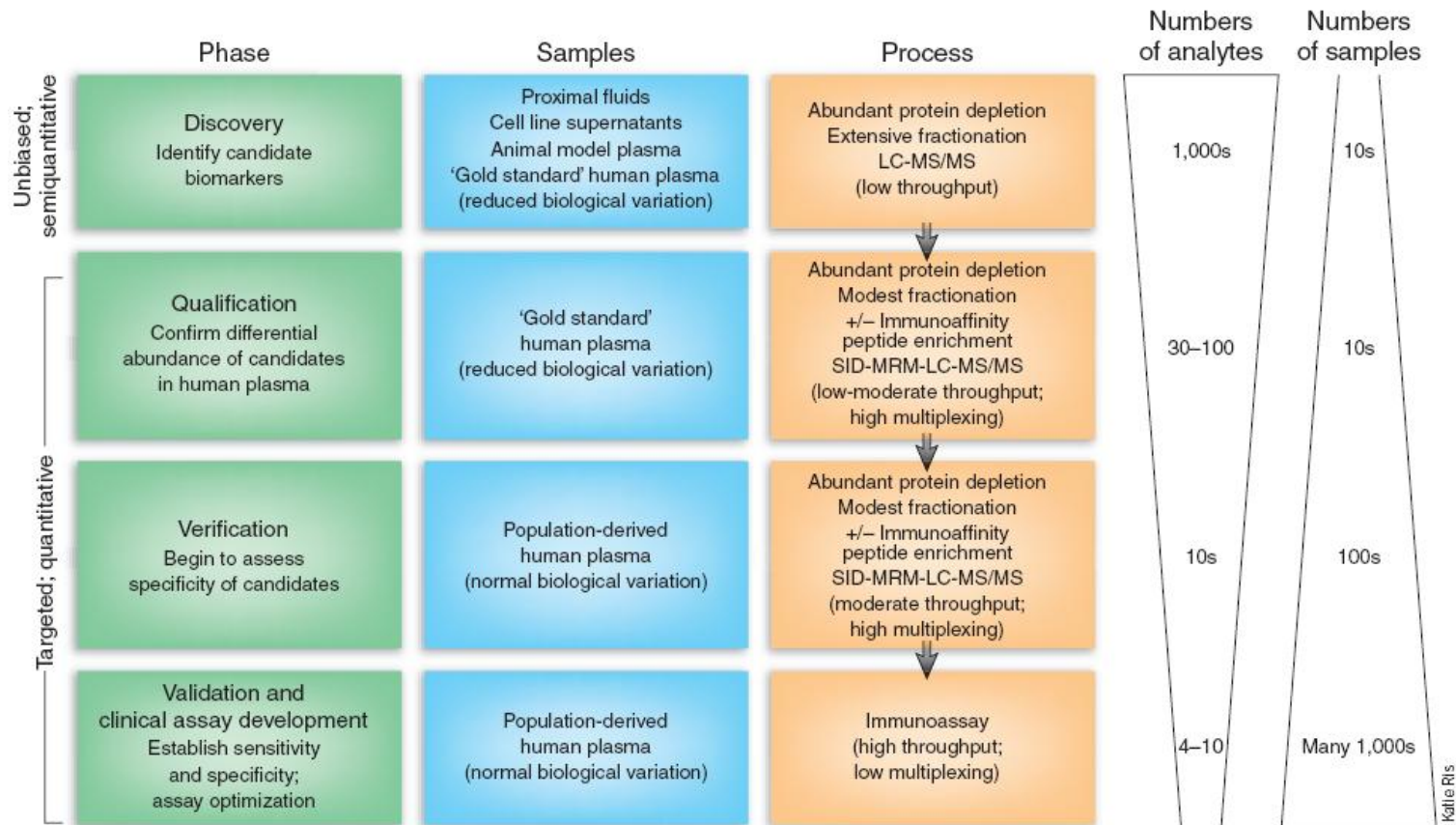


Figure 1 Process flow for the development of novel protein biomarker candidates. 'Numbers of analytes' refers to the number of proteins expected to be evaluated as candidate biomarkers in each phase of development. 'Numbers of samples' refers to the sample requirements for each phase. LC-MS/MS, liquid chromatography tandem mass spectrometry; SID, stable isotope dilution; MRM, multiple reaction monitoring.

Steps towards goal...

- Assays to assess immunogenicity – achieved, measuring IFN γ works
- More complex biosignatures – will come but will take ~3 years? (even longer for newest assays; needs agreement on analysis)
- Assays fit for purpose in trials – will require more work
- *(but it is hard to get different labs and groups to use harmonised assays unless they are forced to do so by funders!)*

Challenges

- Making sense of data from multiplex assays, gene arrays etc – we need to agree how to analyse data
- Deciding which assay formats will be most useful, and assessing how robust they are
- Getting different labs to continue the dialog started by the Working Group – a role for Stop TB and/or other consortia funded to work on assays across different diseases (ie EU funded TRANSVAC)

Thanks to:

Immunoassay Task Force

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