The Journal of Infectious Diseases

MAJOR ARTICLE

Modification of the Association Between T-Cell Immune Responses and Human Immunodeficiency Virus Type 1 Infection Risk by Vaccine-Induced Antibody Responses in the HVTN 505 Trial

Youyi Fong,[1,2,a] Xiaoying Shen,[3,4,a] Vicki C. Ashley,[3,4] Aaron Deal,[3,4] Kelly E. Seaton,[3,4] Chenchen Yu,[1] Shannon P. Grant,[1] Guido Ferrari,[3,5,7] Allan C. deCamp,[1] Robert T. Bailer,[8] Richard A. Koup,[8] David Montefiori,[3,5] Barton F. Haynes,[3,4,6] Marcella Sarzotti-Kelsoe,[3,5,6] Barney S. Graham,[8] Lindsay N. Carpp,[1] Scott M. Hammer,[10] Magda Sobieszczyk,[10] Shelly Karuna,[1] Edith Swann,[9] Edwin DeJesus,[11] Mark Mulligan,[12] Ian Frank,[13] Susan Buchbinder,[14] Richard M. Novak,[15] M. Juliana McElrath,[1] Spyros Kalams,[16] Michael Keefer,[17] Nicole A. Frahm,[1] Holly E. Janes,[1,2] Peter B. Gilbert,[1,2] and Georgia D. Tomaras[3,5,6,7]

[1]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center and [2]Department of Biostatistics, University of Washington, Seattle; [3]Duke Human Vaccine Institute, [4]Department of Medicine, [5]Department of Surgery, [6]Department of Immunology, [7]Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina; [8]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [9]Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [10]Division of Infectious Diseases, Department of Medicine, Columbia University, New York, New York; [11]Orlando Immunology Center, Florida; [12]Department of Medicine, Emory University School of Medicine, Atlanta, Georgia; [13]School of Medicine, University of Pennsylvania, Philadelphia; [14]Departments of Medicine, Epidemiology and Biostatistics, University of California, San Francisco; [15]Division of Infectious Diseases, University of Illinois at Chicago; [16]Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, Tennessee; [17]University of Rochester Medical Center, Rochester, New York

Background. HVTN 505 was a human immunodeficiency virus type 1 (HIV-1) preventive vaccine efficacy trial of a DNA/recombinant adenovirus serotype 5 (rAd5) vaccine regimen. We assessed antibody responses measured 1 month after final vaccination (month 7) as correlates of HIV-1 acquisition risk.

Methods. Binding antibody responses were quantified in serum samples from 25 primary endpoint vaccine cases (diagnosed with HIV-1 infection between month 7 and month 24) and 125 randomly sampled frequency-matched vaccine controls (HIV-1 negative at month 24). We prespecified for a primary analysis tier 6 antibody response biomarkers that measure immunoglobulin G (IgG) and immunoglobulin A (IgA) binding to Env proteins and 2 previously assessed T-cell response biomarkers.

Results. Envelope-specific IgG responses were significantly correlated with decreased HIV-1 risk. Moreover, the interaction of IgG responses and Env-specific $CD8^+$ T-cell polyfunctionality score had a highly significant association with HIV-1 risk after adjustment for multiple comparisons.

Conclusions. Vaccinees with higher levels of Env IgG have significantly decreased HIV-1 risk when $CD8^+$ T-cell responses are low. Moreover, vaccinees with high $CD8^+$ T-cell responses generally have low risk, and those with low $CD8^+$ T-cell and low Env antibody responses have high risk. These findings suggest the critical importance of inducing a robust IgG Env response when the $CD8^+$ T-cell response is low.

Keywords. human immunodeficiency virus type 1 (HIV-1); vaccine; correlate of risk; antibody; CD8 T cells.

The development of a safe and efficacious preventative human immunodeficiency virus type 1 (HIV-1) vaccine is hindered by the lack of known correlates of protection (CoPs) against HIV-1 infection. The identification of vaccine-induced immune response biomarkers as CoPs would enable future vaccine trials to evaluate and rank candidate vaccine regimens based on these early biomarker measurements before directly assessing efficacy based on HIV-1 incidence [1–3]. Discovery of correlates of risk (CoRs) of HIV-1 acquisition in vaccinees contributes to the identification of CoPs in vaccine trials [1, 4].

So far, only the RV144 trial of the ALVAC-HIV prime and AIDSVAX B/E boost vaccine regimen has demonstrated protection against HIV-1 acquisition, estimated at 31.2% at month 42 [5] (36 months after last vaccination). Plasma-binding antibodies to the HIV-1 envelope glycoprotein V1V2 loop correlated with decreased risk of HIV-1 infection [6], whereas plasma immunoglobulin A (IgA) to specific HIV-1 envelope glycoproteins directly correlated with HIV-1 risk [6, 7]. The HVTN 505 trial tested the ability of a vaccine regimen comprising DNA HIV-1 Env, Gag, Nef, and Pol primes followed by a single recombinant

Received 19 October 2017; editorial decision 28 December 2017; accepted 6 January 2018; published online January 8, 2018.

[a]Y. F. and X. S. contributed equally to this work. P. B. G. and G. D. T. contributed equally to this work.

Correspondence. G. D. Tomaras, PhD, Duke University Medical Center, Rm 4079 MSRBII, 2 Genome Ct., Durham, NC 27710 (gdt@duke.edu).

The Journal of Infectious Diseases® 2018;217:1280–8

DOI: 10.1093/infdis/jiy008

adenovirus type 5 (rAd5) vector boost carrying trivalent Env and a subtype B Gag-Pol fusion protein to prevent HIV-1 acquisition in circumcised, Ad5-seronegative men and transgendered persons in the United States who have sex with men [8]. The primary efficacy endpoint was HIV-1 infection diagnosed from month 7 to the final visit at month 24 (18 months after last vaccination). Although the final analysis of vaccine efficacy was completed early after an interim analysis established lack of vaccine efficacy, immune response CoRs can be identified for noneffica-cious vaccine regimens [2, 3]. In this context, these CoRs might correspond to markers of intrinsic risk (but the vaccine had no effect on risk for any subgroup) or serve as tools for identifying subgroups with negative and positive vaccine efficacy.

Cellular immune responses in HVTN 505 vaccinees revealed strong inverse correlations between month 7 Env-specific $CD8^+$ immune responses (both magnitude and polyfunctionality) and subsequent infection risk [9]. Additionally, we found that Env-gp120 sequences from HIV-1–infected vaccinees were significantly more distant than those from placebo recipients to the vaccine strain subtype B insert ($P = .01$); k-mer scanning identified sieve effects in monoclonal antibody contact sets for the CD4 binding site and in CD4-induced epitopes [10]. Regarding humoral responses, we found that the HVTN 505 vaccine regimen elicited a weak response to the V1V2 loop [8], consistent with the observation that V1V2 immunoglobulin G (IgG) responses correlated with decreased risk of HIV-1 infection in the RV144 trial and the lack of protection by the DNA/rAd5 vaccine. In contrast, gp41 IgG antibody responses were elicited by the DNA/rAd5 regimen [8], whereas the RV144 vaccine lacked the full length gp41 as part of the immunogen. The DNA/rAd5 regimen also elicited higher antibody responses to a gp41 protein than to gp120 proteins, which may be partially explained by preexisting responses to the microbiota [8, 11]. These data raise a number of hypotheses, including the following: (1) the gp41 dominant antibody response negatively impacted the protective immune response; (2) antibody responses different from those elicited by the RV144 vaccine correlate with infection risk for the DNA/rAd5 vaccine; (3) antibody responses correlate with infection risk dependent upon the strong Env-specific $CD8^+$ T-cell response correlate previously identified; or (4) antibody responses had no relationship with HIV-1 infection risk in HVTN 505. Here we directly evaluated whether vaccine-elicited humoral immune responses correlated with HIV-1 risk in HVTN 505.

METHODS

HVTN 505 Trial

All participants in HVTN 505 (ClinicalTrials.gov ID: NCT00865566) provided written informed consent [8]. The vaccine regimen is detailed in the Supplementary Material.

Pilot Study of Immune Responses to Vaccination

In the pilot study, immune responses to vaccination were assessed 4 weeks after final vaccination (month 7) in 40 vaccinees and 10 placebo recipients as controls to discern HIV-1–specific vaccine-induced responses. Humoral assays were also performed on baseline/preimmunization samples (Supplementary Material).

Laboratory Methods

HIV-1 envelope binding IgG and IgA were determined by binding antibody multiplex assay [6, 7, 12, 13], linear HIV-1 envelope IgG responses were determined by peptide microarray [14–16], and antibody-dependent cellular cytotoxicity (ADCC) assays and neutralization assays were performed [6].

Statistical Methods

Immune response variables were tested as correlates of HIV-1 infection through month 24 using logistic regression methods [17] (osDesign R package, Comprehensive Archive R Network) based on data collected prior to study unblinding on 22 April 2013. Statistical significance was evaluated by pseudo likelihood and sandwich variance estimates. Variables were mean centered and standard deviation scaled (based on vaccinees). All models included the following baseline covariates to control for HIV exposure: participant age, race (white vs black vs Hispanic/other), body mass index, and a behavioral risk score [8]. *P* values were adjusted either controlling for false discovery rate [18] or family-wise error rate (FWER) [19]. Supplementary Material contains details on the optimization of the primary tier immune response biomarkers.

RESULTS

DNA/rAd5 Immunogenicity

We performed an immunogenicity study on a pilot set of vaccinee samples to evaluate binding antibodies (IgG, IgG3, IgA), linear IgG responses to cross-clade HIV-1 envelope peptides, ADCC, and neutralizing antibody IgG responses (Supplementary Table 1). Env IgG responses were epitope-mapped by peptide microarray (Supplementary Figure 1). Immunoglobulin G responses to C1, C1V1, V3, C4, and C5 in the gp120 region and to linear regions in gp41 were elicited (Supplementary Figure 1). The C-C loop of the immunodominant region was deleted in the vaccine envelope sequences; thus, this vaccine did not elicit linear gp41 responses to this immunodominant region. However, cross-clade responses to the 5' adjacent linear region in the gp41 region (QARVLAVERYLKDQQ) and to a region identified from the virus sieve analysis (C4_427B: WQEVGKAMYAPPIRGQIRCSS) were elicited; therefore, we evaluated IgG antibodies to these epitopes in the case–control study. Immunoglobulin G3 responses were elicited to the HIV envelope (11%–90% response rate) with very low levels of IgG3 V1V2 (7%–26% response rate). Immunoglobulin A responses were also elicited (Supplementary Table 1). There were no significant ADCC responses compared with placebo and low-level tier 1 neutralizing antibody responses (Supplementary Table 1).

Development of Primary Tier Immune Response Variables

Based on the pilot immunogenicity study, we downselected immune response measurements and developed a statistical analysis plan to evaluate HIV-1 antibody immune CoRs in HVTN 505, consistent with the approach taken in the RV144 [6] and HVTN 505 T-cell [9] correlates studies. To minimize potential bias, we divided immune response biomarkers into a prespecified primary analysis tier and an exploratory tier, without knowledge of participant infection/outcome status.

The criteria for including antibody immune response biomarkers in the primary analysis tier were the following: (1) significant CoR in RV144, (2) hypothesized to be a CoR based on knowledge of the HVTN 505 vaccine regimen, and (3) ≥ 20% positive response. Primary variables had to meet criterion (3) and either criterion (1) or (2) (Supplementary Tables 2 and 3); only validated or sufficiently qualified immunological assays were eligible for use. Six antibody immune response biomarkers were included in the primary tier: IgG_V2 score, IgG_V3 score, IgG_Env score, IgG binding to gp41, IgG binding to C4_427B, and IgA_Env score. Each score variable was a weighted combination of a panel of immune response biomarkers (Supplementary Material). The weights (given in Supplementary Table 7) were designed to maximize signal diversity by giving less weight to biomarkers that are more highly correlated with other biomarkers in the panel; such weighting can make the biomarkers more reflective of cross-reactivity to many HIV-1 variants [6]. In addition, 2 T-cell immune response biomarkers were included in the primary tier [9]: CD8_Env (CD8$^+$ Env (intracellular cytokine staining [ICS] polyfunctionality score) and CD4_env (CD4$^+$ Env ICS polyfunctionality score). The CD4 immune response was in the primary tier in the T-cell correlates analysis, and the CD8 immune response was an exploratory tier variable found to be strongly inversely correlated with HIV risk [9].

The 6 primary tier antibody variable scores are illustrated for cases and controls (Figure 1A–D). To ensure the selected measurements for the case–control study evaluated unique immunological space, we determined the correlations among the variables. Figure 2 shows the distributions and Spearman rank correlation coefficients of the primary tier variables. Correlation coefficients ranged from −0.052 to 0.27 between antibody variables and T-cell variables and ranged from 0.29 to 0.61 between pairs of IgG_Env, IgG_V2, IgG_V3 and IgG_gp41, and the correlation between CD8_Env and CD4_Env was 0.43.

Analyses of Primary Tier Immune Response Variables

We first asked whether each primary tier antibody immune response biomarker was associated with the risk of HIV-1 infection in a univariate analysis (although all analyses controlled for potential confounders) (Table 1). Of the 6 primary antibody measurements, IgG_Env and IgG_gp41 had a P value < .05 and a q value < 0.1. Among all individual antibody measurements that comprised the weighted scores used in the primary analysis, 6 had a P value < .05 and a q value < 0.1 (Table 1). These included 4 IgG gp140 responses (group M consensus,

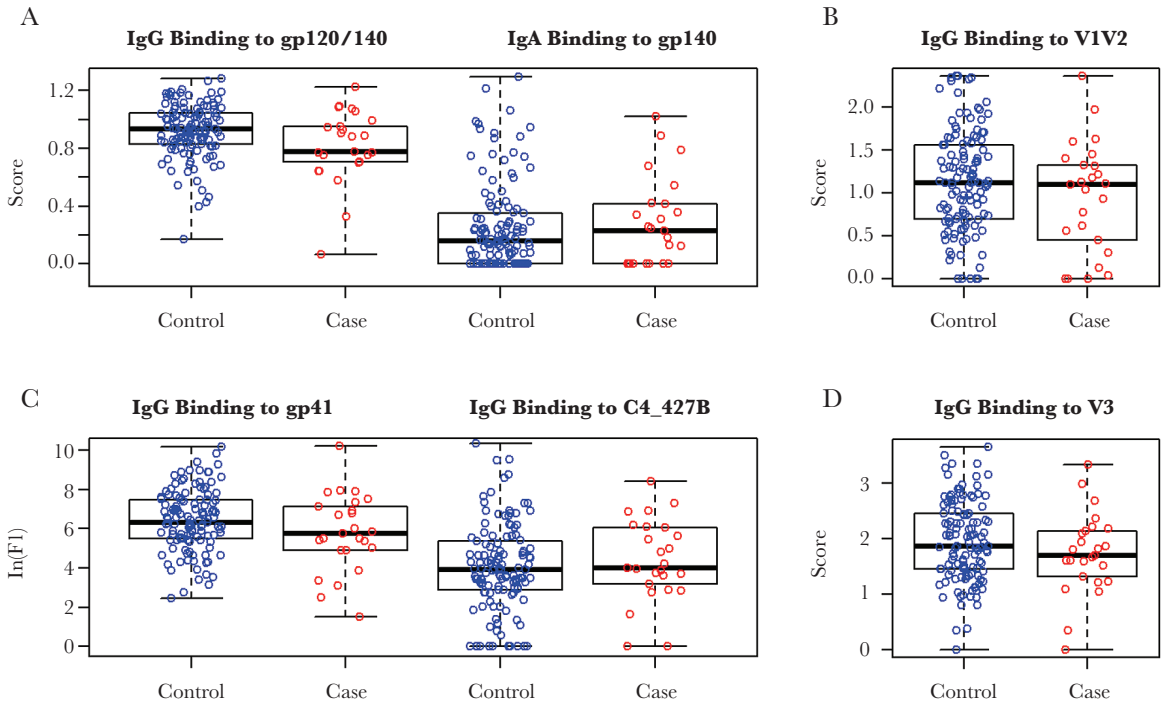

Figure 1. Distributions of the 6 primary tier antibody variables in HVTN 505 human immunodeficiency virus type 1 (HIV-1)–infected cases and HIV-1–uninfected controls in the vaccine group. *A*, IgG binding to gp120/140 and IgA binding to gp140; (*B*) IgG binding to V1V2; (*C*) IgG binding to gp41 and IgG binding to C4_427B; and (*D*) IgG binding to V3. Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G.

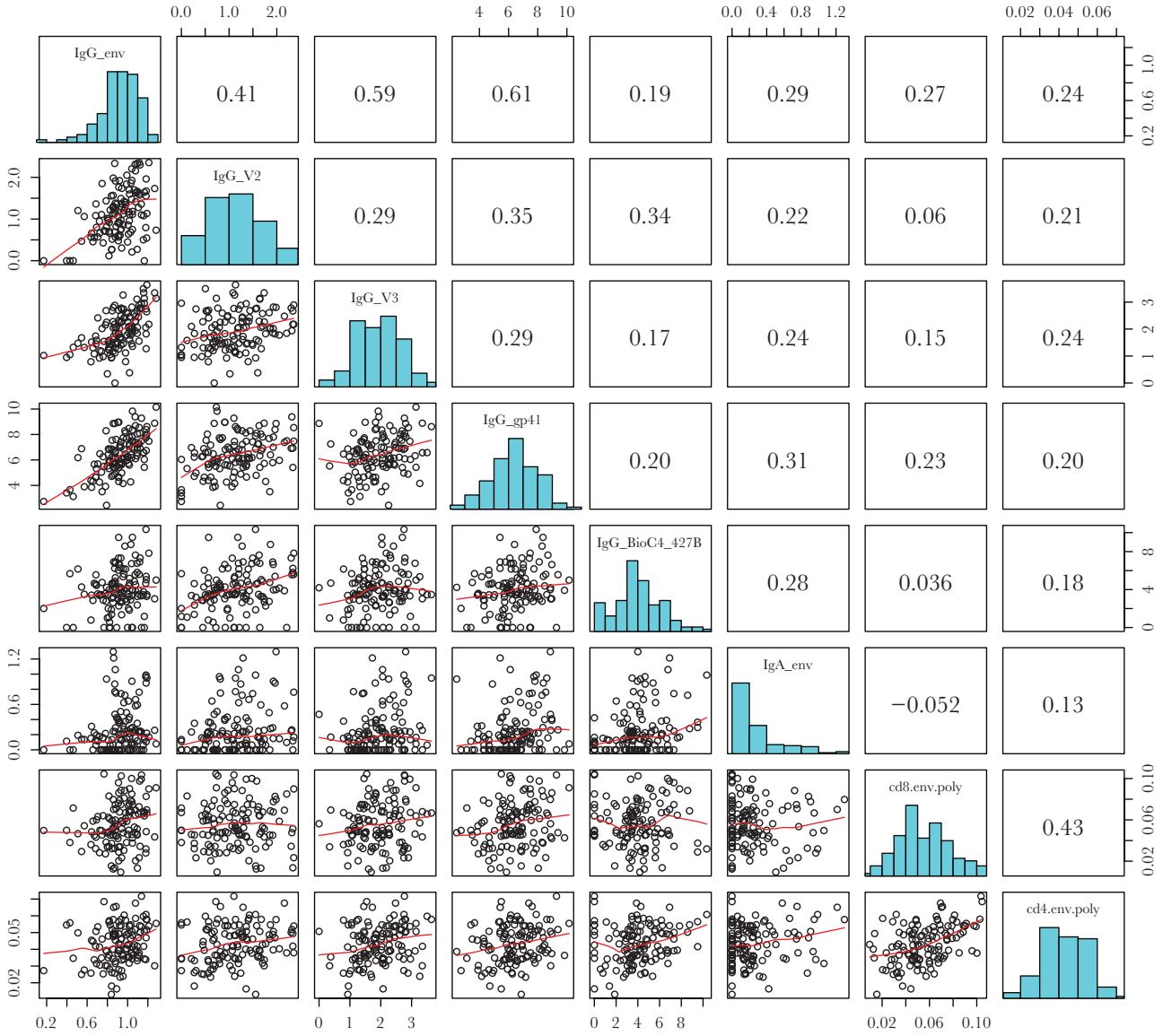

Figure 2. Distributions of the primary immune response variables for the vaccine group. Histograms of each of the primary response variables are shown on the diagonal line. Pairwise scatterplots with loess smoothers are shown in the lower left panels. Spearman correlation coefficients that measure the correlations between the corresponding pairs of immune response variables are shown in the upper right panels. Abbreviation: IgG, immunoglobulin G.

subtype C consensus, and vaccine-matched subtype A and C), IgG to a linear sequence within gp41 (HVTN 505 gp41), and IgG to subtype AE V1V2. We next determined whether these antibody biomarkers interacted with the previously identified CD8$^+$ T-cell CoR in this study—that is, whether the association between CD8$^+$ T-cell CoR and the risk of HIV-1 infection depended on the levels of antibody biomarkers (Table 1). The results suggest strong interaction in the same direction between Env-specific CD8$^+$ T-cell polyfunctionality score and IgG_Env, IgG_V2, IgG_V3 in their association with HIV-1 risk. For example, the *P* value for the interaction between CD8_Env and IgG_Env was <.001, and the adjusted *P* values controlling for either false discovery rate (*q* value) or FWER across 28 interaction tests were both .010, suggesting a complex relationship between infection risk and the immune responses measured by CD8_Env and IgG_Env (Figure 3). The risk changed with IgG_Env when CD8_Env was fixed at 1 of 3 representative values according to the interaction analyses (Figure 3A). Figure 3A shows that when CD8_Env was low, the risk of infection in vaccinees was higher than in placebo recipients and IgG_Env was inversely correlated with risk of infection; when CD8_Env was intermediate, the risk of infection was close to the level of risk in the placebo recipients and was not associated with IgG_Env; when CD8_Env was high, the risk of infection in vaccinees was lower than in placebo recipients and IgG_Env was directly correlated with risk of infection. Figure 3B reverses the role of IgG_Env and CD8_Env and displays how the risk changed with CD8_Env when IgG_Env was fixed at 1 of 3 representative

Table 1. Odds Ratios of the Primary and Select Exploratory Variables (Univariate Model) and Interactions of Antibody and CD8+ Cells

Primary variables						
Month 7 variable	Univariate[a]			CD8_env Interaction[b]		
	OR	*P* value	*q* value	R	*P* value	*q* value
IgG_Env	**0.60**	**.010**	**0.063**	**2.34**	**.000**	**0.010**
IgG_V2[c]	0.73	.129	0.193	**2.00**	**.002**	**0.028**
IgG_V3[c]	0.66	.062	0.124	**1.87**	**.006**	**0.055**
IgG_gp41[d]	**0.59**	**.032**	**0.095**	1.47	.063	0.350
IgG_C4_427B[e]	1.17	.499	0.599	1.45	.190	0.563
IgA_Env[c]	1.06	.747	0.747	1.28	.170	0.563
Exploratory variables[f]						
Month 7 variable		Univariate				
	OR	*P* value	*q* value			
IgG_Cconenv03140CF	0.514	.001	0.021			
IgG_ConSgp140CFI	0.579	.002	0.021			
IgG_VRC_A_gp140	0.534	.002	0.021			
IgG_VRC_C gp140	0.574	.012	0.074			
IgG_C_HVTN505gp41ID	0.578	.010	0.074			
IgG_AE.A244V1V2Tags	0.588	.019	0.097			

Abbreviations: IgG, immunoglobulin G; OR, odds ratio; R, ratio of odds ratios for the interaction term between CD8_env and the B-cell variable.

[a]Six univariate analyses were performed in vaccinees, 1 for each primary B-cell variable, to look at the association between risk of infection and immune response while adjusting for clinical covariates. *P* values < .05 and *q* values < 0.1 are in bold. *q* value: multitesting-adjusted p-values, adjustment occurred on the set of 6 univariate analyses.

[b]Twenty-eight interaction analyses were performed, 1 for each pairwise combination among the 6 primary B-cell and 2 primary T-cell variables. The 6 interaction results between CD8_env and the 6 B-cell primary variables are shown here, which also correspond to all interactions with *P* values < .05 from the entire set of 28 interaction analyses (Supplementary Table 4).

[c]Significant inverse or direct correlate of risk in RV144.

[d]Hypothesized to be a correlate of risk in HVTN 505 based on Williams et al (2015) [11].

[e]Significant sieve effect in HVTN 505.

[f]Only exploratory variables with *P* < .05 and *q* < 0.1 are shown.

values according to the interaction analyses. The risk of infection was inversely associated with CD8_Env at all 3 levels of IgG_Env, but the strength of association was dependent on the IgG_Env level, with lower IgG_Env corresponding to stronger association (Figure 3B).

To examine the robustness of these results to the strong parametric model assumptions of logistic regression models, we undertook a complementary nonparametric approach. We divided vaccinees into 9 strata based on low/medium/high CD8_Env and IgG_Env responses and estimated the empirical risks

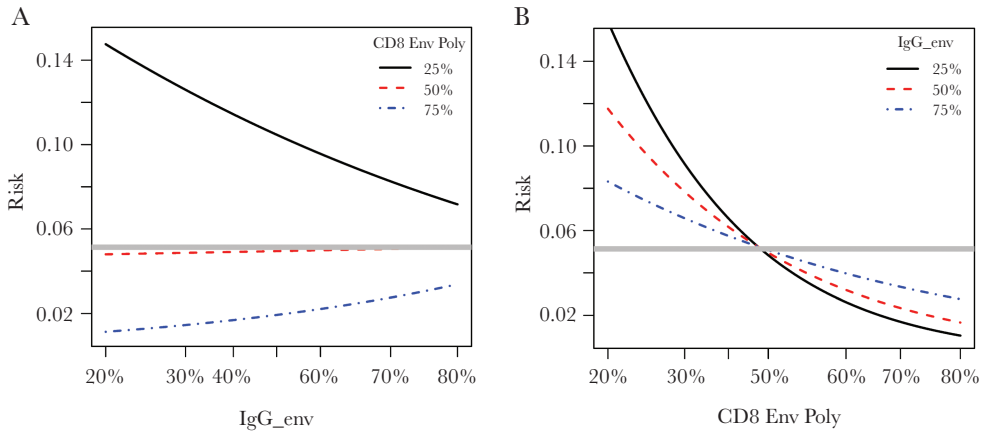

Figure 3. Risk functions estimated parametrically from logistic regression models. *A*, Human immunodeficiency virus type 1 (HIV-1) infection risk of vaccinees (from month 7 through month 24, based on data collected prior to study unblinding) with low, medium, or high CD8_env as a function of the immunoglobulin G (IgG) Env response. The black line represents the risk of a vaccinee with a low CD8_env (25% percentile) as a function of IgG_env level. The red and blue lines correspond to the risks of vaccinees with medium (50% percentile) and high (75% percentile) CD8_env, respectively. *B*, HIV-1 infection risk of vaccinees with low, medium, or high IgG Env responses as a function of CD8_env. The black line represents the risk of a vaccinee with a low IgG_env response (25% percentile) as a function of CD8_env. The red and blue lines correspond to the risks of vaccinees with medium (50% percentile) and high (75% percentile) IgG_env responses, respectively. The clinical covariates in the regression model were set to the following values: median age, median body mass index, median behavior risk, white race. Gray horizontal lines in both panels indicate the risk of a placebo recipient having the same clinical covariates, as estimated by a logistic model fitted to case–control cohort placebo recipients. Abbreviation: IgG, immunoglobulin G.

Table 2. Human Immunodeficiency Virus Type 1 Infection Risk of Vaccinees Estimated Nonparametrically

Month 7	IgG_env		
CD8 Env Poly	Low	Medium	High
Low	0.30 (.19–.45)	0.13 (.05–.29)	0.11 (.03–.32)
Medium	0.00 (.00–.09)	0.10 (.03–.25)	0.15 (.05–.36)
High	0.00 (.00–.29)	0.00 (.00–.11)	0.00 (.00–.07)

Each cell corresponds to 1 stratum of vaccinees formed by crossing CD8_env trichotomized at vaccinee tertiles and IgG_env trichotomized at vaccinee tertiles. In each stratum, the risk and 95% confidence interval were estimated through inverse sampling probability weighting. The analysis adjusts for clinical covariates. Risk was calculated from month 7 through month 24, based on data collected prior to study unblinding.

within each stratum, accounting for sampling weights (Table 2). Because the risks shown in Figure 3 are for subjects of a specific set of clinical covariate values, whereas the risks in Table 2 are population-averaged, the risks are not directly comparable between these 2 analyses; however, the trends can be compared. When CD8_Env was low, the results of the parametric and nonparametric analyses corresponded well—that is, risk decreased as the IgG_Env response increased. When CD8_Env was medium or high, both analyses suggest that the risk of infection did not decrease as IgG_Env increased.

The IgG_Env variable measures IgG binding antibodies to gp120 or gp140. To determine whether similar results are obtained for the Env V2 region, we repeated the analysis for CD8_Env and IgG_V2 (FWER-adjusted *P* value for the interaction = .03). The results of this analysis were qualitatively the same as for IgG_Env in that CD8 response correlated inversely with CoR independent of IgG responses (against V2) whereas IgG response correlated inversely with CoR when CD8 response was low (Supplementary Figure 2, Supplementary Table 5).

To better understand the relationship between risk of infection and the primary immune response variables, we carried out forward stepwise model building, which identifies variables that best predict the study outcome (risk of infection). At each step, we selected the most significant (by *P* value) predictor out of all individual biomarkers and pairwise interactions between biomarkers not yet in the model. When an interaction term was evaluated or added to the model, the main effect terms that make up the interaction were always included. The final model contained 3 main effects—CD8_Env, IgG_Env, and IgG_V2—and 2 interactions—CD8_Env × IgG_Env and CD8 score × IgG_V2 (Table 3). As expected, the estimated ratios of odds ratios for the interaction terms were attenuated toward 1 compared with when they were studied individually (Table 1). The *P* values for both interaction terms were significant ($P < .05$), suggesting that IgG_V2 captured a signal independent of IgG_Env.

Analyses of Exploratory Tier Immune Response Variables

We next studied the 31 exploratory tier antibody response biomarkers, some of which were components of the score variables studied in the primary tier. Others were additional variables

Table 3. Multivariate Model Selected by a Forward Stepwise Model Building Process

Month 7 variable	OR	95% CI	*P* value
CD8.Env.poly	0.26	.15–.45	.000
IgG_Env	1.02	.62–1.68	.936
IgG_V2	1.25	.76–2.04	.379
CD8.Env.poly:IgG_Env	1.88	1.11–3.19	.019
CD8.Env.poly:IgG_V2	1.59	1.03–2.47	.037

The candidate variables of vaccinees included pairwise interactions and main effects. At each step the most significant variable was added. If an interaction term was added, all components of the interaction term were added as well. The process stopped when no variable was significant (unadjusted *P* value < .05) when added to the model. Odds ratio for main effects (rows 1–3) or ratio of odds ratios for interaction terms (rows 4–5), 95% confidence intervals, and *P* values for all immune response variables in the final model are shown. The analysis adjusts for clinical covariates.

Abbreviations: CI, confidence interval; IgG, immunoglobulin G; OR, odds ratio.

that did not fit into the primary tier. Although these analyses were more exploratory, we still computed unadjusted and multitesting-adjusted *P* values. Additionally, a simple filter of >20% positivity was applied unless the variable was a component of a score variable in the primary tier. For each exploratory tier immune response biomarker, we performed 2 analyses, 1 univariate and 1 including interaction with the Env-specific $CD8^+$ T-cell polyfunctionality score.

Of the 6 antibody variables that significantly correlated with decreased HIV-1 risk in the univariate analysis ($P < .05$; $q < 0.1$), 3 of these antibody variables also had a significant interaction with CD8_Env (Table 4, bold). There was an interaction between CD8_Env and IgG_Env for many IgG binding responses to gp120/gp140 proteins (Table 4). Of the 8 variables comprising the IgG_Env score (listed in the Supplementary Material), 7 had interaction *P* values < .10, 4 had interaction q values < 0.05, and all estimated interaction term ratios of odds ratios were >1.4. The same was true for IgG_V2. Of the 4 variables that made up the IgG_V2 score (listed in the Supplementary Material), all 4 variables had interaction *P* values < .10, 2 had interaction *q* values < .05, and all estimated interaction term ratios of odds ratios were >1.4.

Analyses of Baseline gp41 Immune Response Variables

Immunoglobulin G gp41 responses elicited by the DNA/Ad5 vaccine regimen were reported to be derived from preexisting antibody responses to the microbiome [11]. In this study, postvaccination IgG binding to gp41 was found to inversely correlate with HIV-1 risk (Table 1). To further investigate the association between baseline gp41 immune responses and risk of infection, we pooled samples from the vaccine and placebo arms. For each immune response variable, we fit 2 logistic regression models, 1 of which included the treatment indicator and 1 of which did not; both analyses yielded similar results (Supplementary Table 6). Similar HIV-1 risk was observed across different baseline IgG gp41 levels ($P = .27$), indicating that preexisting gp41 reactivity did not predict risk of infection in this study.

Table 4. Odds Ratios of All Exploratory Variables (Univariate Model) and Interactions of Antibody and CD8+ Cells

Month 7	Univariate			CD8 Interaction		
Variable	OR	*P* value	*q* value	R	*P* value	*q* value
IgG_AEA244V1V2Tags	**0.588**	**.019**	**0.097**	**2.798**	**.000**	**0.001**
IgG_Con6gp120B	0.741	.143	0.294	2.656	.002	0.031
IgG_V3A	0.762	.187	0.323	2.058	.006	0.041
IgG_C1086C_V1_V2Tags	0.730	.151	0.294	2.042	.007	0.041
IgG_Cconenv03140CF	**0.514**	**.001**	**0.021**	**2.744**	**.007**	**0.041**
IgG_ConSgp140CFI	**0.579**	**.002**	**0.021**	**2.444**	**.009**	**0.041**
IgG_V3_M	0.729	.166	0.302	1.830	.009	0.041
IgG_V3B	0.633	.038	0.142	1.935	.015	0.058
IgG_VRCBgp140	0.689	.049	0.151	1.774	.037	0.127
IgG_gp70_BCaseA2V1V2169K	0.996	.980	0.980	1.558	.042	0.130
IgA_ConSgp140CFI	1.154	.476	0.595	1.703	.047	0.133
IgG_Bconenv03140CF	0.691	.065	0.168	1.685	.054	0.139
IgG_gp41	0.586	.032	0.140	1.474	.063	0.149
IgG_V3CRF2	0.727	.123	0.272	1.555	.076	0.167
IgG_VRCC_avi	0.574	.012	0.074	1.446	.083	0.167
IgG_VRC_A_gp70V1V2	0.827	.299	0.442	1.482	.089	0.167
IgG_A1conenv03140CF	0.698	.054	0.151	1.609	.091	0.167
IgG_V3C	0.789	.224	0.349	1.573	.105	0.180
IgA_VRC_C gp140	1.105	.630	0.723	1.543	.114	0.187
IgA_VRC_B gp140	1.010	.959	0.980	1.314	.164	0.254
IgG_C4_427B	1.171	.499	0.595	1.450	.190	0.271
IgA_VRC_A gp140	1.025	.915	0.978	1.467	.192	0.271
IgG_VRC_A gp140	0.534	.002	0.021	1.263	.300	0.400
IgG_ABDM_HVTN505gp41ID	0.662	.041	0.142	0.755	.310	0.400
IgA_A1conenv03140CF	1.079	.706	0.782	1.213	.334	0.414
IgG_RV144_C1IgG_BC	0.674	.105	0.251	1.222	.547	0.652
IgA_Cconenv03140CF	0.853	.498	0.595	0.848	.596	0.681
IgG_C_HVTN505gp41ID	0.578	.010	0.074	0.868	.623	0.681
IgA_gp41	1.157	.467	0.595	0.894	.637	0.681
IgA_Bconenv03140CF	1.180	.360	0.507	1.094	.700	0.724
IgG_C1_AE	0.756	.225	0.349	0.894	.730	0.730

Thirty-one univariate analyses were performed in vaccinees, 1 for each exploratory B-cell variable, to look at the association between risk of infection and immune response while adjusting for clinical covariates. Measurements with *P* value < .05 and *q* value < 0.10 for the univariate analysis are also listed in Table 1, and those that are also significant for CD8 interaction are in bold here. Thirty-one interaction analyses were performed, 1 for the interaction between CD8_env and each exploratory B-cell variable. *q* value: multitesting-adjusted *P* values, adjustment occurred on the set of 31 interaction analyses.

Abbreviations: IgG, immunoglobulin G; OR, odds ratio; R, ratio of odds ratios for the interaction term between CD8_env and the B-cell variable.

DISCUSSION

Envelope-specific IgG responses measured after vaccination significantly correlated with decreased HIV-1 risk in HVTN 505 (Table 1, $P = .010$). Moreover, envelope IgG responses together with Env-specific CD8+ T cells demonstrated the strongest correlation with HIV-1 risk, indicating that combined levels of multiple immune responses to HIV-1 could be important for protection. Specifically, we found that vaccinees with a low level of polyfunctional Env-specific CD8+ T cells had their risk modified by their IgG Env response, such that a higher IgG Env response was associated with decreased risk, whereas vaccinees with high Env-specific CD8+ T-cell polyfunctionality had low risk regardless of their IgG Env response. This finding reinforces the previous finding that this Env-specific CD8 response was strongly correlated with low risk [9] and indicates that Env-specific CD8+ T cells were the dominant independent correlate of risk. Moreover, this result indicates that the only subgroup of vaccinees with high risk of HIV-1 infection had low responses for both IgG Env and Env-specific CD8 (ie, no measureable vaccine "take"), such that in a sense vaccinees had 2 chances of low risk (high Env-specific CD8 or low Env-specific CD8 combined with high IgG Env).

For the 1 efficacious vaccine regimen to date, V1V2 IgG inversely correlated with decreased HIV-1 risk, and Env-specific IgA directly correlated with HIV-1 risk [6, 7]. Interestingly, in this trial with nonefficacy, the IgG V1V2 response was substantially lower than that observed in the RV144 trial [8]. However, in vaccinees with detectable V1V2 IgG responses, AE V1V2 IgG responses correlated with decreased HIV-1 risk (P value < .05; q value < 0.1) in vaccinees with low CD8+ Env polyfunctionality scores. The variability of V2 antibody levels was less than in RV144, with 17% response rate to AE V1V2 IgG compared with 95% response rate to the same V2 antigen in RV144. This

observation suggests that V2 antibody levels do not necessarily need to be high to be a marker of HIV-1 risk, at least in some contexts. The Env-specific IgA score in this study was different than that measured in the RV144 study due to the low response rate of the IgA response to some of the HIV-1 antigens in the RV144 score. However, there was no evidence that the IgA responses measured in this correlates analysis associated with HIV-1 risk.

We previously reported that gp41 protein IgG responses were elicited by this vaccine regimen [8], that these responses were higher than antibody responses to gp120 proteins, and that some of the gp41 antibody response can be derived from preexisting responses to the microbiota [8, 11]. These findings generated the hypothesis that gp41 antibodies may divert from a protective response. We found that baseline responses to gp41 did not correlate with HIV-1 risk and postvaccination IgG binding to gp41 was inversely correlated with HIV-1 risk in a univariate model (estimated odds ratio [OR] = 0.59; 95% confidence interval [CI], .36–0.95) and not correlated in a multivariate model that included CD8_Env, IgG_Env, and their interaction (estimated OR = 1.07; 95% CI, .53–2.2), suggesting that there is no evidence that higher gp41 responses are a marker for an elevated risk of HIV-1 infection. Moreover, gp41 responses to a specific linear region in gp41 correlated with decreased HIV-1 risk in the univariate analysis. These results suggest caution in concluding that gp41 responses are undesirable for candidate HIV-1 vaccine regimens.

Our result in HVTN 505 that IgG Env response was only associated with risk in the absence of a CD8 Env response was consistent across measurement of the IgG Env response to different targets (gp120, gp140, V2), but not IgG_C4_427B, a sieve peptide in the CD4 binding site in which sequences from HIV-1–infected vaccinees were significantly more distant from the subtype B vaccine insert than those from placebo recipients (P = .0038) [10]. In this same study [10], we measured the capacity of these vaccine-elicited IgG responses to mediate antibody-dependent phagocytosis of gp140-coated microspheres and found that most vaccinees elicited antibody-dependent phagocytosis with a range of magnitudes. This suggests that follow-up studies to test antibody effector functions as correlates of risk are warranted.

The correlate result in this study with no overall vaccine effect on HIV-1 acquisition [9, 20] is consistent with 2 interpretations that are challenging to discriminate: (1) the vaccine conferred protection in some subgroups balanced by its increase of HIV-1 acquisition risk in other subgroups; versus (2) the vaccine had no effect on acquisition risk in any subgroup (ie, like a placebo vs placebo study), and the correlate merely marked a third underlying factor (eg, a measure of differential exposure to HIV-1) that truly caused the risk gradient. We previously conjectured that the observed strong sieve effects of the DNA/rAd5 vaccine favor explanation (1), where the vaccine generally increased susceptibility to HIV-1 acquisition unless the exposing viruses were genetically similar to the vaccine strains in the CD4 binding site, in which case protection was conferred [10]. If (1) is correct, our findings could indicate that low IgG Env combined with low $CD8^+$ T-cell polyfunctionality marks vaccine-increased risk, whereas high $CD8^+$ T-cell polyfunctionality marks protection. Further studies that combine cellular and humoral analyses to the same antigens, including circulating virus sequences at the time of the trial, will be informative. A finding that the associations are very strong for HIV-1 infection outcomes with the sequences matched or very close to the sequence targets of the immune response, yet are absent for HIV-1 infection outcomes with the sequences divergent from the sequence targets of the immune response, may support interpretation (1). Conversely, a result where the CoRs were independent of the infection outcome sequences or in the unexpected opposite direction may support interpretation (2). Explanation (1) is plausible based on previous data showing increased acquisition risk by an Ad5 vector vaccine [21] and the fact that the correlates analysis controlled for all available HIV-1 behavioral risk factors. However, unmeasured confounding could make explanation (2) correct; future experiments are needed to discriminate the explanations and [if (1) is correct] determine the immune responses responsible for a beneficial versus detrimental vaccine effect on HIV-1 acquisition. The observed interactions between CD8 T cells and antibodies in this study highlight a potential role for eliciting both cellular and humoral responses by an HIV-1 vaccine.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Tara McNair, R. Glenn Overman, Judith T. Lucas, Michael Archibald, Yong Lin, Robert Parks, Ryan Duffy, Krissey Eutsey Lloyd, and Robert Howington for expert technical assistance; Nicole Yates for laboratory oversight; Sheetal Sawant, and April Randhawa for expert data management; Drs H. Liao and Gary Nabel for envelope and V1V2 proteins; and Dr Bette Korber for linear array sequences.

Financial support. Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases (NIAID) US Public Health Service Grants UM1 AI068614 [LOC: HIV Vaccine Trials Network], UM1 AI068618 [LC: HIV Vaccine Trials Network], UM1 AI068635 [SDMC: HIV Vaccine Trials Network], NIH/NIAID Duke Center for AIDS Research (AI064518), and by NIAID award number R37AI054165, R01AI122991. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis **2012**; 54:1615–7.
2. Tomaras GD, Haynes BF. Advancing toward HIV-1 vaccine efficacy through the intersections of immune correlates. Vaccines (Basel) **2014**; 2:15–35.
3. Tomaras GD, Plotkin SA. Complex immune correlates of protection in HIV-1 vaccine efficacy trials. Immunol Rev **2017**; 275:245–61.
4. Qin L, Gilbert PB, Corey L, McElrath MJ, Self SG. A framework for assessing immunological correlates of protection in vaccine trials. J Infect Dis **2007**; 196:1304–12.
5. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al; MOPH-TAVEG Investigators. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med **2009**; 361:2209–20.
6. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med **2012**; 366:1275–86.
7. Tomaras GD, Ferrari G, Shen X, et al. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proc Natl Acad Sci U S A **2013**; 110:9019–24.
8. Hammer SM, Sobieszczyk ME, Janes H, et al; HVTN 505 Study Team. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med **2013**; 369:2083–92.
9. Janes HE, Cohen KW, Frahm N, et al. Higher T-cell responses induced by DNA/rAd5 HIV-1 preventive vaccine are associated with lower HIV-1 infection risk in an efficacy trial. J Infect Dis **2017**; 215:1376–85.
10. deCamp AC, Rolland M, Edlefsen PT, et al. Sieve analysis of breakthrough HIV-1 sequences in HVTN 505 identifies vaccine pressure targeting the CD4 binding site of Env-gp120. PLoS One **2017**; 12:e0185959.
11. Williams WB, Liao HX, Moody MA, et al. HIV-1 vaccines. Diversion of HIV-1 vaccine-induced immunity by gp41-microbiota cross-reactive antibodies. Science **2015**; 349:aab1253.
12. Tomaras GD, Yates NL, Liu P, et al. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. J Virol **2008**; 82:12449–63.
13. Yates NL, Liao HX, Fong Y, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. Sci Transl Med **2014**; 6:228ra39.
14. Shen X, Duffy R, Howington R, et al. Vaccine-induced linear epitope-specific antibodies to simian immunodeficiency virus SIVmac239 envelope are distinct from those induced to the human immunodeficiency virus type 1 envelope in nonhuman primates. J Virol **2015**; 89:8643–50.
15. Gottardo R, Bailer RT, Korber BT, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. PLoS One **2013**; 8:e75665.
16. Tomaras GD, Binley JM, Gray ES, et al. Polyclonal B cell responses to conserved neutralization epitopes in a subset of HIV-1-infected individuals. J Virol **2011**; 85:11502–19.
17. Breslow NE, Holubkov R. Weighted likelihood, pseudo-likelihood and maximum likelihood methods for logistic regression analysis of two-stage data. Stat Med **1997**; 16:103–16.
18. Benjamini Y, Hochberg Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. J Roy Stat Soc B Met **1995**; 57:289–300.
19. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat **1979**; 6:65–70.
20. Gilbert PB, Peterson ML, Follmann D, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis **2005**; 191:666–77.
21. Huang Y, Follmann D, Nason M, et al. Effect of rAd5-vector HIV-1 preventive vaccines on HIV-1 acquisition: a participant-level meta-analysis of randomized trials. PLoS One **2015**; 10:e0136626.