Non-neutralizing Fc-mediated antibody responses:
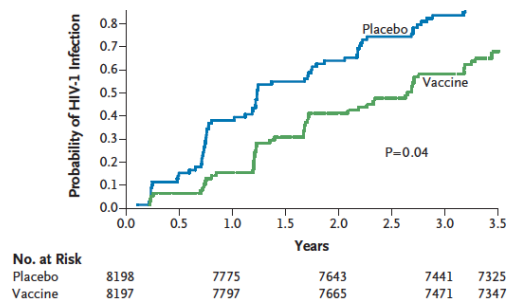## Fcγ Receptor Binding Breadth Of RV144 Antibodies

Amy Chung

University of Melbourne

Doherty Institute

RV144 'Thai' Vaccine Trial

- ALVAC (Pox vector) + AIDSVAX (recombinant Env protein)
- **31.2% efficacy at 42 months (p=0.04)**
  - 60% efficacy at 12 months (Robb et al Lancet Inf Dis 2012)

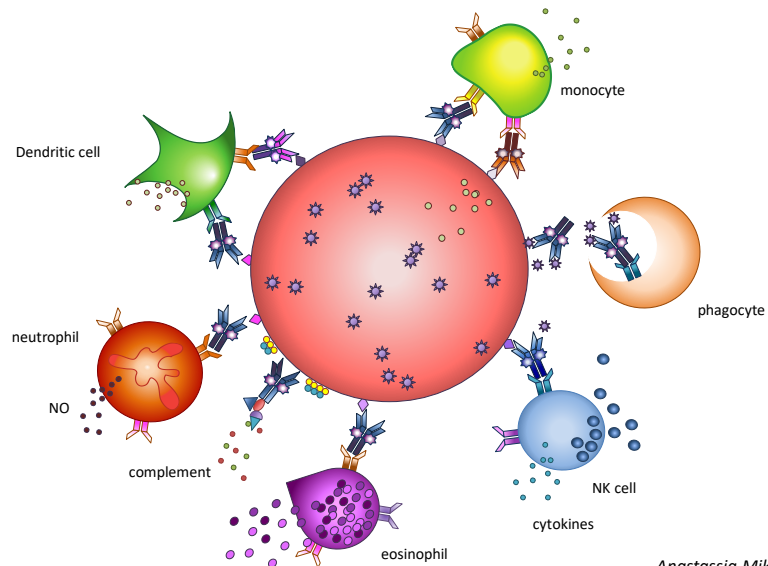*Rerks-Ngarm NEJM 2009*

How was RV144 protective?

- Results
  - Main immune responses induced were binding **non-neutralizing** Antibodies and CD4 responses to gp120

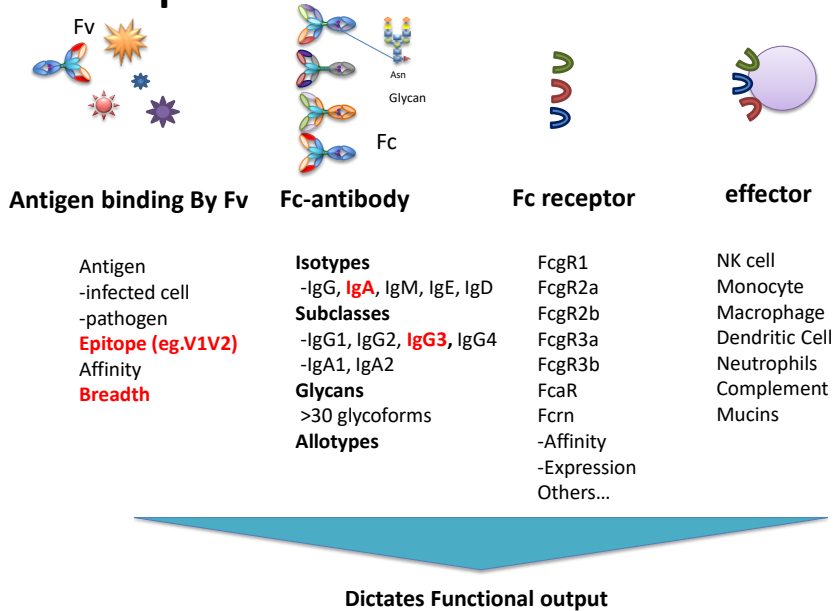| ICS	Vaccine	Placebo
CD8 Gag	7.6%	7.1%
CD8 ENV	11.1%	14.3%
CD4 Gag	1.4%	0
CD4 ENV	34%	3.6%
Binding Ab	**Vaccine**	**Placebo**
gp120 MN	98.6%	0%
gp120 A244	98.6%	0%
p24	52.1%	0%

Rerks-Ngarm NEJM 2009

Beyond Neutralization…
Antibodies are highly functional

Anastassia Mikhailova

Multiple features modulate Ab Functions

Fv

Asn

Glycan

Fc

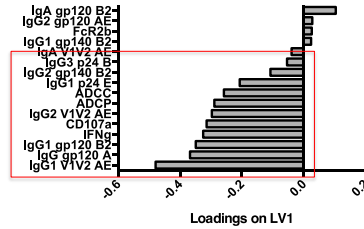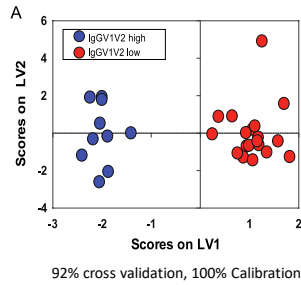| Antigen binding By Fv	Fc-antibody	Fc receptor	effector
Antigen -infected cell -pathogen **Epitope (eg.V1V2)** Affinity **Breadth**	**Isotypes** -IgG, **IgA**, IgM, IgE, IgD **Subclasses** -IgG1, IgG2, **IgG3**, IgG4 -IgA1, IgA2 **Glycans** >30 glycoforms **Allotypes**	FcgR1 FcgR2a FcgR2b FcgR3a FcgR3b FcaR Fcrn -Affinity -Expression Others…	NK cell Monocyte Macrophage Dendritic Cell Neutrophils Complement Mucins

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Dictates Functional output

How was RV144 protective?

- Protection was associated with
  - High levels of IgG binding to V1V2
    - High levels of IgG3 binding to V1V2
  - Low levels of IgA binding to Env
    - High antibody dependent cellular cytotoxicity (ADCC)
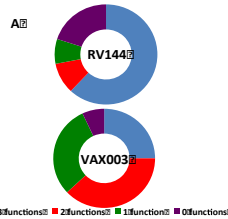   - **Matthew Worley - Oral- 4pm Immunology and Pathogenesis**

Hayes et al NEJM 2012, Yates et al STM 2014

What is so special about V1V2 antibodies?

92% cross validation, 100% Calibration

V1V2 antibodies are associated with polyfunctional non-neutralizing antibody responses

Target virus using multiple different antibody Fc mechanisms

Chung et al. Cell 2015, Chung et al. STM. 2014

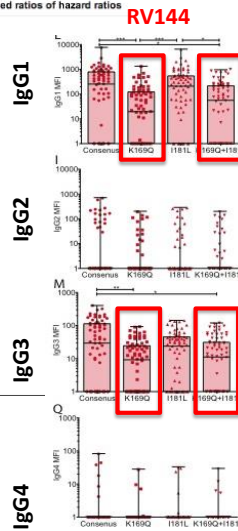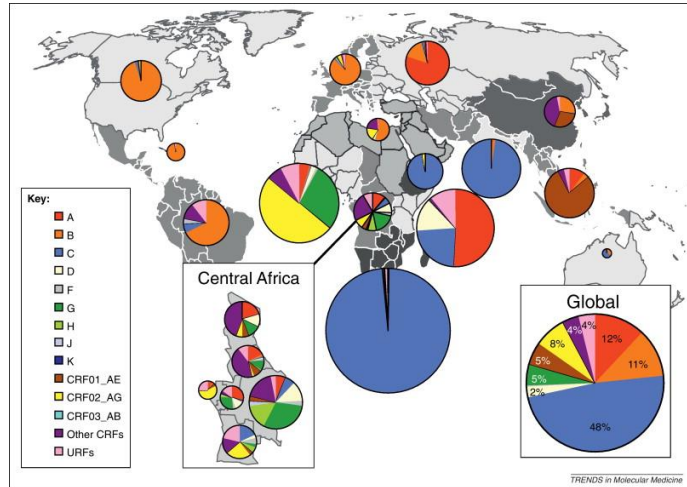# RV144 induced high V1V2, but had low recognition of breakthrough sequences

Rolland et al Nature 2012

Question of non-neutralizing Functional antibody breadth?

Chung et al STM 2014
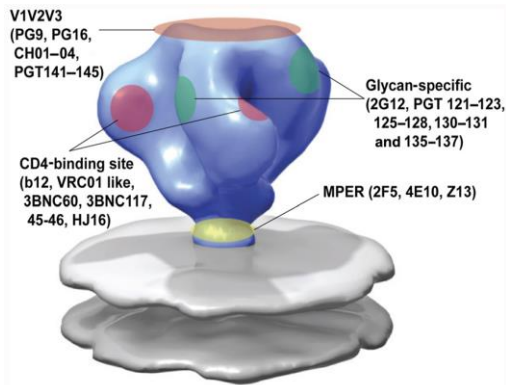
HIV-1 is a highly diverse virus

Hemelaar. Trends Mol Med. 2012

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"Breadth" highly important for HIV antibody neutralization...

**Neutralizing antibody "hot spots"
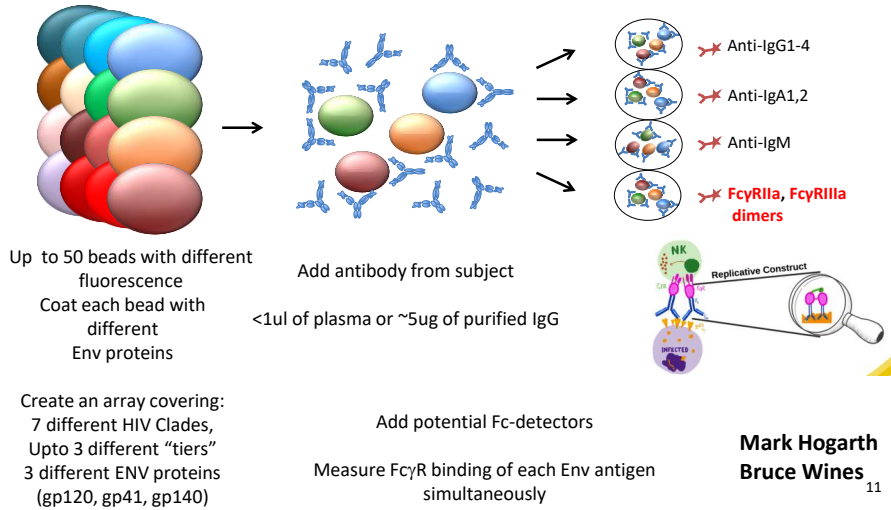-link to viral function**

What about non-neutralizing Fc effector Functional antibody breadth?
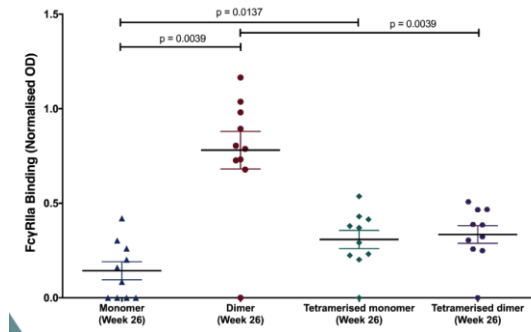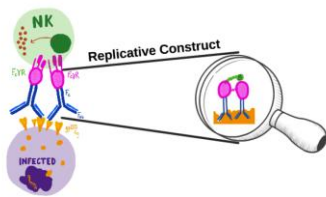
Need high throughput assays to test for non-neutralizing Fc activity across multiple clades

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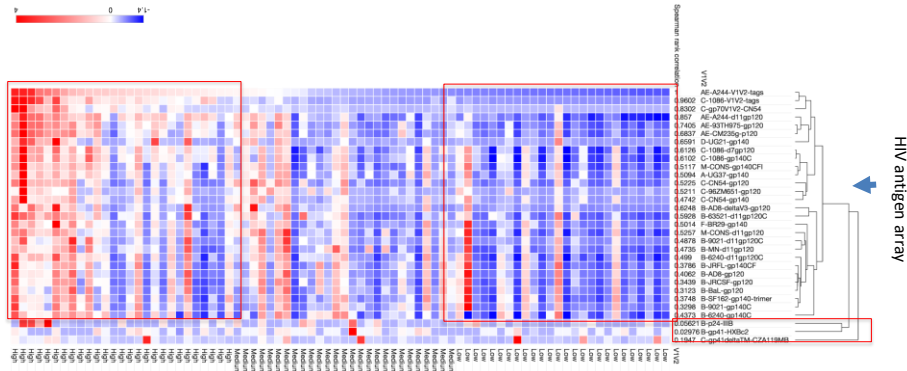# Multiplex Bead Array to predict Fc functional breadth

Up to 50 beads with different fluorescence
Coat each bead with different
Env proteins

Create an array covering:
7 different HIV Clades,
Upto 3 different "tiers"
3 different ENV proteins
(gp120, gp41, gp140)

Add antibody from subject

<1ul of plasma or ~5ug of purified IgG

Add potential Fc-detectors

Measure FcγR binding of each Env antigen simultaneously

Anti-IgG1-4
Anti-IgA1,2
Anti-IgM
FcγRIIa, FcγRIIIa dimers

Mark Hogarth
Bruce Wines
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FcgR dimer detector

Mimics cross linking of FcR
and activation of effector cell

Mark Hogarth
Bruce Wines
Milla McClean

McClean et al. J Immuno. 2017

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Multiplex FcγR dimer array reflects Fc- effector functionality

Anne Kristensen, Fernanda Ana Sosa, Matthew Worley

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RV144 Fc-functional antibody Breadth

32 HIV antigens
7 different HIV Clades, 3 'tiers'
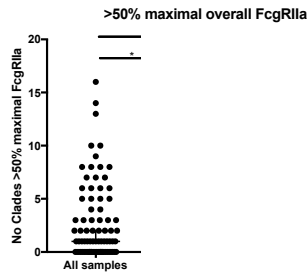3 different ENV proteins (gp120, gp41, gp140)

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RV144 vaccinees with high V1V2 responses have greater non-neutralizing breadth

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Overall FcR breadth from RV144 vaccine is modest

Overall breadth:
Number of HIV Antigens that recognized at least 50% of maximal response (to A244 gp120)

Median # strains	1
Range	0-16

High V1V2 responders (low risk of infection) had greatest breadth

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Summary

- V1V2 correlate of RV144 protection
  - Associated with polyfunctional Fc effector responses
- Fc receptor dimer multiplex array can act as an early screening method for FcR function
- Allows an assessment of FcR binding breadth
  - Modest in RV144 trial
  - Correlates with V1V2 antibodies
  - Breadth of Non-neutralizing antibody may be important for protection?

Acknowledgments