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Analysis of the HIV Vaccine Trials Network 702 Phase 2b–3 HIV-1 Vaccine Trial in South Africa Assessing RV144 Antibody and T-Cell Correlates of HIV-1 Acquisition Risk

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Background. The ALVAC/gp120 + MF59 vaccines in the HIV Vaccine Trials Network (HVTN) 702 efficacy trial did not prevent human immunodeficiency virus-1 (HIV-1) acquisition. Vaccine-matched immunological endpoints that were correlates of HIV-1 acquisition risk in RV144 were measured in HVTN 702 and evaluated as correlates of HIV-1 acquisition.

Methods. Among 1893 HVTN 702 female vaccinees, 60 HIV-1–seropositive cases and 60 matched seronegative noncases were sampled. HIV-specific $CD4^+$ T-cell and binding antibody responses were measured 2 weeks after fourth and fifth immunizations. Cox proportional hazards models assessed prespecified responses as predictors of HIV-1 acquisition.

Results. The HVTN 702 Env-specific $CD4^+$ T-cell response rate was significantly higher than in RV144 (63% vs 40%, $P = .03$) with significantly lower IgG binding antibody response rate and magnitude to 1086.C V1V2 (67% vs 100%, $P < .001$; $P_{mag} < .001$). Although no significant univariate associations were observed between any T-cell or binding antibody response and HIV-1 acquisition, significant interactions were observed (multiplicity-adjusted $P \leq .03$). Among vaccinees with high IgG A244 V1V2 binding antibody responses, vaccine-matched $CD4^+$ T-cell endpoints associated with decreased HIV-1 acquisition (estimated hazard ratios = 0.40–0.49 per 1-SD increase in $CD4^+$ T-cell endpoint).

Conclusions. HVTN 702 and RV144 had distinct immunogenicity profiles. However, both identified significant correlations (univariate or interaction) for IgG V1V2 and polyfunctional $CD4^+$ T cells with HIV-1 acquisition.

Clinical Trials Registration. NCT02968849.

Keywords. HIV-1 vaccine; HVTN 702; RV144; vaccine efficacy trial; T-cell immunogenicity; T-cell polyfunctionality; binding antibodies; correlates of risk; intracellular cytokine staining; vaccine-induced immune response.

An efficacious preventive human immunodeficiency virus-1 (HIV-1) vaccine is important for inducing long-lived immunity and providing a practical, cost-effective method to fight the global epidemic, particularly for regions in sub-Saharan Africa with disproportionately high HIV-1 incidence despite decades of prevention efforts. Eight HIV-1 vaccine candidates have been studied in efficacy trials [1–8]; only the regimen tested in RV144 showed significant HIV-1 acquisition reduction. Estimated vaccine efficacy of the replication-defective

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canarypox vaccine (ALVAC) plus recombinant glycoprotein 120 (gp120) protein (AIDSVAX) vaccine at month 12 was 60% (95% confidence interval [CI], 22%–80%) [9]; however, efficacy waned to 31.2% (95% CI, 1.1%–52.1%; $P = .04$) by month 42 [5].

Given these results, the RV144 regimen was tested in South Africa in the HIV Vaccine Trials Network (HVTN) 097 phase 1b trial, which showed significantly higher cellular and humoral vaccine-induced responses among participants in South Africa than among participants in Thailand [10]. Shortly after the announcement of RV144, the Pox Protein Public–Private Partnership (P5) was formed to develop an RV144-analogous vaccine regimen that would aspirationally improve upon the 30% overall efficacy and overcome the large strain diversity between the focal A/E epidemic in Thailand versus the extremely diverse subtype C epidemic in South Africa [11]. The resultant regimen incorporated regionally adapted HIV-1 subtype C sub-Saharan African strains [7], MF59 adjuvant instead of alum, and a month 12 boost. Safety of this regimen was demonstrated in the HVTN 100 phase 1–2a trial [12], with humoral and cellular immune responses meeting the P5's predefined criteria for studying the regimen's preventive efficacy. HVTN 702, a phase 2b–3 efficacy trial, next evaluated the subtype C regimen in South African HIV-uninfected at-risk adults. However, the immunogenicity signal from HVTN 100 [12–15] did not translate to efficacy, and vaccinations in HVTN 702 were halted after prespecified vaccine efficacy futility criteria were met in January 2020. Participants were unblinded in February 2020. The estimated HIV hazard ratio (vaccine: placebo) for the first 24 months of follow-up was 1.02 (95% CI, .81–1.30, $P = .84$) [7].

Here we evaluated and compared HVTN 702 immune responses to those in RV144, HVTN 097, and HVTN 100 and assessed whether the RV144 correlates of HIV-1 risk would also correlate with HIV-1 risk in HVTN 702.

METHODS

Ethics

For the HVTN 702 trial (NCT02968849) [7], written informed consent was obtained from all participants and all procedures were conducted in accordance with the ethical standards of the Helsinki Declaration. The research ethics committees of the University of the Witwatersrand, University of Cape Town, University of KwaZulu-Natal, Sefako Makgatho University, and the South African Medical Research Council approved the trial.

Study Participants

HVTN 702 enrolled 5404 HIV-uninfected adults (3786 assigned female at birth, 1618 assigned male at birth) at 14 South African sites between 26 October 2016 and 21 June 2019. Participants were randomized to vaccine or placebo within each sex assigned at birth. The vaccine regimen was an ALVAC-HIV vector and an MF59-adjuvanted bivalent subtype C gp120. ALVAC-HIV (vCP2438; Sanofi Pasteur) expresses the subtype C ZM96.C HIV-1 envelope (Env) glycoprotein, along with subtype B LAI gp41 transmembrane sequence, gag, and protease. Bivalent subtype C gp120 (GSK) consists of 100 μg of TV1.C gp120 and 100 μg of 1086.C gp120. Participants received ALVAC-HIV or placebo at months 0 and 1, followed by 4 injections of ALVAC-HIV plus bivalent MF59-adjuvanted subtype C gp120 or placebo at months 3, 6, 12, and 18.

As few male participants acquired HIV-1 in the study [7], we restricted our analyses to female participants. Months 6.5 (2 weeks after fourth vaccination) and 12.5 (2 weeks after fifth vaccination) immune responses among vaccine recipients were used to profile immunogenicity, and were evaluated as predictors of HIV-1 acquisition through month 24, based on follow-up data collected through 18 February 2020. Using a case-control design frequency matched on age, we measured cellular and humoral immune responses in per-protocol females: 60 vaccine cases who acquired HIV-1 between month 6.5 and 24, 60 vaccine noncases who remained HIV-1 negative until month 24, 5 placebo cases, and 5 placebo noncases (Supplementary Text, Supplementary Figure 1).

Immune response data at month 6.5 from per-protocol female and male vaccine noncases from HVTN 100 (n = 184), HVTN 097 (n = 73), and RV144 (n = 201) were compared to HVTN 702 female per-protocol noncases (n = 60). Those who received the first 4 planned immunizations within specified visit windows were considered per protocol. HVTN 100 assessed the same regimen as HVTN 702, without the month 18 boost, enrolling 252 low-risk male and female participants in South Africa in 2015 with randomization to vaccine (n = 210) or placebo (n = 42) [13]. HVTN 097 assessed an RV144-related regimen (see below), enrolling 100 low-risk male and female participants in South Africa in 2013 with randomization to vaccine (n = 80) or placebo (n = 20) [10]. RV144 enrolled 16 402 males and females from the general population in Thailand between 2003 and 2005, with randomization to vaccine (n = 8197) or placebo (n = 8198). Like the heterologous prime-boost combination HVTN 100 and HVTN 702 vaccine regimens, the RV144 and HVTN 097 vaccine regimens consisted of 4 injections at months 0, 1, 3, and 6 of ALVAC-HIV (vCP1521), a canarypox vector expressing clade E Env, clade B gag, and clade B protease with 2 booster injections of alum-adjuvanted AIDSVAX B/E (a bivalent gp120) at months 3 and 6. For HVTN 097 and HVTN 100, immune response data are available on all per-protocol vaccine recipients (n = 73 and 184, respectively) whereas data are available on a subset of n = 201 RV144 vaccine noncases, selected for contemporaneous assaying with HVTN 100 specimens in 2016 [16] to inform whether to proceed with HVTN 702.

Laboratory Methods

All assays were performed blinded in HVTN laboratories utilizing validated methods [16–18]. $CD4^+$ T-cell responses were measured by intracellular cytokine staining [19] and analyzed by flow cytometry (Supplementary Text). Serum HIV-1–specific immunoglobulin G (IgG), IgG3, and IgA binding antibody responses were measured by an HIV-1 binding antibody multiplex assay [4, 16] (Supplementary Text).

Immune Response End Points for Correlates of Risk Assessment

Three primary immune responses were selected based on previous RV144 immune correlates studies [16, 20–24]: (1) the COMPASS Env-specific $CD4^+$ T-cell polyfunctionality score to ZM96 [23], defined as the estimated proportion of antigen-specific cell subsets detected, weighted by degree of functionality using the same 6 markers as the RV144 correlates analysis: CD40L, interferon-γ (IFN-γ), interleukin 2 (IL-2), tumor necrosis factor-α (TNF-α), IL-4, and IL-17a; (2) IgG binding antibody responses to AE.A244 V1V2; and (3) IgG3 binding antibody responses to C.1086 V1V2. Secondary immune responses were $CD4^+$ polyfunctionality score to 1086 and to TV1, $CD4^+$ T cells expressing IFN-γ and/or IL-2 and/or CD40L in response to ZM96, IgA binding antibody score, IgG binding antibody responses to RV144 vaccine-matched antigen (A244 gp120), and IgG binding antibody responses to the consensus antigen (A1.con.env03140CF).

Statistical Methods

Immunogenicity Characterization and Comparison

Given differences in the HVTN 702 versus HVTN 100, HVTN 097, and RV144 study populations (Table 1), we compared immunogenicity under a hypothetical scenario where baseline participant characteristics in the other trials (age, body mass index, South African region, and education level) follow the covariate distribution of the female per-protocol HVTN 702 cohort who were eligible for case-control sampling (Supplementary Text). Targeted minimum loss estimation was applied, with superlearning employed to model the mean (and 95% CI) immune response conditional on baseline covariates [25]. If the estimated response rate exceeded 90%, the Wilson score method was used to calculate the 95% CI as targeted minimum loss estimation can be unstable near the boundary. The Holm method controlled the family-wise error rate at 0.05 across each set of binary and continuous endpoints.

Correlates of Risk Assessment

To evaluate immune responses among HVTN 702 female vaccine recipients as predictors of HIV-1 acquisition within the first 24 months since enrollment, univariate and multivariate Cox proportional hazard models were used. Each model accounted for the case-control sampling design and adjusted for age (≤ 25, >25 years) and a categorical baseline HIV risk score for women to control for potential confounding [7]. Univariate Cox models were fit for each individual categorical and continuous immune response variable at each time point. Four prespecified multivariate models were fit that included all categorical or continuous primary immune response variables at either month 6.5 or 12.5. At each time point, a separate multiplicity adjustment was applied across each set of endpoints. The Holm method controlled the family-wise error rate at 0.05 across the set of 3 primary variables and across the set of 6 secondary variables, with separate multiplicity adjustment for continuous and categorical variables. Q values for the set of exploratory variables were calculated using the Benjamini-Hochberg method [26], with $Q < 0.2$ considered statistically significant. Interactions among Month 6.5 immune responses were considered for their association with HIV-1 acquisition where prespecified criteria were met (Supplementary Text). Separate models were fit, each containing 1 interaction term and associated main effects, adjusting for age and HIV

Table 1. Demographic Characteristics of the Per-Protocol Vaccine Recipient Cohorts from HVTN 097, HVTN 100, RV144 Subset, and the Female Per-Protocol Noncases in the HVTN 702 Case-Control Set for Immunogenicity Analyses

Characteristic	HVTN 097 (n = 73)	HVTN 100 (n = 184)	RV144 (n = 201)	HVTN 702 (n = 60)
Planned treatment				
Placebo	0 (0)	0 (0)	0 (0)	0 (0)
Vaccine	73 (100)	184 (100)	201 (100)	60 (100)
Age in years at randomization				
Mean (SD)	23 (4)	24 (5)	…	24 (4)
Range	18–35	18–40	…	18–35
Age category at randomization				
≤20	27 (37)	44 (24)	56 (28)	10 (17)
21–25	17 (23)	78 (42)	97 (48)	35 (58)
≥26	29 (40)	62 (34)	48 (24)	15 (25)
Sex assigned at birth				
Female	33 (45)	73 (40)	79 (39)	60 (100)
Male	40 (55)	111 (60)	122 (61)	0 (0)
BMI				
Mean (SD)	23 (5)	24 (5)	…	27 (7)
Range	17–37	16–39	…	17–47
Region of enrollment				
Thailand	0 (0)	0 (0)	201 (100)	0 (0)
Central South Africa	50 (68)	100 (54)	0 (0)	34 (57)
KwaZulu-Natal South Africa	0 (0)	59 (32)	0 (0)	19 (32)
WestEast Cape South Africa	23 (32)	25 (14)	0 (0)	7 (12)
Education				
High school	0 (0)	135 (73)	0 (0)	55 (92)
Primary school	0 (0)	6 (3)	0 (0)	0 (0)
Tertiary college university	0 (0)	43 (23)	0 (0)	5 (8)
Prefer not to answer	0 (0)	0 (0)	0 (0)	0 (0)
Not available	73 (100)	…	201 (0)	…

Data are No. (%) except where indicated.

Abbreviation: BMI, body mass index.

risk score, with Holm *P* value adjustment across the multiple models.

RESULTS

Different Immune Response Profiles Elicited in HVTN 702 Versus RV144

The HVTN 702 vaccine regimen induced $CD4^+$ T cells expressing IFN-γ and/or IL-2 and/or CD40L in response to vaccine-matched HIV-1 envelope peptide pools in 72%–87% of vaccine recipient noncases, with similar responses at months 6.5 and 12.5 (Figure 1*A*). $CD8^+$ T-cell response rates were low across HIV-1 peptide pools and time points: ≤12% (data not shown). IgG binding antibody response rates to 1086.C V1V2 were also similar across time points (67% and 63%, respectively; Figure 1*B*).

At month 6.5, the response rate of $CD4^+$ T cells expressing IFN-γ and/or IL-2 to ZM96 was significantly higher in HVTN 702 than in HVTN 100 to ZM96 (63% vs 38%, $P = .03$) and was also significantly higher than that in RV144 to the analogous vaccine-matched envelope, 92TH023 (63% vs 40%, $P = .03$; Figure 1*C*). In contrast, the $CD4^+$ T-cell response rate in HVTN 097 to 92TH023 (74%) was similar to HVTN 702. No significant differences were seen in these magnitudes across trials (all $P > .23$). Although the HVTN 702 IgG binding antibody response rate to 1086.C V1V2 was significantly lower than in RV144 (67% vs 100%, $P < .001$), it was similar to HVTN 100 (77%) (Figure 1*D*) and would have met the prespecified criteria for proceeding to efficacy testing. IgG 1086.C V1V2 magnitudes in HVTN 702 were also significantly lower than those in RV144 ($P < .001$) but similar to those in HVTN 100. Notably, only 3% of HVTN 702 (13% of HVTN 100) vaccine recipients had 1086.C V1V2 magnitudes in the upper tertile of RV144 (Supplementary Figure 2).

Cross-trial immunogenicity comparison across the subset of female per-protocol vaccine recipients in each trial (Supplementary Figure 3) yielded results highly comparable to those shown in Figure 1*C* and 1*D* that included both male and female participants.

No Significant Associations of Primary or Secondary Immune Response Variables With HIV-1 Acquisition in Univariate Correlate of Risk Analyses

Given the lack of overall vaccine efficacy, we performed a limited correlates analysis on down-selected antibody and cellular immune measurements. For antibody measurements, we selected the protein boost clade C V1V2 sequence, which correlated with decreased HIV-1 risk in RV144 [21]. There was no significant association between month 6.5 or 12.5 Env ZM96 $CD4^+$ polyfunctionality and HIV-1 acquisition, regardless of whether the quantitative immune response variable or the categorical variable high-versus-low response indicator and medium-versus-low response indicator was used (Table 2, Supplementary Table 1, Figure 2, and Figure 3). The $CD4^+$ polyfunctionality profile is characterized by subsets that include IFN-γ, IL-2, IL-4, CD40L, and TNF-α (Supplementary Figure 4). Similarly, there was no significant association between month 6.5 or 12.5 IgG binding antibody responses to A244 V1V2 and HIV-1 acquisition when considered as a quantitative variable or a categorical high-versus-low response indicator and medium-versus-low response indicator (Table 2, Supplementary Table 1, Figure 2, and Figure 3). There was also no significant association between month 6.5 or 12.5 IgG3 binding antibody responses to 1086 V1V2 and HIV-1 acquisition when considered as a quantitative variable or a binary positive-versus-negative response indicator (Table 2, Supplementary Table 1, Figure 2, and Figure 3).

No significant associations with HIV-1 acquisition were observed with any secondary immune response variable at either time point, whether considered quantitatively or categorically (Table 1 and Table 2; Supplementary Figures 5–10). Pairwise correlations between primary and secondary endpoints are in Supplementary Figure 11.

No Significant Associations of Primary Immune Response Variables With HIV-1 Acquisition in Multivariate Analyses

When the Env ZM96 $CD4^+$ polyfunctionality score, the IgG binding antibody A244 V1V2 responses, and the IgG3 binding antibody 1086 V1V2 responses were included in a multivariate Cox model, no significant associations with HIV-1 acquisition were observed at either month 6.5 or 12.5, whether continuous or categorical variables were considered (Table 1 and Table 2).

Interactions Between Month 6.5 IgG A244 V1V2 Binding Antibody and $CD4^+$ T-Cell Responses Correlate With Risk of HIV-1 Acquisition

The association between $CD4^+$ T-cell responses and HIV-1 risk qualitatively depended on the level of IgG A244 V1V2-directed binding antibody response (multiplicity-adjusted interaction *P's* ≤ .03; Supplementary Figures 12–15). Among vaccinees with highest tertile IgG A244 V1V2 responses, vaccine-matched $CD4^+$ T-cell endpoints (polyfunctional scores in response to Env-ZM96 and to 1086, triple-functional cells expressing IFN-γ, IL2, and CD40L in response to Env-ZM96) were associated with a 51%–60% lower acquisition risk (estimated hazard ratios = 0.40 to 0.49 per 1-SD increase in the respective $CD4^+$ T-cell endpoint; Table 3). Conversely, among those with lowest tertile IgG A244 V1V2 binding antibody responses, $CD4^+$ T-cell responses were associated with 2.2- to 3.6-fold higher risk of HIV-1 acquisition (Table 3). No significant interactions were observed between any primary or secondary endpoint and IgA score.

Exploratory Immune Response Variables Show Little to No Evidence of a Significant Association With HIV-1 Acquisition in Univariate Analyses

Additional measurements including antigen-specific antibody responses were evaluated in exploratory analyses (Supplementary Text). Among the 206 exploratory immune responses assessed at month 6.5 and/or 12.5, one (IgG3 binding antibodies to

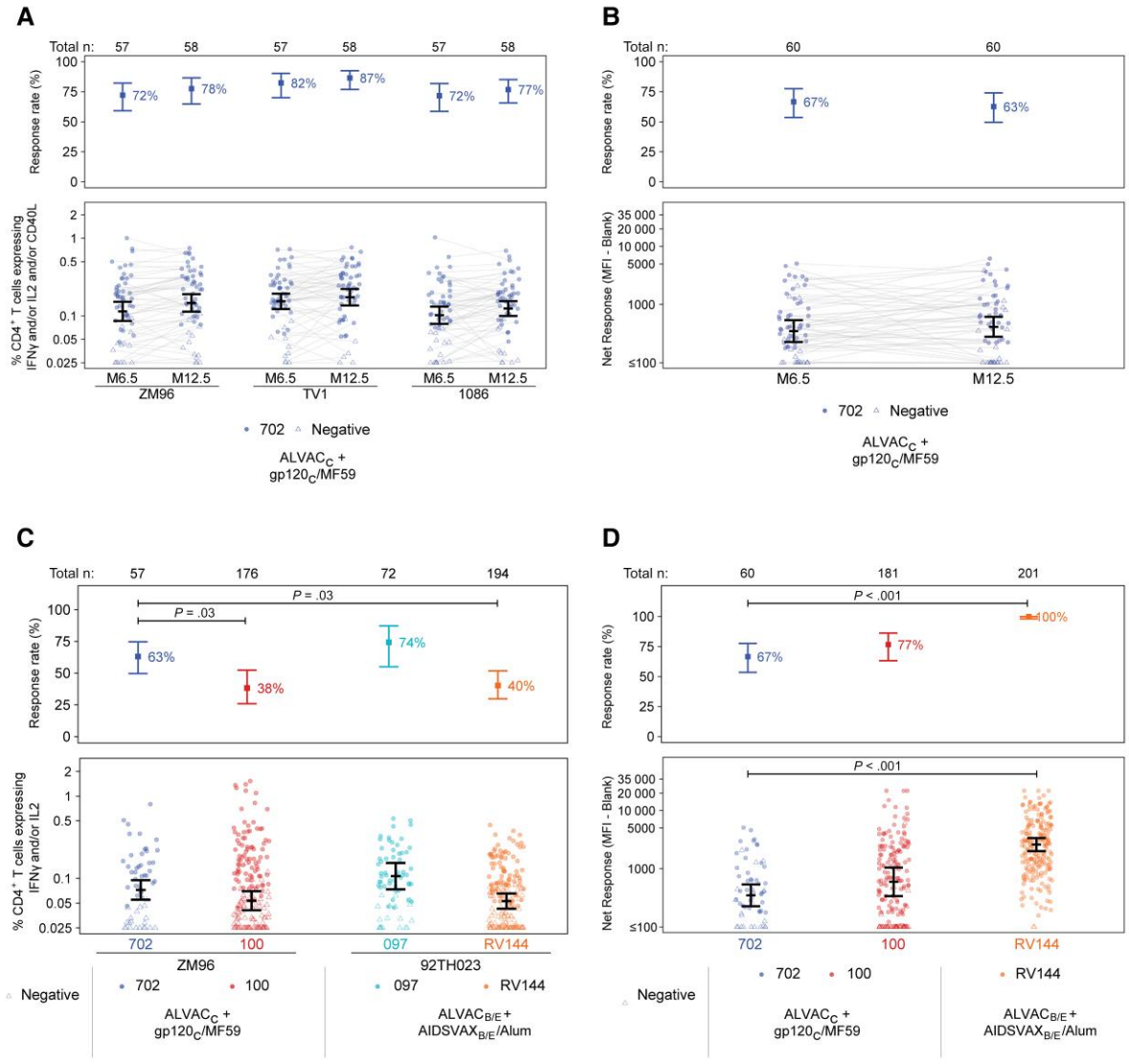

Figure 1. Characterization of HVTN 702 cellular and humoral immune responses among per-protocol vaccinated noncases and comparison with HVTN 100, HVTN 097, and RV144 per-protocol vaccinated noncases. *A*, Response rates and magnitudes of $CD4^+$ T cells expressing IFN-γ and/or IL-2 and/or CD40L among HVTN 702 vaccinated noncases, measured by intracellular cytokine staining at months 6.5 and 12.5. *B*, Month 6.5 and 12.5 IgG binding antibody responses to 1086.C V1V2, HVTN 702. *C*, Response rates and magnitudes of $CD4^+$ T cells expressing IFN-γ and/or IL-2 among HVTN 702 vaccinated noncases compared to those in HVTN 100, HVTN 097, and RV144 at month 6.5, measured by intracellular cytokine staining. *D*, Month 6.5 IgG binding antibody responses to 1086.C V1V2 in HVTN 702 compared to HVTN 100 and RV144 (HVTN 097 data not available). Positive response rates and 95% CIs in the top panels and mean magnitudes and 95% CIs in the bottom panels are estimated by targeted maximum likelihood estimation. All Holm-adjusted *P* values < .05 for HVTN 702 contrasts with earlier trials are displayed. Abbreviations: CI, confidence interval; HVTN, HIV Vaccine Trials Network; IFN-γ, interferon-γ; IgG, immunoglobulin G; IL-2, interleukin 2; MFI, mean fluorescence intensity.

gp70-TV1.GSKvacV1V2/293F, a subtype C vaccine protein-matched V1V2 antigen) was nominally significantly associated with HIV-1 acquisition among vaccine recipients in a univariate model when considered as a binary indicator (hazard ratio [HR] = 5.71; 95% CI, 1.97–16.54; $Q = 0.13$) at month 6.5. However, the number of positive responders was low among both cases (n = 9) and controls (n = 3), and this endpoint would not have passed the more stringent multiplicity correction applied to the primary/secondary correlates analysis. Moreover, the continuous version of the variable at month 6.5 was not significantly associated with HIV-1 acquisition (HR = 1.25; 95% CI, .80–1.93; $Q = 0.82$) and there were too few positive responders at month 12.5 for assessment (2 among the 34 cases, 4 among the 59 noncases; Supplementary Figure 16).

DISCUSSION

Through concerted effort and a significant body of work, immune correlates of HIV-1 acquisition risk in the RV144 trial have been established. However, it has remained an open

Table 2. Results for Month 6.5 Primary and Secondary Immune Responses of Per-Protocol Vaccine Recipients

	Response Type	Variable	HR Scale	HR (95% CI)	Adjusted *P*
Univariate results					
Primary variables	Cytokine-secreting T-cell	$CD4^+$ PFS ZM96	Per 1-SD	1.16 (.84–1.61)	.79
	Binding antibody	IgG A244 V1V2	Per 1-SD	.92 (.58–1.45)	.79
	Binding antibody	IgG3 1086 V1V2	Per 1-SD	1.28 (.83–1.98)	.79
	Cytokine-secreting T-cell	$CD4^+$ PFS ZM96	High vs low	1.67 (.65–4.26)	.19
	Cytokine-secreting T-cell	$CD4^+$ PFS ZM96	Medium vs low	2.62 (.94–7.33)	
	Binding antibody	IgG A244 V1V2	High vs low	1.45 (.54–3.89)	.12
	Binding antibody	IgG A244 V1V2	Medium vs low	.38 (.13–1.12)	
	Binding antibody	IgG3 1086 V1V2	Pos vs neg	2.77 (1.04–7.38)	.12
Secondary variables	Cytokine-secreting T-cell	$CD4^+$ PFS 1086	Per 1-SD	1.23 (.88–1.72)	1.00
	Cytokine-secreting T-cell	$CD4^+$ PFS TV1	Per 1-SD	1.20 (.83–1.73)	1.00
	Cytokine-secreting T-cell	$CD4^+$ IFN-γ^+/IL-2^+/$CD40L^+$ ZM96	Per 1-SD	1.39 (1.00–1.94)	.32
	Binding antibody	IgG A1.con.env03 140 CF	Per 1-SD	1.15 (.74–1.77)	1.00
	Binding antibody	IgG A244 gp120	Per 1-SD	1.08 (.72–1.63)	1.00
	Binding antibody	IgA score	Per 1-SD	1.01 (.64–1.58)	1.00
	Cytokine-secreting T-cell	$CD4^+$ PFS 1086	High vs low	1.32 (.52–3.37)	1.00
	Cytokine-secreting T-cell	$CD4^+$ PFS 1086	Medium vs low	1.22 (.47–3.21)	
	Cytokine-secreting T-cell	$CD4^+$ PFS TV1	High vs low	1.87 (.67–5.21)	1.00
	Cytokine-secreting T-cell	$CD4^+$ PFS TV1	Medium vs low	1.29 (.50–3.32)	
	Cytokine-secreting T-cell	$CD4^+$ IFN-γ^+/IL-2^+/$CD40L^+$ ZM96	High vs low	2.01 (.75–5.37)	1.00
	Cytokine-secreting T-cell	$CD4^+$ IFN-γ^+/IL-2^+/$CD40L^+$ ZM96	Medium vs low	1.11 (.40–3.02)	
	Binding antibody	IgG A1.con.env03 140 CF	High vs low	1.48 (.57–3.82)	1.00
	Binding antibody	IgG A1.con.env03 140 CF	Medium vs low	1.10 (.57–3.93)	
	Binding antibody	IgG A244 gp120	High vs low	1.55 (.61–3.95)	1.00
	Binding antibody	IgG A244 gp120	Medium vs low	1.50 (.40–3.01)	
	Binding antibody	IgA score	High vs low	1.45 (.53–3.97)	1.00
	Binding antibody	IgA score	Medium vs low	1.04 (.40–2.70)	
Multivariate results[a]					
Model 1, primary variables, magnitude	Cytokine-secreting T-cell	$CD4^+$ PFS ZM96	Per 1-SD	1.04 (.71–1.53)	.84
	Binding antibody	IgG A244 V1V2	Per 1-SD	.76 (.42–1.36)	.71
	Binding antibody	IgG3 1086 V1V2	Per 1-SD	1.46 (.78–2.73)	.70
Model 2, primary variables, categorical	Cytokine-secreting T-cell	$CD4^+$ PFS ZM96	High vs low	1.30 (.42–4.07)	.88
	Cytokine-secreting T-cell	$CD4^+$ PFS ZM96	Medium vs low	1.33 (.38–4.61)	
	Binding antibody	IgG A244	High vs low	1.20 (.32–4.53)	.25
	Binding antibody	IgG A244	Medium vs low	.35 (.11–1.16)	
	Binding antibody	IgG3 1086 V1V2	Pos vs neg	1.88 (.52–6.77)	.66

Univariate and multivariate Cox proportional hazards regression results are shown for the primary and secondary immune response variables, adjusted for the baseline covariates age (≤ 25, >25 years) and a previously derived categorical HIV risk score for women.

Abbreviations: CI, confidence interval; HR, hazard ratio; Neg, negative; Pos, positive.

[a]Multivariate models 1 and 2 differ with respect to the immune response variables included: model 1 includes quantitative $CD4^+$ polyfunctionality scores to ZM96, IgG binding antibody magnitude to A244, and IgG3 binding antibody magnitude to 1086 V1V2; model 2 replaces quantitative magnitude and polyfunctionality score variables with categorical low/medium/high or binary positive/negative response indicators.

question whether these correlates are generalizable to other populations and/or vaccine regimens. The HVTN 702 trial provided a unique opportunity to investigate this question in the context of a South African population vaccinated with a similar ALVAC/gp120 regimen. Several possibilities might explain why we did not identify a univariate correlation between any of the prespecified individual primary or secondary ALVAC/gp120 vaccine-induced T-cell or binding antibody immune responses and HIV-1 acquisition at either month 6.5 or 12.5. For instance, the IgG 1086 V1V2 response data are consistent with an explanation that the V1V2-directed binding antibody responses induced in HVTN 702 could have associated with HIV-1 acquisition but were of insufficient magnitude for this association to be detected. In addition, the V1V2 loop region among subtype C isolates has continued diversifying over the last decade, especially compared to the homogeneity in this region during RV144. This strain variation may also be a factor in the inability to identify associations in these nonneutralizing immune responses with HIV-1 acquisition. On the other hand, the CD4 polyfunctionality score and Env gp120-directed IgG binding antibody responses were also correlates of HIV-1 risk in RV144 and, despite high levels of these responses in HVTN 702, vaccine efficacy was not evident and, when considered univariately, these responses did not correlate with risk.

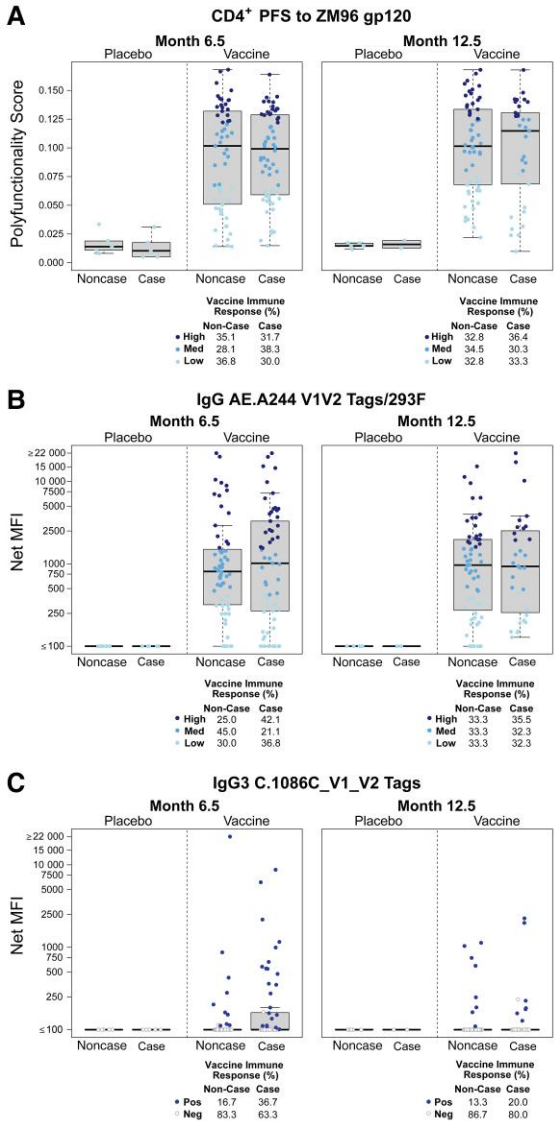

Figure 2. Distribution of primary immune response variables. Boxplots show the primary immune response variable distributions by HIV-1 acquisition status and treatment group: (*A*) CD4$^+$ polyfunctionality score to ZM96; (*B*) IgG binding antibody response to A244 V1V2; and (*C*) IgG3 binding antibody response to 1086 V1V2. *A*, Month 6.5 polyfunctionality score categories were, high, ≥0.121; med, 0.067 to <0.121; low, <0.067. Month 12.5 polyfunctionality score categories were high, ≥0.125; med, 0.080 to <0.125; low, <0.080. *B*, The positive response rates were 88% at month 6.5 and 90.1% at month 12.5. Month 6.5 binding antibody categories were high, ≥1498.83 MFI; med, 421.08 to <1498.83 MFI; low, <421.08 MFI. Month 12.5 binding antibody categories were, high, ≥1603.5 MFI; med, 468.25 to <1603.5 MFI; low, <468.25 MFI. The mid-line of the box denotes the median and the ends of the box denote the 25th and 75th percentiles. The whiskers that extend from the top and bottom of the box extend to the most extreme data points that are no more than 1.5 times the interquartile range or if no value meets this criterion, to the data extremes. Abbreviations: HIV-1, human immunodeficiency virus 1; IgG, immunoglobulin G; Med, medium; MFI, mean fluorescence intensity; Neg, negative; PFS, polyfunctionality score; Pos, positive.

Multivariate analyses of both RV144 and HVTN 505 supported that interactive combinations of antibody and T-cell responses impact HIV-1 risk [27, 28]. Our interaction results in HVTN 702 suggest that high levels of both IgG V1V2-directed binding antibodies and polyfuntional CD4$^+$ T-cell responses may indicate protection from HIV-1 acquisition. This result, however, is partnered with evidence of a significant adverse association of polyfunctional CD4$^+$ T-cell responses with HIV-1 acquisition when IgG V1V2 antibody responses are low.

The RV144 correlates of risk also may not directly translate to the HVTN 702 trial due to differences in vaccine regimen (eg, inserts, adjuvant, booster schedule), populations (eg, HIV incidence, HIV exposure, HLA background), and/or circulating viruses [29]. Different immune response types or specificities may be needed to prevent acquisition of the subtype C viruses that circulated in the HVTN 702 trial. In RV144, vaccine efficacy was greater against viruses with a vaccine-matched (vs mismatched) K169 residue [30], which was less frequent in circulating subtype C viruses than in RV144 placebo recipient CRF01_AE viruses [31]. A planned study of the HVTN 702 viral sequences may inform whether the vaccine applied immune pressure on viral genotypes. Genetic evolution in V1V2 has continued and the HVTN 702 vaccine regimen was less well matched, both overall and for the V2 region, to circulating strains in South Africa than the RV144 vaccine regimen to strains in Thailand [14]. It has also been reported that the A244 vaccine immunogen in the RV144 trial was a unique HIV-1 envelope immunogen in exposure of the V2 loop [32]. Additional study is needed to determine whether the V2-specific antibodies elicited here were to the alpha helical or beta sheet conformation; the former has been associated with decreased simian immunodeficiency virus risk [33]. In a preclinical alum-adjuvanted vaccine model, V2 antibodies correlated with reduced risk of acquisition [34, 35] (as in RV144 [16]). However, when the same preclinical vaccine was adjuvanted with MF59 [36], there were immunogenicity differences and no protection against virus acquisition [37], supporting that vaccine adjuvant significantly shapes the quality of the immune response and may impact vaccine efficacy.

The RV144 and HVTN 702 study populations also differed in the rate of HIV-1 incidence in women in the placebo group (0.3% in RV144 vs 4.2% in HVTN 702) [7], indicating that HIV-1 exposure may have been higher in the HVTN 702 trial. Immune correlates can be abrogated by frequent, heterogeneous, and/or high-pathogen-load exposure [38]. Vaccine efficacy in RV144 was higher in participants considered to be at low/moderate risk (vs high risk) of HIV-1 acquisition [9], whereas protection was not observed in any subgroup defined by baseline covariates in HVTN 702, including participants considered to be at low risk [7].

A limitation of this study was the lack of assessment of other immune biomarkers such as transcriptional signatures, mucosal responses, antibody Fc effector functions, host genetics, and genital inflammatory markers. Future work could examine

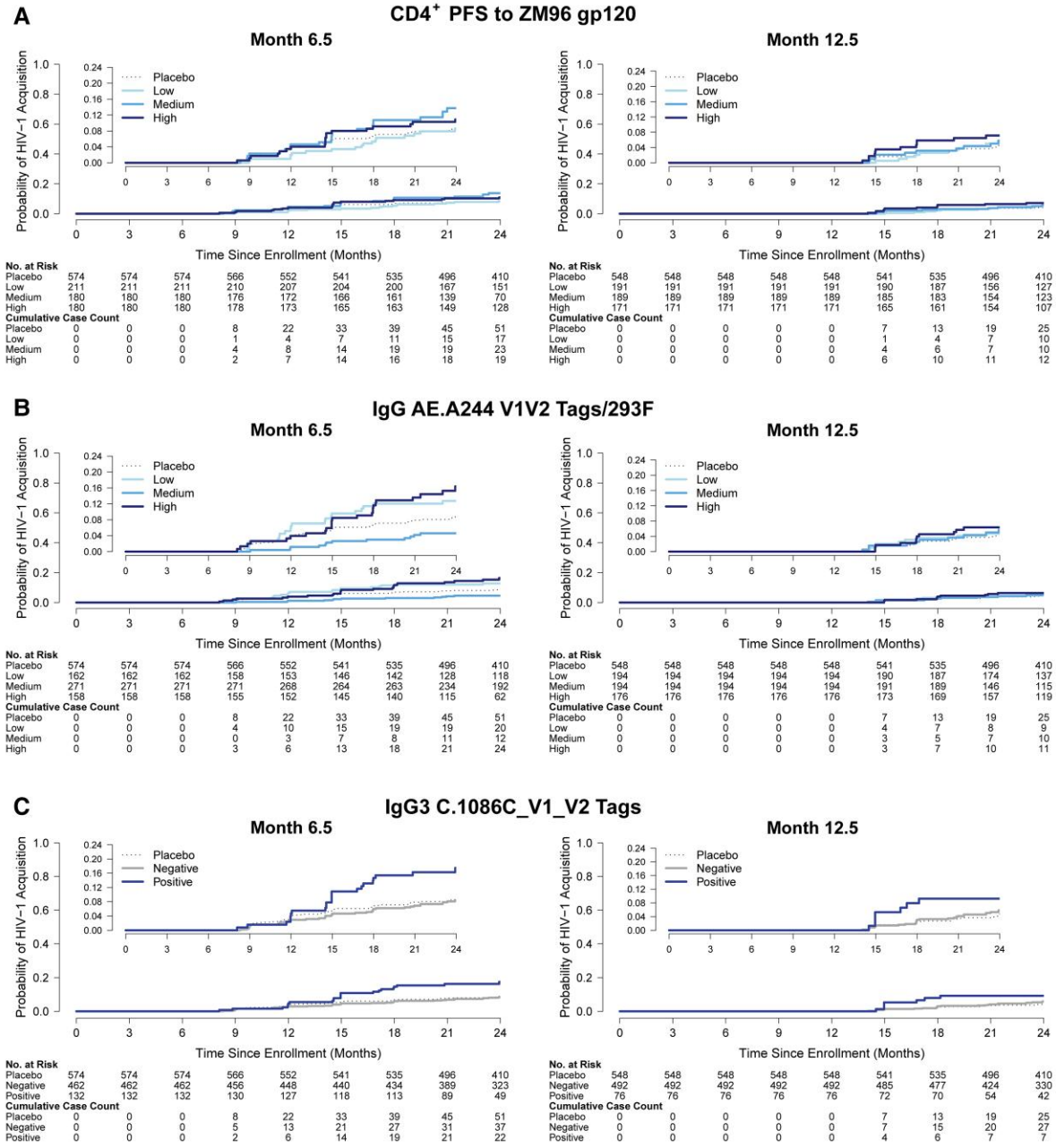

Figure 3. HIV-1 acquisition incidence by vaccine recipient immune response subgroup. Plots show the cumulative incidence of HIV-1 acquisition among per-protocol vaccine recipients by primary categorical immune response variables at month 6.5 or month 12.5: (*A*) $CD4^+$ T-cell polyfunctionality score to ZM96; (*B*) IgG binding antibody response to A244 V1V2; and (*C*) IgG3 binding antibody response to 1086 V1V2. *A*, Month 6.5 polyfunctionality score categories were high, ≥0.121; med, 0.067 to <0.121; low, <0.067. Month 12.5 polyfunctionality score categories were high, ≥0.125; med, 0.080 to <0.125; low, <0.080. *B*, The positive response rates were 88% at month 6.5 and 90.1% at month 12.5. Month 6.5 binding antibody categories were high, ≥1498.83 MFI; med, 421.08 to <1498.83 MFI; low, <421.08 MFI. Month 12.5 binding antibody categories were high, ≥1603.5 MFI; med, 468.25 to <1603.5 MFI; low, <468.25 MFI. Abbreviations: HIV-1, human immunodeficiency virus 1; IgG, immunoglobulin G; PFS, polyfunctionality score.

whether any of these biomarkers associated with HIV-1 acquisition risk in HVTN 702. Additional limitations include the lack of data on HIV-1 exposures, the exclusion of individuals assigned male at birth due to low case counts, and the inability to assess the effect of booster doses [39] due to the limited case accrual after boost receipt.

Despite these limitations, the current study contributes to the HIV-1 vaccine field by demonstrating that the T-cell and binding antibody immune correlates of risk identified in RV144 were not significantly associated with HIV-1 acquisition in HVTN 702 and remain helpful benchmarks. Furthermore, our study raises the hypothesis that moderate to high levels

Table 3. Results for Month 6.5 Immune Response Interaction Analyses of Per-Protocol Vaccine Recipients

Variable, Continuous	IgG A244 V1V2, Categorical	HR Scale for Continuous Variable	HR (95% CI)	Unadjusted *P*	Adjusted *P*
$CD4^+$ PFS ZM96	Low	Per 1-SD	2.20 (1.20–4.04)		
	Medium	Per 1-SD	0.84 (.41–1.74)	<.0001	.0017
	High	Per 1-SD	0.40 (.26–.63)		
$CD4^+$ PFS 1086	Low	Per 1-SD	3.59 (1.99–6.46)		
	Medium	Per 1-SD	1.06 (.55–2.04)	<.0001	.0002
	High	Per 1-SD	0.42 (.23–.76)		
$CD4^+$ PFS TV1	Low	Per 1-SD	2.30 (1.36–3.89)		
	Medium	Per 1-SD	0.83 (.41–1.71)	.0016	.033
	High	Per 1-SD	0.52 (.24–1.12)		
$CD4^+$ $IFNg^+/IL2^+/CD40L^+$ ZM96	Low	Per 1-SD	3.20 (1.84–5.56)		
	Medium	Per 1-SD	1.10 (.64–1.90)	<.0001	.0001
	High	Per 1-SD	0.49 (.28–0.85)		

Cox proportional hazards regression results are shown for the models with significant interactions. Each model included 1 interaction term and associated main effects, adjusted for the baseline covariates age (≤25, >25 years) and a previously derived categorical HIV risk score for women.

Abbreviations: CI, confidence interval; HR, hazard ratio.

of both Env V1V2-directed responses and high polyfunctional $CD4^+$ T cells are needed for protection against HIV-1. These results expand the scientific knowledge from this valuable HIV-1 vaccine efficacy trial and, in concert with future studies including sequence analysis of breakthrough viruses, will further guide HIV-1 vaccine development.

Supplementary Data

Supplementary material is available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author

Notes

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Potential conflicts of interest. M. K. is an employee of the GSK group of companies and holds shares in the GSK group of companies. Z. M., L. N. C., and P. B. G. received a contract from Sanofi Pasteur within the previous 3 years to conduct statistical analysis work (outside the scope of the current work; related to the CYD-TDV dengue vaccine) and submit the results

for publication. P. B. G. is also on the Bill and Melinda Gates Foundation Advisory Boards on Vaccine Development (unpaid). C. A. D. G. was an employee of Sanofi Pasteur at the time of study execution. T. D. received funding from Fred Hutchinson Cancer Center to attend the HVTN regional and full group meetings. M. A. is employed by NIAID, the study sponsor. The coauthors are current recipients of NIAID funding, and the publication is a result of activities funded by NIAID. MA was not involved in the process of funding these awards, not in their administration or scientific aspects, and, in accordance with NIAID policies, is deferred from decisions regarding funding of coauthors for a requisite period. G. D. T. received consulting fees from Janssen, Axon Consulting, and Gilead within the past 36 months; received payment for lectures, presentations, speaker bureaus, manuscript writing or educational events from the UNC Scientific Advisory Board, the NIH Board of Scientific Counselors, and Johns Hopkins within the last 36 months; and received support from her institution for attending meetings/travel to scientific network meetings. N. G. declares that Sanofi Pasteur provided the study product for the trial (ALVAC-HIV), declares that GSK provided study product for the trial (bivalent gp120/adjuvant); and is a CoVPN member representative on Protocol Team and Protocol Safety Review Team for Sanofi's phase 3 COVID-19 vaccine program with funding provided by the NIH via a grant paid to her institution. Y. H. received contracts through her institution from the World Health Organization within the previous 3 years to conduct statistical analysis work (outside the scope of the current work; related to the SARS-CoV-2 vaccine). S. C. D. R. was awarded contracts to his institution from Janssen and Battelle within the past 36 months; and was awarded grants to his institution from the Gates Medical Research Institute and the Paul G. Allen Family Foundation within the past 36 months. M. J. M. received funding from DAIDS for the HVTN Laboratory Center as Principal Investigator within the past 36 months. N. L. Y. received support from the HVTN in the form of registration payment for online conferences (paid directly to the conference). M. N. received support paid to their institution within the last 36 months from DAIDS for attending meetings/travel. J. H. and L. Z. received support from NIH NIAID to support attendance and travel to HVTN Full Group Meeting (paid directly to conference). E. L. received support from the NIH DAIDS HIV Vaccine Trials Unit in the form of salaries, equipment, supplies, and related costs to conduct the HVTN 702 clinical trial protocol at Soweto-Kliptown site. S. K. received support from the HVTN for travel and accommodation related to scientific meetings. K. E. S. received support from NIH NIAID in the form of registration payment for online conferences (paid directly to conference). J. T. is an employee of Sanofi and has performance shares awarded by the company. All other authors report no potential conflicts.

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.

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