

Assessment of the Safety and Immunogenicity of 2 Novel Vaccine Platforms for HIV-1 Prevention

A Randomized Trial

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Background: A prophylactic HIV-1 vaccine is a global health priority.

Objective: To assess a novel vaccine platform as a prophylactic HIV-1 regimen.

Design: Randomized, double-blind, placebo-controlled trial. Both participants and study personnel were blinded to treatment allocation. (ClinicalTrials.gov: NCT01215149)

Setting: United States, East Africa, and South Africa.

Patients: Healthy adults without HIV infection.

Intervention: 2 HIV-1 vaccines (adenovirus serotype 26 with an HIV-1 envelope A insert [Ad26.EnvA] and adenovirus serotype 35 with an HIV-1 envelope A insert [Ad35.Env], both administered at a dose of 5×10^{10} viral particles) in homologous and heterologous combinations.

Measurements: Safety and immunogenicity and the effect of baseline vector immunity.

Results: 217 participants received at least 1 vaccination, and 210 (>96%) completed follow-up. No vaccine-associated serious adverse events occurred. All regimens were generally well-tolerated. All regimens elicited humoral and cellular immune responses in nearly all participants. Preexisting Ad26- or Ad35-neutralizing antibody titers had no effect on vaccine safety and

little effect on immunogenicity. In both homologous and heterologous regimens, the second vaccination significantly increased EnvA antibody titers (approximately 20-fold from the median enzyme-linked immunosorbent assay titers of 30–300 to 3000). The heterologous regimen of Ad26–Ad35 elicited significantly higher EnvA antibody titers than Ad35–Ad26. T-cell responses were modest and lower in East Africa than in South Africa and the United States.

Limitations: Because the 2 envelope inserts were not identical, the boosting responses were complex to interpret. Durability of the immune responses elicited beyond 1 year is unknown.

Conclusion: Both vaccines elicited significant immune responses in all populations. Baseline vector immunity did not significantly affect responses. Second vaccinations in all regimens significantly boosted EnvA antibody titers, although vaccine order in the heterologous regimen had a modest effect on the immune response.

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The development of a prophylactic vaccine for HIV infection is a global health priority. To date, 4 concepts have been assessed for possible efficacy, and only 1 has shown modest and short-lived efficacy (1–5). A significant challenge is how to elicit robust and durable anti-HIV-1 immune responses. Various approaches are being investigated to augment these responses, including repeated vaccine administration, increased dose, cytokine coadministration, vectored delivery systems, and heterologous prime-boost strategies (6–8). This article describes a study of prime-boost regimens using 2 human adenovirus vectors (heterologous vectors), to which most persons have little or no immunity and that differ in their biological characteristics from adenovirus serotype 5 (such as use of a different primary cellular receptor and elicitation of innate cytokine responses). The vaccines each carried an HIV clade A envelope (Env) gene; however, the sequences were not matched.

Certain vaccine strategies may be limited by immunity to the delivery vector, which either is preexisting or is induced by the first immunization. Vaccine safety and tolerability may be affected by experience with the vector (9–12), and immune responses to the vector may impair responses to the vaccine insert. One strategy to avoid or minimize preexisting immunity was to use adenovirus delivery systems based on less common serotypes (13, 14) in heterologous vectored vaccine regimens. Adenovirus-vectored HIV-1 vaccines that are in development include adenovirus serotypes 26 (Ad26) (15, 16) and 35 (Ad35) (17), both of which have been

See also:

Web-Only
Supplement

EDITORS' NOTES**Context**

Although new antiviral regimens have reduced mortality due to HIV infection, an effective prophylactic vaccine is needed to control this global pandemic.

Contribution

Two candidate HIV-1 vaccines in different combinations were tested in a multicountry, randomized, controlled trial in East Africa, South Africa, and the United States.

Implication

The vaccines elicited both humoral and cellular immune responses in all populations and regardless of baseline vector immunity. Second administrations of vaccines boosted immune response, no vaccine-related serious adverse events occurred, and responses across countries varied.

shown to be protective in the nonhuman primate model and safe and immunogenic in initial phase 1 human testing. These platforms are also being developed as vaccine candidates for other pathogens (18–22).

To our knowledge, this is the first assessment of an Ad26 and Ad35 heterologous vaccine regimen, with HIV clade A Env gene inserts, in a randomized, double-blind, placebo-controlled, multicenter, international clinical trial. Our study was done in the United States, Kenya, Rwanda, and South Africa.

METHODS**Design Overview, Setting, and Participants**

This trial was a randomized, double-blind (with respect to vaccine or placebo as well as homologous or heterologous treatment groups but not to schedule), placebo-controlled, multicenter trial to evaluate the safety and immunogenicity of 2 HIV adenovirus-vectored vaccines with HIV clade A envelope inserts, Ad26.EnvA (Ad26.EnvA, Crucell Holland, now Janssen Pharmaceuticals) and Ad35.Env (Transgene), administered at a dose of 5×10^{10} viral particles in homologous (groups G, H, K, and L) and heterologous regimens (groups A to F, I, and J) at 2 schedules (0 and 3 months or 0 and 6 months) (Table). Both participants and study personnel (clinical and laboratory) were blinded to treatment allocation. Participants were healthy adults without HIV infection aged 18 to 50 years who reported low risk for HIV infection, and eligibility was not affected by preexisting natural immunity to Ad26 or Ad35. The groups allowed a comparison of homologous and heterologous regimens at the 0- and 3-month interval among African regions and between the 2 dose schedules at the U.S. clinical research center. The study was conducted at 6 clinical research centers in 4 countries and was approved by all relevant local and governmental ethics and regulatory bodies

for each clinical research center. Written informed consent was obtained from each participant.

The Table presents the modular trial schema. Homologous regimens (Ad26–Ad26 and Ad35–Ad35) had previously been assessed in the United States; therefore, comparison of these regimens was replaced with a comparison of the 0- and 3-month versus the 0- and 6-month schedule at the U.S. site (15–17). All vaccines were administered by intramuscular injection in the deltoid muscle. For full study details, see the Supplement (available at www.annals.org).

Vaccines

The Ad26.EnvA.01 vaccine was a replication-deficient adenovirus type 26 constructed to contain an HIV-1 clade A Env gene encoding a modified envelope gp140 protein (RW020 [GenBank: U08794]). The Ad35.Env vaccine was a recombinant replication-incompetent adenovirus type 35 constructed to contain an HIV-1 clade A Env gene encoding a modified gp140 protein (01TZA173 [GenBank: AY253305]). These vaccines had been developed by independent programs, but given the substantial homology between the EnvA inserts, we designed this prime-boost study. There is a 72.7% amino-acid sequence identity for aligned regions of the Ad26.EnvA and Ad35.Env. The placebo was the final formulation buffer for the Ad35 vaccine.

Safety and Immunogenicity Assessments

The primary objective of this study was to evaluate the safety and tolerability of these vaccines and regimens. Secondary analyses included immunogenicity of the heterologous regimens at 3 versus 6 months and the heterologous versus homologous regimens at the 3-month interval, as well as the effect of antivector immunity on the immune responses elicited by the vaccines and regimens. Systematic safety assessments were done. The Supplement presents details of trial schema and safety assessments. Reactogenicity and adverse events (AEs) were assessed with an adapted version of the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0.

Samples of serum and peripheral blood mononuclear cells were collected at baseline and at weeks 2, 4, 8, 24, 26, 28, 32, and 52 in the 0- and 6-month groups and at weeks 2, 4, 8, 12, 14, 16, 20, 36, and 52 in the 0- and 3-month groups, as described previously (15–17). Details of the immunogenicity assessments are given in the Supplement. In brief, direct enzyme-linked immunosorbent assay (ELISA) was performed to assess EnvA-specific serum-binding antibodies against the vaccine immunogens, with titers of 100 or greater defined as positive for either protein (15–17). T-cell responses were assessed by interferon- γ Enzyme-Linked Immunosorbent Assay (ELISpot) and an intracellular cytokine staining assay, as described previously (15–17). The criterion for positive Ad35 and Ad26 neutralizing antibody (NAb) responses was titers greater than 16. Immunology assays were performed at the following 3 centralized laboratories: the International AIDS Vaccine Initiative Human Immunology Laboratory at Imperial College

Table. Study Schema

Group, by Region	Vaccines Regimens*	Participants, n	Month			Abbreviation
			0	3	6	
United States						
A	Heterologous	11	Ad26	-	Ad35	Ad26-Ad35
B	Heterologous	10	Ad35	-	Ad26	Ad35-Ad26
C	Heterologous	10	Ad26	Ad35	-	Ad26-Ad35
D	Heterologous	9	Ad35	Ad26	-	Ad35-Ad26
East Africa						
E	Heterologous	17	Ad26	Ad35	-	Ad26-Ad35
F	Heterologous	17	Ad35	Ad26	-	Ad35-Ad26
G	Homologous	18	Ad26	Ad26	-	Ad26-Ad26
H	Homologous	16	Ad35	Ad35	-	Ad35-Ad35
South Africa						
I	Heterologous	17	Ad26	Ad35	-	Ad26-Ad35
J	Heterologous	16	Ad35	Ad26	-	Ad35-Ad26
K	Homologous	16	Ad26	Ad26	-	Ad26-Ad26
L	Homologous	16	Ad35	Ad35	-	Ad35-Ad35
Total	-	217	-	-	-	-
Active	-	173	-	-	-	-
Placebo†	-	44	-	-	-	-

Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35.

*All vaccine doses were 5×10^{10} viral particles.

†Placebo recipients were evenly divided among all groups, with 11 in the United States, 17 in East Africa, and 16 in South Africa.

(London, United Kingdom), Beth Israel Deaconess Medical Center (BIDMC) (Boston, Massachusetts), and the HIV Vaccine Trials Network Laboratory at Fred Hutchinson Cancer Research Center (Seattle, Washington). All assays were performed by personnel who were blinded to treatment allocation (23).

Statistical Analysis

All analyses are based on the as-treated population (2 participants were randomly assigned to placebo but received 2 vaccine administrations instead: 1 received Ad26-Ad35, and the other received Ad35-Ad35). All safety and immunology data (except for intracellular cytokine staining) were analyzed by the EMMES Corporation (Rockville, Maryland) with SAS, version 9.3 (SAS Institute). Intracellular cytokine staining data were analyzed by the Fred Hutchinson Cancer Research Center. The proportions of participants with events (safety and immunology) were compared by using the chi-square or Fisher exact 2-tailed test, as appropriate. Simple comparison of the magnitude of ELISpot and ELISA values was done with the Wilcoxon rank-sum test for 2 classification levels and otherwise with the Kruskal-Wallis test. To investigate the simultaneous effects of age, sex, body mass index, region, and regimen on the magnitude of ELISpot and ELISA values at specific visits, multivariable regression models of the \log_{10} response were used. There were no significant pairwise interactions; therefore, final models included only main effects. No imputation was done for missing data, which were treated as missing completely at random. A 2-sided *P* value less than 0.05 was considered significant. For more details, see the Supplement.

Role of the Funding Source

This study was funded by the International AIDS Vaccine Initiative, National Institutes of Health, and Ragon Institute in collaboration with Crucell Holland. The funding source had no role in the design or conduct of the study, analysis of the data, or decisions regarding the manuscript and its publication.

RESULTS

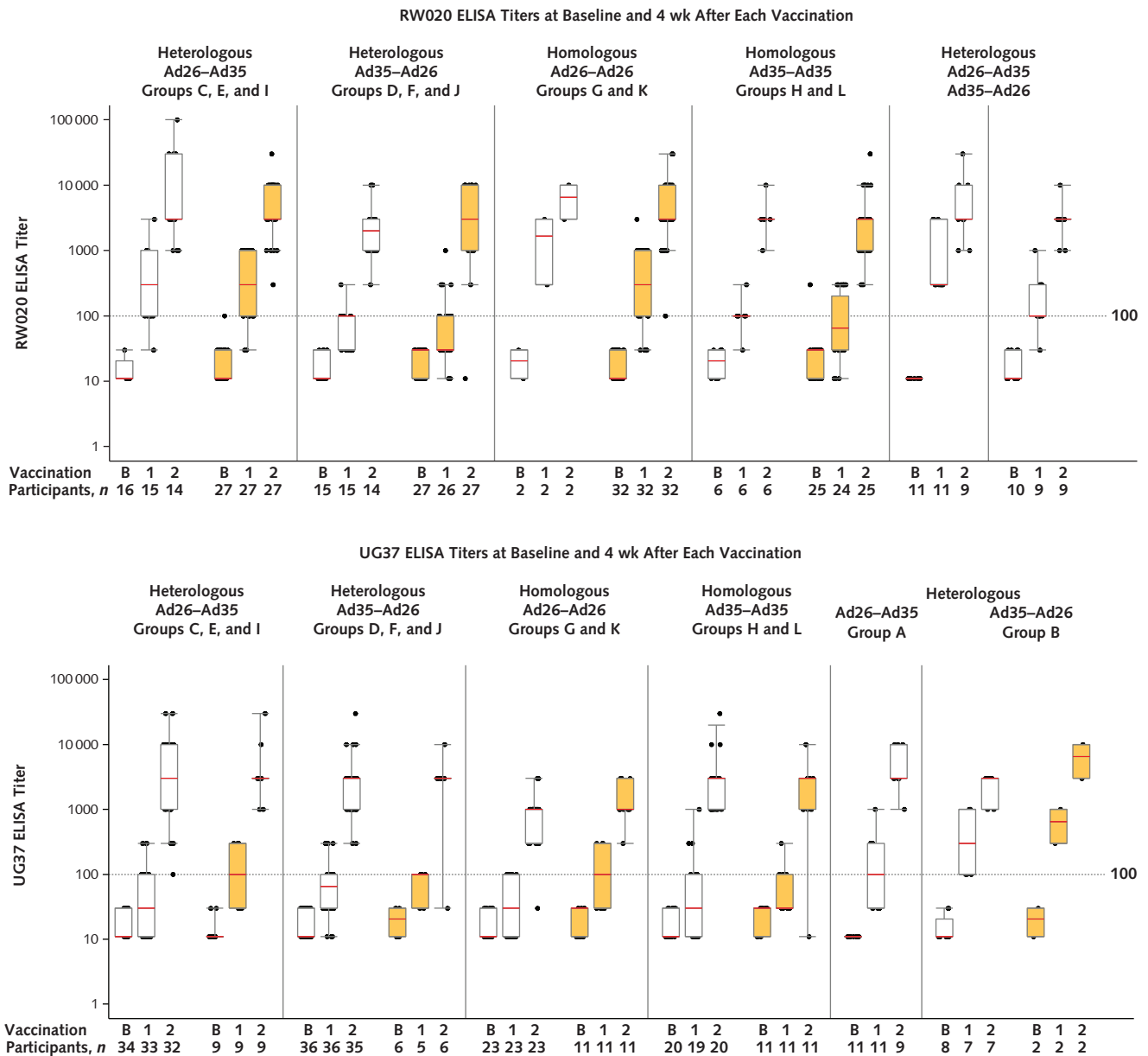
Demographic Characteristics and Disposition of Participants

The trial was conducted between October 2010 and November 2012. Of the 218 participants who were randomly assigned, 217 received at least 1 study vaccination (Figure 1 of the Supplement). Fifty-two participants (23.9%) were enrolled in Boston, 45 (20.6%) in Rwanda, 40 (18.3%) in Kenya, and 81 (37.2%) in South Africa (27 [12.4%] each in Soweto, Cape Town, and Klerksdorp). A total of 107 (49.3%) of the 217 vaccinated participants were women, and the average age was 27.0 years (range, 18 to 50 years). Across all sites, 78.3% of participants were black; in the United States, 66.7% (34 of 51) participants were white, 5 (9.8%) were Asian, and 4 (7.8%) were black (Table 1 of the Supplement). A total of 96.8% (210 of 217) of participants completed follow-up, and 208 (95.9%) received both study vaccinations (165 received the active vaccine, and 43 received the placebo).

Safety and Tolerability

Most local reactions were graded as mild or moderate. The overall frequency of any local reaction was 86.1% (95% CI, 80.1% to 90.9%) in the vaccine groups

Figure 1. Distribution of ELISA titers 4 wk after each vaccination per regimen.



Second vaccinations were at month 6 in groups A and B and at month 3 otherwise. Boxes represent the 1st and 3rd quartiles (interquartile range). Red lines indicate the median titer. Whiskers extend to the 5th and 95th percentiles. White and orange boxes represent negative and positive baseline Ad26 NAb (top) and Ad35 NAb (bottom) titer values, respectively. The dashed lines indicate the assay threshold for positivity. On the x-axis, B represents baseline, 1 represents 4 wk after the first vaccination, and 2 represents 4 wk after the second vaccination. Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35; ELISA = enzyme-linked immunosorbent assay.

and 47.7% (CI, 32.5% to 63.3%) in the placebo group (Appendix Figures 1 and 2 and Table 2 of the Supplement, available at www.annals.org). Twenty-seven of 173 participants (15.6% [CI, 10.5% to 21.9%]) in the vaccine groups and 2 of 44 participants (4.5% [CI, 0.6% to 15.5%]) in the placebo group had local reactions that were graded as moderate or severe by using the chi-square test ($P = 0.054$). The difference between individual regimens in proportion of participants with moderate or severe local reactions was not significant by using the Fisher exact 2-tailed test ($P = 0.181$).

Most systemic reactions were mild or moderate; the overall frequency of any systemic reaction was 80.9% (CI, 74.3% to 86.5%) in the vaccine groups and 68.2% (CI, 52.4% to 81.4%) in the placebo group. According to the chi-square test, there was a significant difference ($P = 0.045$) in moderate or severe systemic reactions between vaccine (63 of 173; 36.4% [CI, 29.2% to 44.1%]) and placebo (9 of 44; 20.5% [CI, 9.8% to 35.3%]) recipients but not between the vaccine regimens (chi-square $P = 0.55$). Approximately 5% (CI, 2.0% to 8.9%) of vaccine recipients ($n = 8$) reported severe

systemic reactions (6 after Ad26 and 2 after Ad35 administration, all of which occurred in participants who were seronegative to the respective adenovirus vectors at baseline). No severe reactogenicity was noted in placebo recipients. The difference between vaccine and placebo groups in the proportion of participants with moderate or more severe unsolicited AEs was not significant. No deaths or vaccine-related serious AEs were reported, and there was no apparent pattern in clinical or laboratory AEs. The Supplement presents further details of safety assessments.

Immunogenicity

HIV-1 Env-Specific Antibody Responses

Samples from 215 participants (excluding 2 who became infected with HIV) from all groups were analyzed at the BIDMC by using 2 HIV clade A envelope proteins, UG37 and RW020. Similar results were seen in analyses with UG37 at the Human Immunology Laboratory; therefore, the data from the BIDMC are reported here. No participants had baseline UG37 antibodies, 2 participants (<1%) had baseline RW020 antibodies, and no placebo recipients had responses to either Env at any time point.

Env ELISA Titer and Response Rates Across Groups.

Figure 1 and Tables 3 and 4 of the Supplement show a summary of RW020 and UG37 ELISA titers across groups, stratified by heterologous or homologous regimens and the presence or absence of Ad26 and Ad35 NAb titers at baseline. At 4 weeks after the first vaccination, median ELISA titers to Env in different groups ranged from 30 to 300 for RW020 and UG37; response rates were 49% to 100% for RW020 and 38% to 100% for UG37. After the second vaccination, titers increased approximately 20-fold to a median of 3000 for RW020 and to 1000 to 3000 for UG37 with response rates greater than 97% across the vaccination regimens.

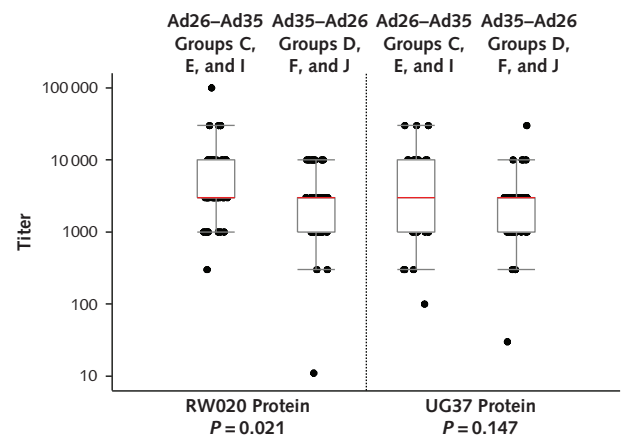
Multivariable Analysis. All vaccine regimens elicited significantly higher anti-HIV Env titers than placebo (all $P < 0.001$). No statistically clear advantage for any of the 4 short-interval regimens was seen by regimen or region. At 4 weeks after the second vaccination, a regimen with an Ad35 vaccination elicited higher UG37 responses than one without an Ad35 vaccination: Ad26-Ad26 (least-squares mean [LSM] titer, 840 [CI, 583 to 1211]) versus Ad26-Ad35 (LSM titer, 2954 [CI, 2218 to 3934]) ($P < 0.001$), Ad35-Ad26 (LSM titer, 2073 [CI, 1561 to 2753]) ($P = 0.002$), and Ad35-Ad35 (LSM titer, 2148 [CI, 1468 to 3143]) ($P = 0.003$). Of note, in the heterologous regimens, Ad26-Ad35 elicited greater RW020 responses than Ad35-Ad26 in South African participants ($P = 0.007$) (Figures 2 to 5 of the Supplement). ELISA response rates and titers across regimens, stratified by schedule, did not differ (Supplement).

Preexisting Immunity and ELISA Responses. No clear effect of preexisting NAb titers to Ad26 or Ad35 on Env ELISA responses (either response rate or magnitude) was identified after the first administration. Responses elicited for UG37 4 weeks after the first vaccination with the Ad35 vaccine (Figure 6, A, of the Supplement) were identified in 33 of 62 (53% [CI, 40% to 66%]) participants who were Ad35-seronegative at

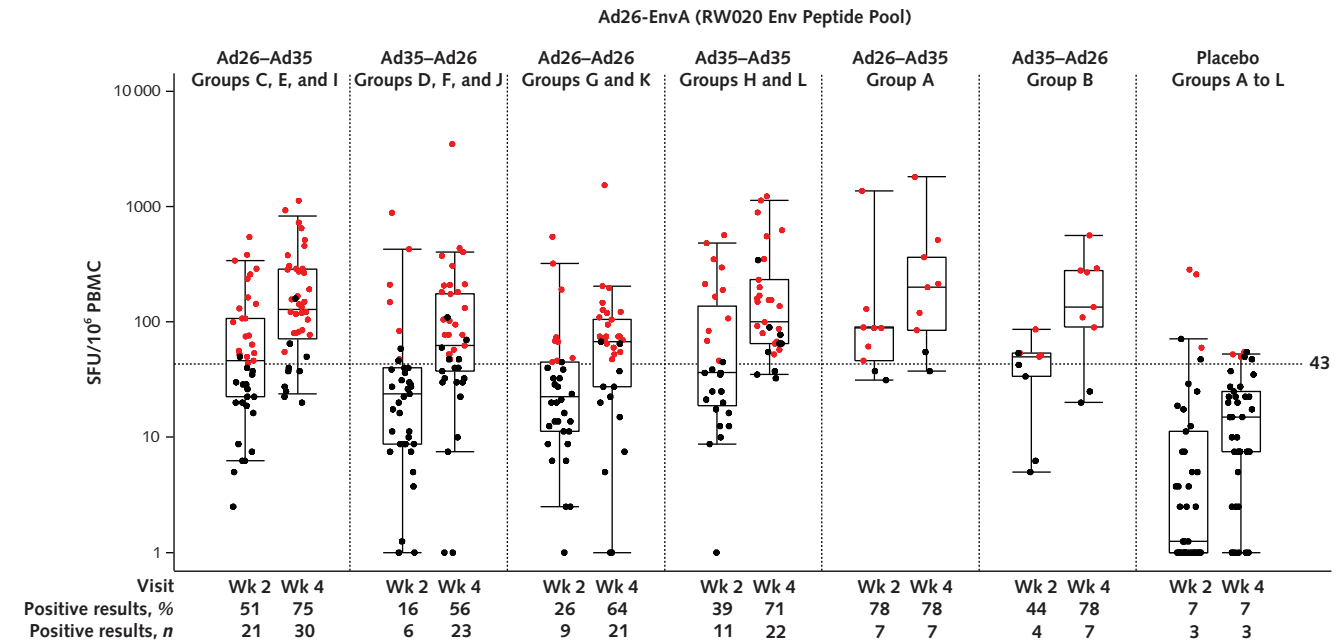
baseline versus 10 of 18 (56% [CI, 31% to 78%]) who were Ad35-seropositive on chi-square testing ($P = 0.86$). Responses elicited for RW020 4 weeks after the first vaccination with the Ad26 vaccine (Figure 6, B, of the Supplement) were identified in 27 of 28 (96% [CI, 82% to 100%]) participants who were Ad26-seronegative at baseline versus 52 of 59 (88% [CI, 77% to 95%]) who were Ad26-seropositive according to the Fisher exact 2-tailed test ($P = 0.43$). Four weeks after the second administration (homologous or heterologous), the immune response was 100% in participants who were Ad26- and Ad35-seronegative and 97% to 99% in those who were Ad35- or Ad26-seropositive on both ELISA assays in all groups (Figure 6, C and D, of the Supplement). A single dose of Ad26 elicited higher Env seroconversion rates than Ad35 (96% vs. 53% for participants who were seronegative [$P < 0.001$] and 88% vs. 56% for those who were seropositive [$P = 0.005$]).

Homologous Versus Heterologous Adenovirus-Vectored Regimens. Among the 34 participants with both samples, the median increase of 2700 (CI, 2700 to 2970) in the Env ELISA titer from the first to second vaccination with Ad26 in the Ad26-Ad26 regimen was significant ($P < 0.001$) against the matched EnvA protein; 32 of the samples were from participants who were Ad26-seropositive at baseline (Table 4 of the Supplement). Similar results were seen with the UG37 titers. The second vaccination with Ad35 in the Ad35-Ad35 regimen resulted in a median increase from baseline of 2800 (CI, 970 to 2970) (signed-rank $P < 0.001$) in ELISA titer (Figure 1, bottom) against the UG37 Env protein, and a significant median increase was seen in both participants who were Ad35-

Figure 2. Distribution of ELISA titers 4 wk after the second vaccination at month 3 in heterologous administration groups.



The 2 left boxes show RW020 ELISA titers (RW020 Env is homologous to the Ad26.EnvA insert), and the 2 right boxes show UG37 ELISA titers (UG37 Env is 81.2% homologous to the Ad35.Env insert). Boxes represent the 1st and 3rd quartiles (interquartile range). Red lines indicate the median titer. Whiskers extend to the 5th and 95th percentiles. The P values are for the comparison of the distribution of titers between the Ad26-Ad35 and Ad35-Ad26 sequences. Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35; ELISA = enzyme-linked immunosorbent assay.

Figure 3. Distribution of interferon- γ ELISpot responses 2 and 4 wk after second vaccination: Ad26.EnvA.

The x-axis shows the study group (treatment), percentage of participants, and frequency of positive responses. Samples at weeks 2 and 4 were analyzed at the Human Immunology Laboratory and the Beth Israel Deaconess Medical Center, respectively. Second vaccinations were at month 6 in groups A and B and at month 3 otherwise. All responses were background-subtracted. Mean responses <1 were set to 1. Boxes show median and interquartile range. Whiskers extend to the 5th and 95th percentiles. Black and red dots represent negative and positive responses, respectively. The dashed line indicates the minimal threshold for positivity for each assay (43 SFU/10⁶ PBMC). Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35; ELISA = enzyme-linked immunosorbent assay; ELISpot = Enzyme-Linked ImmunoSpot; Env = envelope; EnvA = envelope clade A; PBMC = peripheral blood mononuclear cell; SFU = spot forming unit.

seropositive at baseline ($n = 11$) (2700 [CI, 970 to 9900]; $P = 0.002$) and those who were Ad35-seronegative ($n = 19$) (2900 [CI, 989 to 2970]; $P < 0.001$) (Figure 1, bottom, and Tables 3 and 4 and Figure 7 of the Supplement). Similar results were seen for RW020 titers. For early-boost, heterologous regimens, higher titers were seen with the Ad26-Ad35 regimen than with the Ad35-Ad26 regimen; this was statistically significant for the RW020 Env ELISA (matched to Ad26) (geometric mean titer [GMT], 4696 [CI, 3213 to 6865] vs. 2274 [CI, 1507 to 3431]; $P = 0.021$) but not for the UG37 ELISA using Env matched to the Ad35 vaccine (GMT, 2819 [CI, 1888 to 4208] vs. 1959 [CI, 1365 to 2811]; $P = 0.150$) (Figure 2 and Table 3 of the Supplement). Therefore, the regimen with Ad26 prime and Ad35 boost induced more Env-binding antibodies than the reverse order. Further studies are under way to characterize the function of the antibodies elicited.

HIV-1 Env-Specific Cellular Immune Responses

Peripheral blood mononuclear cell samples from all groups were analyzed by ELISpot at the Human Immunology Laboratory (201 participants 2 weeks after the second vaccination) and at the BIDMC (204 participants 4 weeks after the second vaccination). Figure 3 and Appendix Figure 3 (available at www.annals.org) presents the results for each of the 3 peptide pools assessed (2 pools matched to the Ad35-Env, Ad35-Env P1 and Ad35-Env P2 [TZA173], and 1 pool matched to

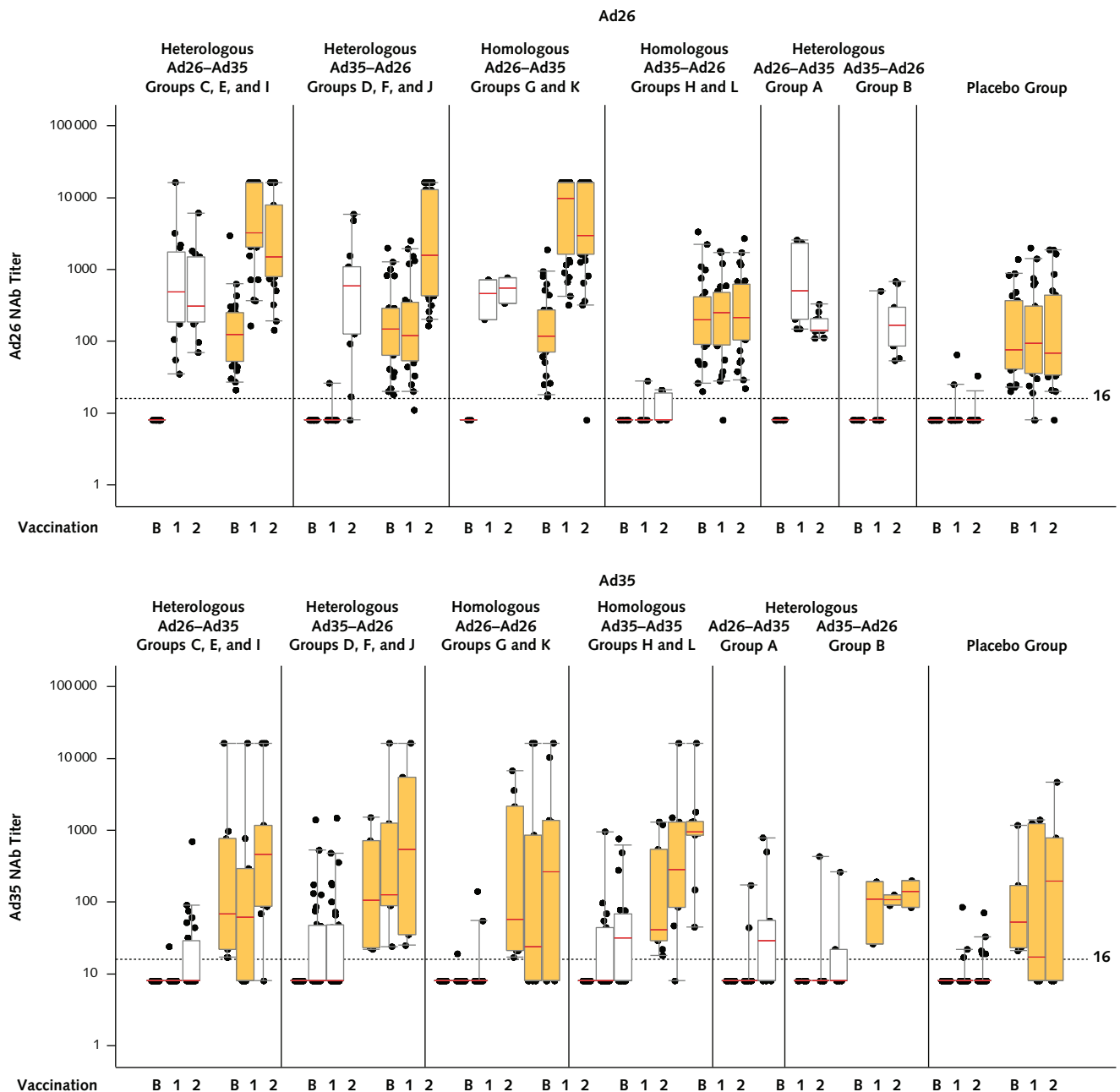
the Ad26-Env [RW020]) (Figure 8 of the Supplement). Overall, the ELISpot magnitude and response rates were higher at 4 weeks than 2 weeks after the second vaccination regardless of homologous or heterologous vaccine regimens or order of the adenovirus vectors. The results across regimens were similar at 2 weeks after the second vaccination, and the results at 4 weeks after the second vaccination are described later. Further analysis of responses to ELISpot and intracellular cytokine staining are described in Table 5 and Figure 9 of the Supplement.

At 4 weeks after the vaccination, all regimens elicited significantly higher ELISpot responses than placebo by all 3 peptide pools (Ad26 EnvA, Ad35 EnvA P1, and Ad35 EnvA P2) ($P < 0.001$) except for the Ad26-Ad26 regimen by the Ad35 pools. Regimens with an Ad35 vaccination had stronger responses detected with the Ad35 peptide pools than the Ad26-Ad26 vaccination, and this was even stronger when 2 doses of Ad35 were given than 1 dose in a heterologous regimen. East African participants had lower responses by all 3 peptide pools (Figures 10 to 12 of the Supplement). The 3- and 6-month regimens did not differ significantly.

NAb Responses to the Adenovirus Vectors

Neutralization of adenovirus was used to assess baseline and vaccine-induced responses to the vector.

Figure 4. Ad26 and Ad35 neutralizing titers.



Individual Ad26 and Ad35 NAb responses from participants at baseline and 4 wk after the first and second vaccination are shown. Second vaccinations were at month 6 in groups A and B and at month 3 otherwise. White and orange boxes represent negative and positive baseline Ad26 (top) and Ad35 (bottom) neutralizing antibodies, respectively. Boxes represent the median, interquartile range, and 5th and 95th percentiles of the titer distribution. The dashed lines indicate the assay threshold for positivity. On the x-axis, B represents baseline, 1 represents 4 wk after the first vaccination, and 2 represents 4 wk after the second vaccination. Placebo responses represent the proportion of participants with a positive titer at any time. Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35; NAb = neutralizing antibody.

Of the 215 samples analyzed, 134 participants (62%) had positive Ad26 titers (GMT, 144 [CI, 117 to 177]) and 46 (21%) had positive Ad35 titers (GMT, 115 [CI, 67 to 199]) at baseline (Figure 4 and Table 6 of the Supplement). At 4 weeks after the first vaccination, Ad26 NAb titers were detected in more samples to Ad26 (100%) than Ad35 (64%) ($P < 0.001$). At 4 weeks after

the second vaccination, the proportions with detectable titers did not differ significantly, and 90% or more were positive in all active vaccine groups or regimens (Figure 4). After a single vaccination, the magnitude of Ad26 NAb titers was higher than that of Ad35 NAb titers (GMT, 2270 vs. 184) ($P < 0.001$). After the second vaccination in African participants, 2 Ad26.EnvA vacci-

nations induced a higher Ad26 NAb titer (GMT, 3815) than a single Ad26 vaccination, regardless of whether it was the first (GMT, 1320) or second (GMT, 1210) vaccination (Kruskal-Wallis, $P = 0.003$). At 4 weeks after the first Ad35.Env vaccination, Ad35 NAb titers were detected in 46% of participants who were Ad35-naive (GMT, 181) (Figure 4). At 4 weeks after the second vaccination, the highest proportion of positive Ad35 NAb titers was in the homologous Ad35 group (71% after the second vaccination), which was significantly greater ($P = 0.010$) than the proportion in the groups with homologous Ad26 or heterologous regimens; the differences among these 3 groups was not significant).

DISCUSSION

To our knowledge, this is the first heterologous, adenovirus-vectored HIV-1 vaccine study conducted in the United States and sub-Saharan Africa. All vaccine regimens were well-tolerated and elicited both humoral and cellular immune responses. Both heterologous and homologous regimens significantly increased humoral Env responses. Presence of baseline antivector (Ad26 or Ad35) NAb titers did not significantly influence the anti-Env immune responses elicited. Safety was similar across the 3 geographic regions.

The 5×10^{10} -viral particle dose of the Ad26.EnvA and Ad35.Env-vectored vaccines given individually and in combination was generally well-tolerated. Reactions reported in the vaccine groups increased slightly compared with placebo but with no evidence for serious AEs, increased AEs, or laboratory abnormalities related to the vaccine. Severe reactions were seen in 8 participants, and all occurred in participants who were seronegative for the respective adenovirus vaccine vector serotypes at baseline and were brief and self-limited. These findings are consistent with prior adenovirus-based vaccines, which led to some severe but transient systemic reactions, primarily at doses of 10^{11} viral particles (3, 10, 12, 15, 17).

The 3-month regimen seems to have similar humoral and cellular immunogenicity as the 6-month regimen. A shorter vaccination schedule could have substantial benefits clinically and in vaccine development. Thus, further evaluation of a shorter vaccination schedule should be studied, including evaluating the durability of immune responses.

Both homologous and heterologous vector boosting of humoral immune responses was seen (approximately 20-fold), which was not significantly affected by the presence of adenoviral vector immunity to the homologous serotype at baseline. Of note, increased Env antibody titers were elicited in the Ad26-Ad35 compared with Ad35-Ad26 heterologous regimens. This effect of vector order on antibody responses is similar to that seen in preclinical models and raises important considerations in defining the optimal regimen for candidate vaccines in human trials.

T-cell response magnitudes by ELISpot and flow cytometry were modest, with some differences across regimens and dependent on whether matched or mis-

matched peptides were used. This was most apparent for the Ad35 homologous groups in which both higher ELISpot magnitude and response rates were found. This can partly be explained because Ad26.EnvA in a homologous prime-boost regimen has been shown to induce T-cell responses to an average of only 1 Env epitope per participant, whereas an average of 2 Env epitopes per participant was seen after Ad35.Env homologous boosting. Epitope mapping from samples would be required to confirm whether responses are boosted or whether new epitopes are recognized after the second vaccination in the different homologous and heterologous regimens. The differences between the 2 EnvA inserts used in the heterologous vaccine regimens may have amounted to 2 primes rather than a prime boost. East African participants had lower ELISpot responses; this requires further study and highlights the importance of assessing vaccine candidates in target populations.

Both vectored vaccines consistently elicited EnvA humoral and cellular immune responses after a single vaccination, which was not significantly impaired by preexisting antivector NAb titers. In addition, a homologous boost significantly increased humoral responses despite eliciting significant vector-specific NAb titers after the first vaccination. The Ad26-vectored vaccine elicited detectable ELISA responses both in participants who were Ad26-seropositive and in those who were Ad26-seronegative at baseline more often than the Ad35-vectored vaccine. The lack of detectable effect of baseline Ad26 and Ad35 NAb titers on the elicitation of EnvA responses is different from what has been reported for adenovirus serotype 5-vectored vaccines. The reasons for this difference are unclear; it may be due to the biological differences between these adenovirus serotypes or the lower NAb titers to these less common adenovirus serotypes (Ad26 or Ad35). Taken together, neither preexisting nor vaccine-elicited antivector immune response seemed to blunt elicitation of insert-specific responses.

These data demonstrate that humoral HIV-1 responses after an adenovirus-based vaccine can be boosted with a homologous or heterologous (vector and insert) adenovirus vaccine, preexisting vector immunity does not seem to affect safety or immunogenicity, and vaccine order can significantly affect immune responses. T-cell response rates and magnitudes by ELISpot and flow cytometry were modest and similar across regimens, with some differences depending on whether matched or mismatched Env peptides were used and by region. Both heterologous and homologous adenovirus vaccine regimens are promising vaccination strategies.

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Reproducible Research Statement: *Study protocol:* See the Protocol section of the **Supplement** (available at www.annals.org). *Statistical code and data set:* Available from Dr. Baden (e-mail, lbaden@partners.org), Dr. Laufer (e-mail, dlauffer@iavi.org), or Mr. Dally (e-mail, ldally@emmes.com) for those who meet criteria for access to the data.

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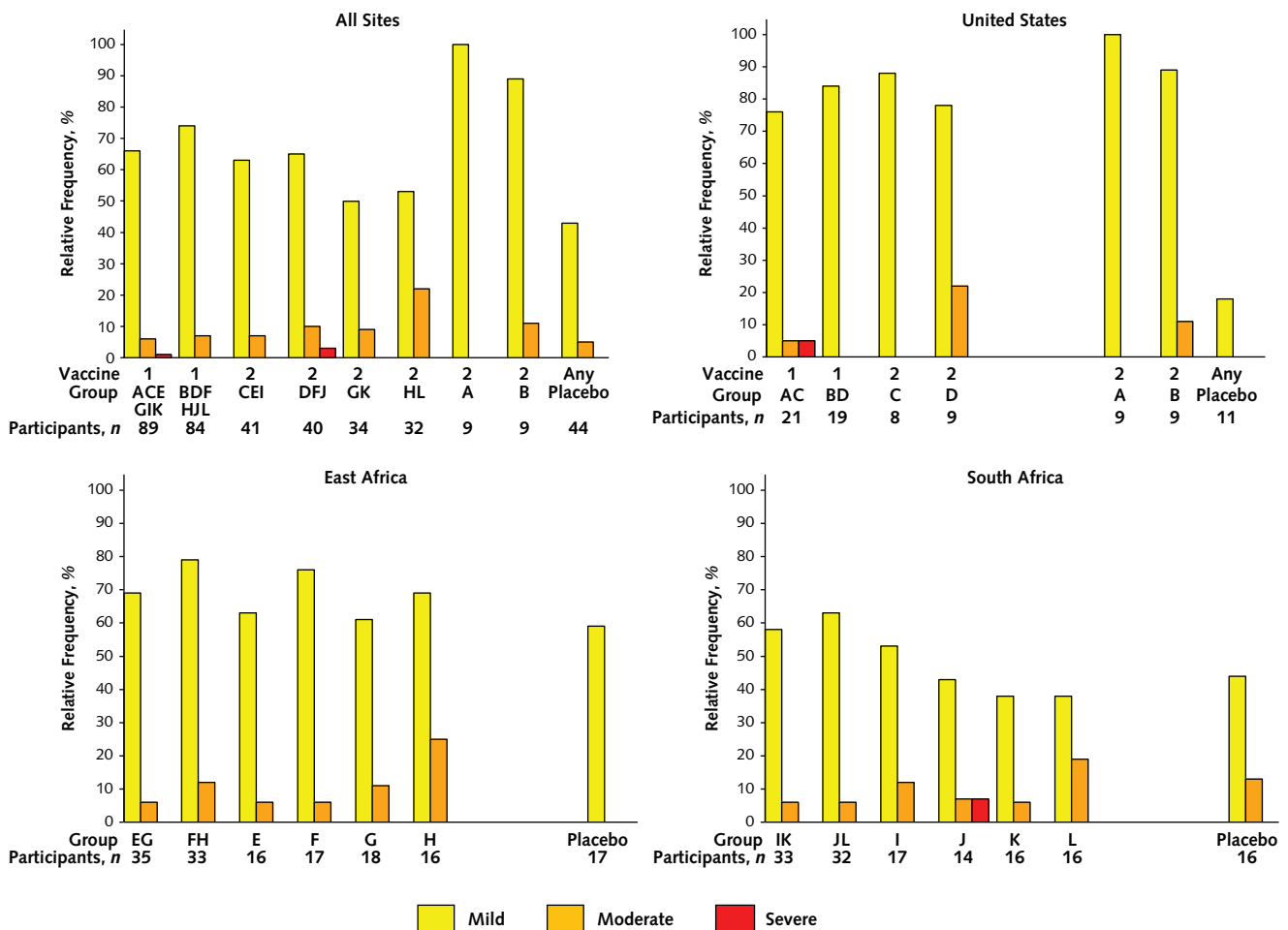
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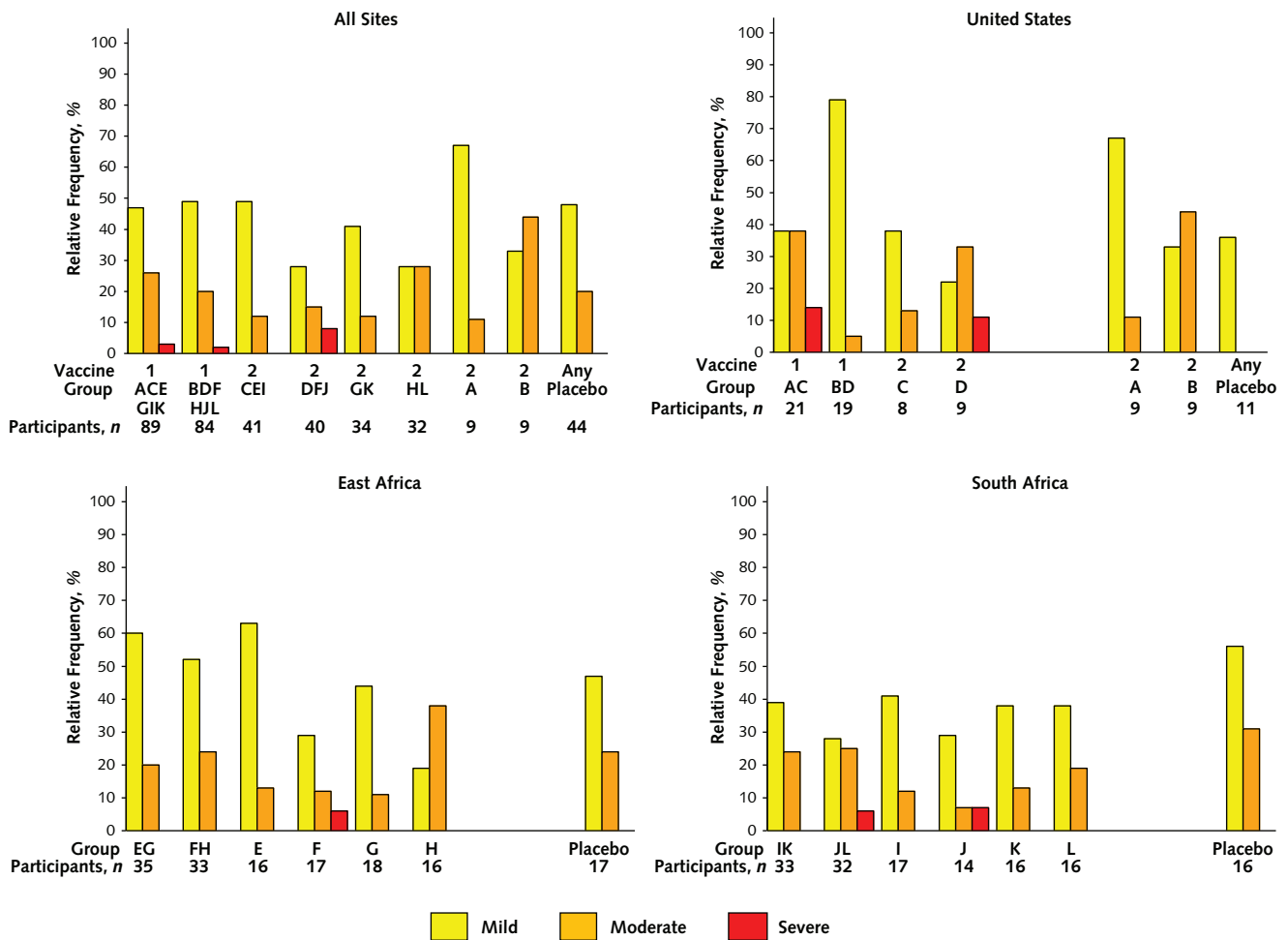
Appendix Figure 1. Maximum local reactions.



Group	Regimen	Time of 2nd Dose
A	Ad26-Ad35	6 mo
B	Ad35-Ad26	6 mo
C, E, I	Ad26-Ad35	3 mo
D, F, J	Ad35-Ad26	3 mo
G, K	Ad26-Ad26	3 mo
H, L	Ad35-Ad35	3 mo

Data from all first vaccinations were pooled on the basis of the vaccine received and then respective homologous and heterologous regimens were examined. The y-axis represents the percentage of participants having reactogenicity events and the x-axis the study groups. Reactions are shown through day 7 after each vaccination by study group for all sites combined and by region. Participants self-assessed reactogenicity with a memory aid on day 0 (evening of vaccine/placebo administration) and daily through day 7. Safety data for placebos by study group for all sites combined and by region are shown in the far right column of each quadrant. The maximum severity assessment grade is shown. The severity grade of the reactogenicity events is indicated by colors (yellow indicates mild, orange indicates moderate, and red indicates severe). Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35.

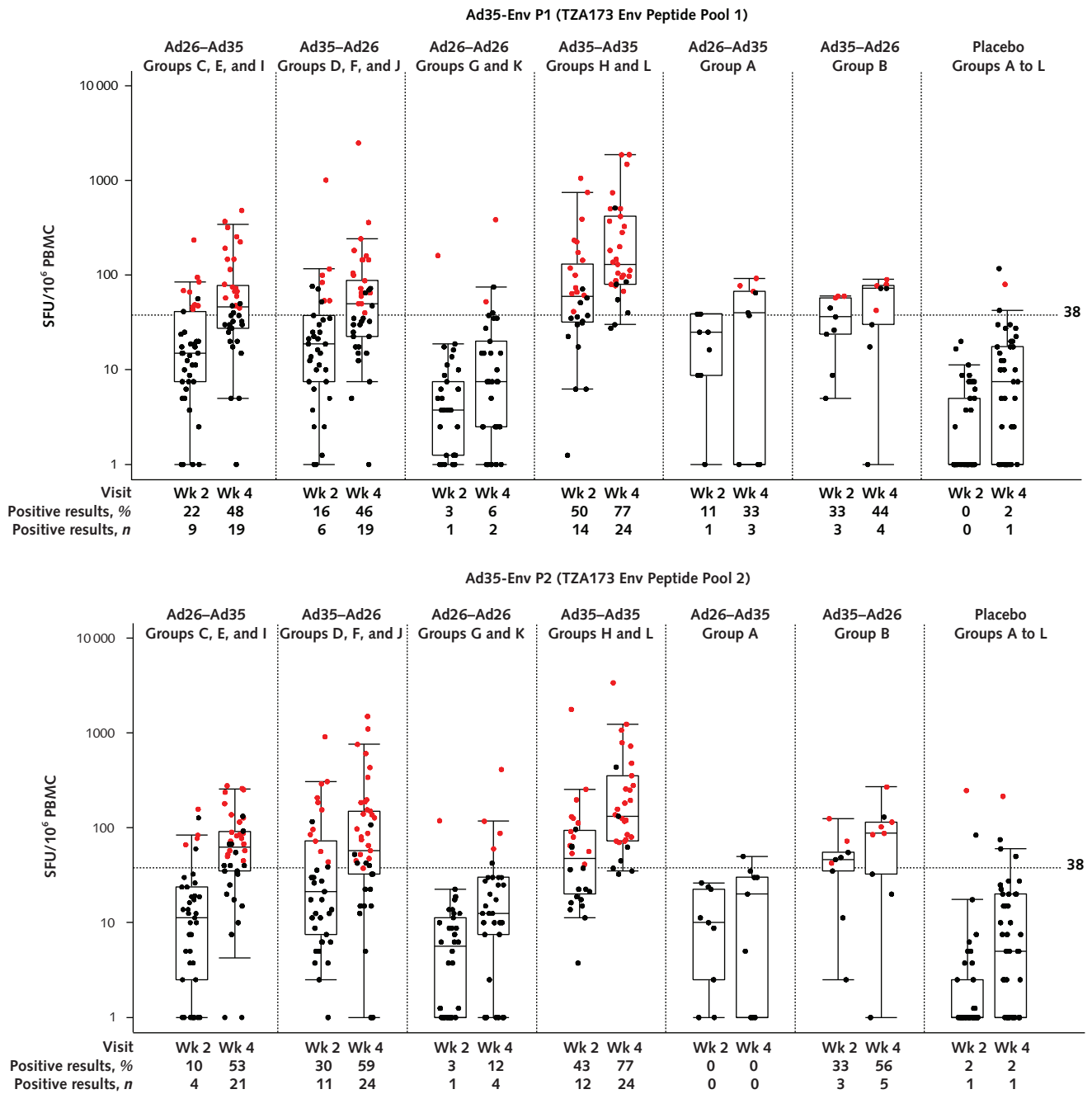
Appendix Figure 2. Maximum systemic reactions.



Group	Regimen	Time of 2nd Dose
A	Ad26-Ad35	6 mo
B	Ad35-Ad26	6 mo
C, E, I	Ad26-Ad35	3 mo
D, F, J	Ad35-Ad26	3 mo
G, K	Ad26-Ad26	3 mo
H, L	Ad35-Ad35	3 mo

Data from all first vaccinations were pooled on the basis of the vaccine received and then respective homologous and heterologous regimens were examined. The y-axis represents the percentage of participants having reactogenicity events and the x-axis the study groups. Reactions are shown through day 7 after each vaccination by study group for all sites combined and by region. Participants self-assessed reactogenicity with a memory aid on day 0 (evening of vaccine/placebo administration) and daily through day 7. Safety data for placebos by study group for all sites combined and by region are shown in the far right column of each quadrant. The maximum severity assessment grade is shown. The severity grade of the reactogenicity events is indicated by colors (yellow indicates mild, orange indicates moderate, and red indicates severe). Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35.

Appendix Figure 3. Distribution of interferon- γ ELISpot responses 2 and 4 wk after second vaccination: Ad35.Env P1 (top) and Ad35.Env P2 (bottom).



The x-axis shows the study group (treatment), percentage of participants, and frequency of positive responses. Samples at weeks 2 and 4 were analyzed at the Human Immunology Laboratory and the Beth Israel Deaconess Medical Center, respectively. Second vaccinations were at month 6 in groups A and B and at month 3 otherwise. All responses were background-subtracted. Mean responses <1 were set to 1. Boxes show median and interquartile range. Whiskers extend to the 5th and 95th percentiles. Black and red dots represent negative and positive responses, respectively. The dashed lines indicate the minimal threshold for positivity for each assay (38 SFU/10⁶ PBMC). Ad26 = adenovirus serotype 26; Ad35 = adenovirus serotype 35; ELISA = enzyme-linked immunosorbent assay; ELISpot = Enzyme-Linked ImmunoSpot; Env = envelope; PBMC = peripheral blood mononuclear cell; SFU = spot forming unit.