Module 8:
Evaluating Immune Correlates of Protection

Instructors: **Ivan Chan, Peter Gilbert, Paul T. Edlefsen, Ying Huang**

Session 7:
RV144 Thai Trial Case Study (HIV Vaccine Efficacy Trial)

Summer Institute in Statistics and Modeling in Infectious Diseases
University of Washington, Department of Biostatistics

July 15-17, 2013

Statistical Center for HIV/AIDS Research & Prevention SCHARP

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Outline of Module 8

Session 1 (Chan)	Introduction to Vaccines and Basic Concepts
Session 2 (Gilbert)	Introduction to Immune Correlates of Protection
Session 3 (Chan)	Evaluating Correlates of Protection using Individual, Population, and Titer-Specific Approaches
Session 4 (Gilbert)	Continuation of Session 2; plus Evaluating a Correlate of Risk (CoR)
Session 5 (Chan)	Use of Statistical Models in Assessing Correlates of Protection
Session 6 (Edlefsen)	Introduction to Sieve Analysis
Session 7 (Gilbert)	**Thai Trial Case Study (Including Sieve Analysis)**
Session 8 (Chan)	Validation using Prentice Criteria, Design Considerations
Session 9 (Gilbert)	Evaluating a Specific Surrogate of Protection Part I (Gilbert and Hudgens, 2008)
Session 10 (Huang)	Evaluating a Specific Surrogate of Protection Part II (Huang and Gilbert, 2011; Huang, Gilbert and Wolfson, 2013)

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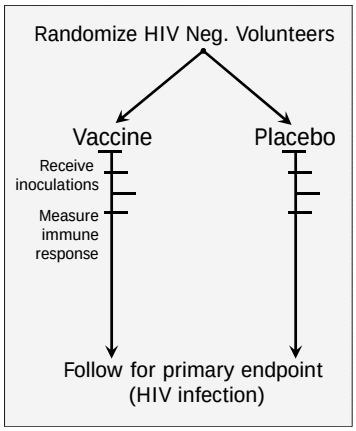
Preventive HIV Vaccine Efficacy Trial



- **Primary Objective**
  - Assess VE: Vaccine Efficacy to prevent HIV infection
- **Secondary Objective**
  - Assess vaccine-induced immune responses as ***immune correlates of protection***

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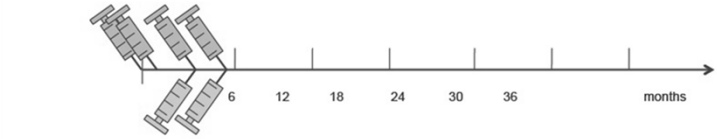
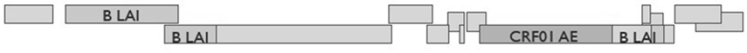
Vaccination Schedule: RV144 Thai Trial



- Four priming injections of a recombinant canarypox vector (ALVAC-HIV vCP1521) at week 0, 4, 12, 24:
  - HIV-1 Gag and Pro (subtype B LAI)
  - CRF01-AE HIV-1 gp 120 (92TH023) linked to gp41-TM (LAI)



- Two booster injections of a recombinant gp120 subunit vaccine (AIDSVAX B/E) at week 12, 24:
  - Subtype E HIV-1 strain A244 (CM244)
  - Subtype B HIV-1 strain MN

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Thai Trial Primary Results

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 3, 2009 VOL. 361 NO. 23

Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand

Supachai Rerks-Ngarm, M.D., Punnee Pitisuttithum, M.D., D.T.M.H., Sorachai Nitayaphan, M.D., Ph.D., aranit Kaewkungwal, Ph.D., Joseph Chiu, M.D., Robert Paris, M.D., Nakorn Premsri, M.D., Chawetsan Namwat, M.D. Mark de Souza, Ph.D., Elizabeth Adams, M.D., Michael Benenson, M.D., Sanjay Gurunathan, M.D., Jim Tartaglia, Ph.D., John G. McNeil, M.D., Donald P. Francis, M.D., D.Sc., Donald Stablein, Ph.D., Deborah L. Birx, M.D., Supamit Chunsuttiwat, M.D., Chirasak Khamboonruang, M.D., Prasert Thongcharoen, M.D., Ph.D., Merlin L. Robb, M.D., Nelson L. Michael, M.D., Ph.D., Prayura Kunasol, M.D., and Jerome H. Kim, M.D., for the MOPH–TAVEG Investigators*

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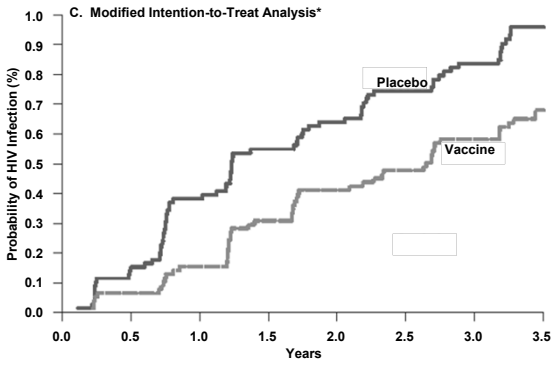
Partial VE to Prevent HIV Infection



*N=16,395 assessed; 51 Vaccine, 74 Placebo HIV-1 infected
Estimated VE = 31% [95% CI 1−51%], p=0.04

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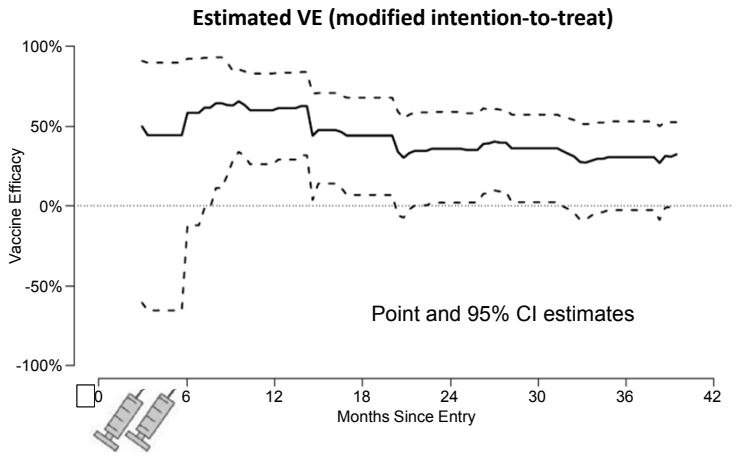
VE Waned Over Time



VE = [1 - cumulative incidence ratio (vaccine/placebo)] × 100%

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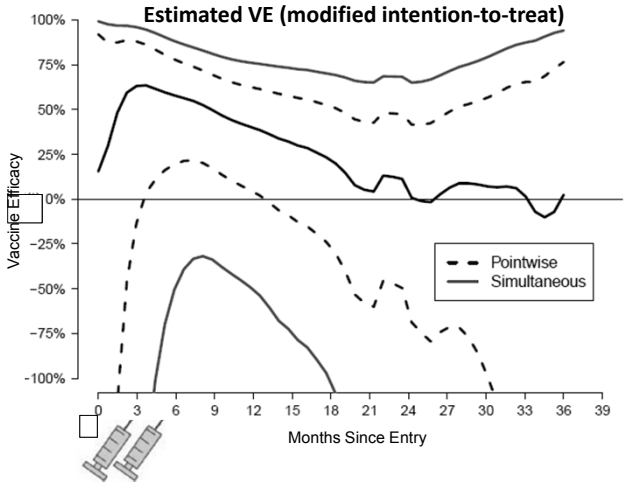
VE Waned Over Time



VE = [1 – instantaneous incidence ratio (vaccine/placebo)] × 100%

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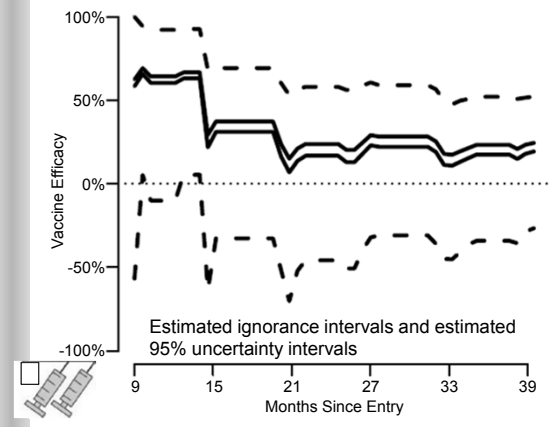
VE Waned Over Time [Causal Per-Protocol (PP) Analysis]



- PP = Complete full immunization series on schedule and HIV-free
- Causal PP VE measured in the subgroup who would be PP under either treatment assignment
- Sensitivity analysis method of Gilbert, Shepherd, and Hudgens*

*Assuming adherence monotonicity
(No subject would be PP under placebo but not under vaccine)

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- The observed partial VE stimulated an "immune correlates" collaborative project to explore how the vaccine may have worked

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Importance of an "Immune Correlate of Protection"

- Developing an immune correlate is a central goal of vaccine research
  - One of the 14 Grand Challenges of Global Health of the NIH & Gates Foundation (for HIV, TB, Malaria)
- Immune correlates useful for:
  - Shortening trials and reducing costs
  - Guiding iterative development of vaccines between basic and clinical research
  - Guiding regulatory decisions
  - Guiding immunization policy
  - **Bridging efficacy of a vaccine observed in a trial to a new setting**
    - Pearl (2011, *International Journal of Biostatistics*) suggests that bridging is the critical use

Two Major Concepts/Paradigms for Immune Correlates of Protection (CoPs)

- Statistical prediction paradigm (Qin et al., 2007, *J Infect Dis*)

  CoP = immune response biomarker that **reliably predicts** the level of VE

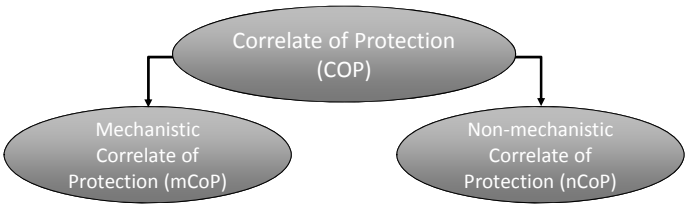
- Causal mechanism paradigm (Plotkin, 2008, *Clin Infect Dis*)

  Mechanistic CoP = immune response that **mechanistically causes** VE

- Both extremely useful for vaccine development, but assessed differently
  - **CoP** a statistical concept assessed via statistical analysis of efficacy trials (CoP = good surrogate endpoint based on statistical validation)
  - **Mechanistic CoP** assessed via holistic science

Terminology on Immune Correlates of Protection

- Terminology on immune correlates has been contradictory (e.g., Qin et al. 2007 vs. Plotkin 2008)
- Plotkin and Gilbert (2012, *Clin Inf Dis)* proposed new terminology



- CoP, mCoP defined on previous slide
- nCoP = CoP that is *not* a mechanism of protection

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RV144 Immune Correlates Analysis

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 5, 2012 VOL. 366 NO. 14

Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial

Barton F. Haynes, M.D., Peter B. Gilbert, Ph.D., M. Juliana McElrath, M.D., Ph.D., Susan Zolla-Pazner, Ph.D., Georgia D. Tomaras, Ph.D., S. Munir Alam, Ph.D., David T. Evans, Ph.D., David C. Montefiori, Ph.D., Chitraporn Karnasuta, Ph.D., Ruengpueng Sutthent, M.D., Ph.D., Hua-Xin Liao, M.D., Ph.D., Anthony L. DeVico, Ph.D., George K. Lewis, Ph.D., Constance Williams, B.S., Abraham Pinter, Ph.D., Youyi Fong, Ph.D., Holly Janes, Ph.D., Allan DeCamp, M.S., Yunda Huang, Ph.D., Mangala Rao, Ph.D., Erik Billings, Ph.D., Nicos Karasavvas, Ph.D., Merlin L. Robb, M.D., Viseth Ngauy, M.D., Mark S. de Souza, Ph.D., Robert Paris, M.D., Guido Ferrari, M.D., Robert T. Bailer, Ph.D., Kelly A. Soderberg, Ph.D., Charla Andrews, Sc.M., Phillip W. Berman, Ph.D., Nicole Frahm, Ph.D., Stephen C. De Rosa, M.D., Michael D. Alpert, Ph.D., Nicole L. Yates, Ph.D., Xiaoying Shen, Ph.D., Richard A. Koup, M.D., Punnee Pitisuttithum, M.D., D.T.M.H., Jaranit Kaewkungwal, Ph.D., Sorachai Nitayaphan, M.D., Ph.D., Supachai Rerks-Ngarm, M.D., Nelson L. Michael, M.D., Ph.D., and Jerome H. Kim, M.D.

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What the Immune Correlates Study Assessed

- The analysis sought to discover **Correlates of Risk:**
  Immune response variables measured 2 weeks after the immunizations that predict whether vaccinees become HIV infected
- Thus, the study is designed to generate hypotheses that certain immune response variables are **CoP** and/or **mCoPs**, that would need validation in future research

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Participating Institutions and Sponsors

- AFRIMS: U.S. and Thai components
- Faculty of Tropical Medicine, Mahidol University
- Global Solutions for Infectious Diseases
- Ministry of Public Health, Thailand
- Sanofi Pasteur
- Center for HIV/AIDS Vaccine Immunology (CHAVI)
- Royal Thai Army
- HIV Vaccine Trials Network (HVTN)
- Fred Hutchinson Cancer Research Center
- U.S. Military HIV Research Program
- Henry M. Jackson Foundation for the Advancement of Military Medicine
- Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH
- The Bill and Melinda Gates Foundation's Collaboration for AIDS Vaccine Discovery (CAVD)

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Two Tiers of Studies

- Pilot immunogenicity studies (2010-2011)
  - Open process inviting immunology labs to perform assays on sample-sets from **HIV uninfected** RV144 participants
  - Conducted standardized comparative analyses of all candidate assays, to down-select the best performing assays and to optimize the immune response variables to study as correlates
- Case-control study (2011)
  - Assessed the selected immune response variables as correlates of HIV infection risk

Pilot Studies: Criteria for Advancing Assays to the Case-Control Study

Criterion	
1. Measures a unique immunological function (not highly correlated with other assays)	✓
2. Low false positive rate (judged in placebo recipients and pre-immunization responses of vaccinees)	✓
3. Vaccine-induced responses with broad variability	✓
4. Relatively low noise (e.g., high reproducibility on replicate samples)	✓
5. Relatively low specimen volume requirement	✓
6. Previously supported as a correlate of infection in the North American VaxGen trial of AIDSVAX	✓

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Summary of Outcome of Pilot Studies

- Evaluated assays from 47 proposals & 20 immunology labs
- 17 assay types passed criteria, and performed on case-control samples
- 6 "best performing" immune variables covering 6 immunological classes selected for primary analysis
- 152 other qualifying immune variables assessed in secondary and exploratory analyses

Down-Selected Primary Immune Variables (n=6)

Primary Variable	Principal Investigator
• Plasma IgA binding (14 Envelope panel)	Georgia Tomaras
• IgG avidity score to A244 gp120	Munir Alam
• Antibody-dependent cellular cytotoxicity (ADCC)-AE-92TH023. HIV infected CD4 T cells	David Evans Michael Alpert
• Neutralization of Tier 1 viruses (6 Envelope panel)	David Montefiori Rungpeung Sutthent Chitraporn Karnasutra
• IgG binding to scaffolded gp70-V1V2*	Susan Zolla-Pazner
• CD4 T cell intracytoplasmic cytokines (IFNγ, IL-2, TNFα, CD154) stimulated by AE-92TH023 peptides	Julie McElrath

*gp70-V1V2 from Abe Pinter (1998, *Vaccine*); gp70 from murine leukemia virus

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152 Secondary Immune Variables Assessed from 17 Assay Types

Assay Type	Investigators
• gp120 V2 Env Binding	N. Karasavva (AFRIMS), M. Rao (USMHRP), G. Tomaras(Duke), S. Zolla-Pazner (NYU), P. Berman (UCSC),
• gp120 V3 Env Binding	S. Zolla-Pazner (NYU)
• HIV-1 Neutralization	R. Sutthent (Siriraj Hsptl), C Karnasuta (AFRIMS), D. Montefiori (Duke)
• CD4 Induced Epitope Ab Env Binding	G. Lewis (UMD)
• IgA Env Binding-Luminex	G. Tomaras (Duke Univ.)
• IgG Env Binding-Luminex	G. Tomaras (Duke Univ.)
• IgG Avidity	S. M. Alam (Duke Univ.)
• Overlapping Peptide Microarray	D. Montefiori (Duke Univ.), R. Koup (VRC/NIH)
• Blocking of CD4 Binding to Env	B. Haynes (Duke Univ.), P. Berman (UCSC)
• Blocking of MAb A32	A. DeVico (UMD), B. Haynes (Duke Univ.)
• ADCC	G. Ferrari (Duke Univ.)
• IgG3 Env Binding	G. Tomaras (Duke Univ.)
• Env-specific CD4 T Cell ICS	J. McElrath (FHCRC)
• Env-stimulated PBMC Luminex	J. McElrath (FHCRC)
• Env Stimulated CFSE	J. McElrath (FHCRC)
• Env Stimulated B Cell ELISpot	J. McElrath (FHCRC)
• NK cell phenotyping	J. McElrath (FHCRC)

Case-Control/2-Phase Design Study

- **Question (Correlates of Risk)**: What immunologic measurements 2 weeks after the immunizations predict whether vaccinees subsequently become HIV infected over 3 years follow-up?
- Addressed by measuring responses from:
  - 41 infected vaccinees (all available)
  - 205 uninfected vaccinees (stratified random sample)
  - 40 placebo recipients (simple random sample)
- Balanced random sampling for vaccinees: 5:1 ratio within covariate strata:
  Gender × Number of vaccinations × Per-protocol status

Analysis Approach

- Statistical analysis plan (SAP) finalized before conducting the primary analysis
  - SAP is openly available
- Each immune variable definition finalized before unblinding the data
  - Primary data-set set in stone and then the analysis was carried out
- Primary results validated by an independent statistical team (Emmes)

Analysis Approach

- Primary analysis code vetted on practice data and applied in an automated way to the real data without post-hoc adjustment
- Lab work separated from statistical analysis work
- Centralized statistical analysis ensured uniform treatment of all evaluated immune assays

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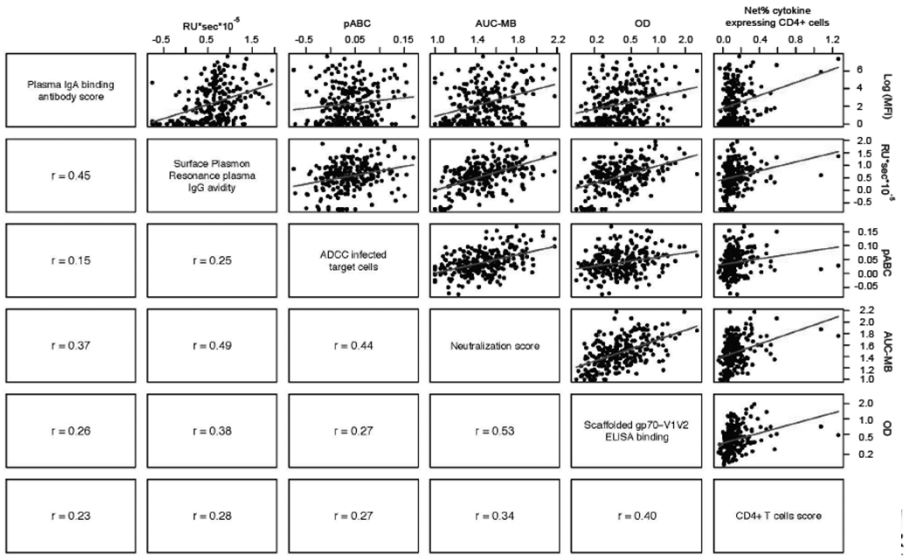
Statistical Assessment of Week 26 Immune Biomarkers as Correlates of Risk

- Two regression models that accounted for the 2-phase sampling design
  - Logistic regression full maximum likelihood*
  - Cox proportional hazards partial likelihood § (yielded ~ the same results)
- Confounding control
  - Adjust for gender, baseline behavioral risk (low, medium, high)
  - Evaluate the 6 primary variables together in multivariate models, and as single variables

* Breslow and Holubkov (1997, *Biometrika*)

§ Borgan et al. estimator II (2000, *Lifetime Data Analysis*)

Pairwise Scatterplots of the 6 Primary Variables (Effectively Chose Only Weakly-Correlated Variables)



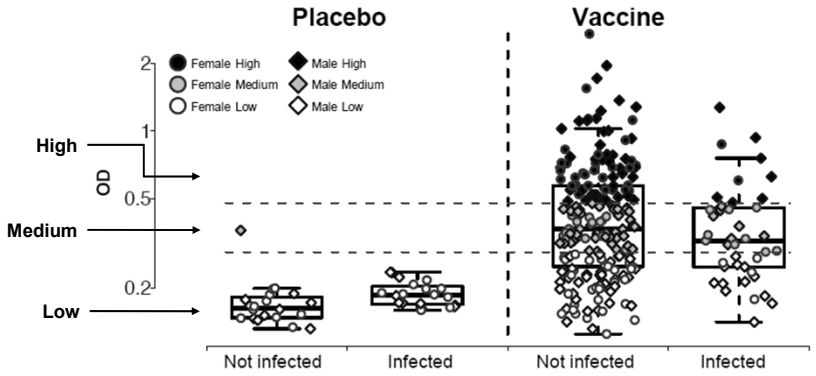
Multivariate Logistic Regression:

Quantitative Variables

Variable	Relative risk per SD	P-value	Q-value
IgA Binding to Envelope Panel	**1.54**	**0.027**	**0.08**
IgG Avidity A244 gp120	0.81	0.37	0.56
ADCC AE.HIV-1 Infected CD4 Cells	0.92	0.68	0.68
Tier 1 Neutralizing Antibodies	1.37	0.22	0.45
IgG Binding to gp70-V1V2	**0.57**	**0.015**	**0.08**
CD4+ T Cell Intracellular Cytokines	1.09	0.61	0.68

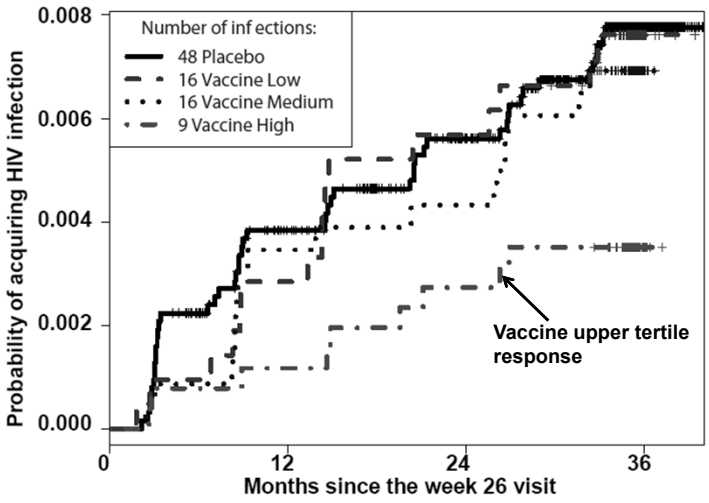
- All 6 variables together in multivariate analysis: p=0.08
- The 2 correlates in multivariate analysis: p=0.01

Binding Antibodies to Scaffolded gp70-V1V2 (ELISA)



	Low	Med	High
Vaccine not Infected	32.2%	32.2%	35.6%
Vaccine Infected	39.0%	39.0%	22.0%

Cumulative HIV Infection Rates by gp70-V1V2 Response Tertiles



Estimated Relative Risk High vs. Low Response = 0.29

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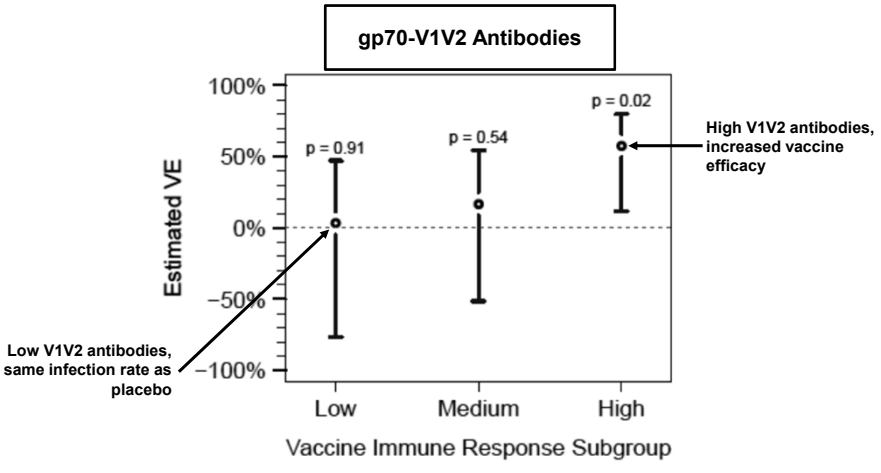
Estimated VE by gp70-V1V2 Response Tertiles



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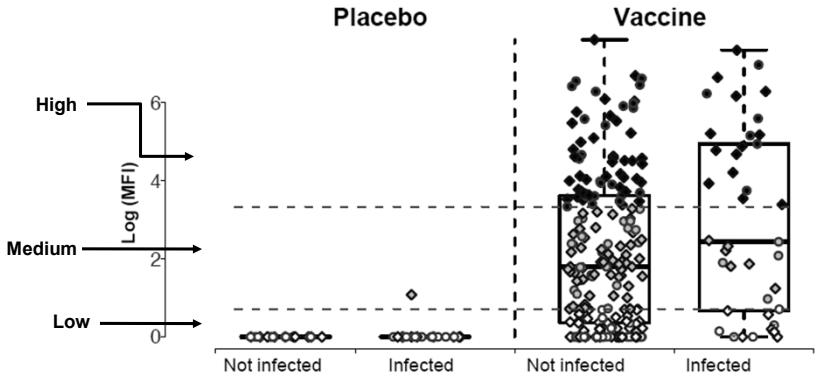
Plasma IgA Binding To Envelope Panel



	Low	Med	High
Vaccine not Infected	33.7%	36.1%	30.2%
Vaccine Infected	29.3%	24.4%	46.3%

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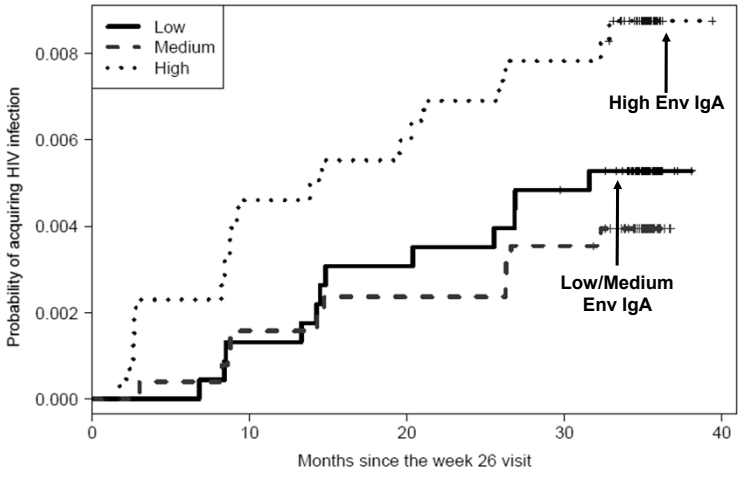
Cumulative Infection Rates with IgA Env Binding Assay



Estimated Relative Risk High vs. Low = 1.89

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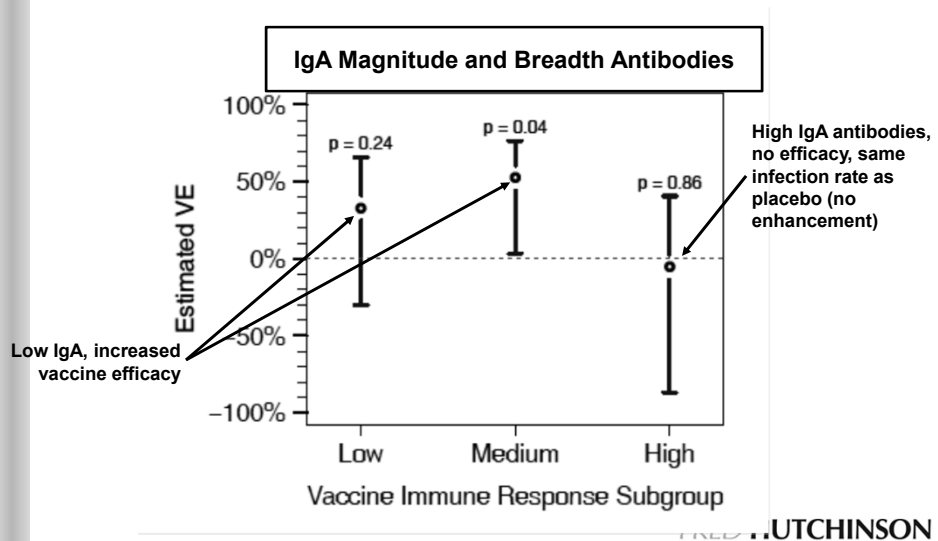
Vaccine Efficacy for Vaccine Subgroups:

By IgA Env Response



These Results Generate Two Hypotheses About Potential CoPs

- Vaccinees with high plasma IgG gp70-V1V2 antibodies received protection from vaccination, whereas those with low responses received no protection
- Vaccinees with low plasma IgA binding responses to envelopes received protection from vaccination, whereas those with high responses received no protection

(Note: These CoP hypotheses are in the language of statistical prediction, not mechanism)

These Results Generate Two Hypotheses About Potential CoPs

- Vaccinees with high plasma IgG gp70-V1V2 antibodies received protection from vaccination, whereas those with low responses received no protection
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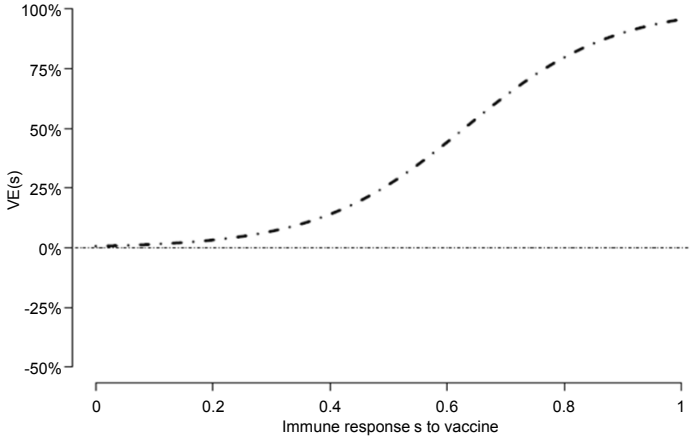
(Note: These CoP hypotheses are in the language of statistical prediction, not mechanism)

How to Interpret the gp70-V1V2 Correlate Result?

What it Could Mean

Most Useful for Vaccine Development

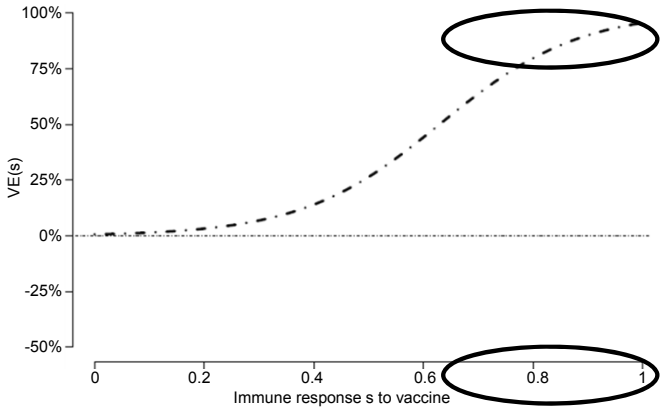
- The following scenario is possible



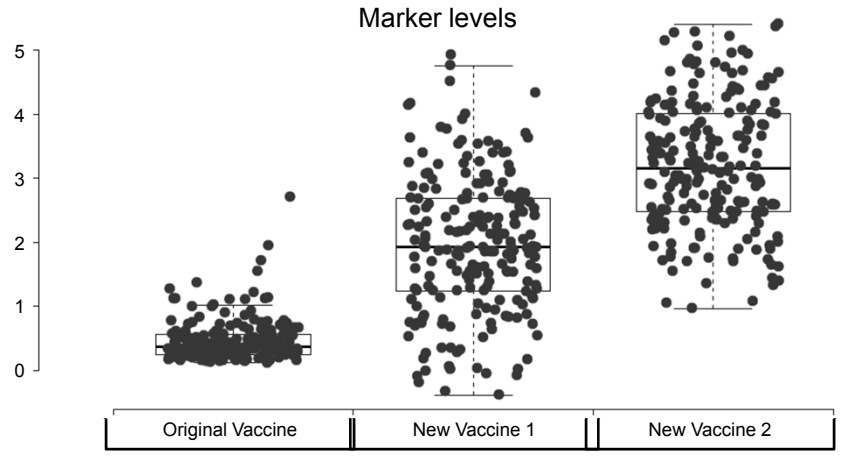
- Would indicate an excellent CoP

This Would Set a Target for Improving the Prime-Boost Regimen

- **Target:** Improve the vaccine regimen by increasing the percentage of vaccinees with high V1V2 antibody responses



Using a CoP for Improving the Vaccine Regimen



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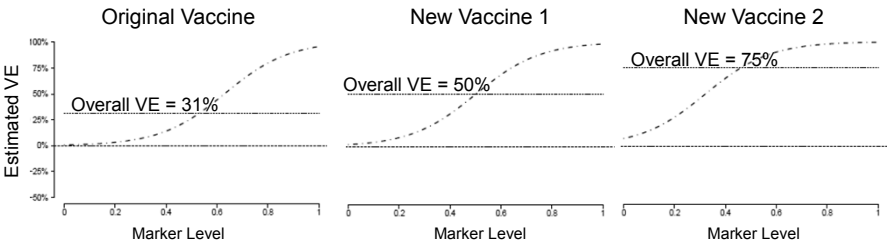
Using a CoP for Improving the Vaccine Regimen

- Suppose each new vaccine is tested in an efficacy trial
- Under a bridging hypothesis we expect the following efficacy results:



- Idealized model for using a CoP to iteratively improve a vaccine regimen

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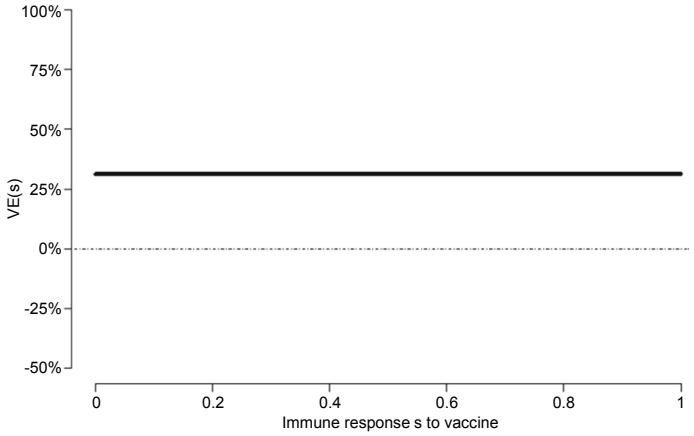
However, a Correlate of Risk (CoR) may not Predict VE (i.e. CoR ≠ CoP)

- The following scenario is also possible



Two Ways the CoR Could Fail to be a CoP

- **Potential Failure 1:** Vaccinees with highest gp70-V1V2 antibody levels had the least amount of HIV exposure, and the regression model inadequately controlled for exposure
- **Potential Failure 2:** Vaccinees with highest gp70-V1V2 antibody levels had a host factor that conferred natural biological resistance to HIV acquisition, but this factor did not affect VE

Strategies to Assess CoRs as CoPs and as Mechanistic CoPs

- Reproduce the results by re-running the assay on the case samples and on new control samples
- Collect the requisite data for correcting the CoR analysis for potential exposure confounding
- Collect the requisite data for directly assessing the utility of the CoR as a CoP
- Conduct sieve analysis of HIV sequences to assess whether the vaccine applied pressure on the HIV Env target(s) specific to the immune correlate
- Design follow-up efficacy trials to test the generated hypotheses
- Collaborate with basic scientists, such that the statistical results lead to the design of experiments to test the generated hypotheses

Strategies to Assess CoRs as CoPs and as Mechanistic CoPs

Approaches beyond clinical efficacy trials are needed

- Basic science:
  - Understand specificity/functionality of the immune response biomarkers
  - Understand all the effects of vaccination and the exposure-infection process
- Laboratory validation studies:
  - Understand measurement/variability characteristics of biomarkers
- Causal manipulation studies in animal trials
  - E.g., repeated low-dose challenge studies comparing vaccine regimens with and without induction of the immune response biomarker
  - Passive biomarker (e.g., gp70-V1V2 antibody) transfer repeated low-dose challenge studies in macaques
    - Use R5 SHIVs derived from RV144 breakthrough infections

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Four Frameworks for Surrogate Endpoints (Joffe and Greene, 2008, *Biometrics*)

- **Causal-effects paradigm**

  "for a good surrogate, the effect of treatment on the surrogate, combined with the effect of the surrogate on the clinical outcome, allow prediction of the effect of treatment on the clinical outcome"

  1. Prentice/statistical surrogate — *Valid replacement endpoint*
  2. Controlled natural direct and indirect effects — *Mediation*

- **Causal-association paradigm**

  "for a good surrogate, the effect of treatment on the surrogate is associated with its effect on the clinical outcome"

  3. Principal stratification — *Association of individual-level treatment effects*
  4. Meta-analysis — *Association of group-level treatment effects*

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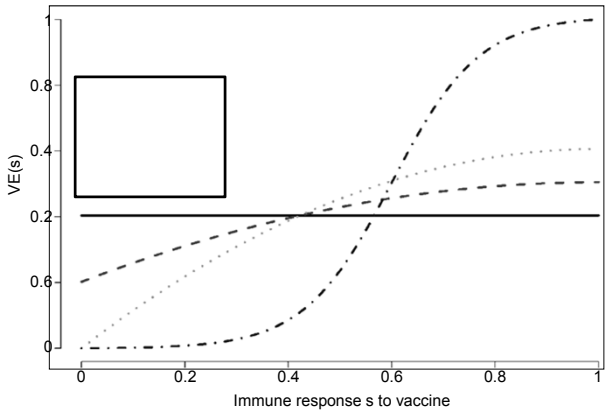
Principal Stratification Approach

- Goal is estimation and inference about the 'vaccine efficacy curve'



Black marker: worthless as surrogate

Green and blue markers satisfy average causal necessity

Blue marker: very good surrogate

Definition of a Good CoP (Principal Stratification Framework*)

- Define the vaccine efficacy curve as

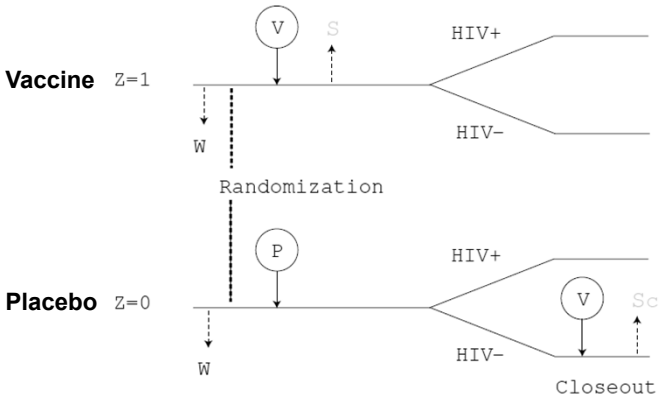
$$VE(s) = 1 - \frac{\text{Risk of HIV infection for vaccinees for subgroup with marker } s}{\text{Risk of HIV infection for placebos for subgroup with marker } s}$$

- **Interpretation:** Percent reduction in infection risk for a vaccinated subject with markers **s** compared to if s/he had not been vaccinated
- **Definition:** A good CoP is a marker with large variability of VE(s) in s

*Proposed by Frangakis and Rubin (2002, *Biometrics*)

Challenge to Estimating VE(s): Missing Data on Vaccine-Induced Response of Placebo Recipients

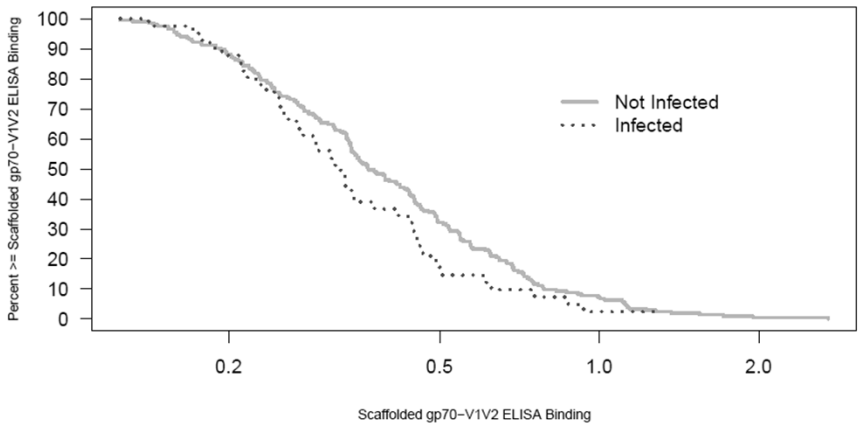
- Follmann (2006, Biometrics) proposed two techniques:
  - BIP = baseline immunogenicity predictors
  - CPV = close-out placebo vaccination



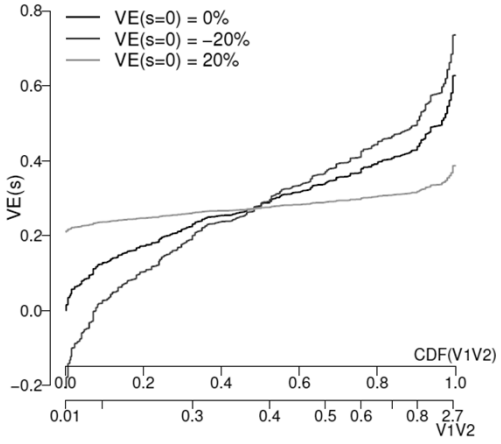
Literature on Statistical Methods for Estimating the VE Curve via BIP and/or CPV

Article	Remarks
1. Follmann (2006, Biometrics)	Binary outcome; BIP&CPV; Estimated likelihood
2. Gilbert and Hudgens (2008, Biometrics)	Binary outcome; BIP; Estimated likelihood; 2-phase sampling
3. Qin, Gilbert, Follmann, Li (2008, Ann Appl Stats)	Time-to-event outcome (Cox model); BIP&CPV; Estimated likelihood; 2-phase sampling
4. Wolfson and Gilbert (2010, Biometrics)	Binary outcome; BIP&CPV; Estimated likelihood; 2-phase sampling; relaxed assumptions
5. Huang and Gilbert (2011, Biometrics)	Binary outcome; BIP&CPV; Estimated likelihood; 2-phase sampling; relaxed assumptions; compare markers
6. Huang, Gilbert, Wolfson (2013)	Binary outcome; BIP&CPV; Pseudolikelihood; 2-phase sampling; relaxed assumptions; marker sampling design
7. Miao, Li, Gilbert, Chan (2013)	Time-to-event outcome (Cox model); BIP; Estimated likelihood with multiple imputation; 2-phase sampling
8. Gabriel and Gilbert (2013, submitted)	Time-to-event outcome (Weibull model); BIP+CPV; Estimated likelihood and pseudolikelihood; 2-phase sampling; threshold models

Reverse Cumulative Distribution Curves



Application to the Thai Trial: gp70-V1V2 Antibodies (Without BIP or CPV)*



*Analysis by Dr. Ying Huang at the Fred Hutchinson Center

- Curve estimated using probit risk models $P(Y=1|Z,S) = \Phi(\beta + \beta Z + \beta S + \beta ZS)$ treating β as a fixed sensitivity parameter

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Sieve Analysis

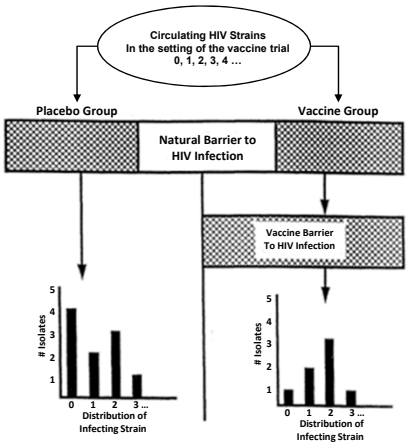

Figure 1 from Gilbert, Self, Ashby (1998, *Biometrics*)

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Sieve Analysis for Helping Interpret the V1V2 Antibody CoR

- The correlates analysis showed V1V2 antibodies predicted infection in the **vaccine group only**
- In contrast, sieve analysis examines evidence for a difference in the sequences of viruses infecting **vaccine vs. placebo** recipients
  - Observed differences attributable to the vaccine (it's a randomized trial)
  - Detection of a 'sieve effect' may suggest that the vaccine blocks infection with some types of exposing HIVs
  - In particular, if a sieve effect is detected in regions of V1V2 to which the RV144 vaccine directed antibodies, it may suggest these antibodies had a role in protection (as a CoP and as a mechanistic CoP)

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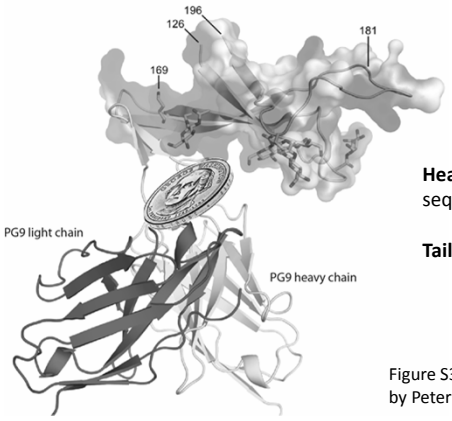
Sieve Analysis an Integral Part of Immune Correlates Assessment: Other Side of the Same Coin



Heads: Vaccine-induced responses to specific HIV sequences

Tails: HIV sequences in infected subjects

Figure S3 from Rolland, Edlefsen et al. (2012, *Nature*) constructed by Peter Kwong's Group at the Vaccine Research Center NIH

- If certain epitope-specific responses block HIV infection, then expect to see a relative absence of these epitope sequences in infected vaccinees vs. infected placebos

Statistical Approach to Sieve Analysis

- Local sieve analysis (high-dimensional)
  - Assess Env amino acid (AA) sites as '**signature sites**'
    - **Signature** = site with different distribution of residues vaccine vs. placebo relative to a vaccine-insert-residue*
  - Assess immunologically relevant sets of Env AA sites as '**signature sets**'
- Global sieve analysis (low-dimensional)
  - Assess if and how VE depends on the distance of the exposing virus to a vaccine-insert-sequence
  - Distance of a breakthrough HIV summarized by 1−3 numbers

*3 vaccine-insert-sequences: ALVAC-AE.92TH023, rgp120-AE.CM244, rgp120-B.MN

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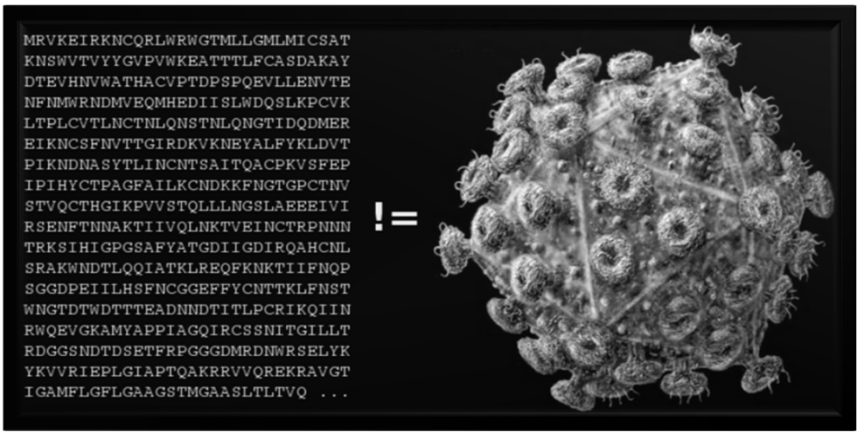
Caveat: AA Sequences ≠ Conformational Structure

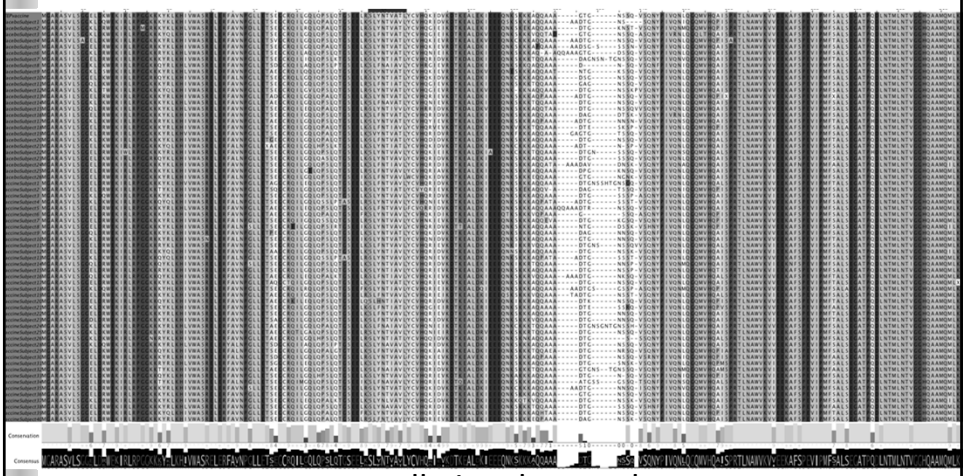


Local Sieve Analysis (Site Scanning)

V3 loop amino acid sequence of reference GNE8 strain		...TRPNNNTRRSIHIG-PGR-AFYATGEIIGDIRQ...
Vaccine group V3 loop sequences	1.	...TRPNNNTRRRIHLG-PGR-AFYATG-IIGDIRQ...
	2.	...TRPNNNTRKGIHIG-PGR-AFYATGEIIGNIRQ...
	.	
	.	
	.	
	217.	...TRPSNNTRKGIHIG-PGR-AFYATEEITGDIRQ...
Placebo group V3 loop sequences	1.	...TRPNNNTRTGVHLG-PGR-VWYATGDIIGDIRQ...
	2.	...TRPNNNTRRSIHIQ-PGR-AFYAT-DIIGDIRK...
	.	
	.	
	.	
	119.	...TRPNNNTISKIRIR-PGRGSFYATNNIIGDIRQ...

Gilbert, Wu, Jobes (2008, *Biometrics*)

Looking for Sequence Differences: Vaccine vs. Placebo



… a needle in a haystack …

Focus the Sieve Analysis on Statistically x Biologically Relevant AA Sites

- To maximize power, pre-filter sites based on treatment-blinded data
  - Exclude difficult-to-align sites and too-conserved sites
  - Restrict analysis to the 85 V1V2 AAs constituting the gp70-V1V2 reagent
  - Restrict analysis to sites potentially part of reactive antibody epitopes

3 types of biological input on 'antibody important' sites
- Env reactivity hotspots of RV144 vaccine-induced binding antibodies (David Montefiori *et al.*)
- Published monoclonal antibody-gp120 contact sites (Peter Kwong *et al.*)
- Potential antibody epitopes based on structural biology (Bill Schief *et al.*)

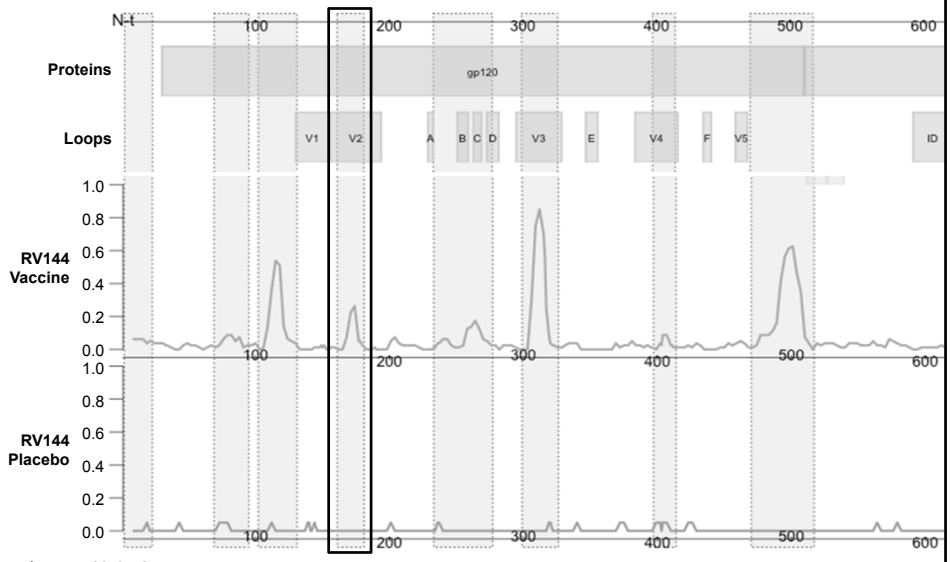
- Rolland, Edlefsen et al. (2012, *Nature)* focused on the sites meeting all of the above criteria (n=9 Env V2 sites)

Env Binding Reactivity Hotspots Measured with Linear Peptide Microarrays*

- Montefiori et al. measured binding to 1453 linear peptides tiling Env (almost all 15-mers)
- Peptides from 7 HIV-1 subtypes
- Identified 4 reactivity hotspots spanning multiple peptides
- For each hotspot, an immune variable was defined as the average of the normalized intensities for all peptides on the array centered on the **hotspot-region summit**, and evaluated as a CoR

*Analysis led by Raphael Gottardo

Linear Peptide Microarray Analysis of Env: Identified 4 Binding Reactivity Hotspots*



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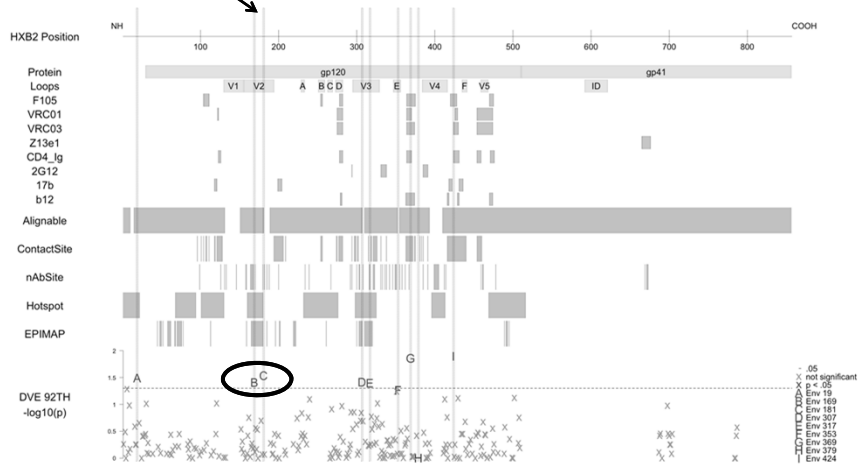
*Karasavvas et al. (2012, *AIDS Res Hum Retroviruses*); Raphael Gottardo

Results of Sieve Analysis (Env)

Focused sieve analysis



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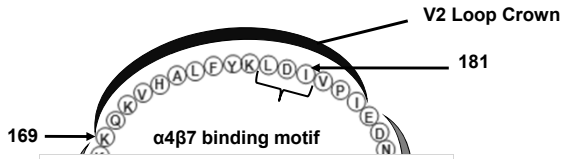
Results of Focused Sieve Analysis*

- 2 sites with evidence (q-value < 0.2) of a different rate of AA mismatch to the insert residue: Sites 169, 181



*Rolland, Edlefsen et al. (2012, *Nature)*

Vaccine Efficacy by HIV Genotype (Defined by Site 169, 181)

HIV-1 Genotype	Number AE Infections	Estimated VE*	95% CI	P-value
169 match	87	48%	18% to 66%	0.0036
169 mismatch	23	-55%	-258% to 33%	0.30
181 match	88	17%	-26% to 45%	0.38
181 mismatch	22	78%	35% to 93%	0.0028

*Estimated with a Cox model (Prentice et al., 1978, *Biometrics*)

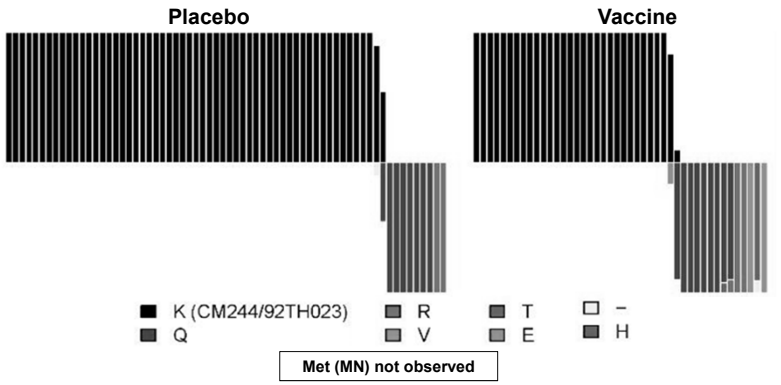
- VE greater against 169-matched than mismatched HIV-1: p = 0.034**
- VE greater against 181-mismatched than matched HIV-1: p = 0.024**

**Differential VE assessed with a re-coded Cox model (Lunn and McNeil, 1995, *Biometrics*)

Position 169

MMBootstrap: p =.044, q =.199
Gilbert, Wu, Jobes: p =.019, q =.087

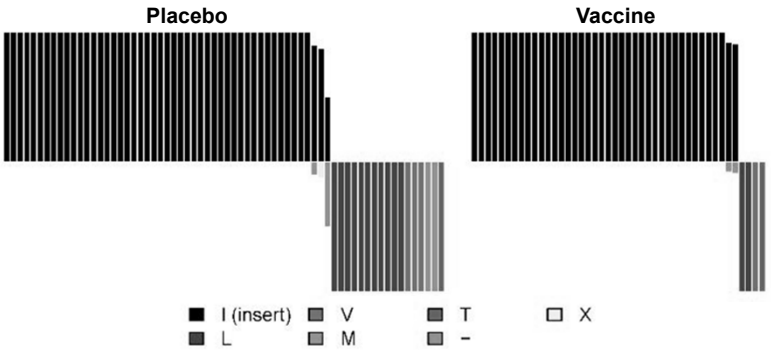
Model-Based Sieve:
P(sieve|data) = .67, p =.016, q = .1288



Key:
Each subject is represented by a bar
Bars all have equal height. Insert AA residue, in black, is shown above the midline
Within a bar, colors depict the fraction of the subject's sequences with that AA residue

Position 181

MMBootstrap: p=.039, q =.199
Gilbert, Wu, Jobes: p=.022, q =.087



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Frequencies of Infection with HIV-1 Genotypes Defined by V2 Sites 169 & 181*

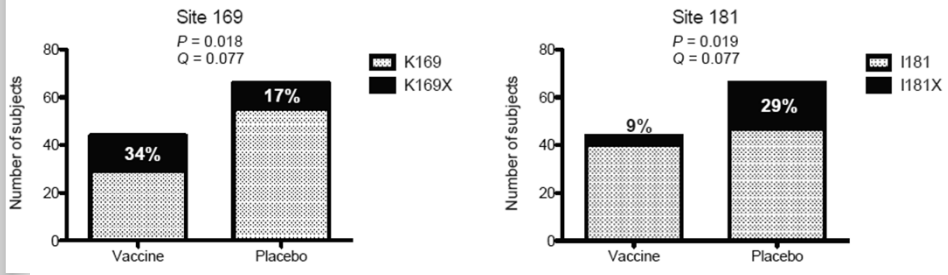

*Figure 2 from Rolland, Edlefsen et al. (2012, *Nature*)

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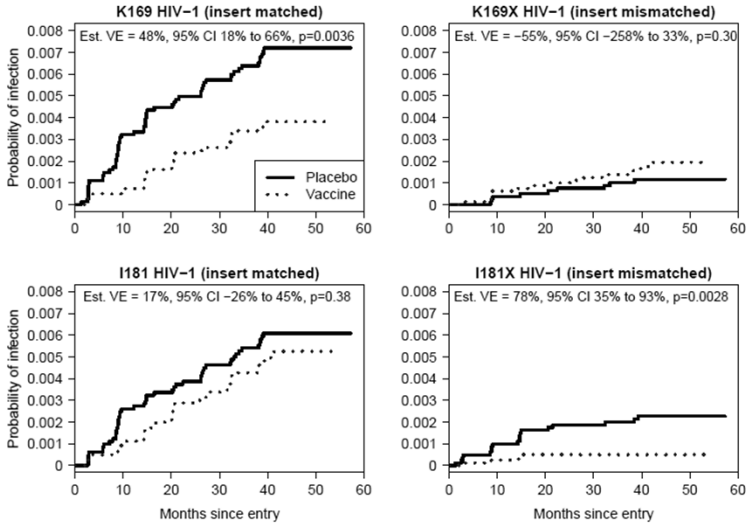
Cumulative Incidences of Infection with HIV-1 Genotypes Defined by V2 Sites 169 & 181*



*Supplementary Figure 3 from Rolland, Edlefsen et al. (2012, *Nature*)

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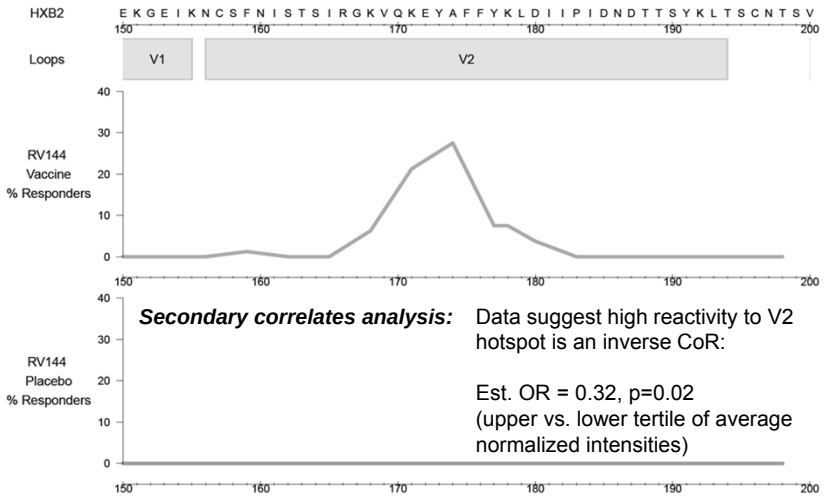
Moreover, Antibodies Binding to the V2 Hotspot* are an Inverse CoR



Secondary correlates analysis: Data suggest high reactivity to V2 hotspot is an inverse CoR:

Est. OR = 0.32, p=0.02
(upper vs. lower tertile of average normalized intensities)

*Karasavvas et al. (2012, *AIDS Res Hum Retroviruses*); Raphael Gottardo

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Interpretation of the Local Sieve Effect at Sites in the V2 Region of Core Vaccine-Induced Antibody Reactivity

- The signature sites discriminating vaccine vs. placebo sequences are in the V2 region of core vaccine-induced antibody reactivity, and this reactivity correlates with a low infection rate
- Confluence of the correlates result and the sieve result support the hypotheses that V2 antibodies are a CoP and a mechanistic CoP better than either of these results alone
- Thus, the sieve results provide a measure of independent corroboration of the hypothesis that V2 antibodies have a role in protection (as a CoP and possibly as a mechanistic CoP)

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Functional Questions (Liao, Haynes et al., 2013, *Immunity*)

- What are the binding sites of V2-directed antibodies induced by the vaccine?
  - E.g., epitope mapping via alanine scanning
- What are the candidate antibody effector functions that could mediate protection?
- What conformations of V2 can the vaccine-induced antibodies recognize?

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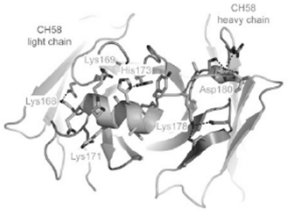
Functional Data Indicating Immune Pressure on Site 169 and Other V2 Hotspot Sites (Liao, Haynes et al., 2013, *Immunity*)

Cell PRESS

Immunity
Article
Vol. 38, 176–186
January 24, 2013



Vaccine Induction of Antibodies against a Structurally Heterogeneous Site of Immune Pressure within HIV-1 Envelope Protein Variable Regions 1 and 2

Hua-Xin Liao,[1,15,*] Mattia Bonsignori,[1,15] S. Munir Alam,[1,15] Jason S. McLellan,[2,15] Georgia D. Tomaras,[1] M. Anthony Moody,[1] Daniel M. Kozink,[1] Kwan-Ki Hwang,[1] Xi Chen,[1] Chun-Yen Tsao,[1] Pinghuang Liu,[1] Xiaozhi Lu,[1] Robert J. Parks,[1] David C. Montefiori,[1] Guido Ferrari,[1] Justin Pollara,[1] Mangala Rao,[3] Kristina K. Peachman,[3] Sampa Santra,[4] Norman L. Letvin,[4] Nicos Karasavvas,[5] Zhi-Yong Yang,[2] Kaifan Dai,[2] Marie Pancera,[2] Jason Gorman,[2] Kevin Wiehe,[1] Nathan I. Nicely,[1] Supachai Rerks-Ngarm,[6] Sorachai Nitayaphan,[5] Jaranit Kaewkungwal,[7] Punnee Pitisuttithum,[8] James Tartaglia,[9] Faruk Sinangil,[10] Jerome H. Kim,[3] Nelson L. Michael,[3] Thomas B. Kepler,[11] Peter D. Kwong,[2] John R. Mascola,[2] Gary J. Nabel,[2] Abraham Pinter,[12] Susan Zolla-Pazner,[13,14] and Barton F. Haynes[1,*]

- **Approach:** Probed the specificities and effector functions of 4 mAbs isolated from RV144 vaccine recipients that recognize Env V2, and determined the crystal structures of 2 of these (CH58, CH59) with V2 peptides containing site 169
- **Results:** Showed that the V2 mAbs mediated the effector function ADCC against RV144 trial breakthrough Env-target cells, and this ADCC activity was dependent on site 169
- **Interpretation:** These data directly demonstrate the plausibility of these types of V2 antibodies to mediate immune pressure targeted at site 169 of Env V2

Functional Data Indicating Immune Pressure at Site 169 and Other V2 Hotspot Sites

- The 4 mAbs also neutralized lab-adapted (tier 1) HIV-1 strains
- Mutations at 169 and other V2 hotspot sites are associated with significant alterations in neutralization by mAbs including PG9, PG16, CH01-04, PGT141-145, CH58, CH59*
- **Conclusion:** The potential mechanisms of antibody-mediated immune pressure at site 169 include:
  1. Virus neutralization of susceptible CRF01_AE HIV-1 strains (not likely)
  2. Binding HIV-1-infected CD4+ T cells and mediation of ADCC
  3. Other as yet undefined effector mechanisms

*Tomaras et al. (2011, *J Virology)*; Moore et al. (2011, *J Virology)*

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Global Sieve Analysis

- Summarize the distance, V, between an infected subject's set of sampled sequences and a vaccine insert sequence
- Assess mark-specific vaccine efficacy, VE(t,v)

  VE(t,v) = 1 – hazard ratio (vaccine/placebo) of infection at time t with a virus of distance v

- Statistical methods* for estimation of VE(t,v) and testing of the null hypotheses:

  H_{00}: VE(t,v) = 0 Assess any VE at all

  H_0: VE(t,v) = VE(t) Assess differential VE (a 'sieve effect')

*Gilbert, McKeague, and Sun (2008, *Biostatistics*), Sun, Gilbert, and McKeague (2009, *Ann Stat*), Sun and Gilbert (2012, *Statistica Sinica*), Sun, Li, and Gilbert (2013, *Biostatistics*), Juraska and Gilbert (2013, *Biometrics*)

Global Sieve Analysis

- Global sieve analyses were conducted for RV144, for a set of weighted Hamming distances V
  - To each of the 3 vaccine reference sequences
  - For the whole gp120 protein and for the V1V2 protein on the gp70-V1V2 reagent
  - Using the PAM-25 and Blosum-90 amino acid substitution matrices
  - Restricting to antibody-relevant sets of sites using the same screens as for the local sieve analysis

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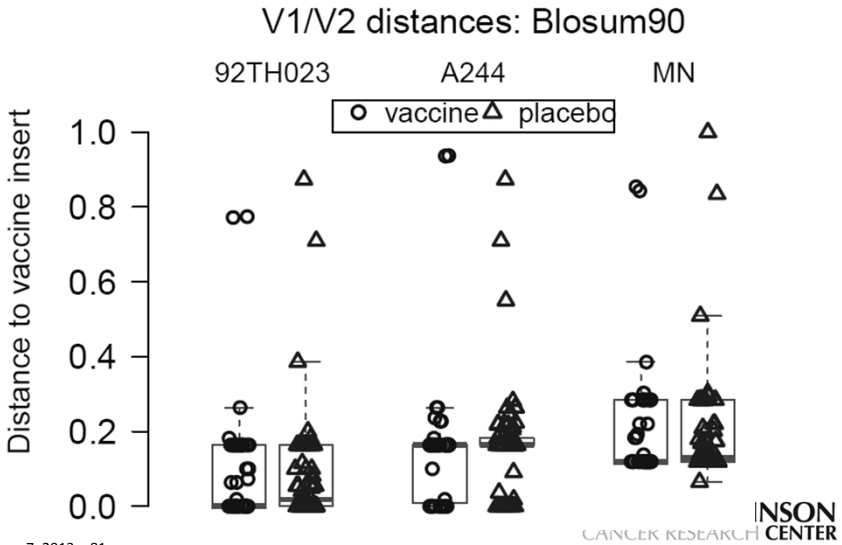
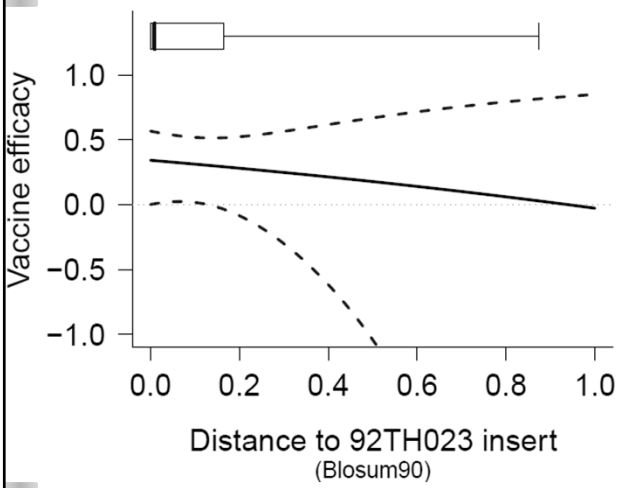
Global Sieve Analysis: Blosum90 Distances (Mab-gp120 Contact Sites) V1V2



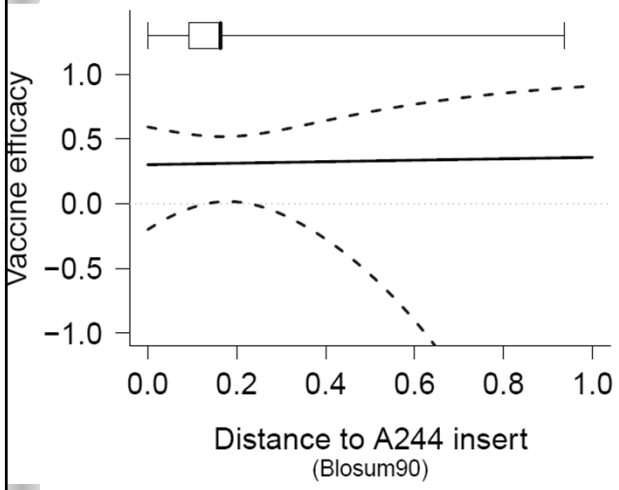
Estimated VE(t,v) Under Mark-Specific Proportional Hazards Model V1V2



Null hypothesis	p-value
$VE(v) = 0$	0.018^a
$VE(v) = VE$	0.68^b

[a] weighted Wald test
[b] likelihood ratio test

Estimated VE(t,v) Under Mark-Specific Proportional Hazards Model V1V2



Null hypothesis	p-value
$VE(v) = 0$	0.022^a
$VE(v) = VE$	0.94^b

[a] weighted Wald test
[b] likelihood ratio test

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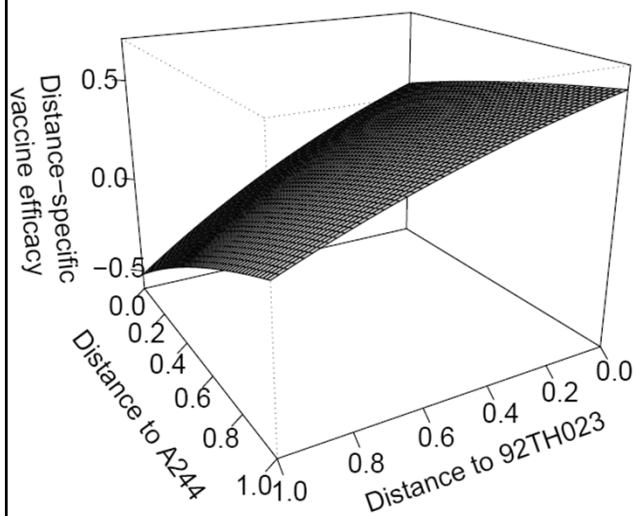
Estimated VE(t,v) Under Mark-Specific Proportional Hazards Model V1V2



Null hypothesis	p-value
$VE(v) = 0$	0.020^a
$VE(v) = VE$	0.85^b

[a] weighted Wald test
[b] likelihood ratio test

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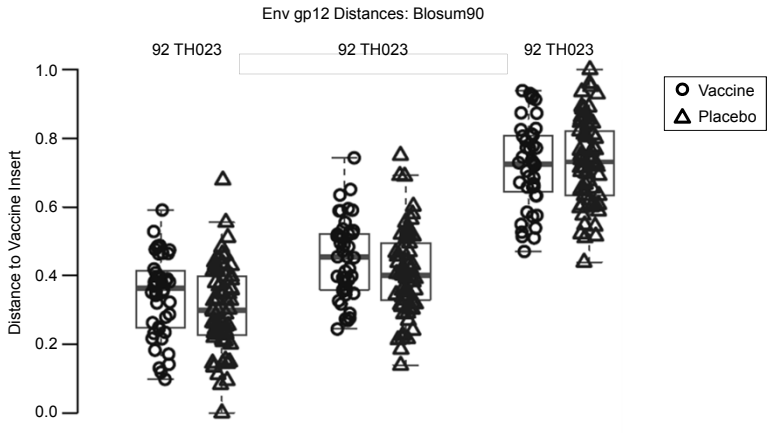
Global Sieve Analysis: Blosum90 Distances (Mab-gp120 Contact Sites) gp120



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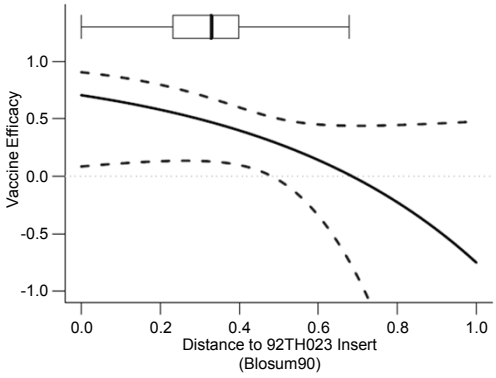
Estimated VE(t,v) Under Mark-Specific Proportional Hazards Model* gp120



Null Hypothesis	*p*-value
VE(v) = 0	0.011[a]
VE(v) =VE	0.10[b]

[a]weighted Wald test
[b]likelihood ratio test

*Juraska and Gilbert (2013, *Biostatistics*)

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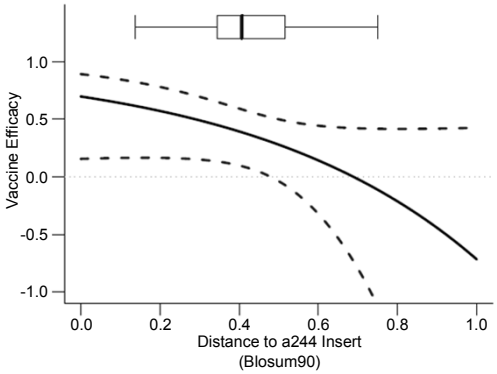
Estimated VE(t,v) Under Mark-Specific Proportional Hazards Model* gp120



Null Hypothesis	*p*-value
VE(v) = 0	0.010[a]
VE(v) = VE	0.085[b]

[a]weighted Wald test
[b]likelihood ratio test

*Juraska and Gilbert (2013, *Biometrics)*

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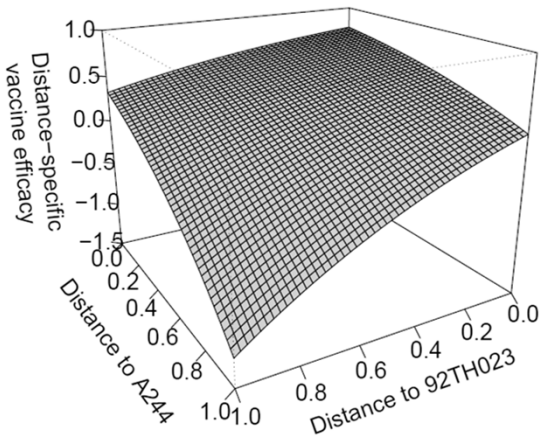
Estimated VE(t,v) Under Mark-Specific Proportional Hazards Model* gp120



Null Hypothesis	*p*-value
VE(v) = 0	0.012[a]
VE(v) = VE	0.17[b]

[a]weighted Wald test
[b]likelihood ratio test

*Michal Juraska (2012, PhD dissertation)

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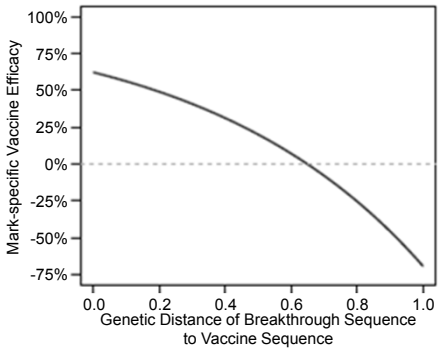
Interpretation of Global Sieve Analysis Results

- Non-significant trend toward decreasing vaccine efficacy against viruses with more mutations in MAb-gp120 contact sites
  - P = 0.10 not compelling
  - But RV144 only has 63% power to detect a large sieve effect:



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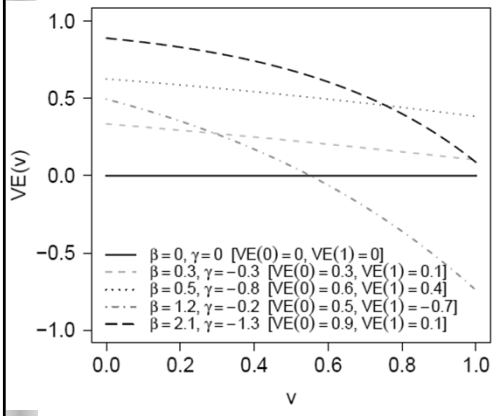
Expanded Calculations by Michal Juraska: Power to Detect Various VE(v) Curves (RV144-Sized Trial and 2 Larger Trials)



Curve	# Inf. Vx	# Inf. Plc	Power*
Green	75	57	0.07
	100	76	0.08
	200	152	0.13
Black	75	57	0.54
	100	76	0.61
	200	152	0.89
Purple	75	57	0.66
	100	76	0.80
	200	152	0.97

*Likelihood ratio test from Juraska and Gilbert (2013)

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Interpretation of Global Sieve Analysis Results

- What is the relative influence of different antibody contact sites on the apparent sieve effect?
  - Driven by certain monoclonal antibodies with certain specificities?
- No evidence of differential vaccine efficacy when restricted to the 19 Mab-gp120 contact sites in the V1V2 region (not shown)

Conclusions

1. A Prime-Boost HIV Vaccine Regimen Can Prevent HIV Infection

- In 2009, some interpreted the Thai trial result as a false positive
- Evidence supporting real VE > 0%
  - The identification of a target-specific immune correlate of risk, combined with a sieve effect in the targeted region and the functional work of Bart Haynes et al.
  - Estimated VE was highest during the period of maximal vaccine-induced immune responses and waned with immune responses
  - Estimated VE was at least as high in the fully immunized/per-protocol cohort compared to the intention-to-treat cohort (when analyzed with a causal method)

2. The Inter-Collaborative Team Approach Was Effective

- Factors aiding the ability to identify immune CoRs and CoPs
  - Pilot studies for down-selecting immune assays and for optimizing immune response biomarkers
  - Centralized and standardized statistical analysis of lab data
- This approach constitutes a model for consideration in other vaccine efficacy trial settings, auspicious when:
  - Samples are stored from key time-points in all trial participants, making it possible to measure immune responses in most cases
  - There are a large number of potential immune response assays to assess as correlates

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3. The Results are Informing the Next HIV Vaccine Efficacy Trials

- The HIV Vaccine Trials Network is planning follow-up efficacy trials of prime-boost vaccine regimens
- Some regimen factors under consideration
  - Choice of vector prime (e.g., ALVAC, NYVAC, Adenovirus)
  - Whether to add DNA to the prime regimen
  - Choice of protein boost, including optimal HIV sequences
  - Choice of adjuvant
  - Add an extra protein boost to improve durability of immune responses
- The future trials will provide tests of whether vaccine regimens with improved V2-directed antibody responses have better VE

4. Future Efficacy Trial Designs Aim to Deepen and Extend Integrated Immune Correlates and Sieve Analyses

- Enhance programs of assay validation/qualification/ optimization and standardized assay comparisons
- Assess VE of multiple vaccine regimens with differing mechanisms of action in the same efficacy trial
- Enhance resolution of HIV infection timing, to control for potential bias in the sieve analysis due to early T cell escape of HIV before the sequences are sampled
- Enhance inter-collaborative nature of statistical sieve analysis, as incorporating immunological information is crucial for maximizing power of the immune correlates/sieve analyses
- These lessons may transport to other vaccine fields

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