Mosaic HIV-1 Vaccines Expand the Breadth and Depth of Cellular Immune Responses in Rhesus Monkeys

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Desired Features of a Next Generation T Cell-Based HIV-1 Vaccine Candidate

- **In the post-STEP era, key features that would be desired in a next generation T cell-based HIV-1 vaccine include:**
  - **Vectors that avoid pre-existing vector-specific NAbs and that can be combined into a heterologous prime-boost regimen**
  - **Antigens that improve cellular immune breadth and that optimize coverage of global virus diversity**
- **In the post-Thai trial era, development of a T cell-based vaccine with global coverage (or at least a T cell-based component of a vaccine) remains an important priority**

Desired Features of a Next Generation T Cell-Based HIV-1 Vaccine Candidate

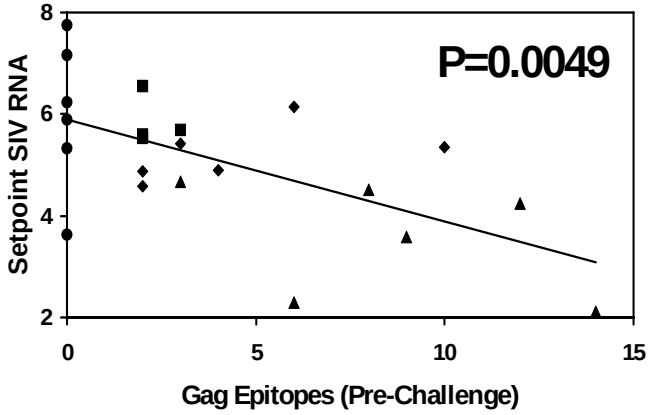
- **Importance of cellular immune breadth increasingly clear**
  - **Gag breadth critical for vaccine control of SIV challenge in NHPs (Liu et al. Nature 2009; 457: 87-91)**
  - **Gag breadth critical for immune control of HIV-1 in humans (Kiepiela et al. Nat Med 2007; 13:46-53)**
  - **In the STEP study, limited breadth with Merck rAd5-Gag/Pol/Nef vaccine (J. McElrath, N. Frahm, D. Casimiro):**
    - **Median of only 2-3 epitopes (1 Gag epitope) per vaccinee**
    - **Reduced Gag breadth in subjects with pre-existing Ad5 NAbs**

- **Critical to improve Gag-specific cellular immune breadth in a next-generation T cell-based HIV-1 vaccine**

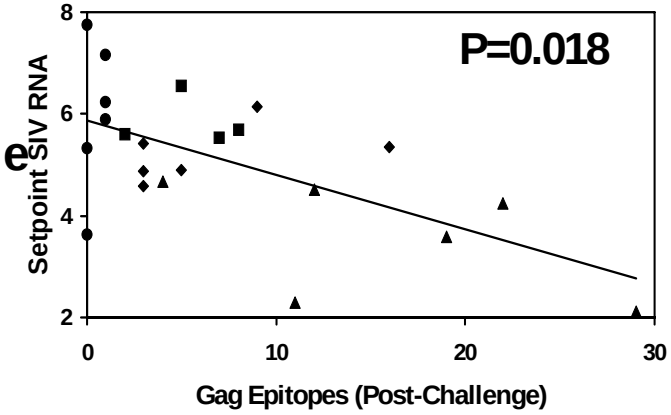
Augmented Gag Cellular Immune Breadth Improves Protective Efficacy Against SIV in Rhesus Monkeys

Gag Epitope Breadth

Pre-Challenge



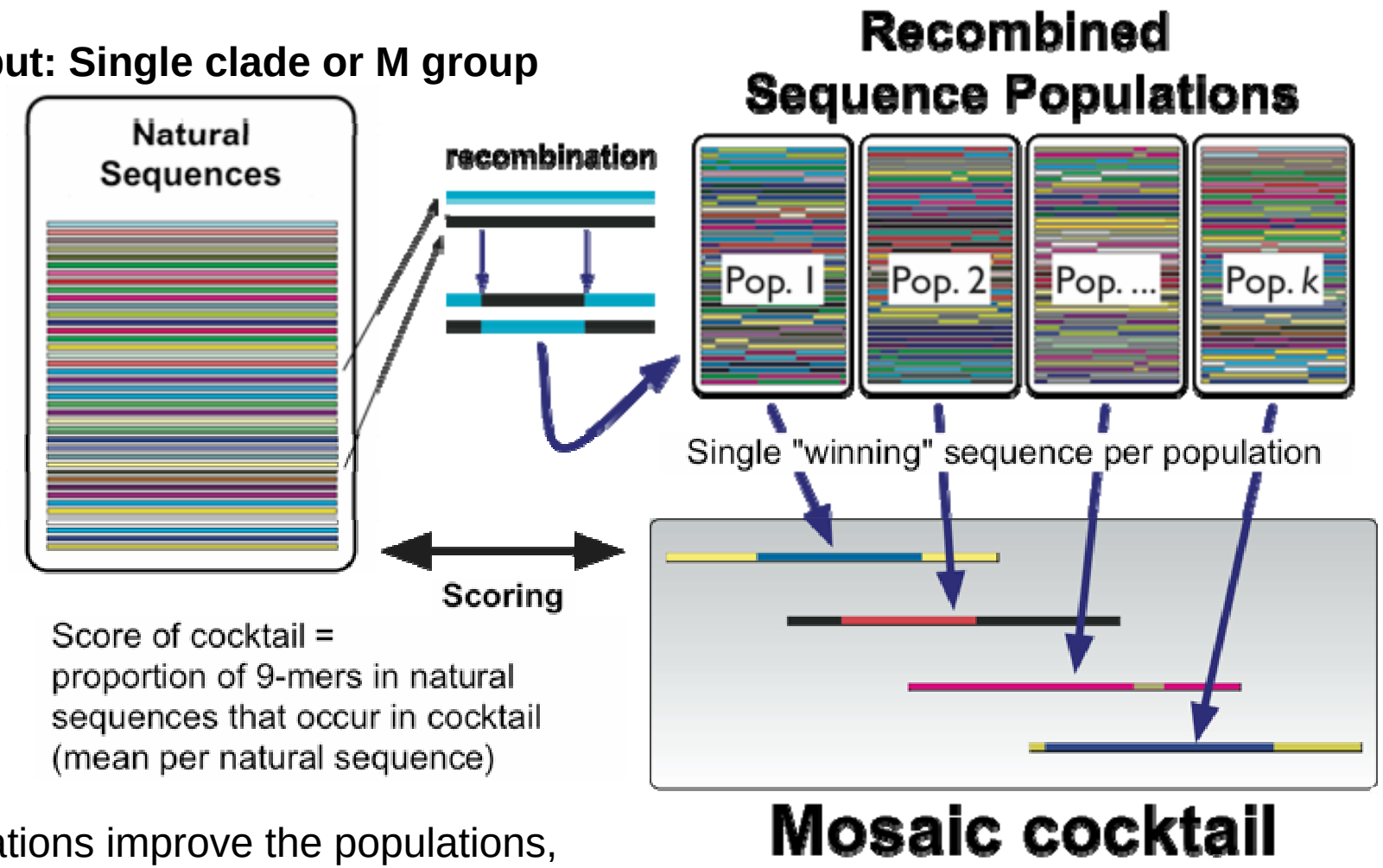
Post-Challenge



Liu et al. Nature 457: 87-91 (2009)

What are Mosaic Antigens?
Algorithm to Generate a *k*=4-Valent Mosaic Vaccine

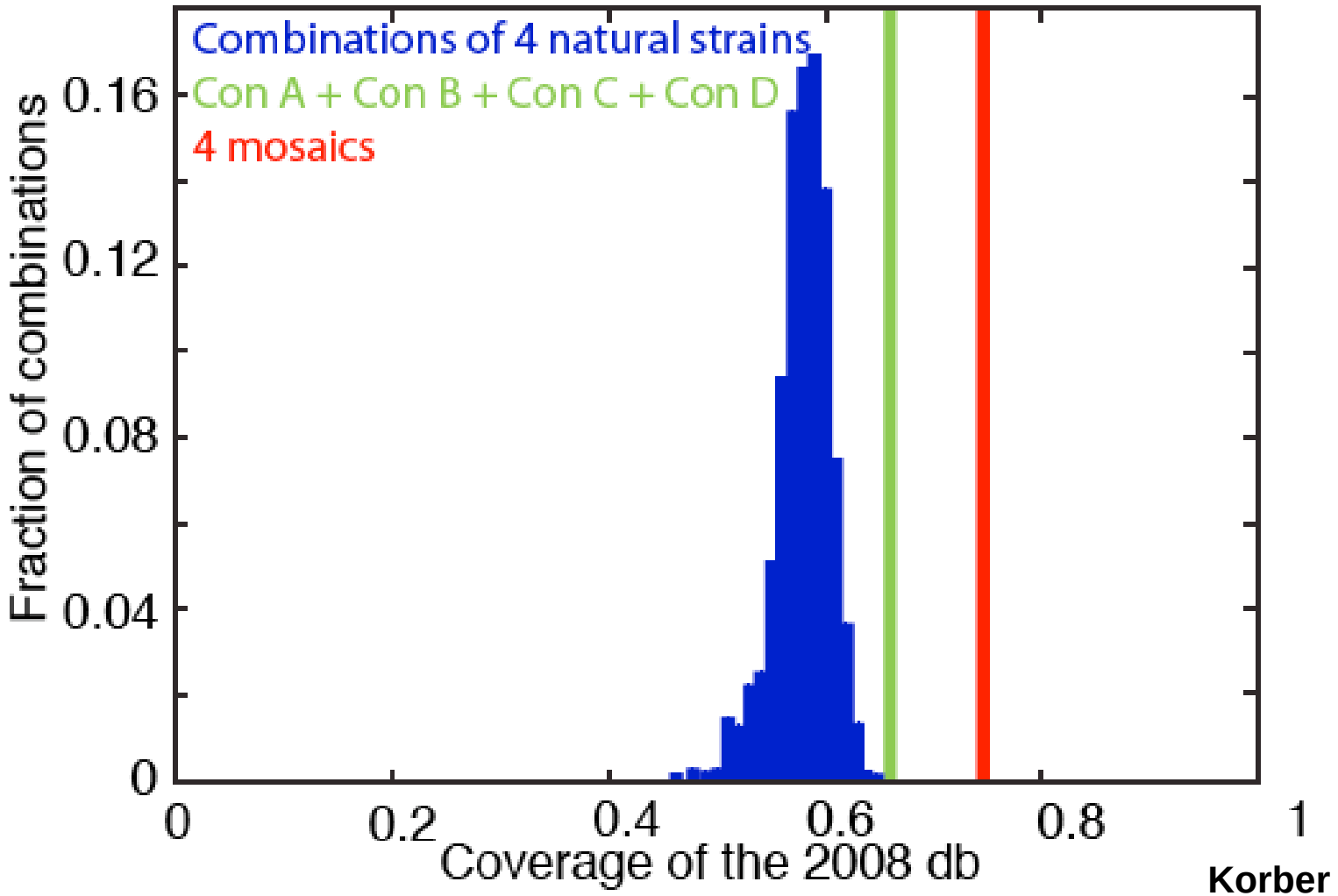
Input: Single clade or M group



Iterations improve the populations, improve the cocktail

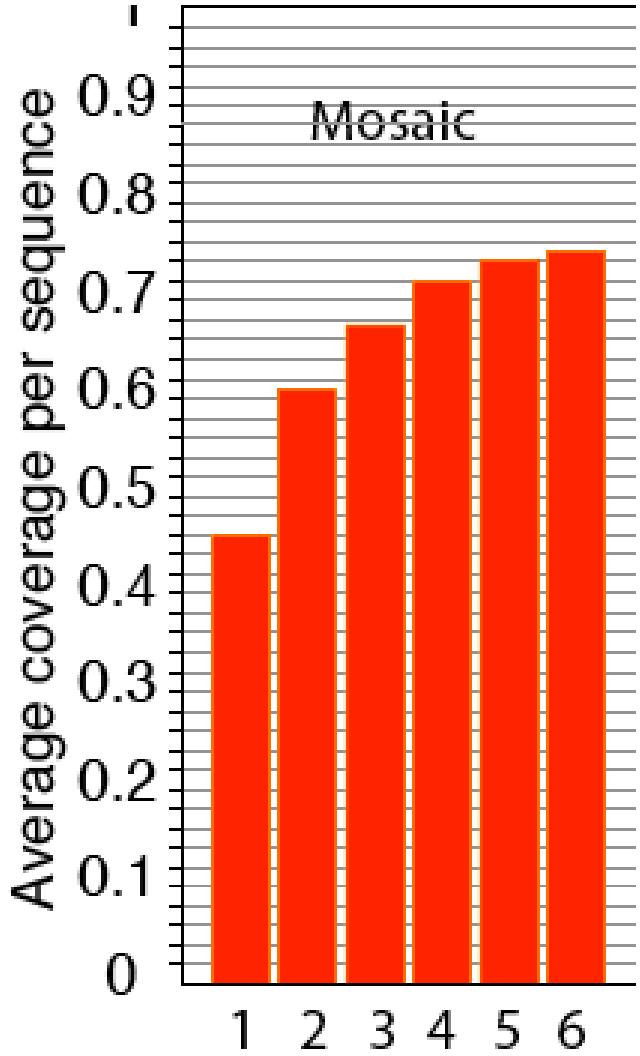
Fischer, Korber et al. Nat. Med. 13: 100-106 (2007)

A 4-Valent Gag Mosaic Vaccine Covers More Potential Epitopes than Any 4 Natural or Consensus Sequences



Korber et al. 2009

Increasing Valency (Number of Mosaic Variants) Increases Coverage but Has Diminishing Returns



Korber et al. 2009

Immunogenicity of HIV-1 Mosaic Antigens in Rhesus Monkeys (Collaboration with Bette Korber, LANL)

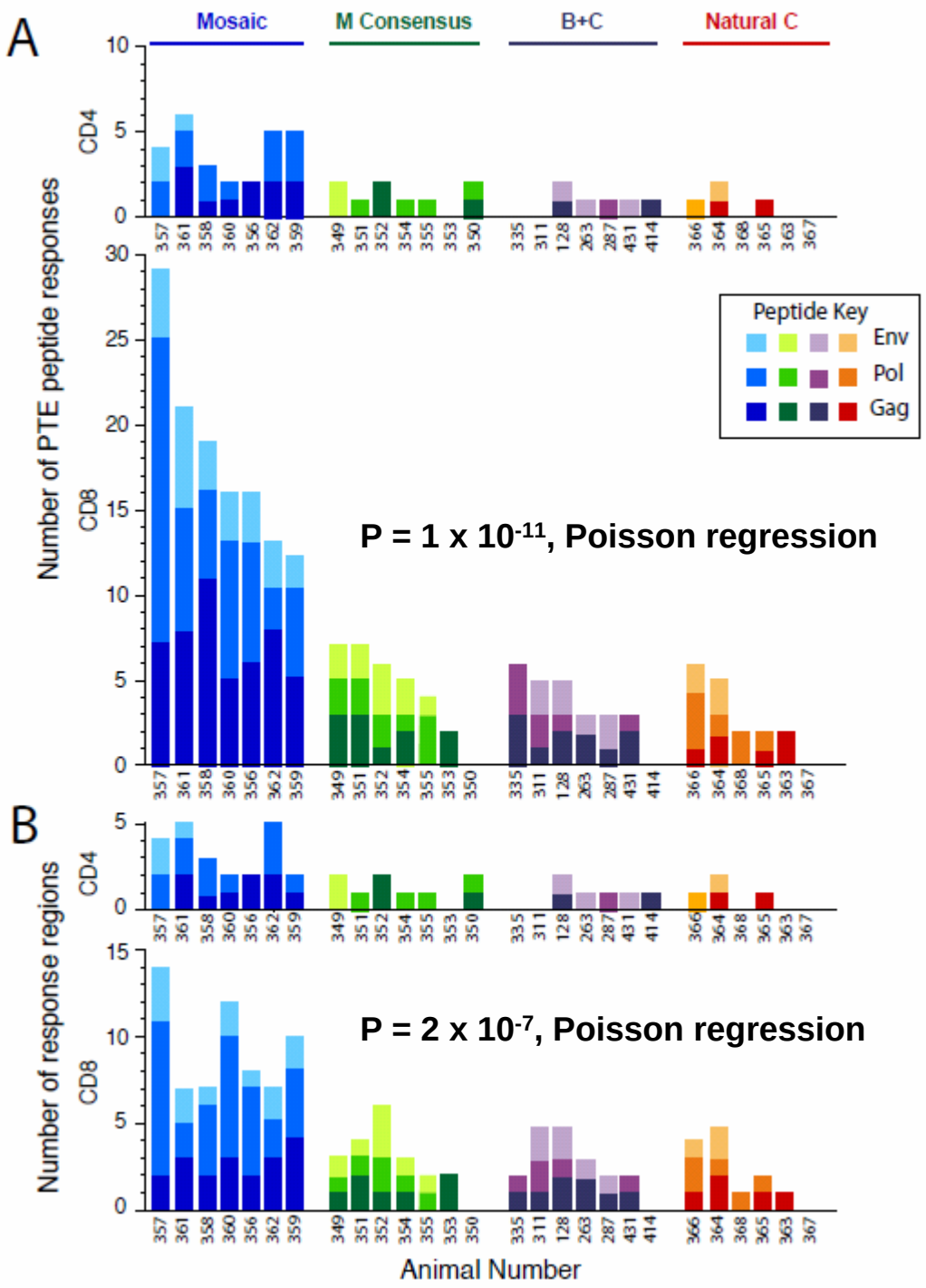
- **2-valent M mosaic antigens – balance between theoretical and practical considerations**

- **27 rhesus monkeys immunized once with $3x10^{10}$ vp rAd26 vectors expressing HIV-1 Gag, Pol, Env from:**
  - **2-valent M mosaic sequences (N=7)**
  - **M consensus sequences (N=7)**
  - **2-valent clade B + clade C sequences (N=7)**
  - **Optimal natural clade C sequences (N=6)**

- **Cellular immune breadth assessed by ELISPOT assays:**
  - **Global potential T cell epitope (PTE) peptides that represent 85% of worldwide viral sequences designed by HVTN**
  - **PTE peptide pools and subpools to assess magnitude of responses**
  - **Comprehensive mapping of CD4 and CD8 epitopes with individual 15-mer PTE peptides to assess breadth of responses**
  - **Breadth also assessed with 5 Gag proteins from clades A, B, C**
  - **Criteria for positivity: 55 SFC / 10^6 PBMC, 4x background**

Expanded Cellular Immune Breadth by Mosaic Antigens

The mosaic vaccine yielded many more Gag, Pol, and Env (A) epitope-specific T lymphocyte responses as well as conservatively (B) the number of epitope response regions to PTE peptides than did an M consensus vaccine, a 2-valent clade B + clade C vaccine, or an optimal natural C clade vaccine



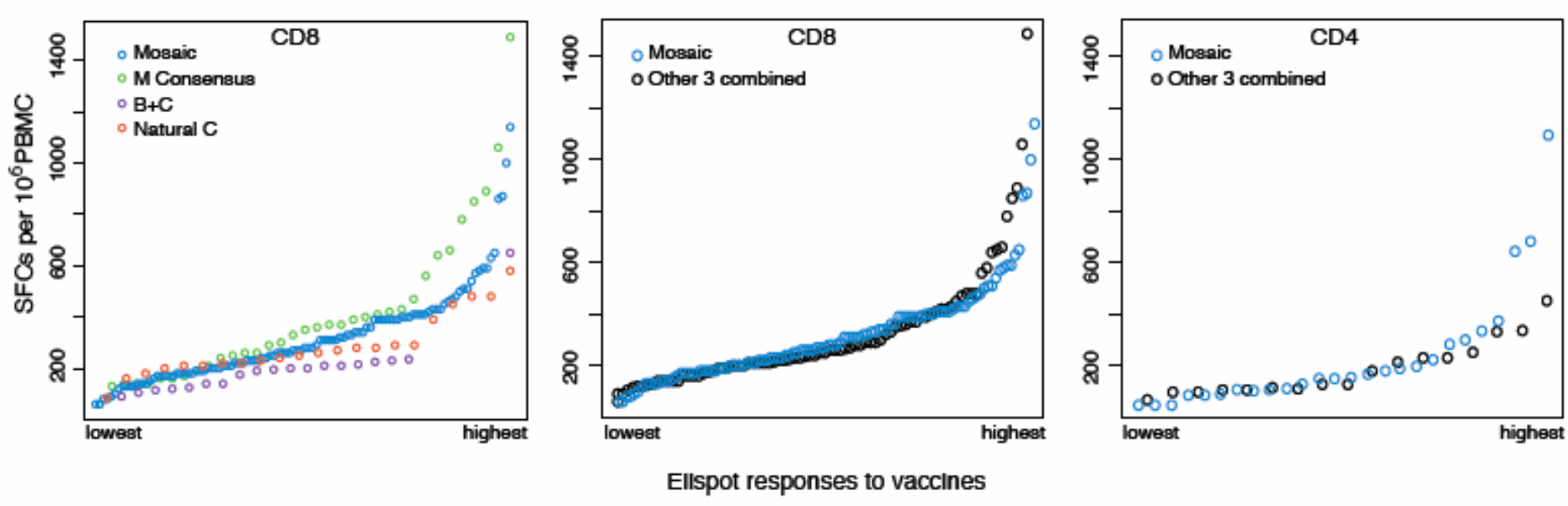
Poisson Regression Statistical Models
PTE Epitope Analysis

- **There were more CD8 than CD4 epitope-specific T lymphocyte responses by a factor of 4.3 ($P < 2 \times 10^{-16}$)**
- **There were fewer responses to Env than to Gag and Pol by a factor of 0.5 ($P < 0.0007$)**
- **The mosaic vaccine elicited more epitope-specific responses than the other vaccines by a factor of 3.8 ($P = 1 \times 10^{-11}$)**
- **No significant differences among epitope-specific responses for the M consensus, the clade B + clade C, or the optimal natural clade C vaccines**

Poisson Regression Statistical Models
PTE Response Region Analysis

- **There were more CD8 than CD4 epitope-specific T lymphocyte responses by a factor of 2.8 ($P = 2 \times 10^{-7}$)**
- **There were fewer responses to Env than to Gag and Pol by a factor of 0.5 ($P < 0.0008$)**
- **The mosaic vaccine elicited more epitope-specific responses than the other vaccines by a factor of 3.1 ($P = 2 \times 10^{-7}$)**
- **No significant differences among response regions for the M consensus, the clade B + clade C, or the optimal natural clade C vaccines**

Comparable Magnitude of Individual Epitope-Specific Cellular Immune Responses



The mosaic vaccine yielded epitope-specific CD8+ and CD4+ T lymphocyte responses of a comparable magnitude to the other vaccines (P = 0.58 and 0.99, respectively, Kolmogorov-Smirnov tests).

Thus, the mosaic vaccine expanded cellular immune breadth without compromising the magnitude of individual epitope-specific responses.

No detectable problems of antigenic competition or immunodominance.

Epitope-Specific Responses to Consensus and Natural Sequence Vaccines: Solid Matches of Responding PTE Peptides with Vaccine Sequences

OptC 366-07:

5 CD8 responses:

OptC	I**VQQQSNLLRAIEAQ**Q			
E54	VVQQQSNLLRAIEAQ	Env	548	562
E72	-VQQQNNLLRAIEAQH	Env	549	563
OptC	A**VFIHNFKRKGGIGG**Y			
P22	AVFIHNFKRKGGIGG	Pol	894	908
P236	-VLIHNFKRKGGIGGY	Pol	895	909
OptC	**MAICEEMEKEGKIT**K			
P224	TAICEEMEKEGKITK	Pol	190	204
OptC	**CTHGIKPVVSTQLLL**			
E15	CTHGIKPVVSTQLLL	Env	247	261
OptC	**GGPSHKARVLAEAMS**			
G76	GGPSHKARVLAEAMG	Gag	354	368

1 CD4 response:

OptC	**IIGQVRDQAEHLKTA**			
P86	LIGQVRDQAEHLKTA	Pol	876	890

Good matches with solid stretches of identity between vaccine and target PTE peptide

Epitope-Specific Responses to Mosaic Vaccines: Local Variant Responses, No Apparent Antigenic Competition

Mos1	IC**TTTVPWNASWSNK**SL	
Mos2	IC**TTAVPWNTSWSNK**SQ	
E334	ICTTTVPWNASWSN**R**	T...A
E214	-CTTTVPWN**S**SWSNKT	T...S
E158	--TTAVPWNASWSNKSL	A...A
E290	--TTAVPWNTSWSNKSL	A...T

1) Four variable PTE peptides were recognized
2) In the region of overlap both mosaic forms were recognized, as well a combination of the two
3) A new form (S) was also recognized

Typical pattern of CD8+ PTE peptide responses in a mosaic vaccinated animal (361-07)

22 PTE peptides

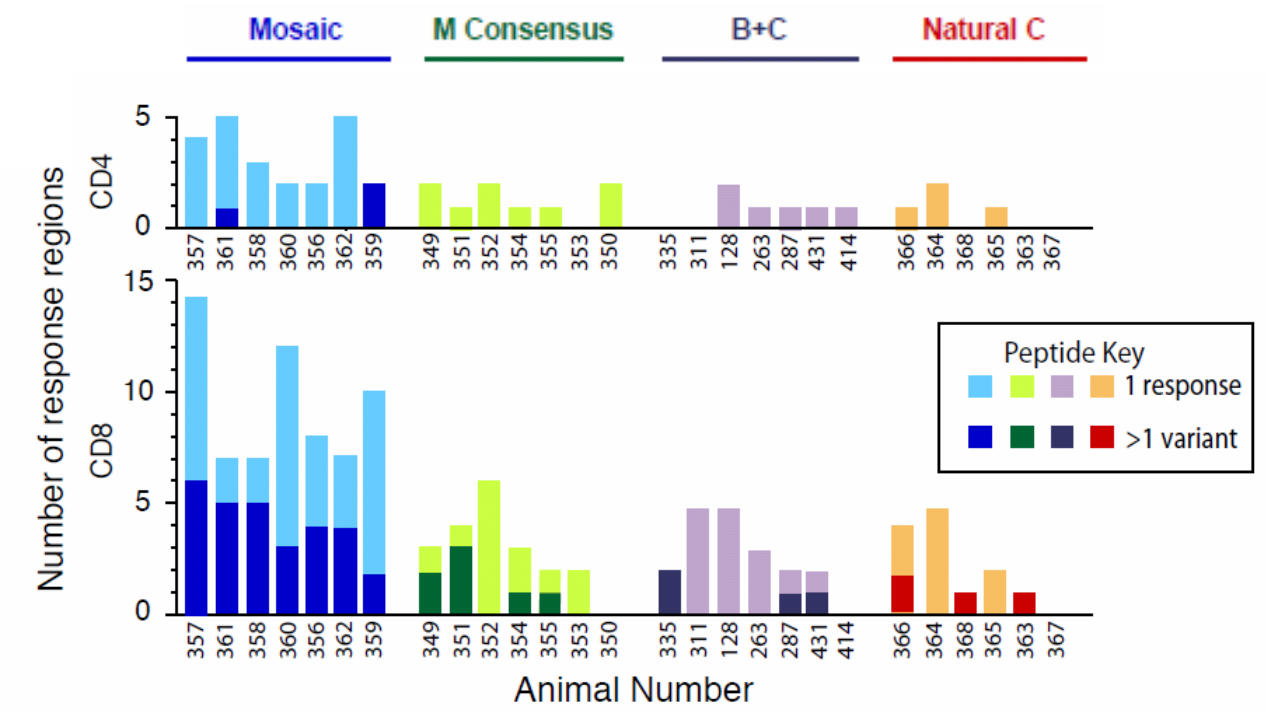
8 responsive regions

5 regions included variant peptides that match amino acids *in one or the other* of the mosaic vaccine sequences

8 CD8 responses:

Mos1	EQLIKK**ERVYLSWVP**AHKGIG			
Mos2	EQLIKK**EKVYLAWVP**AHKGIG			
P221	EELIKKEKVYLAWVP	Pol	678	692
P250	EPLIKKEKVYLSWVP	Pol	678	692
P316	-KLIEKDKVYLSWVPA	Pol	679	693
P223	--LIKKERVYLSWVPAH	Pol	680	694
P169	------EKVYLAWVPAHKGIG	Pol	684	698
P83	------EKVYLSWVPAHKGIG	Pol	684	698
Mos1	**CTHGIRPVVSTQLLL**			
Mos2	**CTHGIKPVVSTQLLL**			
E15	CTHGIKPVVSTQLLL	Env	247	261
E47	CTHGIRPVVSTQLLL	Env	247	261
Mos1	IC**TTTVPWNASWSNK**SL			
Mos2	IC**TTAVPWNTSWSNK**SQ			
E334	ICTTTVPWNASWSN**R**	Env	603	617
E214	-CTTTVPWN**S**SWSNKT	Env	604	618
E158	--TTAVPWNASWSNKSL	Env	605	619
E290	--TTAVPWNTSWSNKSL	Env	605	619
Mos1	ACQGV**GGPGHKARVL**AEAMS			
Mos2	ACQGV**GGPSHKARVL**AEAMS			
G166	ACQEVGGPGHKARVL	Gag	349	363
G76	-----GGPSHKARVLAEAMG	Gag	354	368
Mos1	AAEW**DRVHPVHAGPIA**PGQ			
Mos2	AAEW**DRLHPVHAGPVA**PGQ			
G319	AAE-DRLHPVHAGPIP	Gag	209	225
G242	-ADWDRLHPVHAGPVA	Gag	210	224
G44	-AEWDRLHPVHAGPIA	Gag	210	224
G277	---WDRVHPVHAGPNPPG	Gag	212	226
G102	----DRVHPVHAGPIPPGQ	Gag	212	226
Mos1	**HSNWRAMASDFNLPP**			
Mos2	**HSNWRAMASEFNLPP**			
P93	HSNWRAMASDFNLPP	Pol	731	745
Mos1	**KGRPGNFLQNRPEPT**			
Mos2	**KGRPGNFLQSRPEPT**			
G86	KGRPGNFLQNRPEPT	Gag	442	456
Mos1	**SRELERFAVNPGLLE**			
Mos2	**SRELERFALNPGLLE**			
G39	SRELERFALNPGLLE	Gag	38	52

Expanded Cellular Immune Depth with Mosaic Antigens



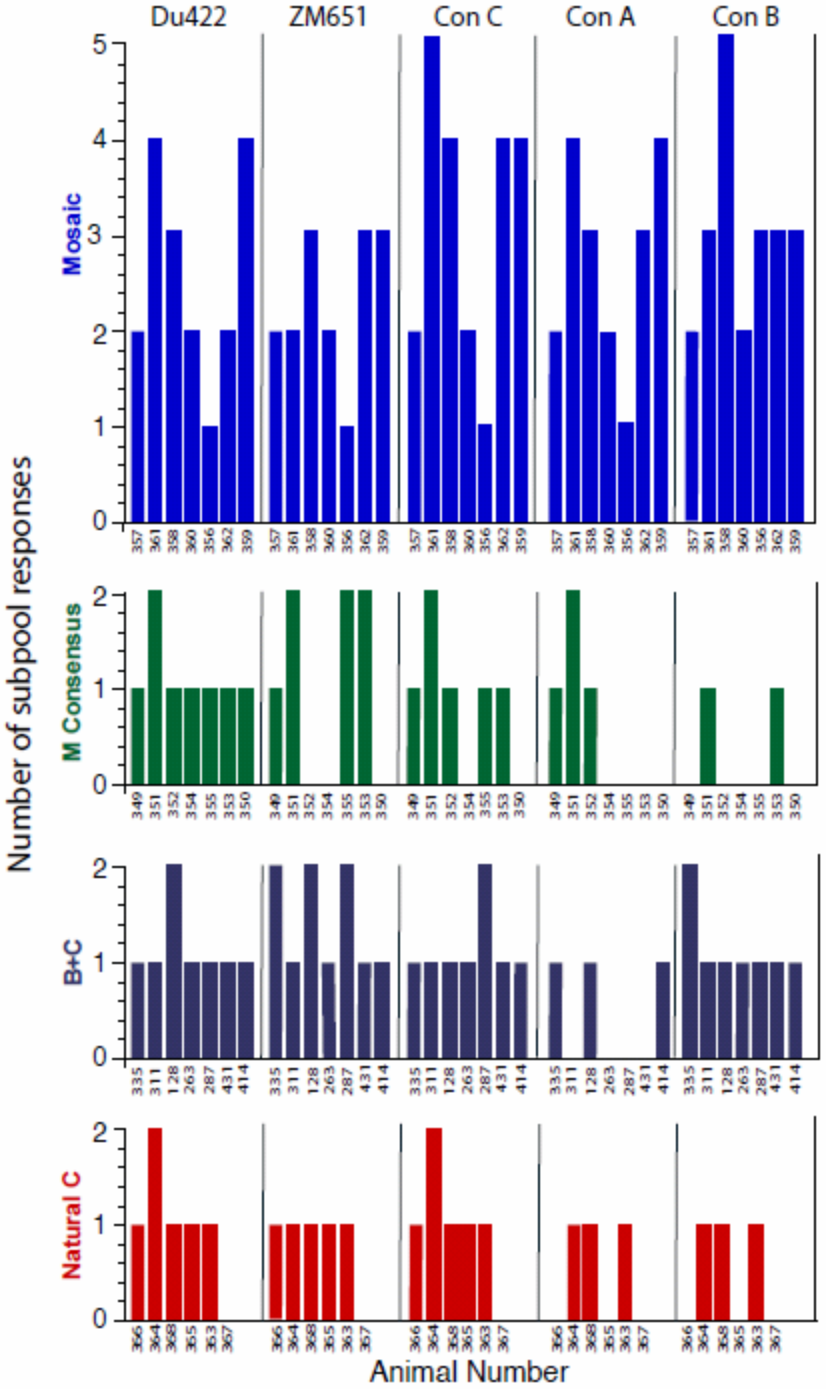
The mosaic vaccine yielded increased depth of CD8+ T lymphocyte responses compared with an M consensus vaccine, a 2-valent clade B + clade C vaccine, or an optimal natural C clade vaccine (P = 0.001, Wilcoxon rank-sum test)

Expanded Cellular Immune Breadth by Mosaic Antigens

T cell responses elicited by the mosaic vaccine recognized more peptide subpools spanning *actual* Gag proteins from clades A, B, C ($P = 1x10^{-7}$, binomial regression)

Mosaic vaccine did better at eliciting responses against natural C clade proteins than did the optimal natural C clade vaccine

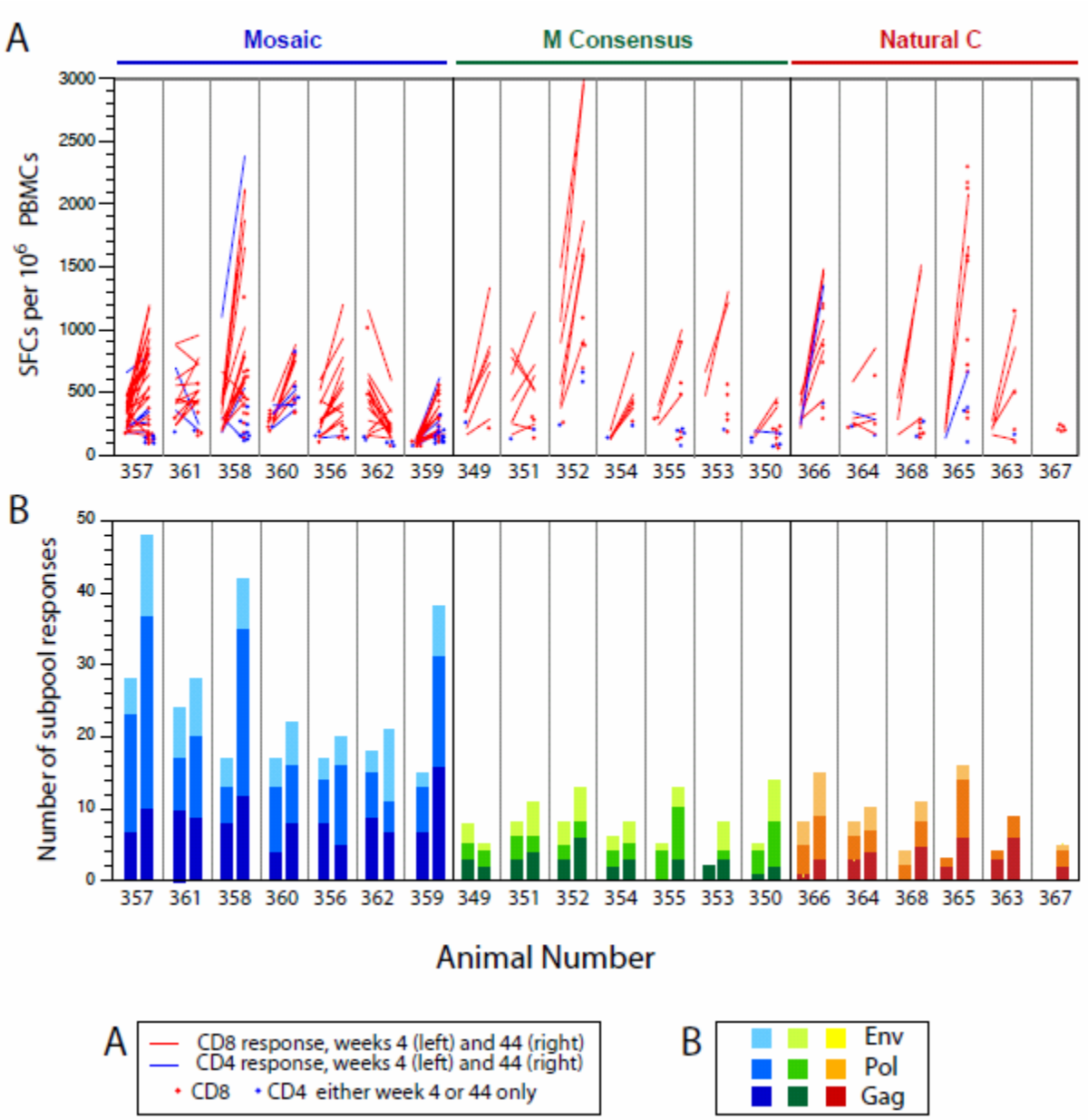
Mosaic vaccine elicited comparable cross-clade responses against Gag proteins from clades A, B, C



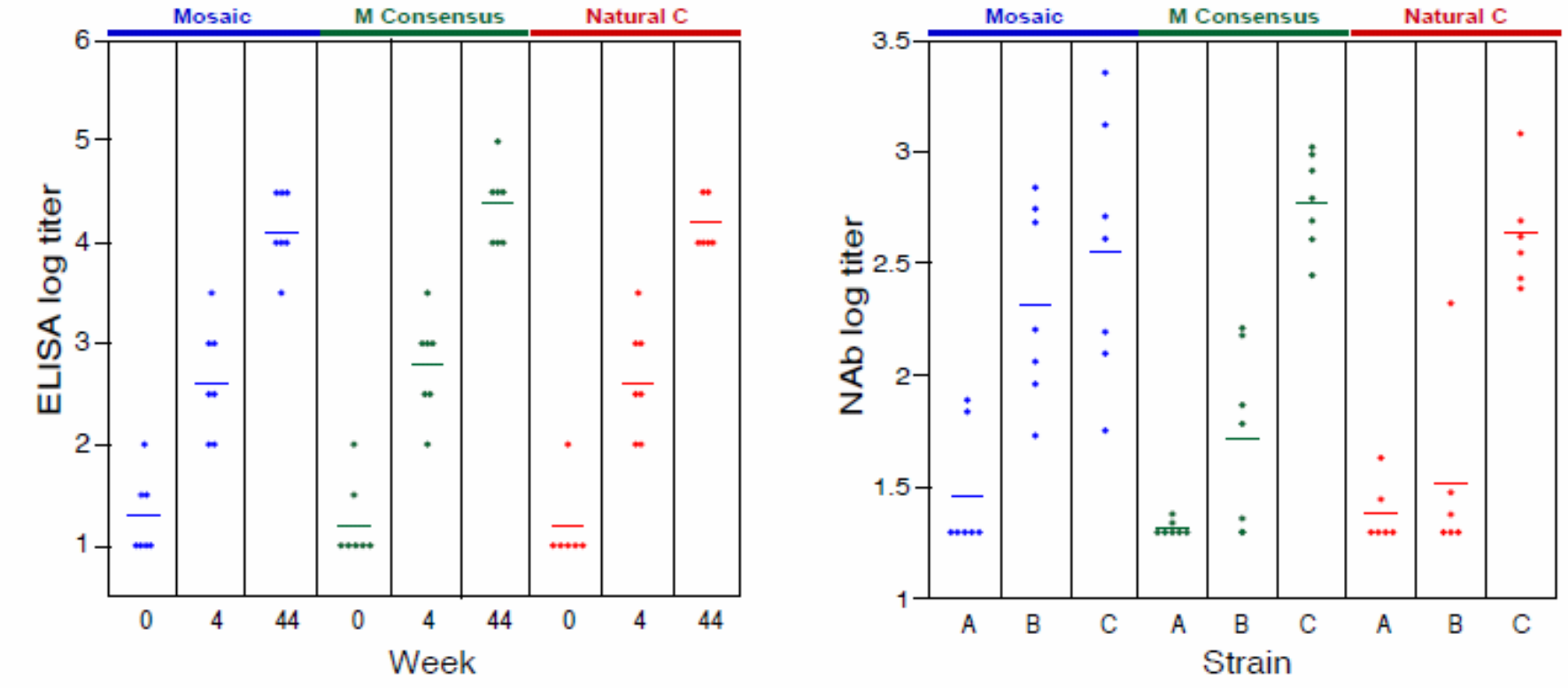
Breadth and Magnitude Following Boost Immunization

Following a boost immunization with rAd5HVR48 vectors, the mosaic vaccine continued to show (A) comparable magnitude but (B) increased breadth of cellular immune responses compared with the other vaccine regimens.

In each column, pre-boost (left) and post-boost (right) responses are shown



Mosaic Env Antigens Elicit Noninferior ELISA and NAb Responses Compared with Consensus or Natural Sequence Env Antigens



The mosaic vaccine elicited comparable ELISA and Tier 1 C (MW965.26) NAb titers (P = NS) and slightly increased Tier 1 B (SF162.LS) NAb titers compared with the M consensus and optimal natural C clade vaccine (P = 0.02, Wilcoxon rank-sum test)

Immunogenicity of HIV-1 Mosaic Antigens in Rhesus Monkeys

- **Improved breadth of coverage by mosaic antigens as compared with consensus or natural antigens**
  - **May improve coverage of global virus diversity**
  - **Possibility of a globally relevant T cell-based vaccine**

- **Improved depth of coverage by mosaic antigens in terms of simultaneous induction of responses to variant epitopes**
  - **May limit T cell escape or force the virus down suboptimal escape routes that incur higher fitness costs**
  - **Possibility of a more effective T cell-based vaccine**

- **2-valent mosaic antigens attractive for clinical evaluation**
  - **Substantial enhancement of breadth and depth in NHPs**
  - **May prove superior to the natural sequence antigens that have been utilized in most clinical trials to date**
  - **Logistically and practically feasible for development**

HIV-1 Vaccine Clinical Development Strategy

1. **Develop “prototype” novel rAd vectors expressing a single test antigen (VRC EnvA) for a rapid assessment of vector safety and immunogenicity in humans**

2. **Develop “complete” vaccine products involving an optimal heterologous rAd prime-boost regimen expressing multiple HIV-1 antigens for further clinical development**

Adenovirus Serotype 26 (Ad26)

- **Ad26 is a rare Ad serotype that we selected for clinical studies following preclinical evaluation of group D (Ad26, Ad28, Ad48, Ad49) and group B (Ad11, Ad34, Ad35, Ad50) Ads**

- **Ad26 is substantially different than Ad5 in terms of:**
  - **Baseline seroprevalence (Abbink et al. J. Virol. 2007; 81:4654-4663; Thorner et al. J. Clin. Microbiol. 2006; 44:3781-3783)**
  - **Cellular receptors (Abbink et al. J. Virol. 2007; 81:4654-4663)**
  - **Tropism (Waddington et al. Cell 2008; 132:397-409)**
  - **Innate immune profile (Barouch et al., unpublished data)**
  - **Adaptive immune phenotype (Liu et al. J. Virol. 2008; 82:4844-4852)**
  - **Protective efficacy against SIV (Liu et al. Nature 2009; 457:87-91)**

- **First-in-human Ad26 safety and immunogenicity**

A phase 1 randomized, double-blind, placebo controlled dose escalation clinical trial to evaluate the safety and immunogenicity of recombinant adenovirus serotype 26 HIV-1 vaccine (Ad26.ENVA.01) in healthy, HIV-1 uninfected adults

PI: Lindsey Baden, Brigham & Women's Hospital

- **Late breaker oral abstract presentation earlier today (OA05-06LB)**
- **Ad26 safe and immunogenic in humans at doses of 10^9, 10^{10}, and 10^{11} vp**
- **Ad26 is a promising vector for further clinical development**

Proposed Next-Generation HIV-1 Vaccine Candidates
NIH IPCAVD U19 AI078526 Program

- **Our IPCAVD program aims to develop a global HIV-1 vaccine candidate for clinical evaluation**
  - **Vectors that avoid pre-existing vector-specific NAbs and that can be combined into a heterologous prime-boost regimen**
    - **Heterologous rare serotype rAd prime-boost regimen**
  - **Antigens that improve cellular immune breadth and that optimize coverage of global virus diversity**
    - **2-valent mosaic Gag/Pol/Env antigens**
- **Rare serotype Ad vectors expressing mosaic HIV-1 antigens are currently being manufactured for clinical studies by Crucell**

Proposed Next-Generation HIV-1 Vaccine Candidates IPCAVD-MHRP Collaboration

- **IPCAVD-MHRP collaboration to develop rPox/rAd regimens**
  - **Protective efficacy of rMVA/rAd26 regimens against heterologous SIV challenges in NHPs**
  - **Immunogenicity of rMVA/rAd26 regimens expressing mosaic HIV-1 Gag/Pol/Env antigens in NHPs and humans**
- **MVA vectors expressing matching mosaic HIV-1 antigens are currently being manufactured for clinical studies by MHRP**
- **We propose parallel clinical development of heterologous rare serotype rAd/rAd as well as rMVA/rAd vaccine regimens expressing matching mosaic HIV-1 Gag, Pol, Env antigens**

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