AERAS

In Pursuit of the Unknown: Standardizing Endpoint Assays for Evaluating Complex Immunological Signatures

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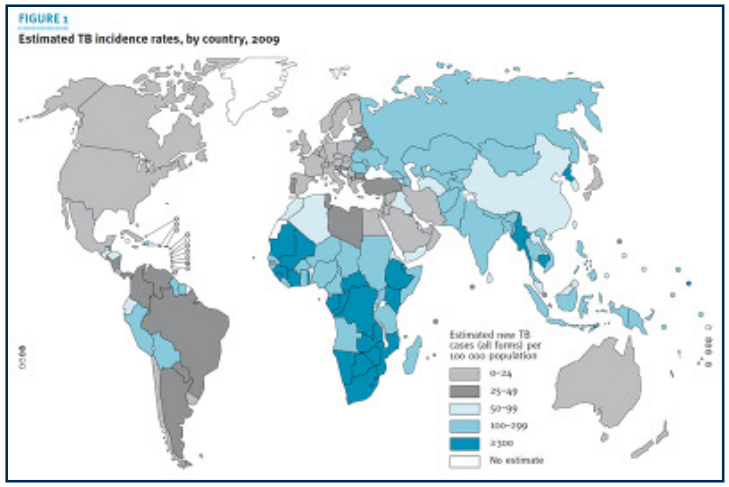
Tuberculosis: a devastating epidemic

- 8.7 million new cases and 1.4 million deaths in 2011
- WHO declared global public health emergency
- Over 2 billion people or 1/3 of the world's population is infected with *M. tuberculosis*
- TB/HIV co-epidemic – TB is the leading cause of death for people living with HIV
- MDR/XDR/TDR on the rise



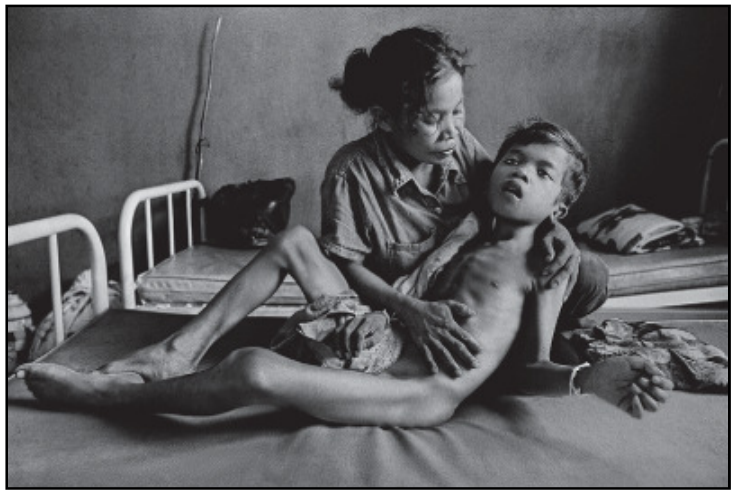


Photo by James Nachtwey xdrtb.org

Key challenges in vaccine development

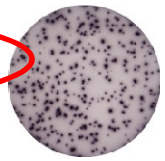
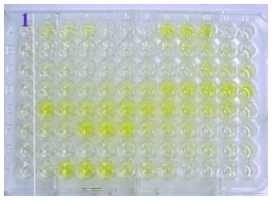
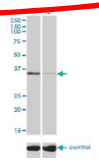
- Unknown correlation between animal models and human protection
- Animal challenge studies are long and expensive
- Lack of immune correlate of protection
- Optimal antigen identification unclear
- Protective human immunity not well understood: ~10% develop active disease
- Very large sample sizes required for Phase III efficacy studies

Progress

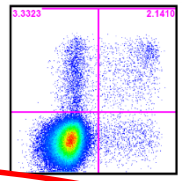
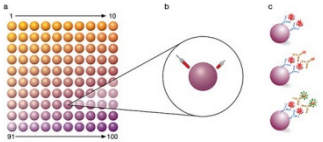
- We have identified immune responses that help to control TB
  - CD4+ T cells (from HIV+ patients)
  - CD8+ T cells (NHP models)
  - IFN-γ pathways (human deficiencies)
  - TNF (TNF blocking antibodies)
- Refining animal models
- Expanding immunological assays to look at more facets of the immune response (looking beyond Th1)
- Expansion of the clinical pipeline to test novel platforms and gain more knowledge on human immune responses and their impact on human disease

Immune assays for measuring responses to TB vaccines

- Simple assays (measuring single variables)
  - ELISA (cytokines, antibodies, etc)
  - ELISpot (cytokines, B cell responses)
  - Western blot (antibodies)
  - Proliferation assays
  - MGIA

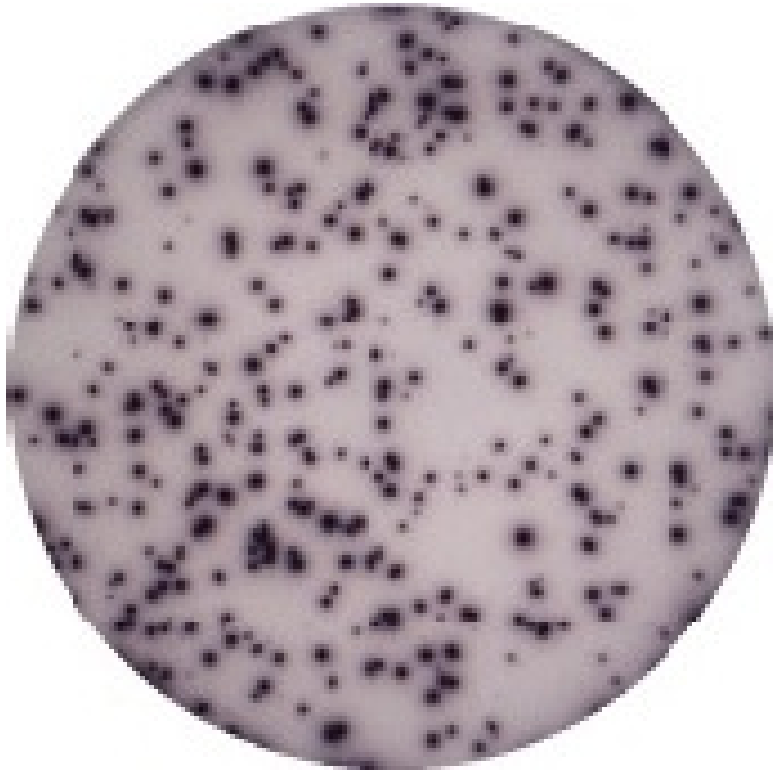





- Complex assays
  - Tetramers
  - ICS (whole blood or PBMC ICS by flow cytometry)
  - Multiplex (measure multiple cytokines or antibodies)

IFN-γ ELISpot

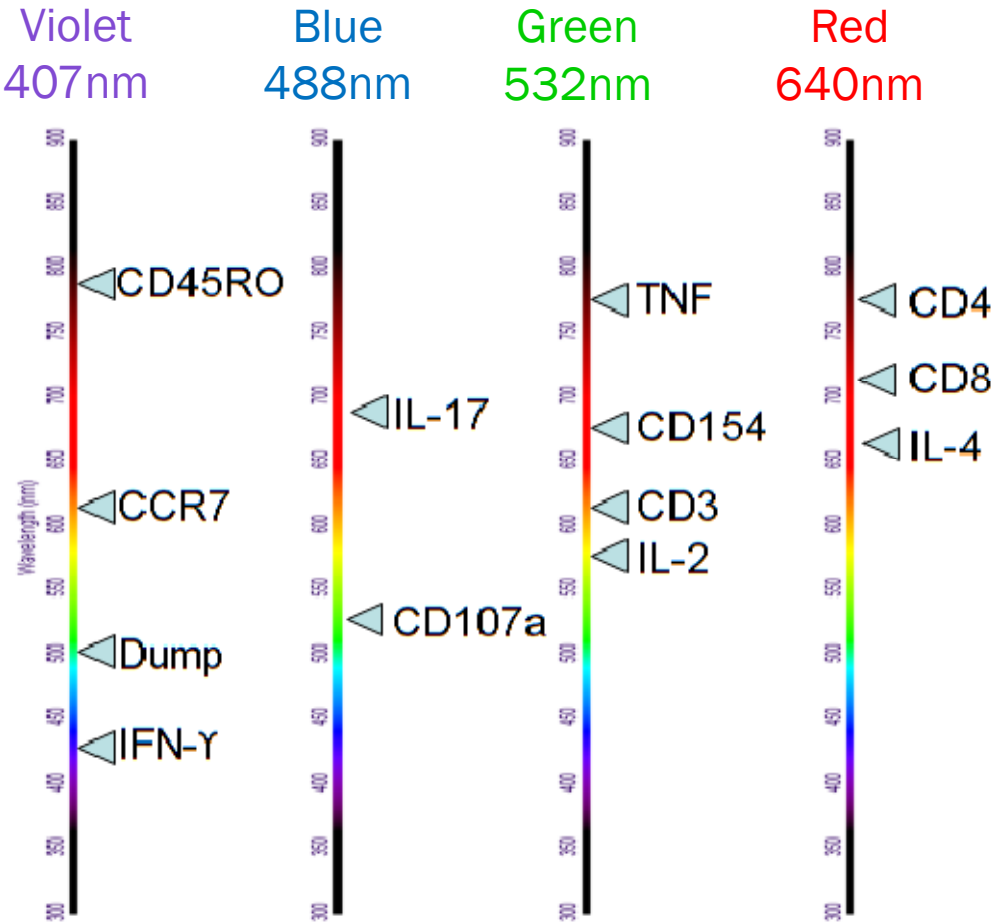
- Our ELISpot assay is based on the assay currently in use by the HVTN
- ELISpot is a simple assay that is relatively easy to qualify and validate
- Ideal as an immunogenicity assay in efficacy trials



- Highly sensitive
- Extremely limited assessment of immune responses compared to other assays such as ICS

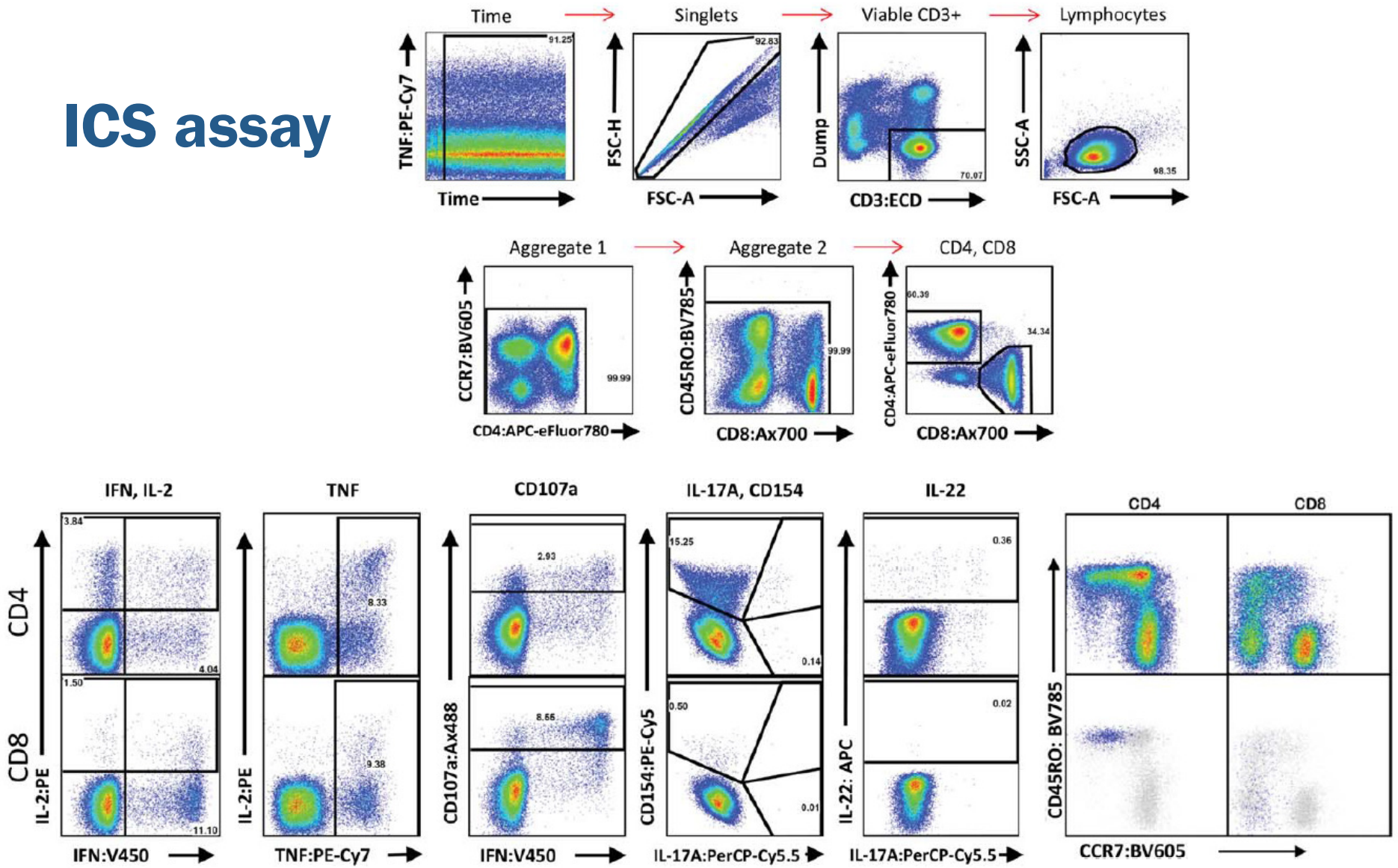
13-color ICS assay

The Panel



Purpose	Marker
Dump	Viability
	CD14
	CD19
Lineage	CD3
	CD4
	CD8
Degranulation	CD107a
B cell help	CD154
Th1 cytokines	IFN-γ
	IL-2
	TNF
Th17 cytokine	IL-17
Th22 cytokine	IL-22
Memory	CCR7
	CD45RO

ICS assay



OMIP-22
A. Graves et al. Cytometry Part A. 2014

How do you adequately control such a complex assay?

Strategy for assay controls

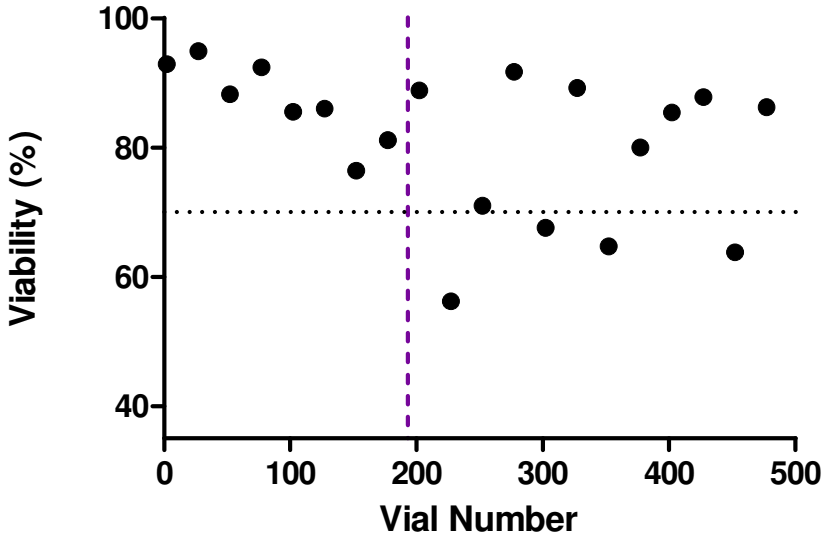
- We obtained leukapheresis samples from CMV-reactive donors for use as assay controls in the IFN-γ ELISpot

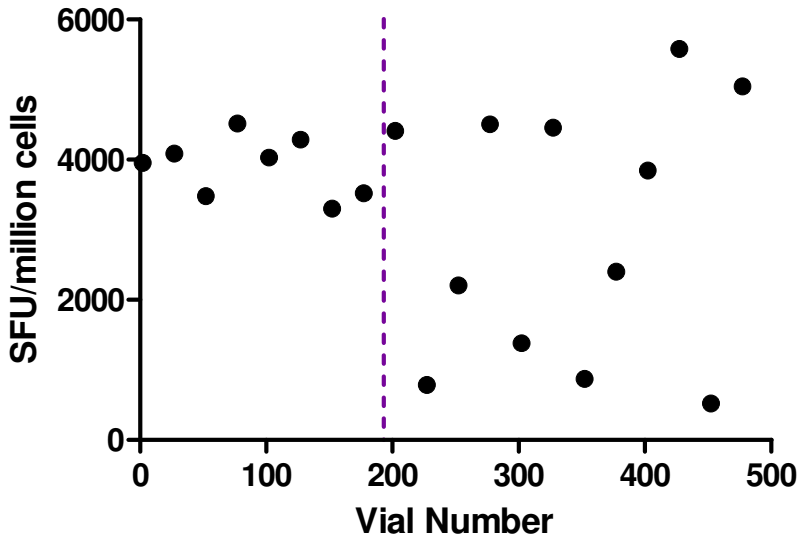
- Each leukapheresis sample generated 400-700 vials of PBMC (25 x 10e6 cells per vial)

- Obtained every 25th vial from the series for evaluation of stability of the first leukapheresis sample following the freezing process

Trending of the first leukapheresis sample

- We started freezing using Cool Cells, but then had to switch to Mr. Frosty containers (indicated below by the dotted line)

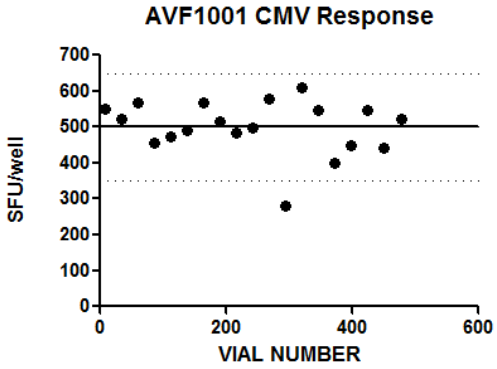


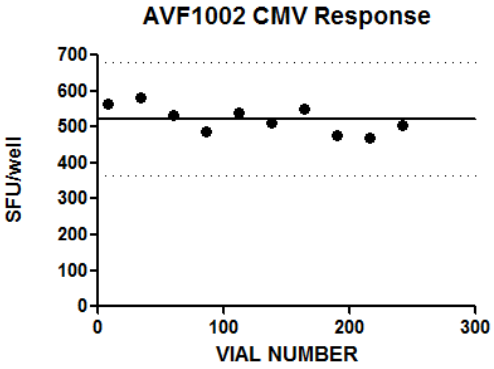


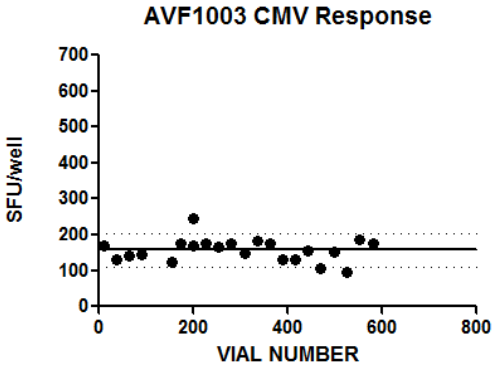
- There was a clear issue with the Mr. Frosty containers – subsequently we obtained sufficient Cool Cells to freeze an entire series of vials

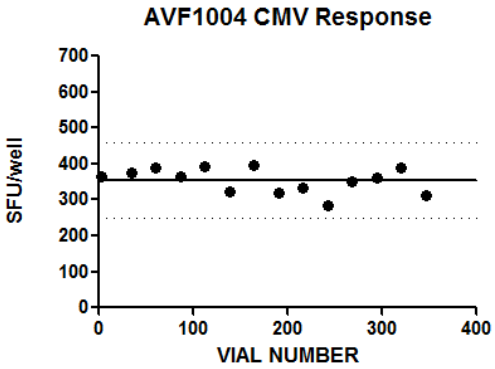
Subsequent samples with Cool Cells

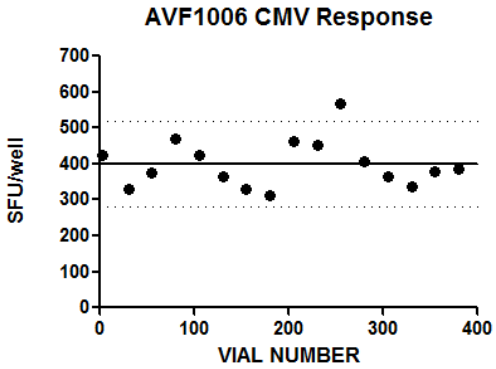
- Following the first donor, more Cool Cells were obtained and we collected more leukapheresis samples which were then trended





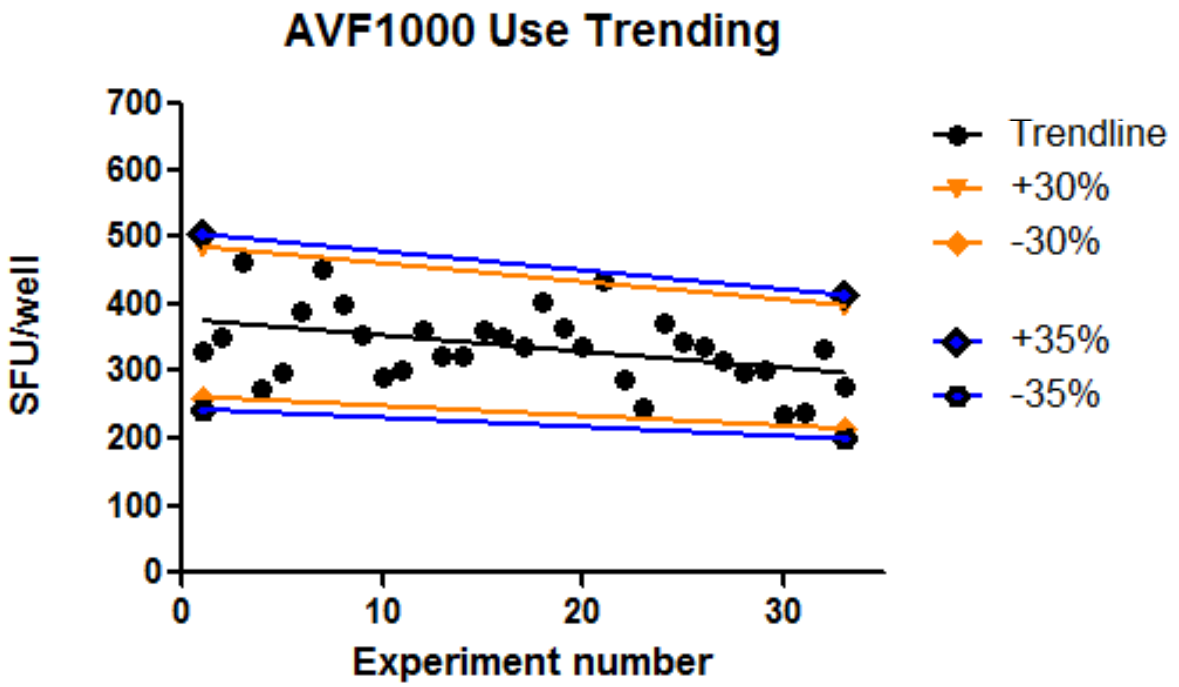






Long-term trending

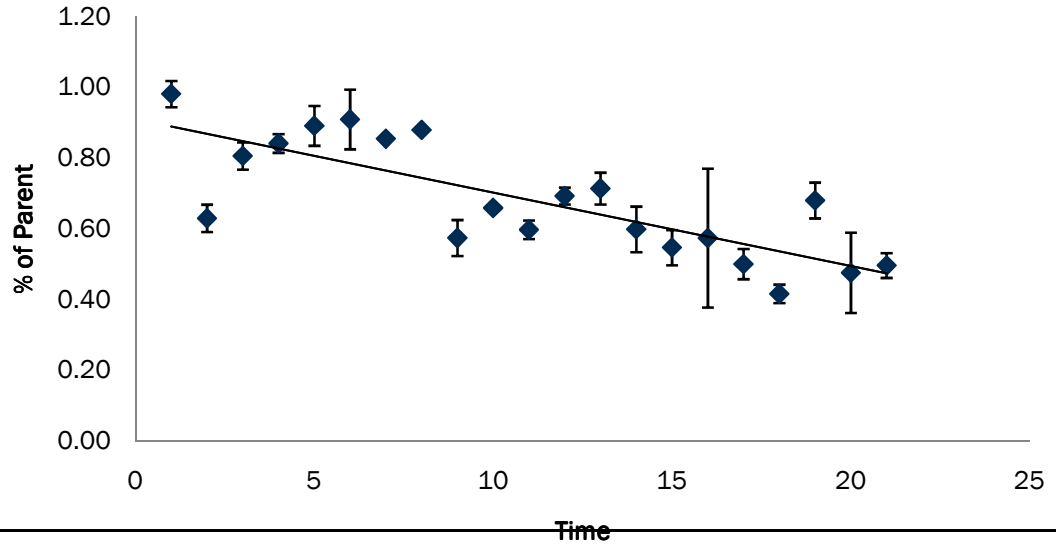
- The first donor has been used in multiple assays for more than a year
- We have examined the trending of this donor as a factor of time



Addressing the downward trend

- The primary concern with the trend was that the assay may be losing sensitivity over time

- In order to assess whether the observed loss of response is due to the donor sample or possibly due to an issue with the sample storage or freezing, we examined the responses that have been observed in the ICS assay over the same time period and observed a very similar trend



Conclusions regarding assay stability

- Based on the consistency of the data between two different assays, we believe that the observed decline is due to a loss in functionality of the control donor rather than a decrease in assay sensitivity

- In addition to the experiments shown, we have also generated data with other laboratories comparing assay results and obtained strikingly consistent data

- The loss in functionality of the control donor is likely due to either the length of time the cells were in freezing solution during the freezing process or due to long-term storage in LN2 (or both!)

- Despite the loss of function, real-time trending allows for detection of assay fluctuations that call into question the validity of the generated data (we currently use +/- 30% for cellular assays)

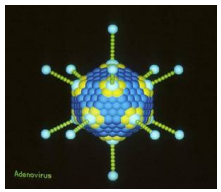
Assay performance

- Both the ELISpot and ICS assays have been used in multiple clinical trials
  - Protein/adjuvant combinations
  - Viral vectors
    - Adenovirus
    - MVA
  - Recombinant mycobacteria
  - Mycobacterial lysates (soon!)

- Generally, responses are low in the TB field
- The largest responses seen to date was generated using a heterologous prime/boost regimen

AERAS-402/MVA85A

- AERAS-402
  - Adenovirus serotype 35 (Ad35) vaccine
  - Encodes a fusion protein of Ag85A, Ag85B, and TB10.4
  - Dominant response is CD8+ T cells largely against Ag85B



- MVA85A
  - Modified Vaccinia Ankara (MVA) vector
  - Encodes Ag85A
  - Dominant response is CD4+ T cells against Ag85A

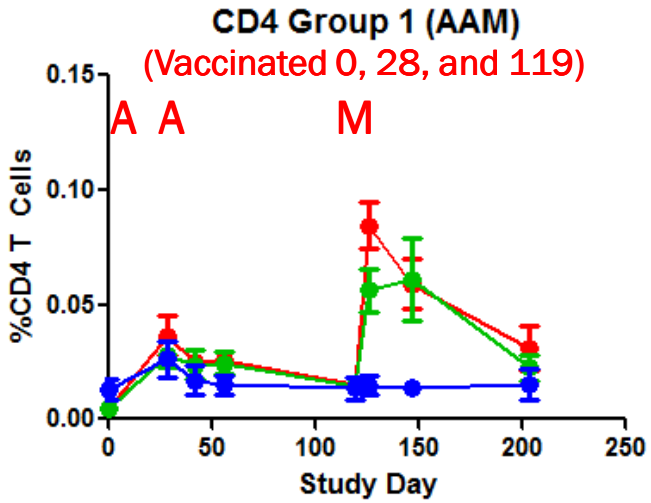
- Both have been shown to be safe and immunogenic in adults with lower immunogenicity in infants

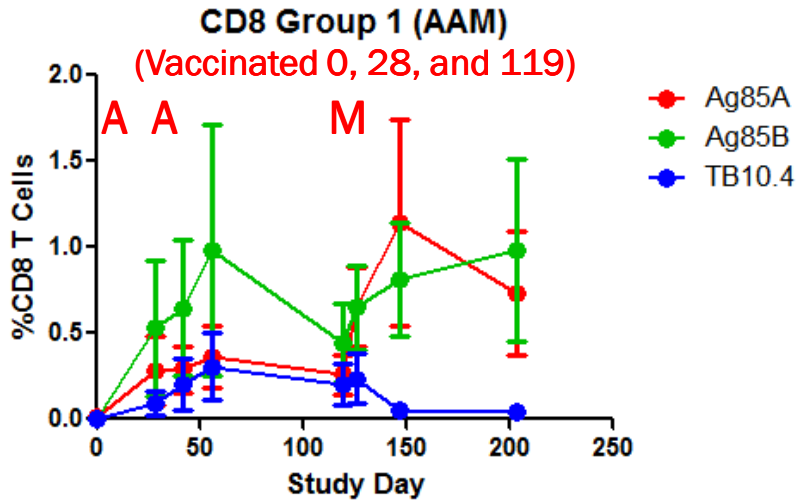
AERAS-402/MVA85A study design

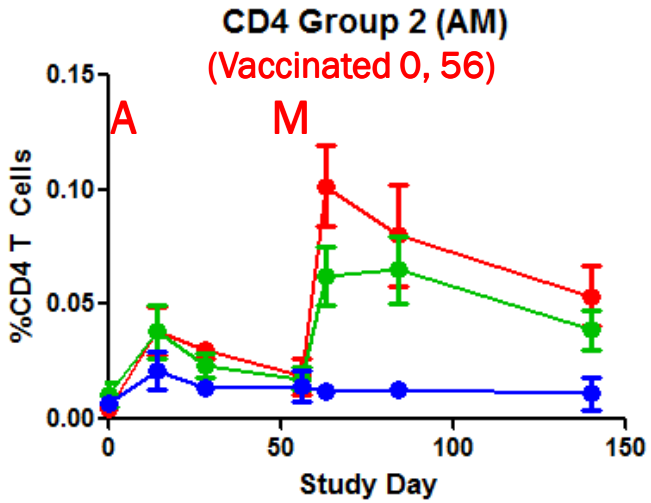
Group	Injection #1	Injection #2	Injection #3	Total
1	AERAS-402 1×10^{11} vp	AERAS-402 1×10^{11} vp	MVA85A 1×10^{8} pfu	15
2	AERAS-402 1×10^{11} vp	MVA85A 1×10^{8} pfu	N/A	15
Total Number of Subjects				30

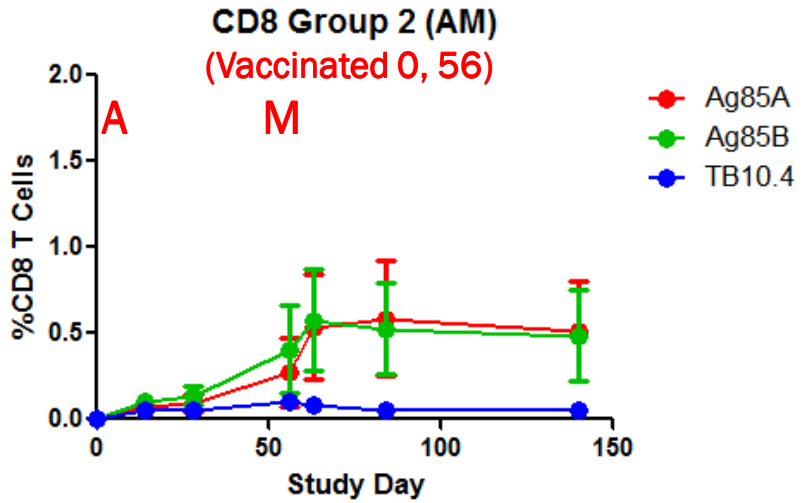
- Group 1: AAM (Vaccinated on days 0, 28, and 119)
- Group 2: AM (Vaccinated on days 0 and 56)

AERAS-402/MVA85A

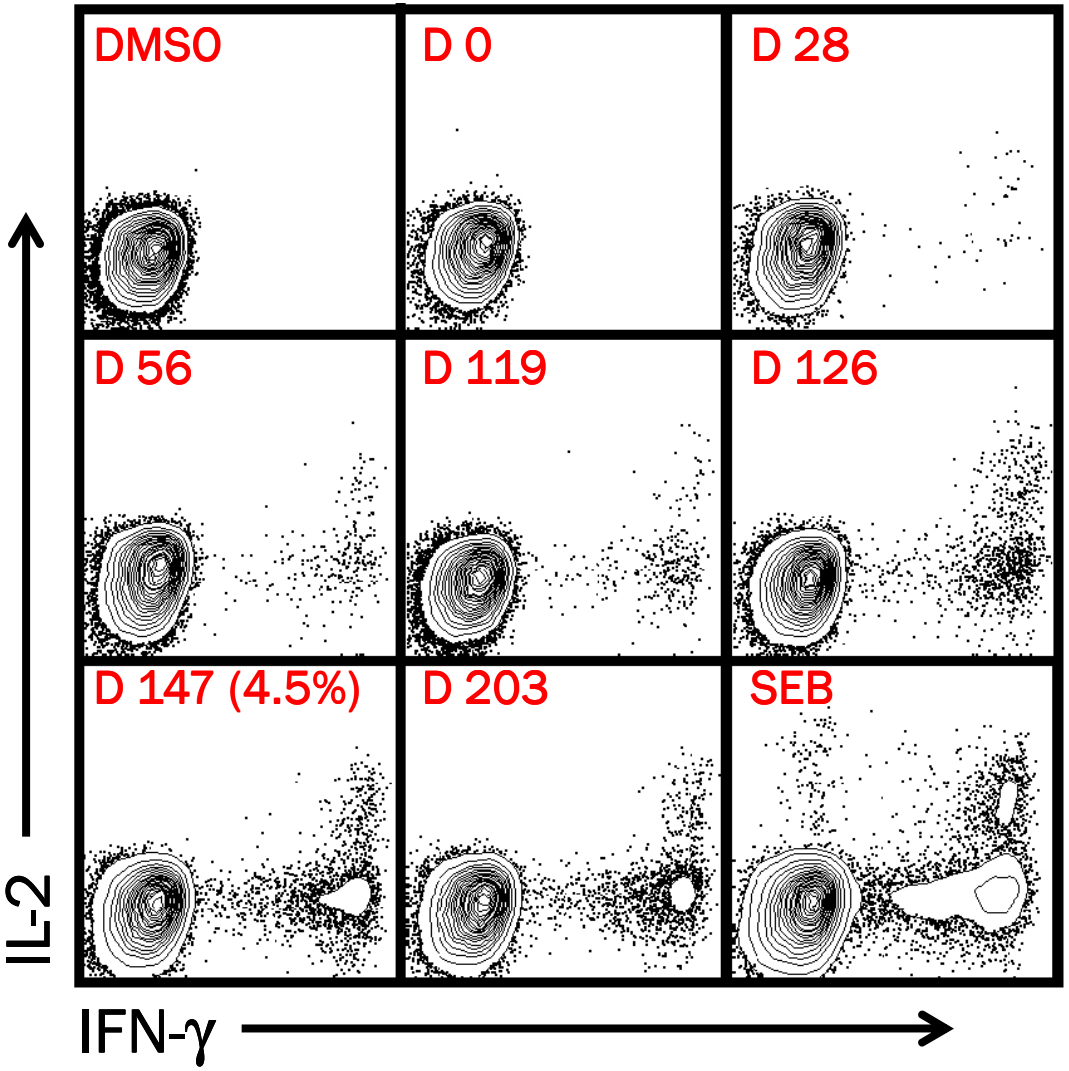








CD8+ Ag85A responses (AAM group)

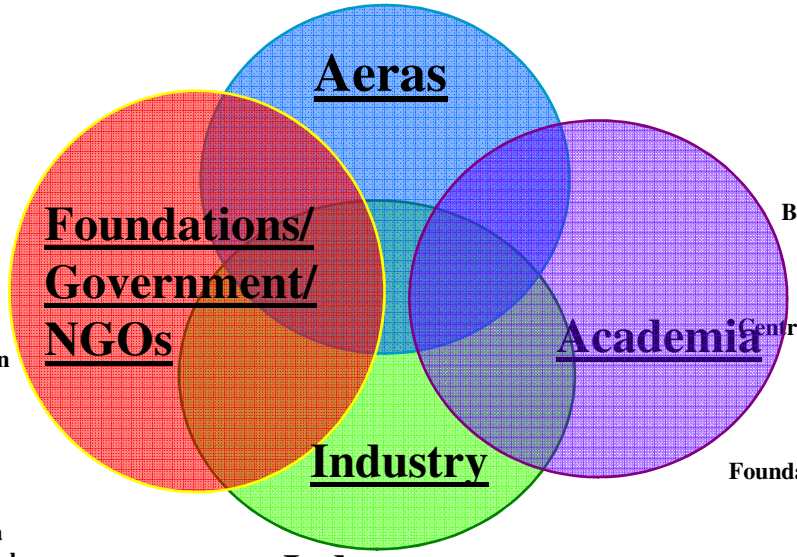


AERAS-402 on days 0 and 28

MVA85A on day 119

Largest vaccine-induced responses in TB uninfected adults observed to date

Aeras global partners



Foundations/ Governments/ NGOs

Bill & Melinda Gates Foundation, U.S.
Ministry of Foreign Affairs of Denmark
The Netherlands Ministry of Foreign Affairs, the Netherlands
Centers for Disease Control and Prevention (CDC), U.S.
Fogarty International Center and NIAID, National Institutes of Health, U.S.
Research Council of Norway, Norway
AIDS Fondet, Denmark
Cambodian Health Committee, Cambodia
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LHL/ The Norwegian Association of Heart and Lung Patients, Norway
Planeta Salud, Spain
Manhiça Health Research Centre, Mozambique
Medicine in Need (MEND), U.S.
Stop TB Partnership, Switzerland
TB-Alert, United Kingdom
Tuberculosis Vaccine Initiative (TBVI), Netherlands
Wellcome Trust, United Kingdom

Industry

GlaxoSmithKline Biologicals, Belgium
Crucell, the Netherlands
Statens Serum Institute, Denmark
ImmunoBiology, United Kingdom
Wuhan Institute of Biological Products, China
Serum Institute, India
Thymed, Germany
Alphalyse, Denmark
Japan BCG Laboratory, Japan
Korean Institute of TB, Korea
Cyncron, Denmark
Cellestis, Australia
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Larimer, U.S.
Sanofi Pasteur, France
Smittskyddsinstitutet, Sweden
BIOCON, U.S.
Emergent BioSolutions, U.S.
Intercell, Austria
Spring Valley Laboratories, U.S.
Statens Serum Institute, Denmark
Bioland, Korea

Academia

Oxford University, United Kingdom
South African TB Vaccine Initiative (SATVI), South Africa
St. Johns Research Institute, India
Makerere University, Uganda
Kenya Medical Research Institute, Kenya
Karolinska Institute, Sweden
Wuhan University, China
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Arizona State University, U.S.
Biomedical Primate Research Center, the Netherlands
Boston University
Case Western Reserve University, U.S.
Central Institute for Tuberculosis, Russia
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Dartmouth University
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Max Planck Institute for Infection Biology, Germany
McGill University, Canada
National Cancer Institute (NKI), the Netherlands
New York University, U.S.
Oregon Health Sciences University, U.S.
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Stanford University, U.S.
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University of California-Davis, U.S.
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E D C T P

European and Developing Countries Clinical Trials Partnership

FDA

US Food and Drug Administration

National Institute of Allergy and Infectious Diseases
National Institutes of Health

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Thank You.