

The STEP Trial

A Pathway Toward Understanding
the Biological Basis
for the Vaccine Efficacy Results

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Major Scientific Questions

What are the reasons for the lack of vaccine efficacy?

and

What are potential biological mechanisms for apparent increased HIV-1 acquisition in the Ad5 immune vaccine group?

Reasons for Lack of Vaccine Efficacy?

Review the immunogenicity of the vaccine in the STEP trial

- Magnitude of the immune responses
- Frequencies of the vaccine responders
- Quality of the immune responses
- Migration of Immune cells to sites of HIV transmission

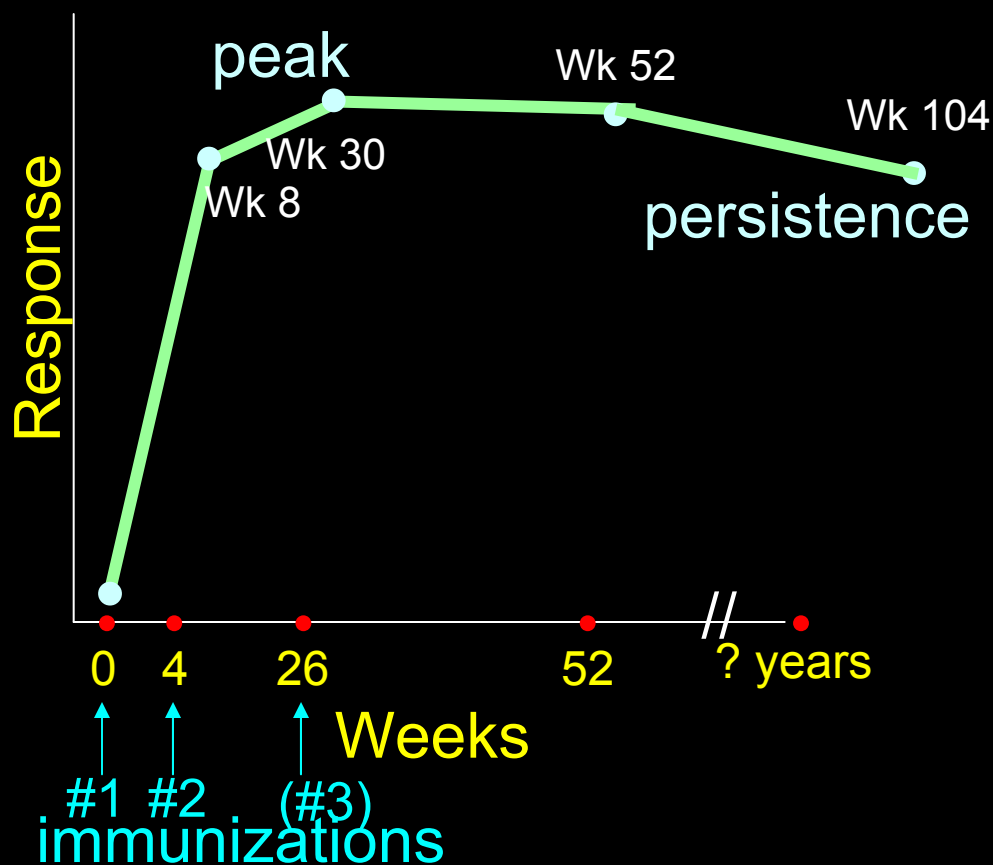
Was HIV-1 able to evade the immune responses elicited by the vaccine?

Consideration for future studies:

Was the concept of a T-cell-based HIV-1 vaccine clinically not valid or is the lack of efficacy specific to the Ad5 vaccine candidate?

Immunogenicity Analyses of T Cell Responses

Time points: baseline
peak - 30 weeks
durability - 52 weeks



Validated Assays

IFN- γ ELISpot:
PBMC secreting IFN- γ

and/or

8-color ICS:

CD3+CD4+ secreting IL-2 \pm IFN- γ
CD3+CD8+ secreting IL-2 \pm IFN- γ

PBMC cryopreserved within
8 hours of venipuncture

Reasons for Lack of Vaccine Efficacy?

1. Did the vaccine induce expected T cell responses?

Yes

Were these similar to findings in previous phase I studies?

Yes

STEP Study: Vaccine Immunogenicity

ELISpot Data at Week 8 (4 Weeks after 2 Doses)

Ad5	Study	N	Gag		Pol		Nef	
			% Response	GM	% Response	GM	% Response	GM
≤200	STEP*	148	76%	264	73%	471	70%	239
	Phase I	23	61%	113	48%	179	57%	90
>200	STEP*	175	55%	168	47%	243	52%	163
	Phase I	12	100%	202	33%	153	58%	98

*For the STEP study, assay was run on random ~25% of the subjects

Gag, Pol, Nef Values not mock subtracted

ELISPOT responder: ≥ 55 SFC/ 10^6 PBMC and ≥ 4 -fold over negative control

GM = geometric mean of all subjects

Reasons for Lack of Vaccine Efficacy?

2. Were the magnitude and frequencies of the immune responses lower in the vaccine cases than in the overall study cohort?

No

How do the magnitudes compare with those in HIV-infected cohorts?

Analysis pending (HVTN)
Comparison with LTNP, seropositive control cohort

STEP Results: Male Cases vs Male Non-Cases

ELISpot Data for Male **PP Vaccine** Recipients (Week 8)

			Gag		Pol		Nef	
Ad5	Cases	N*	% Response	GM	% Response	GM	% Response	GM
≤200	Yes	19	74%	354	63%	627	74%	327
	No	102	75%	264	74%	493	68%	248
>200	Yes	13	46%	181	38%	296	46%	149
	No	87	51%	150	47%	229	46%	146

*For the NON-CASES, N represents data from assays run on random ~25% of the STEP male subjects

Gag, Pol, Nef Values not mock subtracted

ELISPOT responder: ≥ 55 SFC/ 10^6 PBMC and ≥ 4 -fold over negative control

GM = geometric mean of all subjects

STEP Results: Immunogenicity by Gender

ELISpot Data for Vaccine Recipients (Week 8)

Ad5	Gender	N*	Gag		Pol		Nef	
			% Response	GM	% Response	GM	% Response	GM
≤200	F	41	78%	250	71%	396	76%	212
	M	107	76%	270	74%	504	68%	250
>200	F	86	58%	188	47%	254	57%	184
	M	89	52%	151	47%	232	47%	146

*Assay was run on random ~25% of the STEP subjects

Gag, Pol, Nef Values not mock subtracted

ELISPOT responder: ≥ 55 SFC/ 10^6 PBMC and ≥ 4 -fold over negative control

GM = geometric mean of all subjects

Reasons for Lack of Vaccine Efficacy?

3. Was the breadth of T cell responses adequate? In the cases, did the vaccine provide antigenic coverage against the infecting virus?

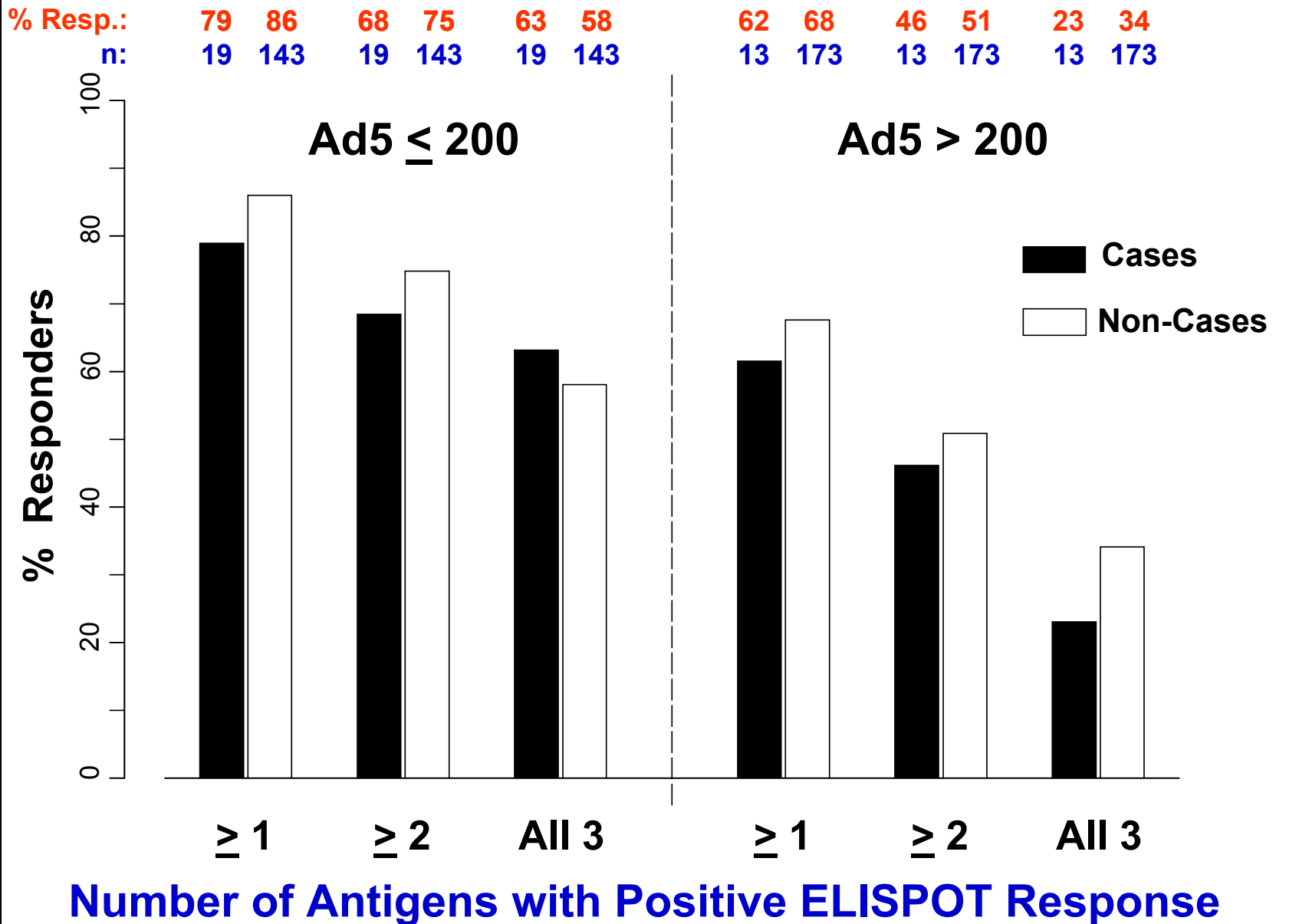
To be determined

Studies to address:

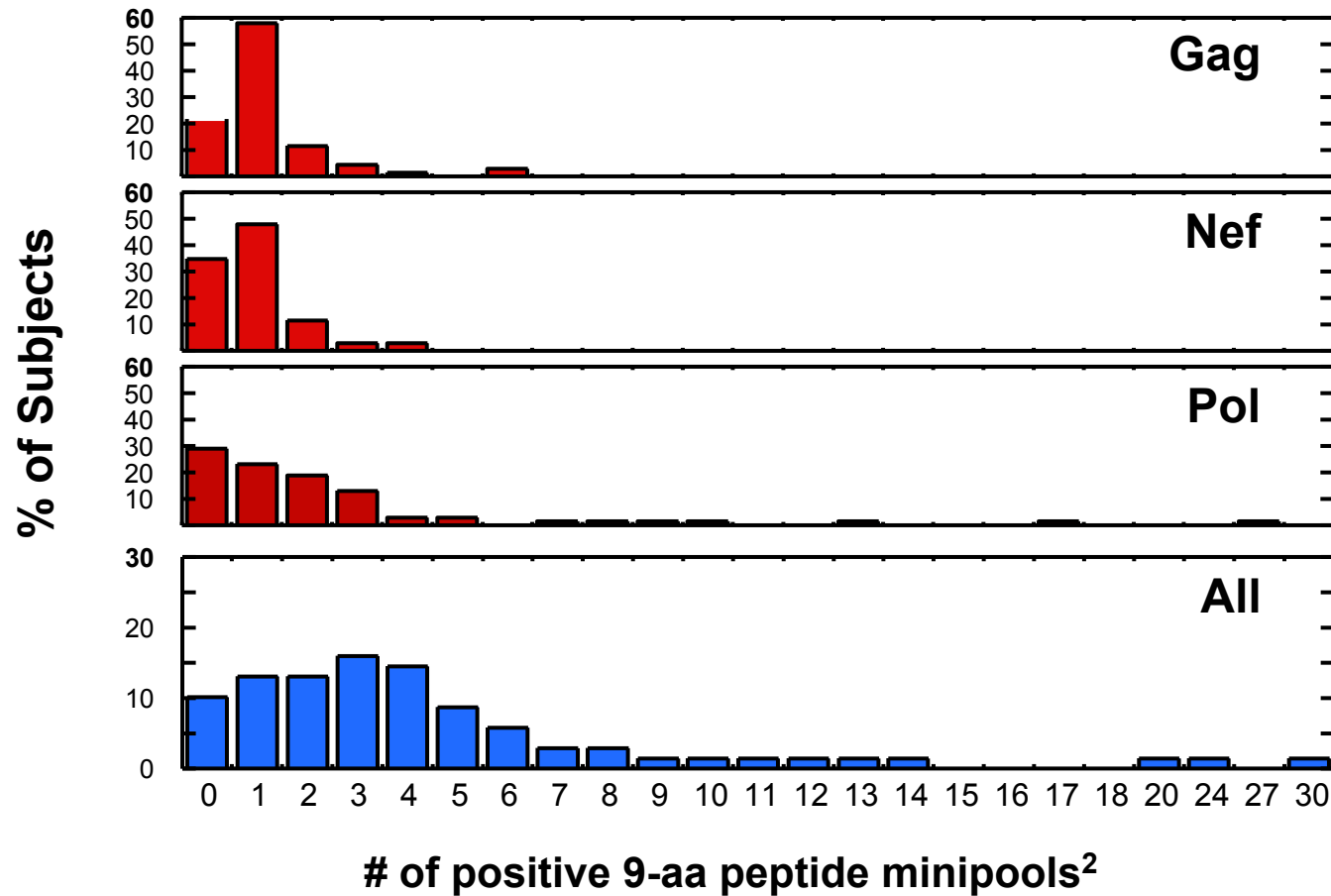
- 1) Responses by protein
- 2) Epitope mapping at week 8 after 2 doses
- 3) HLA typing
- 4) Viral sequencing from early and later-stage infection
- 5) Confirmatory immunologic analyses

T Cell Responses for STEP Subjects Receiving Vaccine

Comparison of cases vs. non-cases



Breadth of CTL responses induced by MRKAd5 Trivalent Vaccine from Phase I trial using 9-mer peptide pools¹



Median #

1

1

1

3

¹Other epitopes (helper T) are likely not detected in this screen
²Gag: 62 peptide minipools; Pol: 105 minipools; Nef: 27 minipools;
 each minipool consists of eight 9-aa peptides (with 8-aa overlaps)

Planned Experiments to Map Epitopes

I. Pre-Infection (Week 8 time point)

- When positive to the full protein peptide pool, screen against small pools of 15 aa for that protein
 - Gag, 24 pools; Pol, 42 pools, Nef, 10 pools
 - Advantages (over mapping with 9-aa peptides)
 - Detection of both CD8 and CD4 epitopes
 - Detection of longer CD8 epitopes
- Further deconvolution of smaller pools to individual peptides (for responses >100 SFC/ 10^6 PBMC); cross references with HLA and LANL database
- Flow cytometric analyses to determine T cell subset (CD4/CD8) if needed

TIMELINES: T-2Q2008

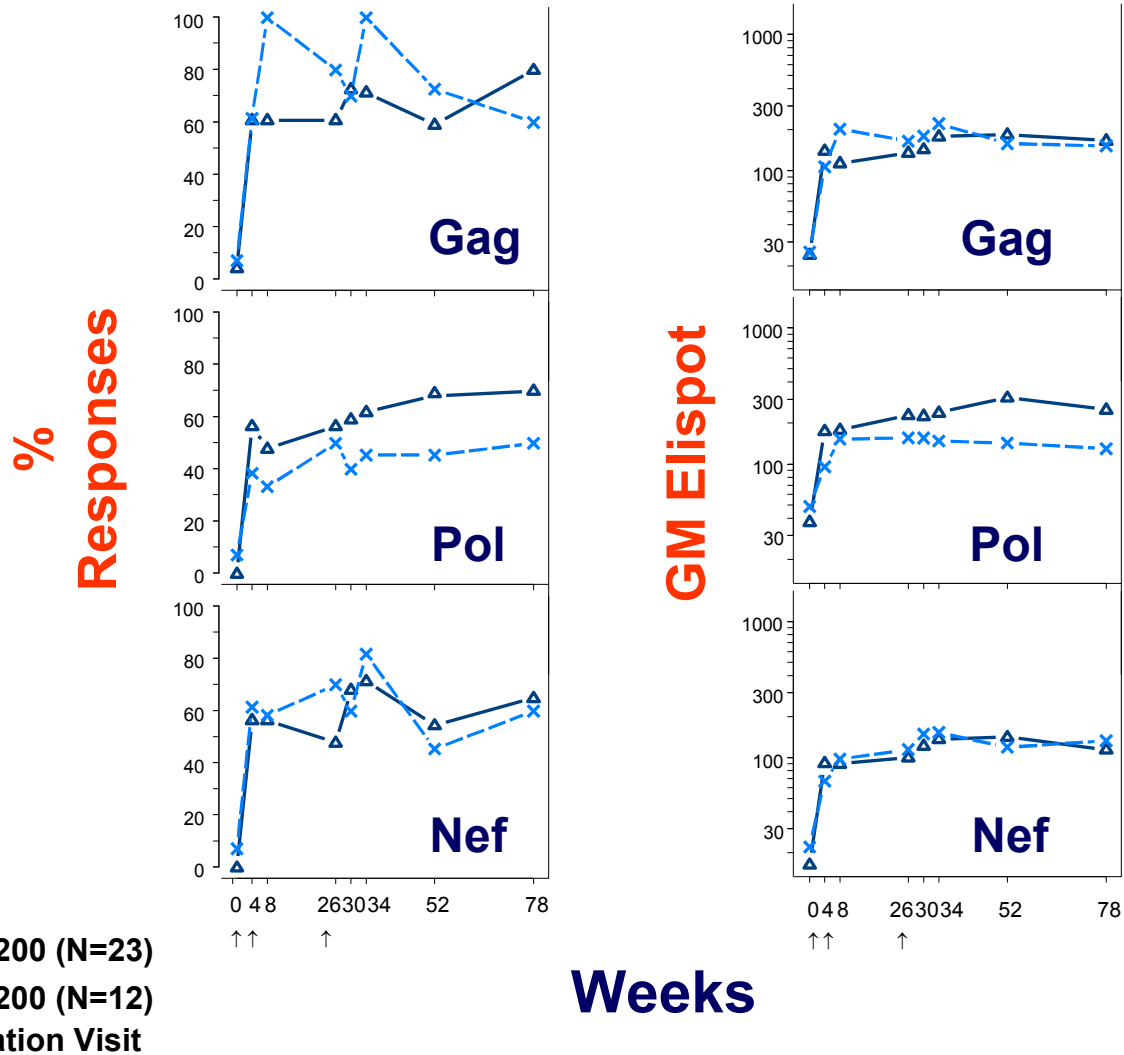
II. Post-infection Samples (same)

Reasons for Lack of Vaccine Efficacy?

4. Is the vaccine-induced immunity long-lived?

Yes

Phase I Study Results: Immune Responses are Long-lived (3×10^{10} vp Dose Level)



Reasons for Lack of Vaccine Efficacy?

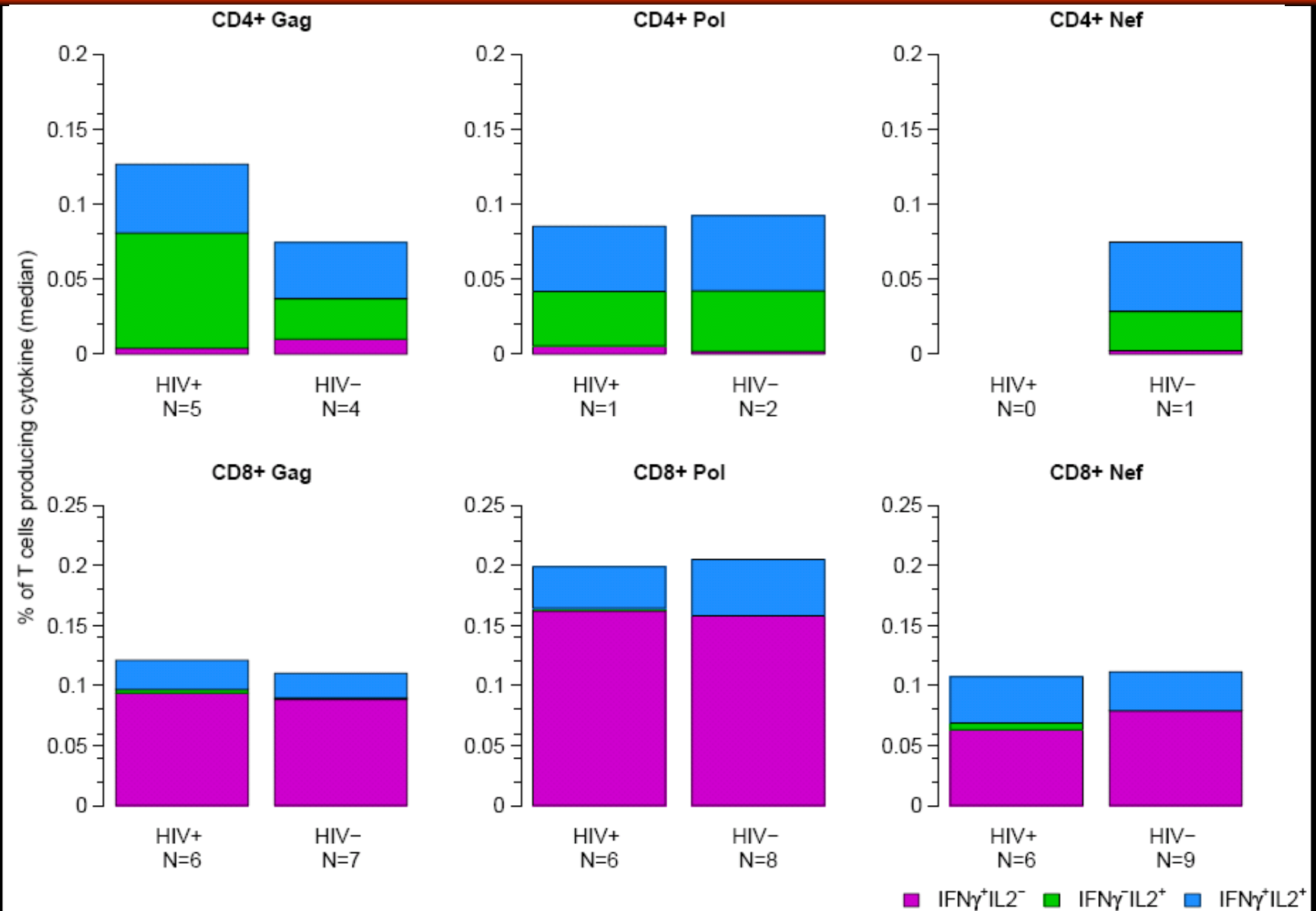
5. Was the Ad5 HIV vaccine unable to provide the optimal quality of T cell responses to induce HIV protection?

unclear

Merck Ad5/HIV Vaccine-induced T Cells Secreting IFN- γ and IL-2 (week 30, 8-color ICS)

T cell subset	HIV status	Response Rate	95% CI
CD4+	HIV+	5/10 (50%)	23.7%-76.3%
	HIV-	4/11 (36.4%)	15.2%-64.6%
CD8+	HIV+	8/10 (80%)	49%-94.3%
	HIV-	10/11 (90.9%)	62.3%-98.4%

IFN- γ /IL-2-Secreting T Cells, Stratified by T Cell Subset, Protein Recognized and HIV-1 Serostatus



Further investigations* to define immune function and anti-viral activities of vaccine-induced T cells

Functional Phenotype of Epitope-specific T Cells

functional avidity
central/effector memory phenotypes
anti-viral cytokines/chemokines
proliferative capacity (Ki67+, CFSE, tetramer staining)
TCR repertoire

Anti-HIV Activities

T-cell viral neutralization
Cytolytic potential (perforin, granzymes)

Transcriptional and Proteomic Analysis

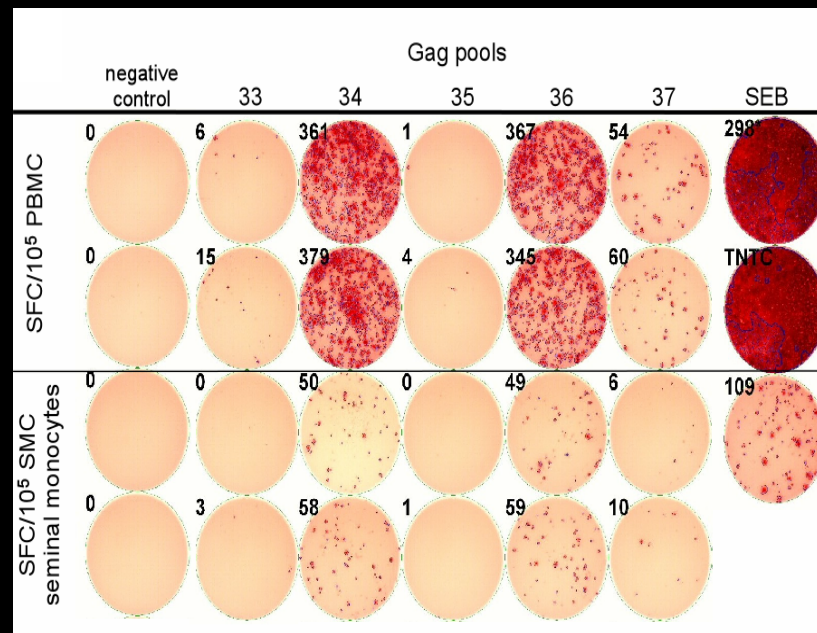
Microarray
Bead array

* Prioritization will be essential because of limited specimen quantities

Reasons for lack of vaccine efficacy?

6. Can vaccine-induced T cells migrate to sites of HIV transmission?

Yes, in male genital tract



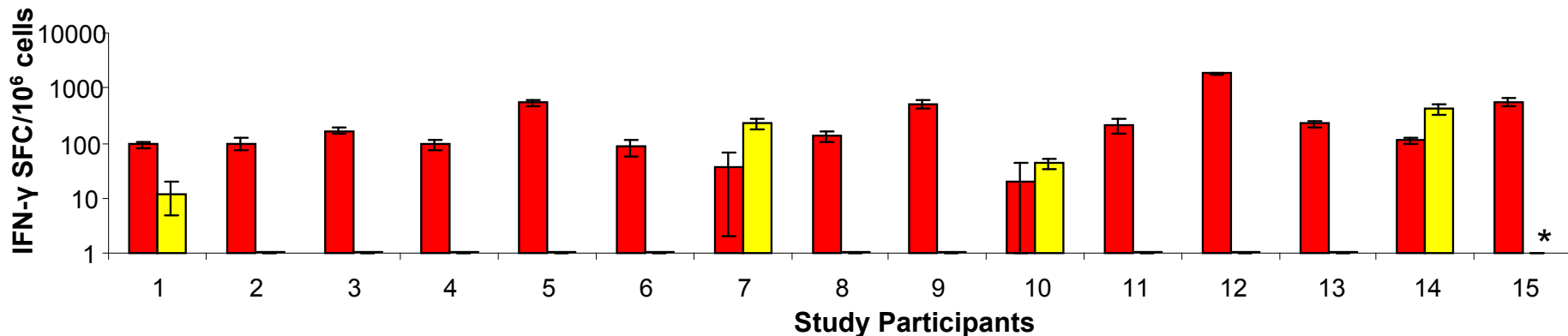
Representative IFN- γ ELISpot of Seminal Mononuclear Cells



Gag-specific T cell responses detected in blood and semen 4 and 26 weeks after 3rd Mrk Ad5 vaccination

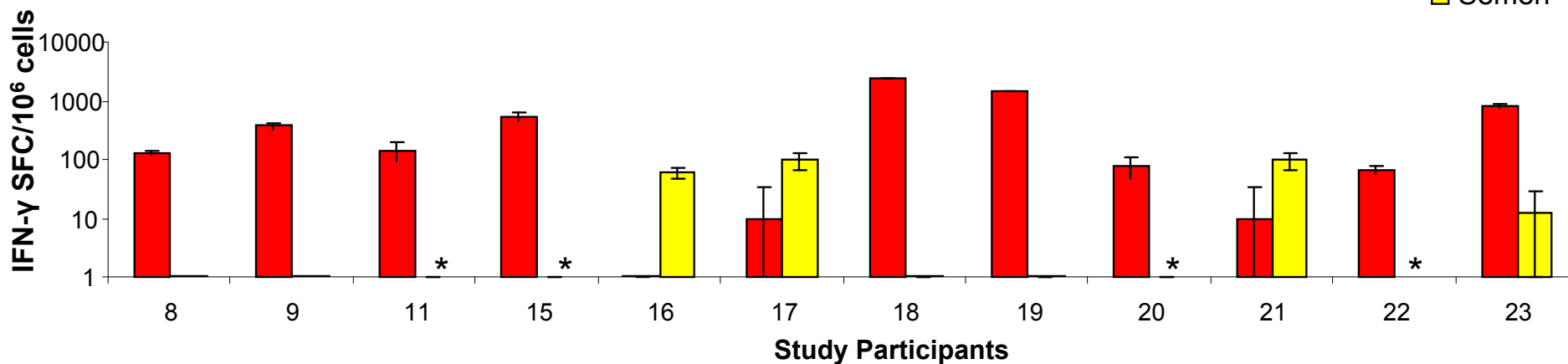
4 weeks post 3rd vaccination

■ Blood
■ Semen



26 weeks post 3rd vaccination

■ Blood
■ Semen



* not determined due to too few T cells in samples



Major Scientific Questions

What are the potential biological mechanisms for the apparent increased HIV-1 acquisition in the Ad5-immune vaccine group?

“The vaccine does not cause HIV/AIDS”

Specific Scientific Questions

Can this observation
(apparent increased acquisition)
be explained by
general immune activation?

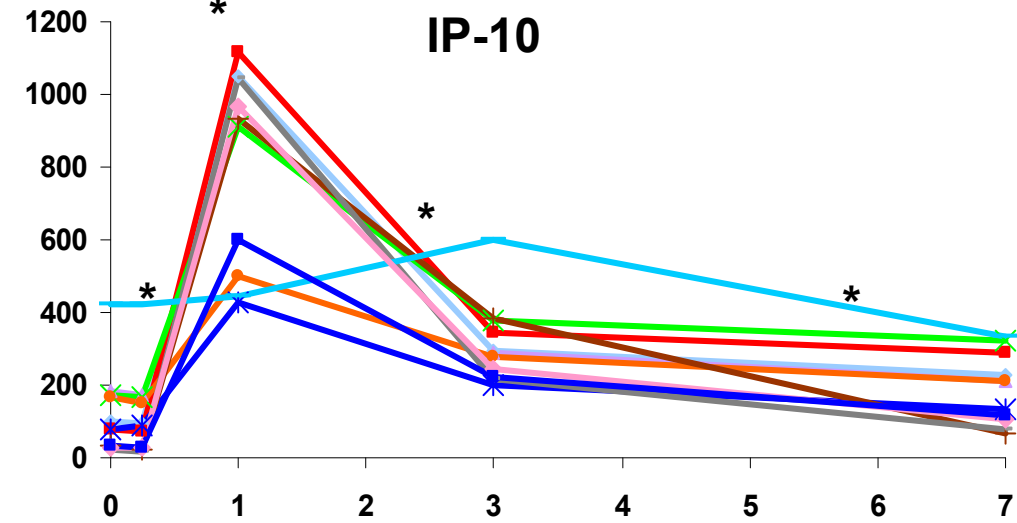
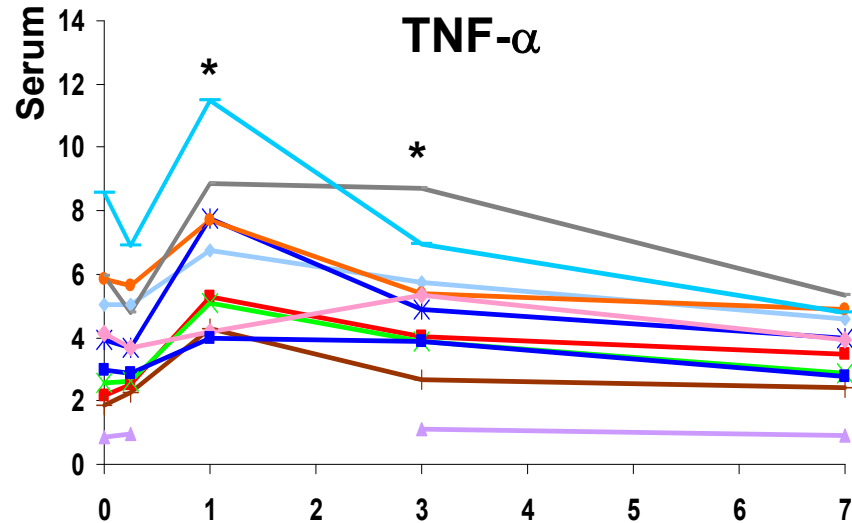
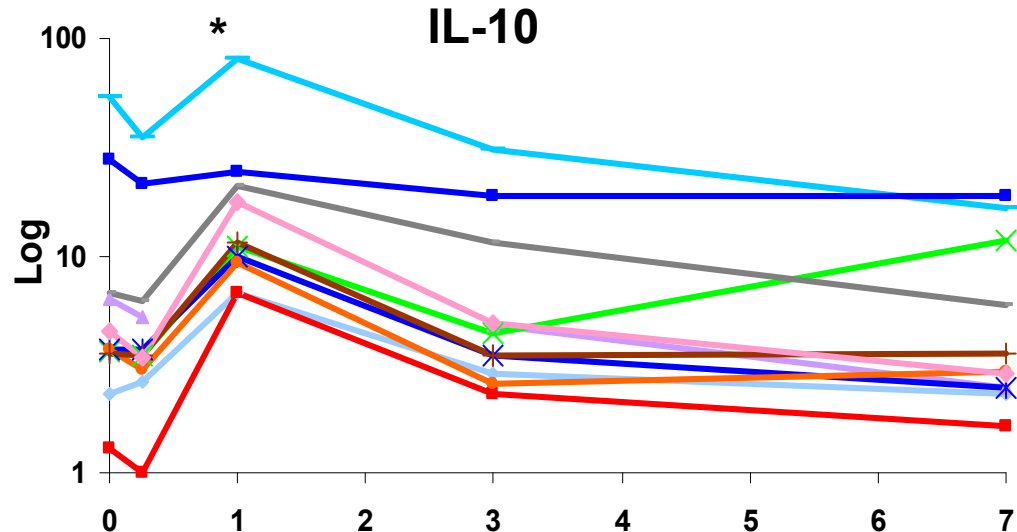
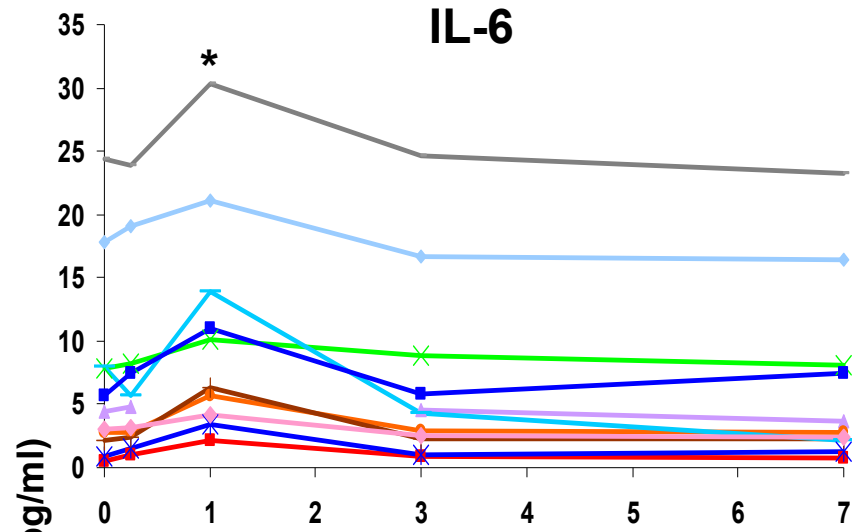
Or responses to the
Ad5 vector or **HIV-1 inserts** or **both?**

and

Are the vaccine effects altered with
repeated doses?



Innate Immune Responses after One Dose of Merck Ad5-HIV Vaccine (HVTN 071)



Responses fall to baseline within one week Days Post Vaccination

* P<0.05 by paired T test



In the Ad5>200 group,
does Ad5-HIV vaccination enhance the
susceptibility of target cells to HIV?

by

increasing CD4+ T cell
activation and CCR5 expression?

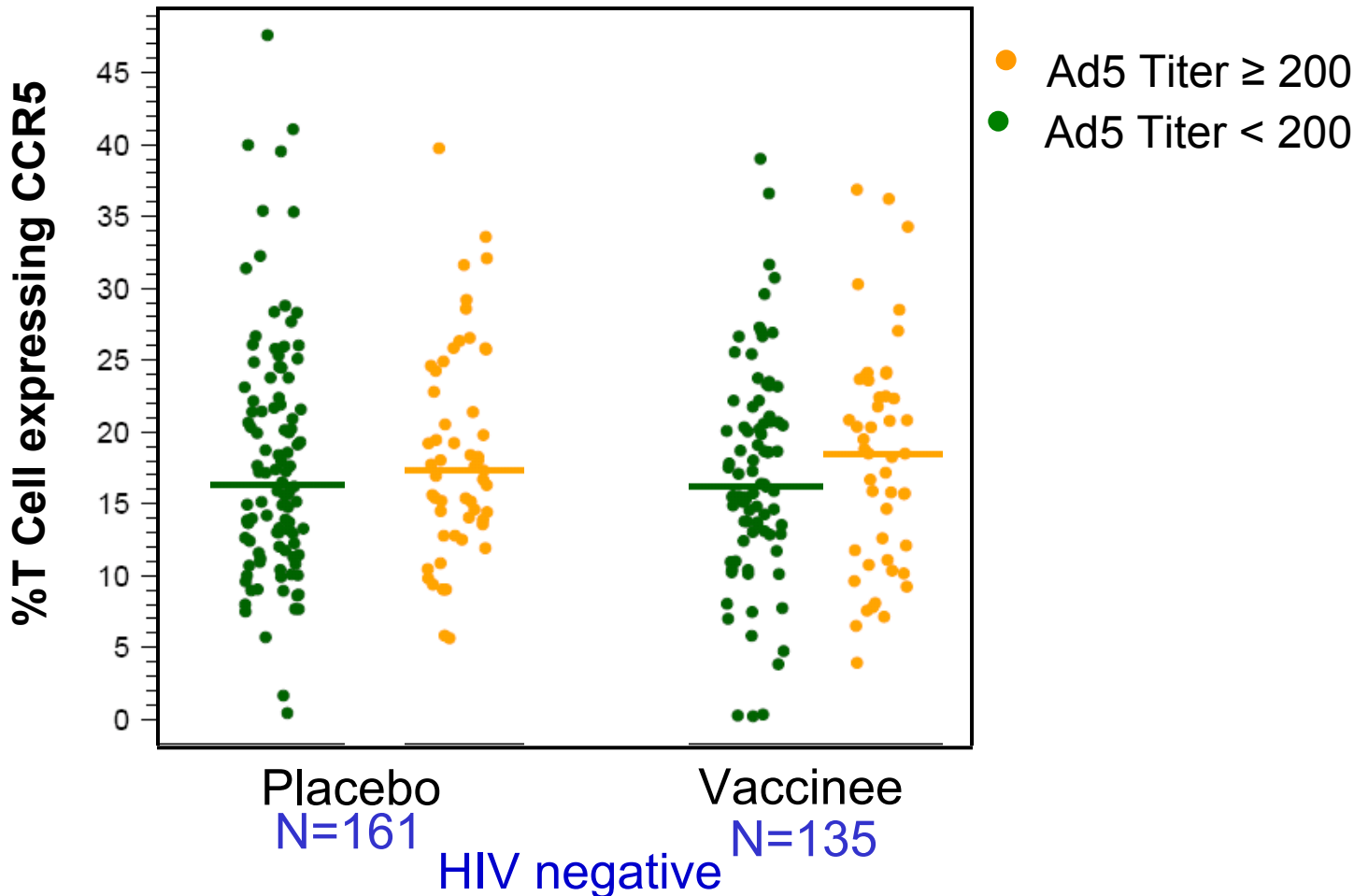


Blood CD4⁺ T Cells Expressing the HIV-1 Co-receptor CCR5

STEP trial week 30, Preliminary

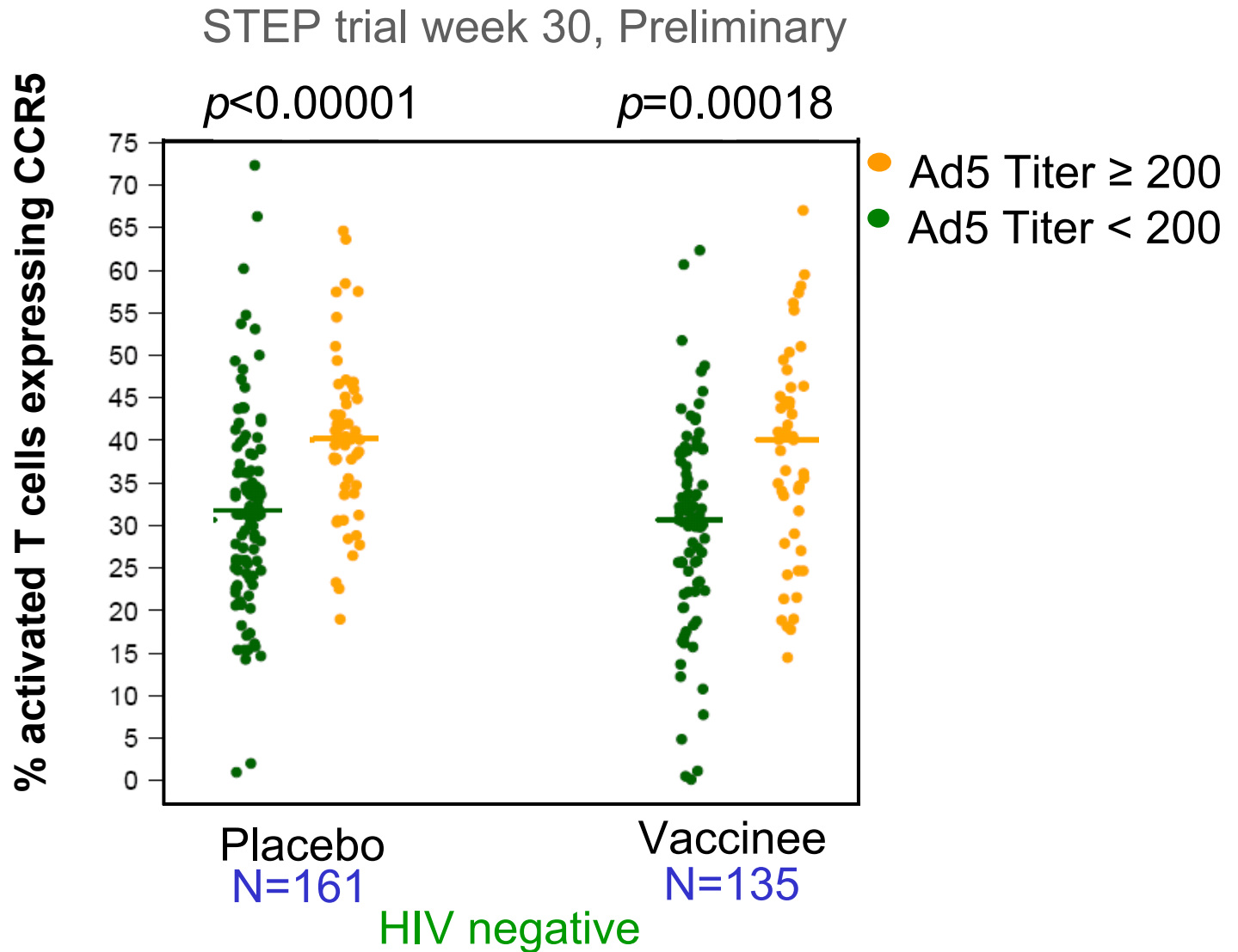
$p=0.56115$

$p=0.37$





Activated Blood CD4+ T Cells (Ki67⁺/BcL2⁻) Expressing the HIV-1 Co-receptor CCR5

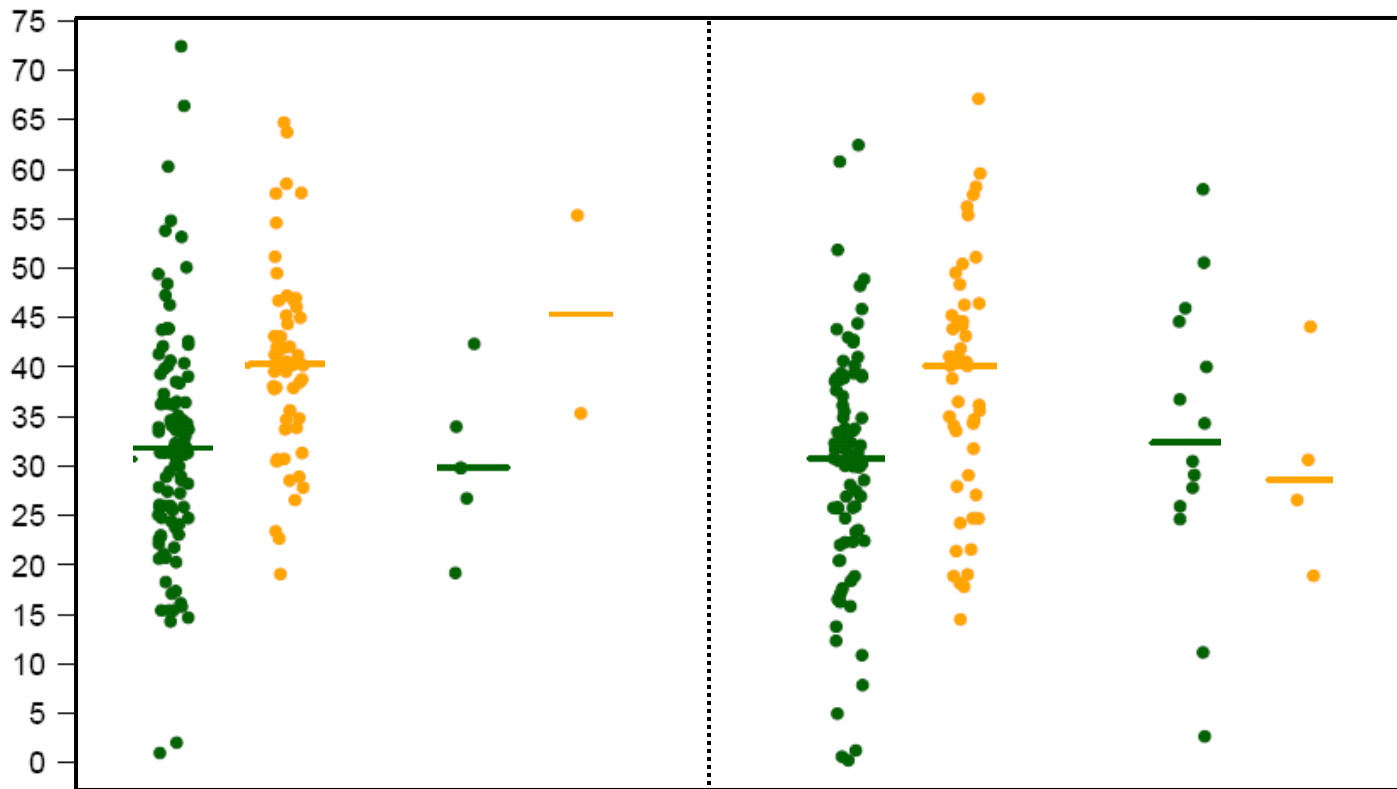




Activated Blood CD4+ T Cells (Ki67⁺/BcL2⁻) Expressing the HIV-1 Co-receptor CCR5

STEP trial week 30, Preliminary

% activated T cells expressing CCR5



HIV negative
N=161

HIV positive
N=8

Placebo

HIV negative
N=135

HIV positive
N=18

Vaccinee

● Ad5 Titer > 200 ● Ad5 Titer < 200

Summary of Preliminary Findings

At week 30, 4 weeks after 3 doses of Mrk Ad5 Trivalent HIV vaccine, *no difference* is observed in blood CD4+ T cell immune activation or CCR5 expression between Ad5 <200 vaccinees and placebos, and Ad5 ≥200 vaccinees and placebos.

However, a greater number of *activated* blood CD4+ T cells expressing CCR5 are observed in the Ad5 ≥ 200 group than in the Ad5 <200 group.

Further studies are required in greater numbers and additional time points to confirm.

What are potential biological mechanisms for apparent increased acquisition in the Ad5-immune vaccine group?

Future Studies

- 1) Define Ad5-specific immune responses (T cell and neut Ab) and possible association with increased acquisition
- 2) Examine potential effect of immune responses with repeated doses
- 3) Examine effect of immunization on CD4+ T cell number, activation, CCR5 expression in rectum and lower genital tract
- 4) Explore relationship of specific Ad5 gene deletions with increased CD4+ T cell activation
- 5) Assess *in vitro* susceptibility of CD4+ T cells (DCs, macrophages) in Ad5 low vs Ad5 high titer vaccinees

What are potential biological mechanisms for apparent increased acquisition in the Ad5-immune vaccine group?

Future Studies

Is this observation specific to Ad5 or also characteristic of other adenovirus vectors?

Will this be a similar issue with use of highly immunogenic viral vectors in previously immune study participants?
(e.g., pox vectors?)

Did vaccination alter the course of HIV infection?

Were HIV-1 epitopes recognized with vaccination present in the infecting strain? If not, were variants in the infecting strain recognized?

Does immunization with MrkAd5 HIV vaccine alter the early immune adaptive response to HIV-1? The rate of viral diversification?

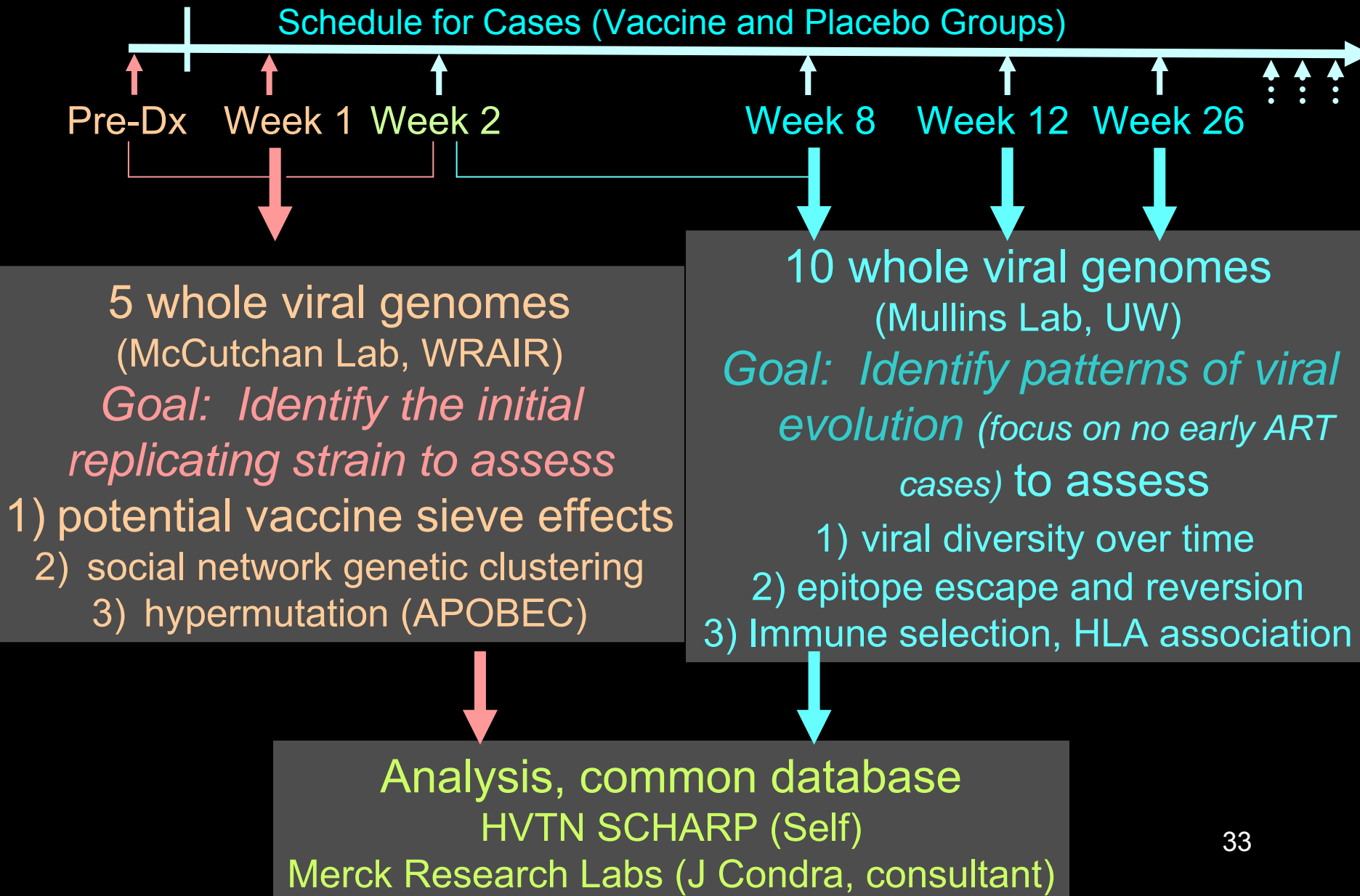
Is there an anamnestic immune response to vaccine-induced T cells, and how does this impact VL set point?

Were CD4+ T cell memory cells preserved at set point?

Does escape mutation occur early within the vaccine-induced T cell epitopes? Is this associated with higher VL set point?

Do some epitope-specific T cells better control viremia?

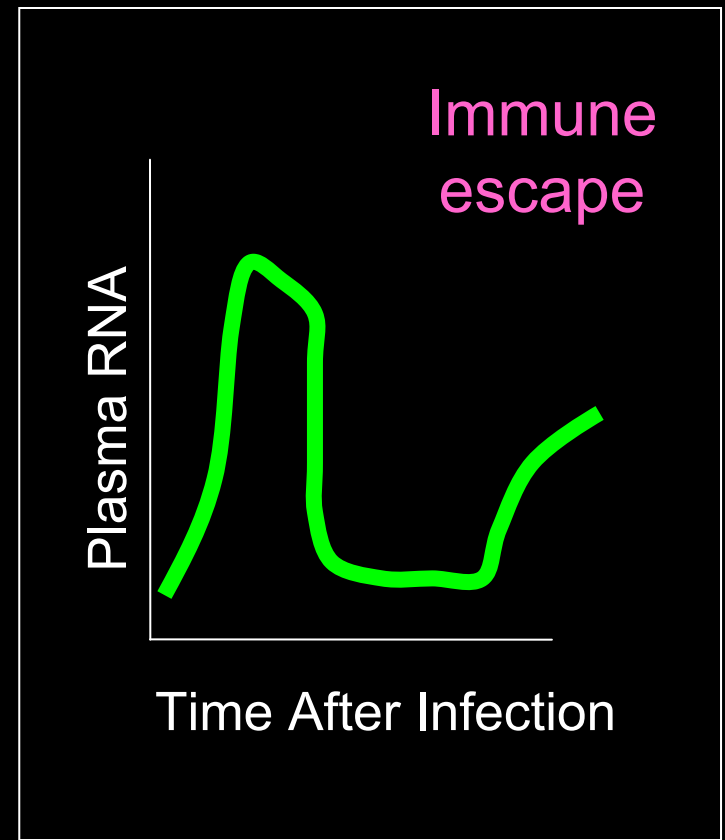
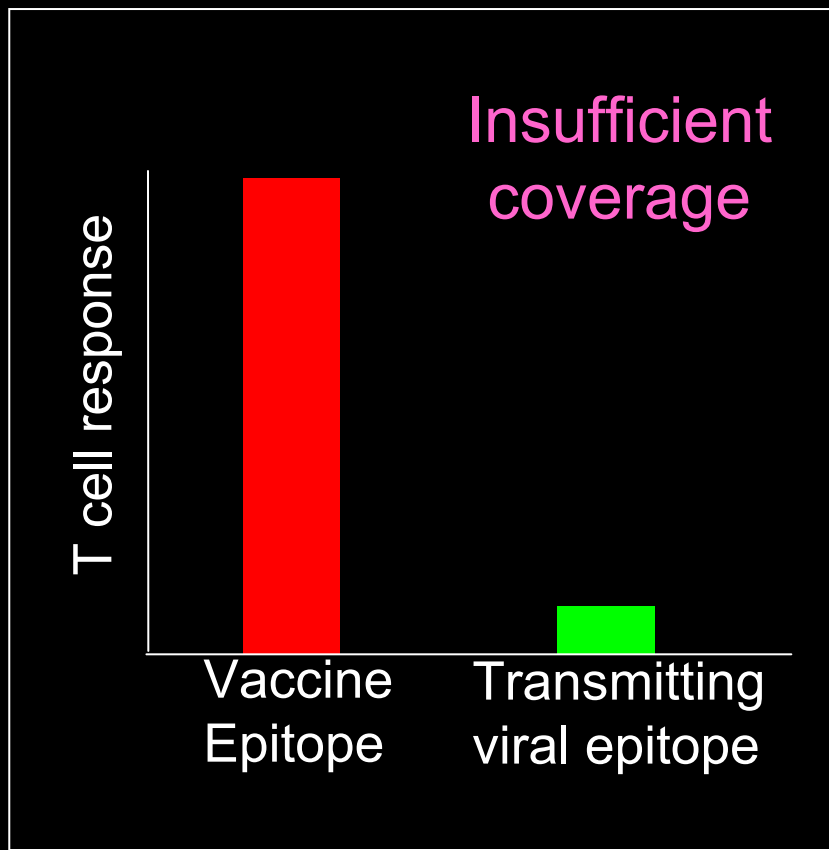
Viral Sequencing and Analysis Plan



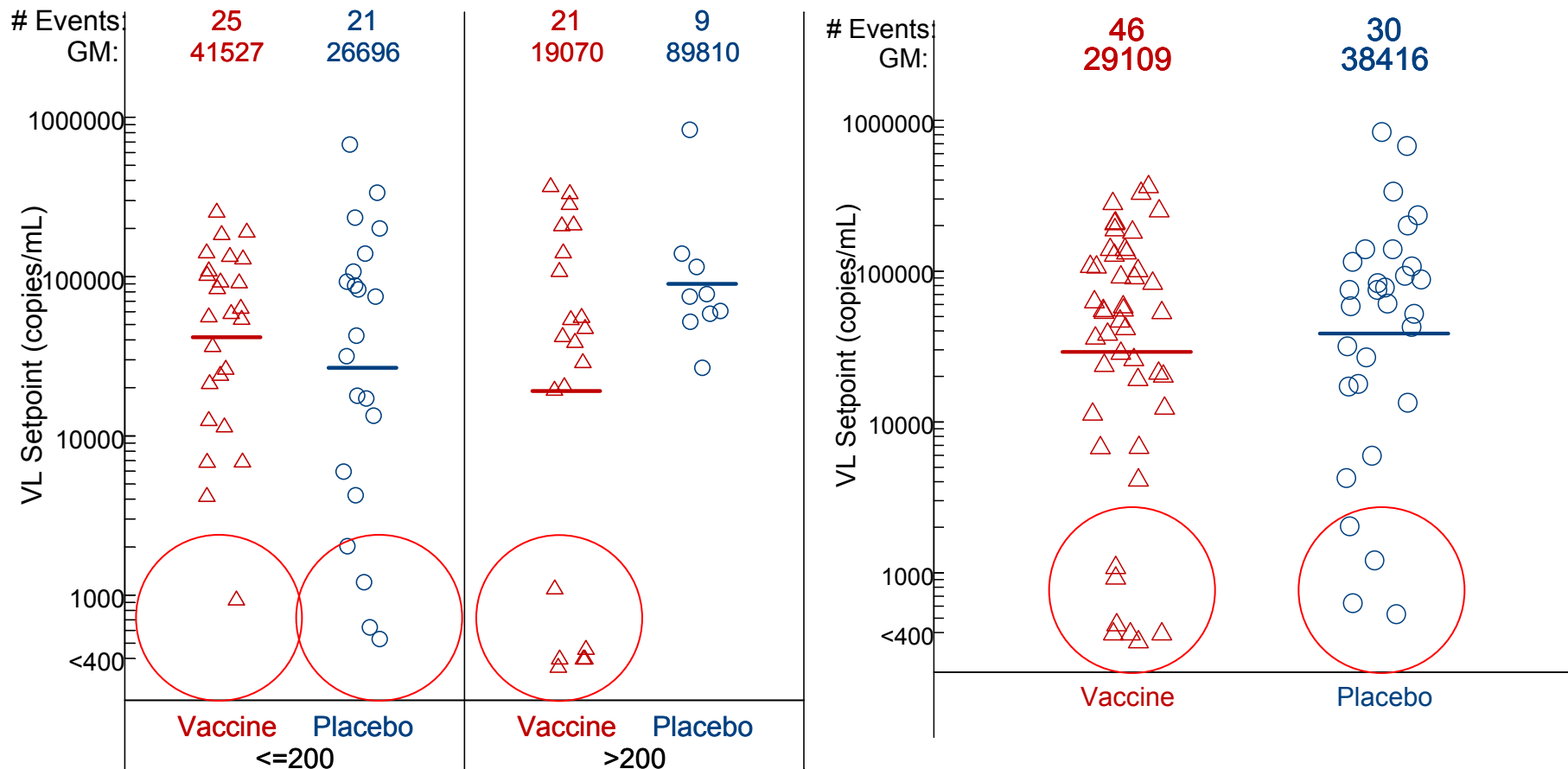
No vaccine efficacy observed
in reducing viral load set point

Was this a failure of CD8+ T cells

to recognize incoming virus or HIV-1 escape from
vaccine-induced responses early in infection?



Summary of Viral Load Setpoint MITT population (males)



Adapted from Mike Robertson's
slide shown earlier

HLA typing in progress,
(3 of 5 with VL < 2000 have HLA B27, B57, B58)

For subjects with VL setpoint data available as of 10/17/07

Were intrinsic host factors responsible for altered susceptibility to infection or differences in viral load?

Not known

Future Studies

Whole host genome sequence analysis

Target gene analysis:

- 1) immune response genes potentially associated with HIV acquisition/disease progression:
HLA class I and II, KIR, TLR, FcR, etc.
- 2) Genes whose products are associated with HIV-1 replication and life cycle:
CCR5, CCR5 promoter, CCR2, APOBEC3G, etc.

Questions for the Vaccine Field

Will previous vector immunity impact efficacy and safety? Is this a problem for all Ad5 and non-Ad5 vaccines?

Can other T cell-based vaccines provide immune protection?

Can non-human primate vaccine challenge studies predict efficacy in humans?

Will mucosal protection occur with systemic immunization?

Can sufficient antigenic coverage be provided in a single vaccine candidate?

Defining Potential Mechanisms to Further Address the Scientific Questions

Our goal is to provide an expeditious yet outside reviewed process to address these issues

- Establish a scientific committee of investigators from Merck, HVTN, NIAID and the scientific community to develop a scientific agenda to explain the vaccine's lack of efficacy and apparent increased acquisition
 - Bruce Walker will serve as chair, and members of HVTN Lab Sciences Advisory Committee will be included
 - Laboratories of HVTN, Merck, CHAVI, USMRP and VRC will be enlisted to help in these endeavors
 - Contract funds for outside laboratories will also be sought
- Use HVTN web site for unsolicited proposals for ancillary studies involving trial specimens and related studies

SUMMARY

- The design and conduct of the STEP trial was a success.
- The MRKAd5 HIV vaccine is as immunogenic in the STEP study as in previous Phase I trials.
- The issues of lack of vaccine efficacy and differential infection rates between vaccine and placebo groups with previous Ad5 immunity are complex ones and require careful consideration
- Numerous laboratory investigations are underway to understand the trial results, and we will enthusiastically engage the scientific community to join us in answering the key scientific questions

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