Vaccine-Induced Immunity in T-Cell Based Candidate HIV Vaccines

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AIDS Vaccine 2008

Cape Town

In the Step Study,
the MRKAd5 HIV-1 gag/pol/nef vaccine
did not lower HIV-1 infection rates
or post-infection plasma viremia.

HIV-1 incidence was higher in
vaccine-treated than placebo-treated
uncircumcised males with pre-existing
adenovirus serotype 5 (Ad5) immunity.

What threshold immune response
must future HIV vaccines elicit
if T cell immunity is critical
in vaccine-induced HIV protection?

Step Trial: After 3 immunizations with MRKAd5 HIV-1 gag/pol/nef vaccine, HIV-specific T cells were detected in 89%; response frequencies were similar to those in previous phase I trials

Protein	% (# positive/# tested)
Total	89.6% (206/230)
Gag	83.0% (191/230)
Pol	67.8% (156/230)
Nef	78.3% (180/230)

*analysis by IFN-γ ELISpot in PBMC at week 30 in stratified random sample

Step Trial: HIV-specific T cell responses Case-cohort comparison study*

a. CD4+ T helper cells mounted in ~41% of vaccinees, median ~0.2% of circulating CD4+ cells, recognizing predominantly Gag epitopes,

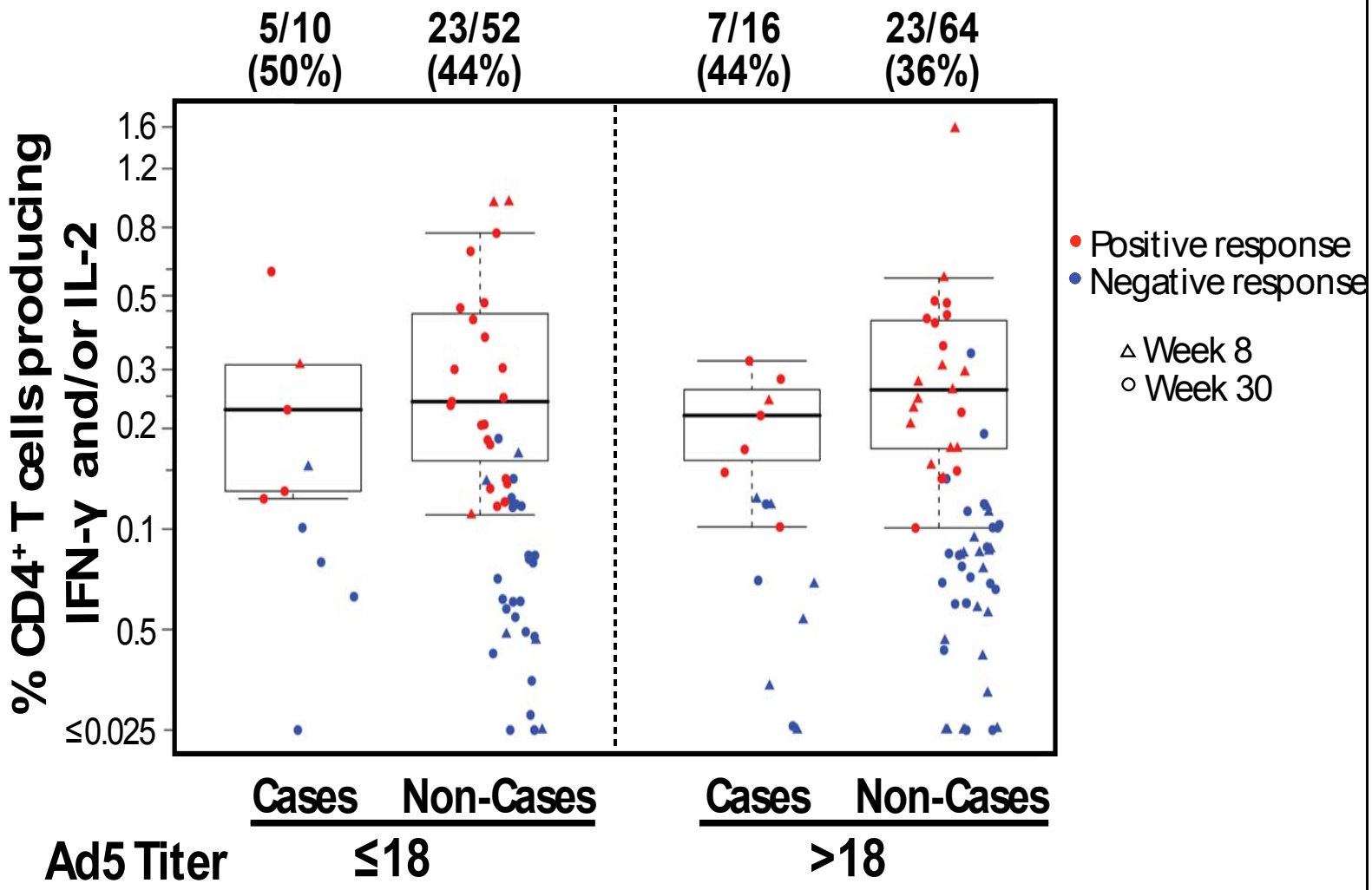
no differences apparent between cases vs. non-cases

b. CD8+ T cells elicited in the majority of vaccinees, 0.5-0.8% of circulating CD8+ cells, magnitude and frequency greater in Ad5≤18 group,

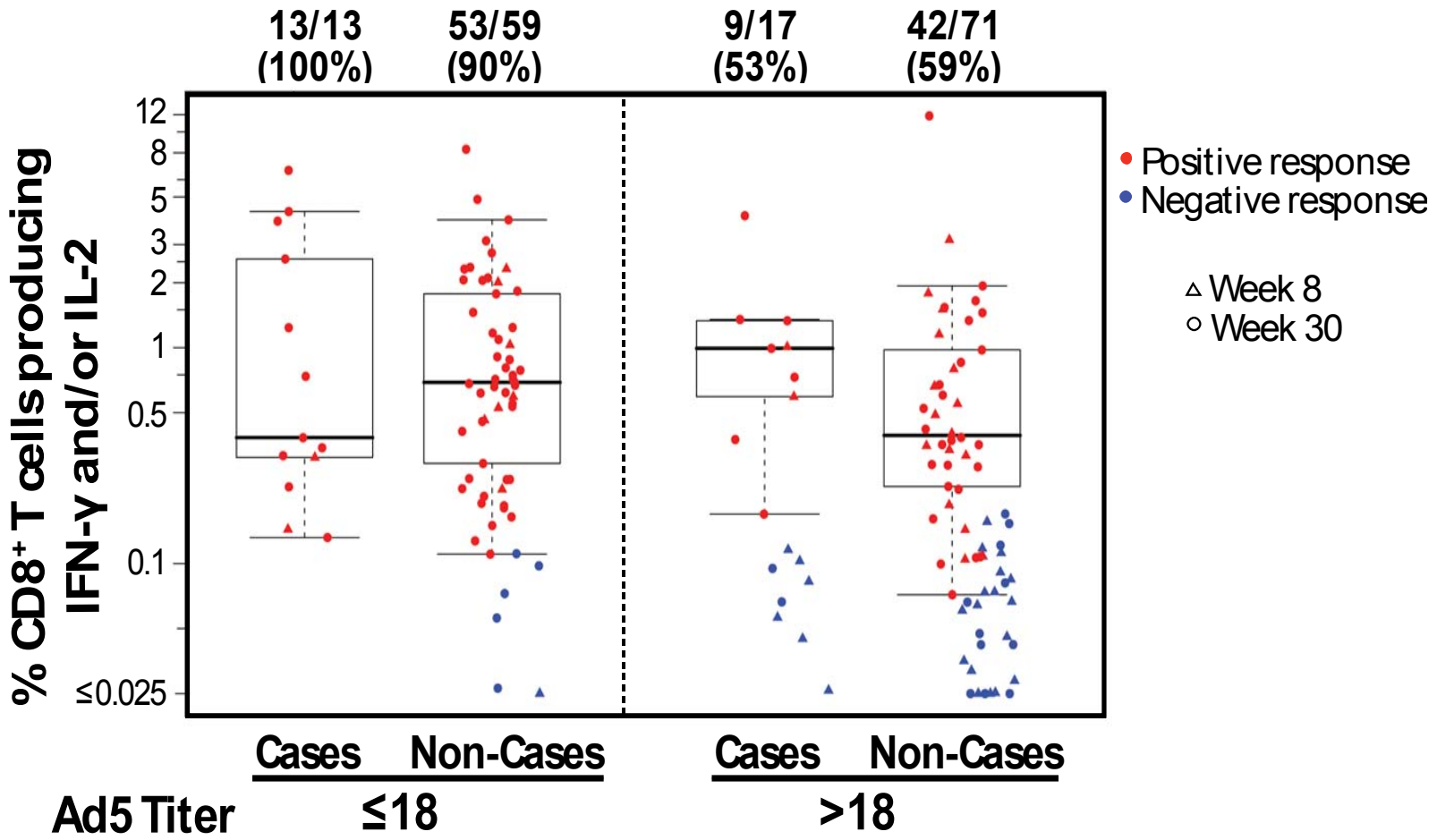
no differences apparent between cases vs. non-cases

*male per-protocol cases, non-cases matched 2-4:1 by age, region, Ad5 titer, treatment

Magnitude of vaccine-induced CD4+ T cells in cases and non-cases 4 weeks after the second (week 8) or third (week 30) vaccination



Magnitude of vaccine-induced CD8+ T cells in cases and non-cases 4 weeks after the second (week 8) or third (week 30) vaccination



HIV VACCINE
TRIALS NETWORK

While the overall frequency of
T cell responses was high,
only 31% (CI: 24-41%)
of vaccine recipients mounted
both CD4+ and CD8+ HIV-specific
T cell responses after three doses.

Step Trial: HIV-specific T cell responses*

"Polyfunctional" CD4 and CD8 T cells elicited:

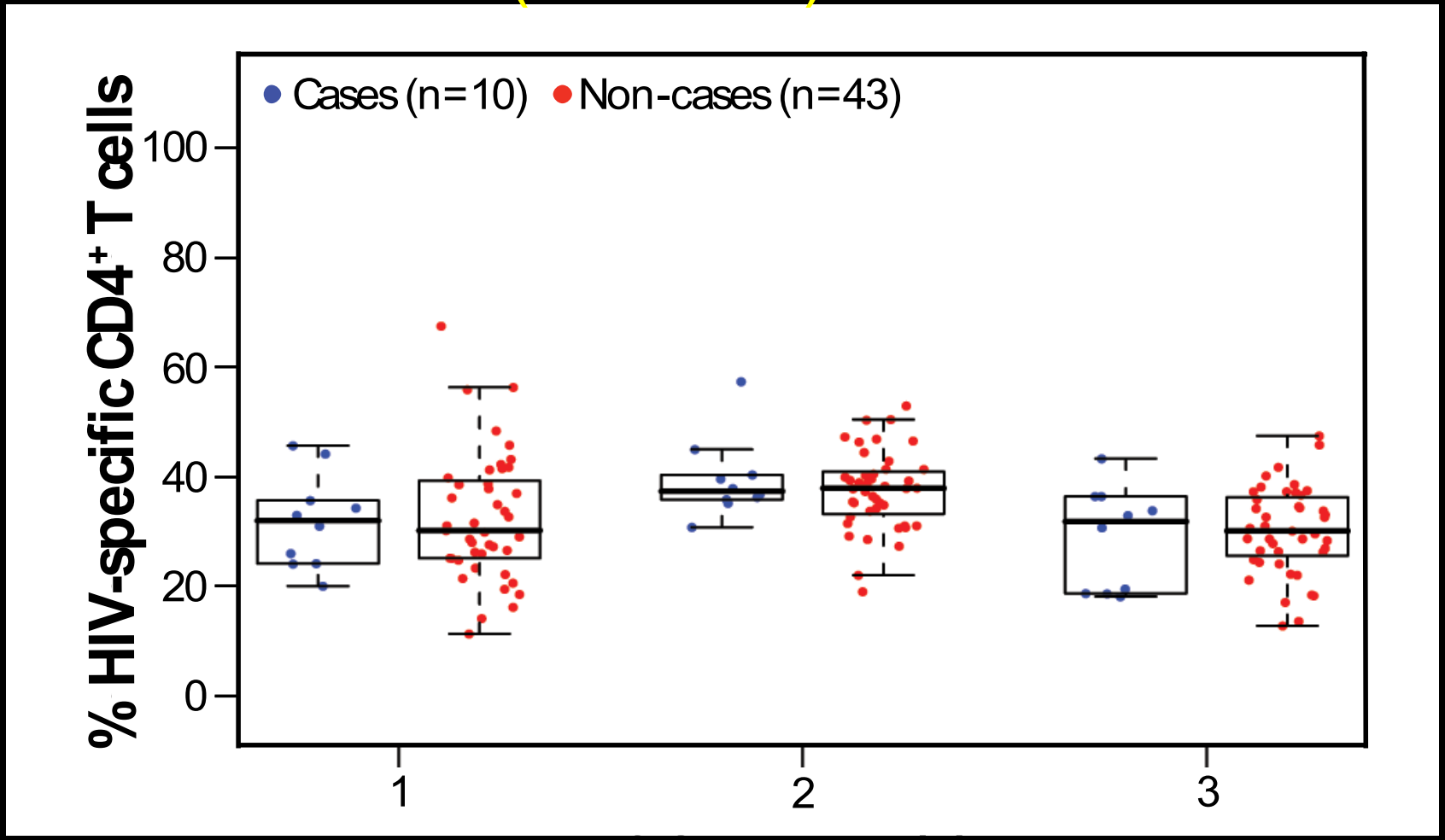
1) CD4+ T cells: IL-2+ predominated, majority secreted 2-3 cytokines
2) CD8+ T cells: IFN-γ (TNF) predominated, majority secreted 1-2 cytokines

no differences apparent between cases vs. non-cases

*Case-cohort study

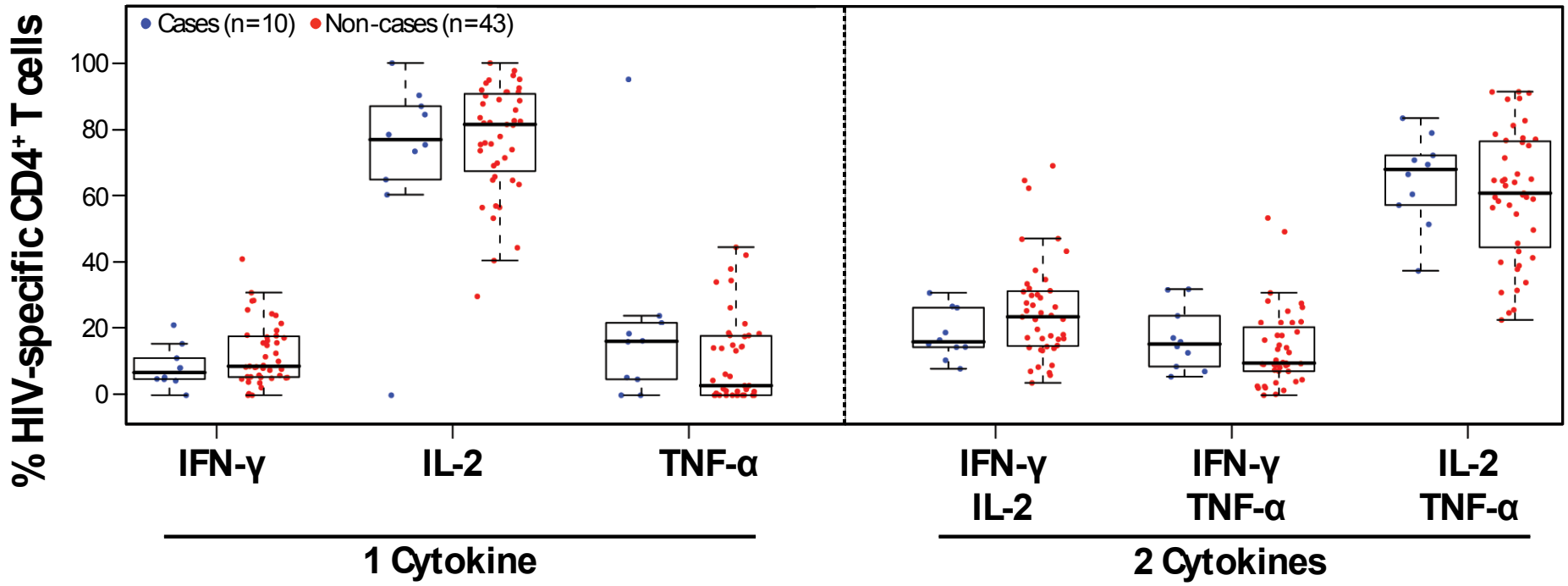
HIV VACCINE TRIALS NETWORK

Polyfunctional CD4+ T cell responses in cases and non-cases 4 weeks after the third (week 30) vaccination



HIV VACCINE TRIALS NETWORK

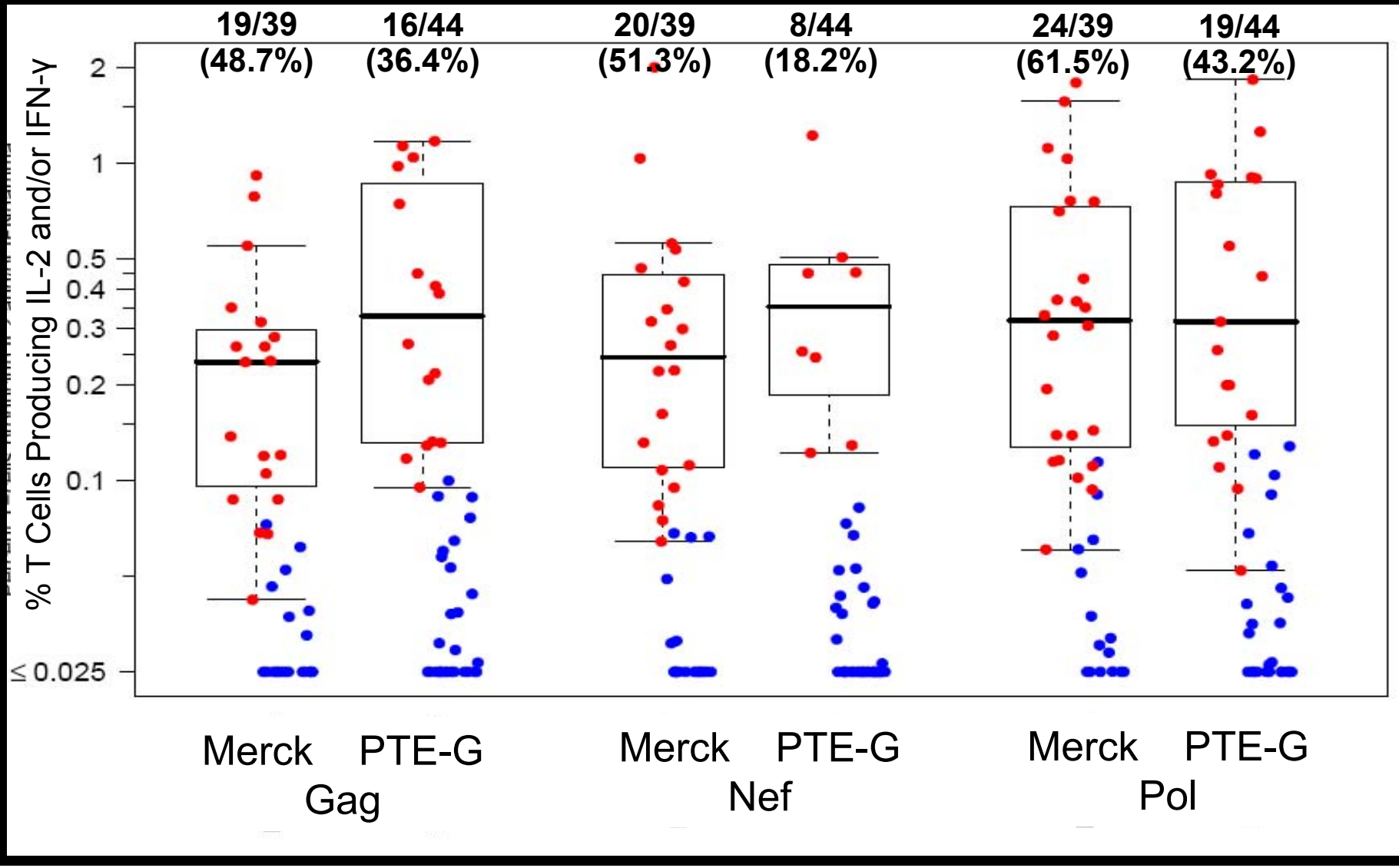
Polyfunctional CD4+ T cell responses in cases and non-cases 4 weeks after the third (week 30) vaccination



CD4+ T Cells

Did the MRKAd5 HIV-1 gag/pol/nef vaccine elicit sufficient magnitude and breadth of T cell responses?

HVTN 502: Comparison of CD8+ T Cell Responses Using Merck vs Global PTE Peptides (ICS), U.S. male vaccinees



Comparing CD8+ T cell responses
using the same
PTE-Global peptide panel (Gag, Pol and Nef)
in the ICS assay,
the median percentage of vaccine-induced
CD8+ T cells in the Step study was
43% lower than in
HIV-infected long-term non-progressors

Prior Ad5 Immunity Impacts Breadth and Potency of HIV-specific T Cell Responses

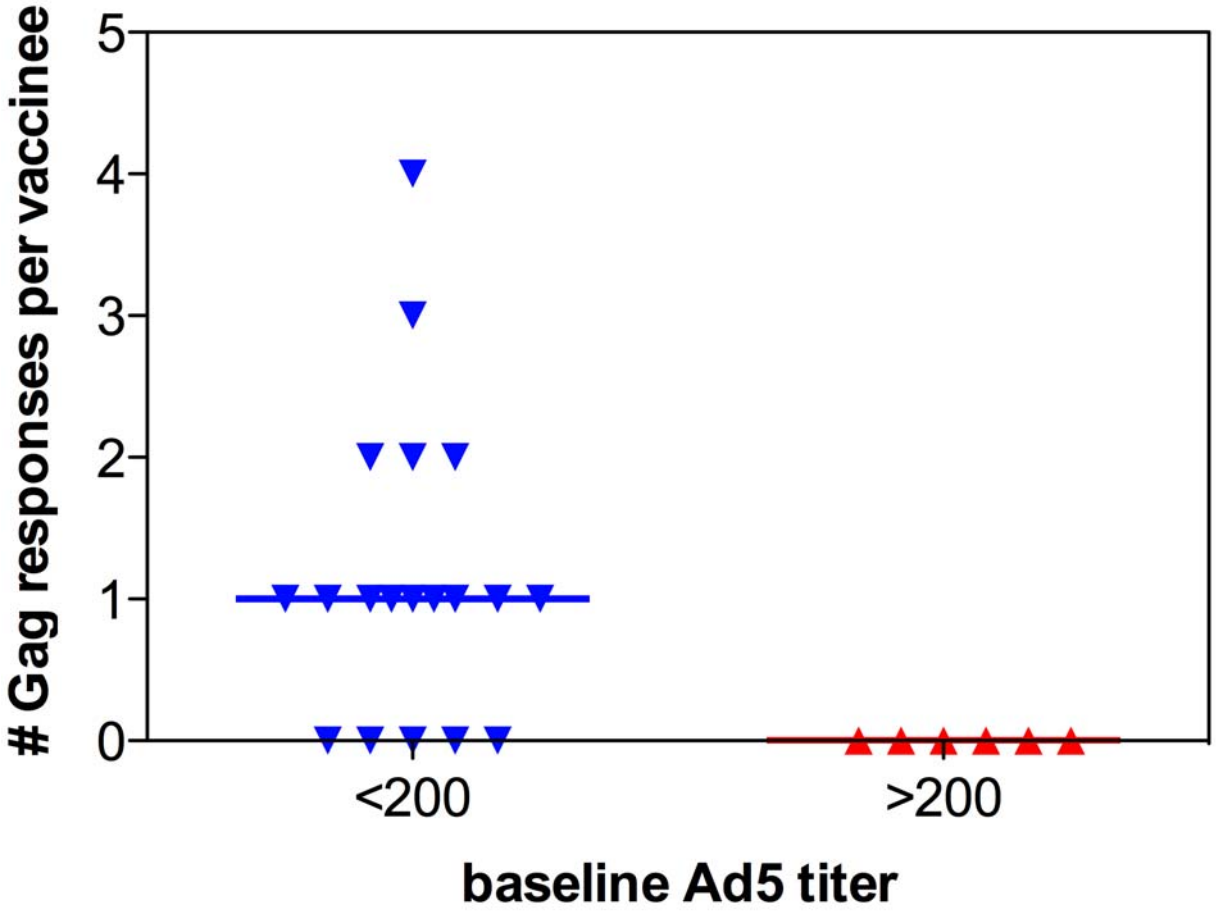
IFN-secreting T cells from *Ad5 naïve* (58%) were more likely than Ad5 immune (34%) vaccinees to recognize epitopes within all 3 HIV proteins expressed in the vaccine (Gag, Pol and Nef)

There was a higher probability of CD8+ responders among the Ad5 naïve vaccinees than the Ad5 immune group (Odds ratio = 5.76; p=0.0006).

Gag response rates were significantly reduced in vaccinees with high baseline Ad5 titers

Gag response rates were significantly reduced in vaccinees with high baseline Ad5 titers

Response rates: **14/19 (74%)** **0/6 (0%)** **p = 0.0026**



N Frahm

Three preliminary findings in the Step trial cases suggest that a threshold CD8+ T cell response may be reached with a T-cell based vaccine that could reduce viremia

D. Casimiro, S. Dubey, D. Mehrotra (Merck Research Labs)
N. Frahm, F. Li, D. Friedrich, D. Geraghty (HVTN Lab)
H. Janes, Z. Moodie, P. Gilbert, S. Self (HVTN/SCHARP)
(P08-10)

Three preliminary findings in Step trial cases suggest that a threshold CD8+ T cell response could be reached with a T-cell based vaccine that could reduce viremia

1) Level of IFN-γ-secreting T cells (total, Gag) correlate inversely with viral load in Ad5 naive vaccinees

2) Vaccinees with more Gag CD8+ T cells trend toward lower viral load (minipools, epitope mapping)

3) Beneficial effect of protective HLA alleles observed in HIV+ cohorts confirmed in Step Trial cases; this effect was more pronounced in vaccine recipients than in placebo recipients

(P08-10)

What immune function will most reliably provide indicators of vaccine-induced protection?

control of HIV replication?
memory phenotype?
Cytokine/chemokine secretion?

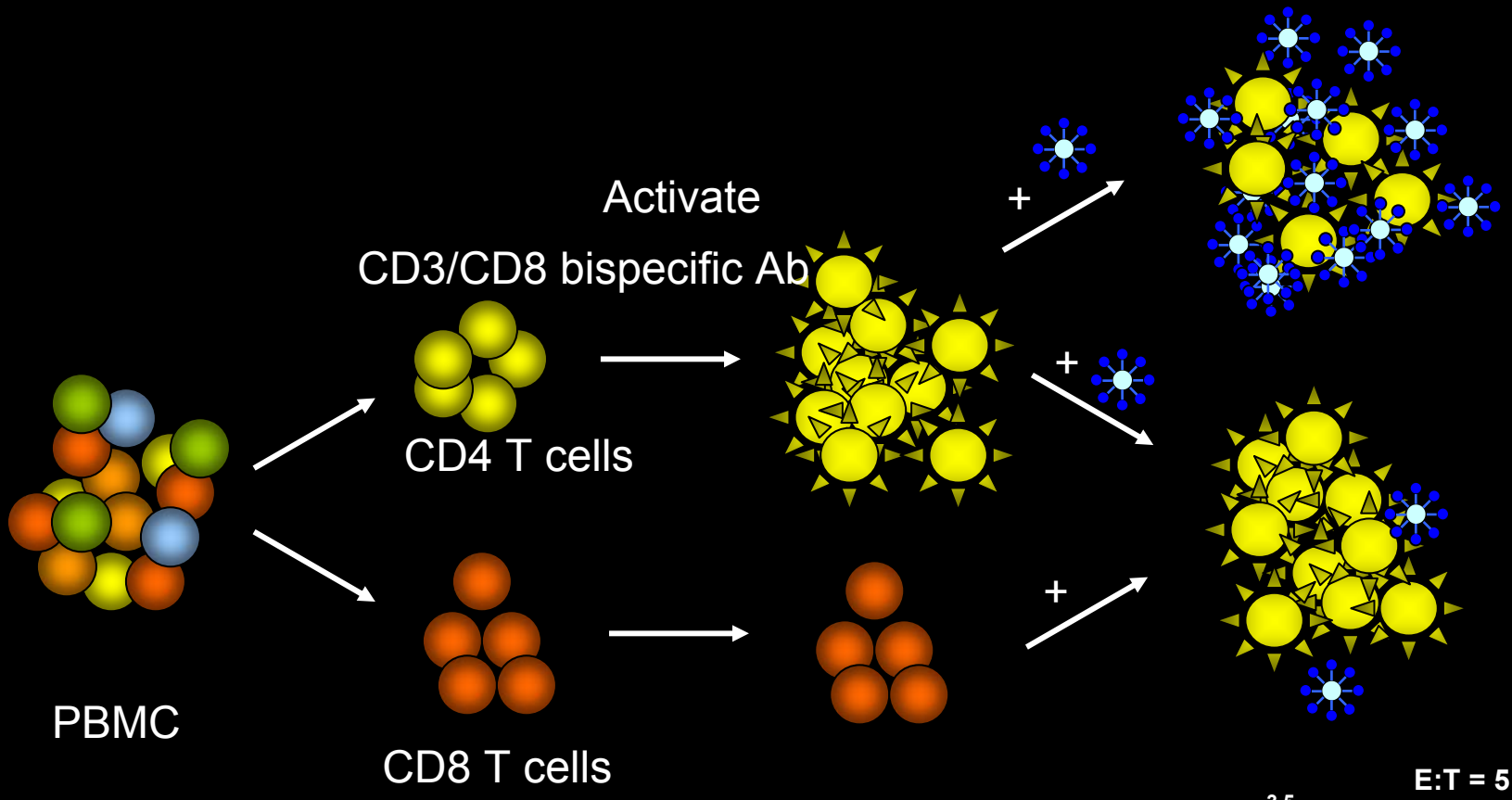
Do vaccine-induced T cells have antiviral activities?

Viral Inhibition Assay Working Group

Jill Gilmour, Peter Hayes (IAVI)
Bruce Walker, Boris Juelg (Harvard)
Georgia Tomaras (Duke HVTN)
Otto Yang (UCLA)
Julie McElrath, Natalie Zheng (HVTN)
David Watkins (U Wisconsin)

Patricia D'Souza (DAIDS) and Holly Janes (SCHARP)

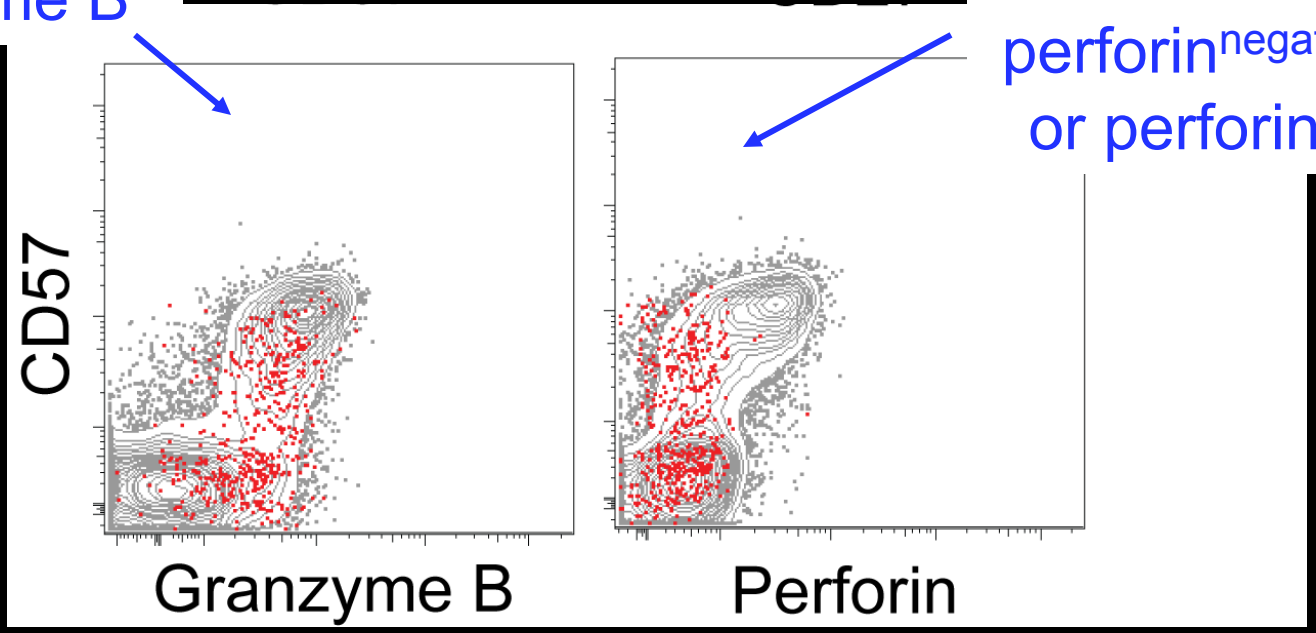
Viral inhibition assay method



At 6 months post boost, most vaccine-induced *ex vivo* CD8+ T cells express low-level granzyme B and little/no perforin

Many express low-level granzyme B

And are perforin^{negative} or perforin^{lo}



Steve De Rosa (0A05-03, Wed AM)
Boris Juelg (P15-22)

HIV VACCINE TRIALS NETWORK

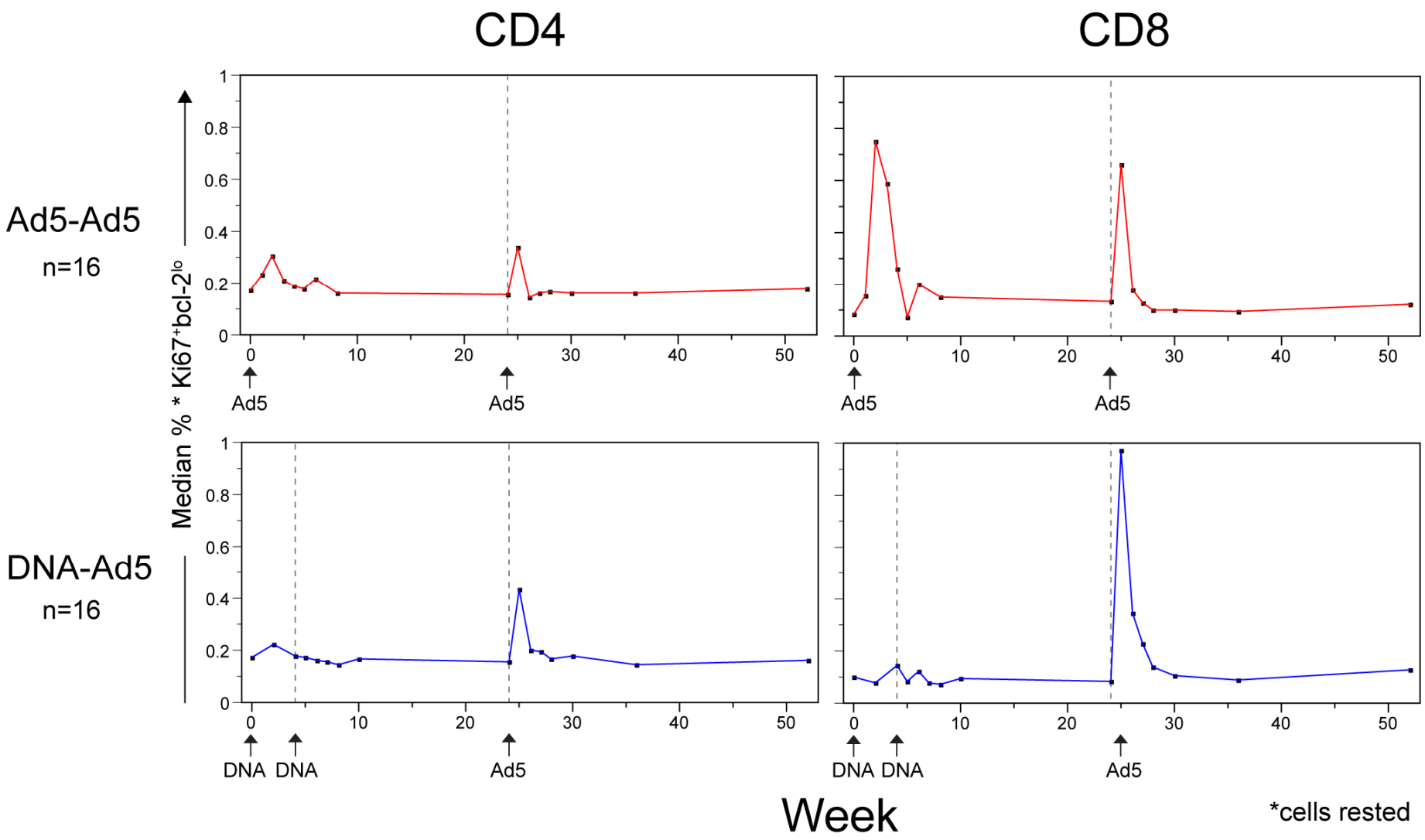
What additional antiviral, anti-inflammatory activities should we capture?

Approach: bead array, transcriptional arrays

In the Step Study,
HIV-1 incidence was higher
in vaccine-treated than placebo-treated males
with pre-existing Ad5 immunity.

What are the kinetics of T cell activation following priming and boosting with candidate Ad5/HIV vaccines?

Short-lived peaks of activated cells appear after vaccination, and kinetics are accelerated after boosting



Step Trial: Enhancement of HIV Infection

Immune Activation
Increase activated CCR5+ CD4+ T cells in high Ad5 group (week 30 and week 52), unrelated to treatment arm
(vaccine vs. placebo)

Step Trial: Enhancement of HIV Infection

Ad5-specific T cell immune responses:

Vaccine recipients mount both CD4+ and CD8+ T cell responses, stronger in low Ad5 group

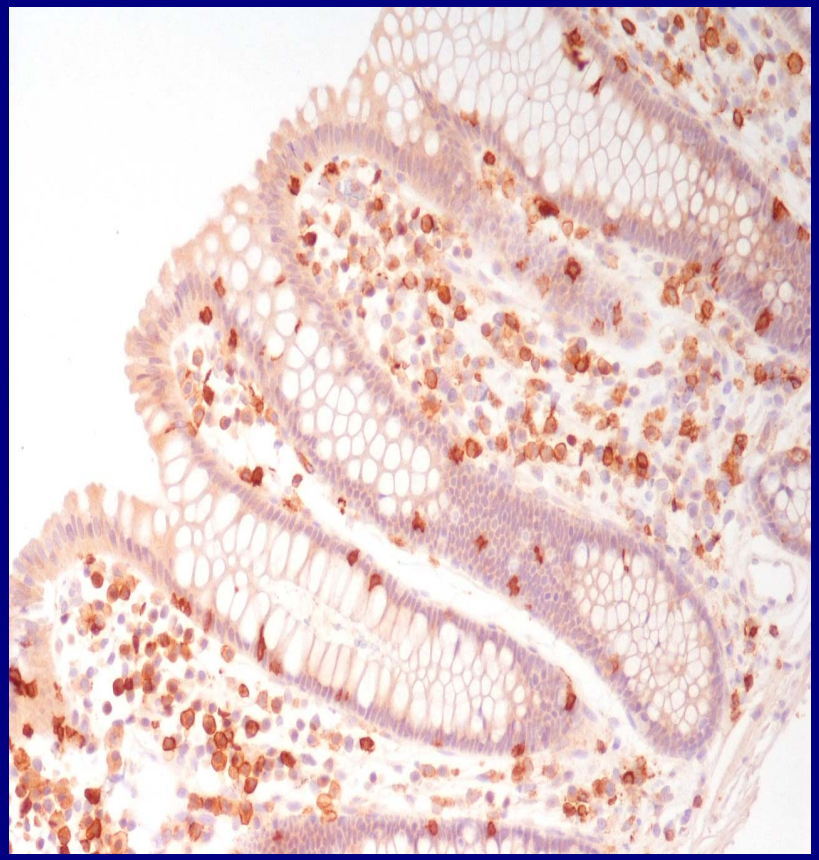
Lower CD4+ T cell response rates in cases in some subgroups

*(*N Frahm, late-breaker presentation)*

Possible migration to mucosal sites?

Evaluation of immune cell infiltration in mucosal HIV transmission sites in vaccine trials

Intestinal CCR5-staining cells



Staining panel

		1st Ab	2nd Ab
T cells	HIV receptors	CD3	CD4
		CD3	CCR5
		CD4	CCR5
	Activation	CD3	HLA-DR
		CD3	Ki67
	Regulatory Tc	CD3	FoxP3
Macrophages		CD68	CCR5
Dendritic cells		DC-SIGN	CCR5
		CD1a	CCR5
		HLA-DR	CCR5
		S-100	CCR5

Florian Hladik

Perspectives

1) Further progress has been made in defining threshold responses for T-cell based vaccines
2) Potential insight for post-infection endpoints may be gained comparing vaccine- vs placebo-treated cases in <18 Ad5 subgroup
   - Next 6 months: completion of viral sequence analysis of transmitting isolate, epitope mapping to understand epitope breadth/coverage, associations with HLA class I, II and KIR
3) T cell specificities: number of epitopes recognized and coverage relative to circulating strains may be inadequate. Improved strategies to elicit these may be necessary: 1) protein-adjuvant prime, vector boost (R Seder, 0503-05, Wed pm); 2) heterologous prime boost: different vectors, or different inserts (L Corey, SP01-01, Tues pm)

Perspectives

Potency of response: unclear what is needed, NHP studies could lend insight

Ad5 vectors as currently constructed may not provide the “correct” memory response

Ad5 effect requires further exploration: dampening responses to HIV-1 transgene, potential migration of Ad5-specific T cells to mucosa, Ad5 persistence in the mucosa

Ad5 innate signatures: transcriptional profiles of activation may lead to altered vaccine response.

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T Cell Responses to Ad5 Vector Two Approaches

1. Ad5 Empty Vector: VRC and Merck

2. 1773 15-mers overlapping by 11 a.a. spanning the 11 proteins/ORFs

Ad5 100K:	282 peptides
Ad5 E2 DNA polymerase:	300 peptides
Ad5 E2 pre-terminal protein:	186 peptides
Ad5 E2 ssDNA binding protein:	146 peptides
Ad5 E3 gp19K:	43 peptides
Ad5E4 Orf6:	78 peptides
Ad5 fiber:	161 peptides
Ad5 hexon:	266 peptides
Ad5 penton base:	159 peptides
Ad5 pV:	100 peptides
Ad5pVII:	52 peptides

Peak Gag-specific T cell responses in Ad5-naïve (≤18) compared to Ad5-immune (≥200) vaccinees

Higher in Ad5-naïve (n=28)

Gag	p
IL-1α	0.06
IP-10	0.02
IL-15	0.09

Higher in Ad5-immune (n=28)

Gag	p
IL-6	0.05
IL-8	0.03
IL-10	0.09
TGF-α	0.05

Remaining analytes showed no appreciable difference between Ad5-immune and Ad5-naïve test groups

Mean background-subtracted concentration of positive responders, two-tailed t-test.

Sam Pine

Intracellular Cytokine Analysis

Laser	Channel	8-Color	10-Color
Violet 407nm	V450	ViViD[1]	CD57 (Alx 405)
	V525		AViD[2]
Blue 488nm	FITC	CD4	TNF-α
	PerCP-Cy5.5	CD8	CD8
Green 532nm	PE	IL-2	IL-2
	PE-Tx Rd	CD3	CD3
	PE-Cy7	IFN-γ	IFN-γ
Red 633nm	Alexa 647	Perforin	Perforin
	Alexa 700	TNF-α	Granzyme B
	APC-H7		CD4

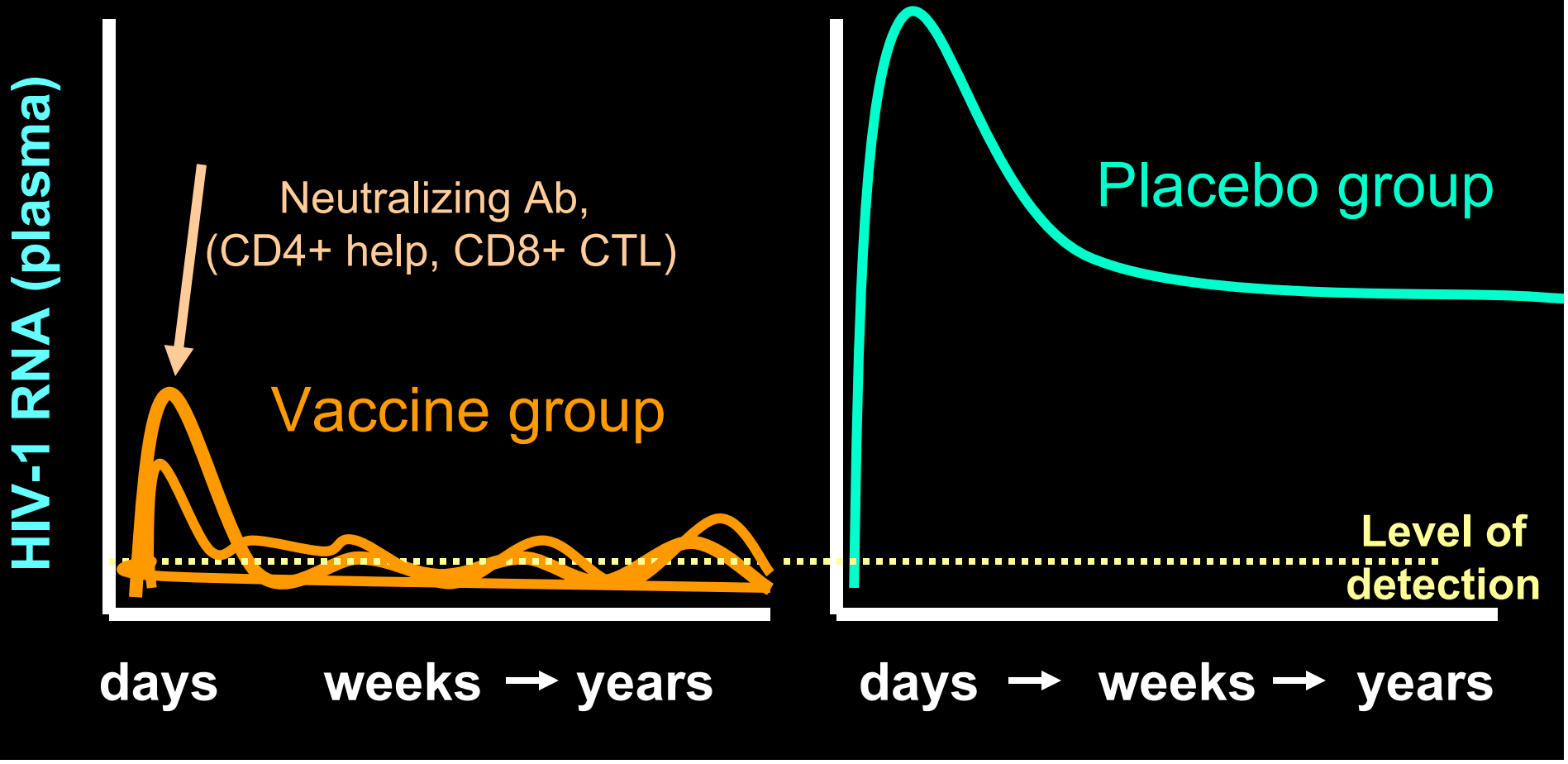
Assay (8, 10-color) validated for IL-2 and IFN-γ

(Horton H, De Rosa S, J Immunol Methods, 2007)

How can vaccine-induced HIV-specific T cells provide protection against HIV infection and disease?

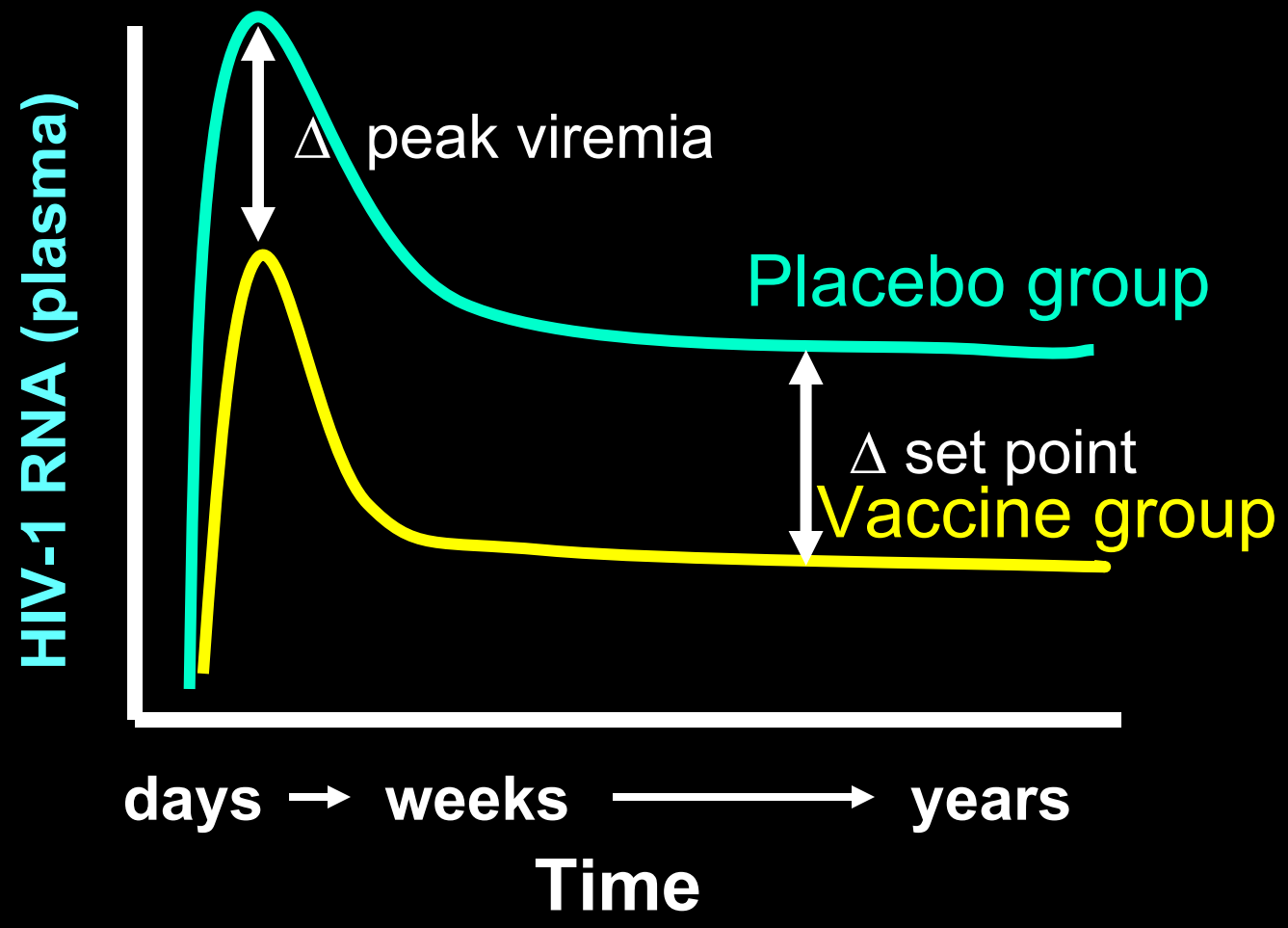
Providing vaccine-induced immune protection against HIV infection

Need rapid effector response at site of exposure



Providing protection against HIV disease

Need central memory T cells to maintain immunity and to efficiently expand to effector cells



Magnitude of CD8+ T Cell Responses in Long Term Nonprogressors (PTE-Global Peptides)