Safety and Immunogenicity of a Replication-Incompetent Adenovirus Type 5 HIV-1 Clade B *gag/pol/nef* Vaccine in Healthy Adults

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Background. The safety and immunogenicity of the MRK adenovirus type 5 human immunodeficiency virus type 1 clade B *gag/pol/nef* vaccine, a replication-incompetent adenovirus type 5–vectored vaccine designed to elicit cell-mediated immunity against conserved human immunodeficiency virus proteins, was assessed in a phase 1 trial.

Methods. Healthy adults not infected with human immunodeficiency virus were enrolled in a multicenter, dose-escalating, blind, placebo-controlled study to evaluate a 3-dose homologous prime-boost regimen of the trivalent MRK adenovirus type 5 human immunodeficiency virus type 1 vaccine containing from 3×10^6 to 1×10^{11} viral particles per 1-mL dose administered on day 1, during week 4 and during week 26. Adverse events were recorded for 29 days after each intradeltoid injection. The primary immunogenicity end point was the proportion of study participants with a positive unfractionated Gag-, Pol-, or Nef-specific interferon-γ enzyme-linked immunosorbent spot response measured 4 weeks after administration of the last dose.

Results. Of 259 randomized individuals, 257 (99%) received $\geqslant$1 dose of vaccine or placebo and were included in the safety analyses. Enzyme-linked immunosorbent spot results were available for 217 study participants (84%) at week 30. No serious vaccine-related adverse events occurred. No study participant discontinued participation because of vaccine-related adverse events. The frequency of injection-site reactions was dose dependent. Vaccine doses of $\geqslant 3 \times 10^9$ viral particles elicited positive enzyme-linked immunosorbent spot responses to $\geqslant$1 vaccine component in >60% of recipients. High baseline antibody titers against adenovirus type 5 diminished enzyme-linked immunosorbent spot responses at all doses except the 3×10^{10} viral particle dose.

Conclusions. The vaccine was generally well tolerated and induced cell-mediated immune responses against human immunodeficiency virus type 1 peptides in most healthy adults. Despite these findings, vaccination in a proof-of-concept trial with use of this vaccine was discontinued because of lack of efficacy.

The HIV pandemic continues to cause devastating morbidity and mortality [1, 2]. Spread of multidrug-resistant HIV increasingly threatens the usefulness of current antiretroviral regimens [3–10]. Safe and effective vaccines for both prevention and treatment are urgently needed [11–14]. Even if a vaccine only slowed disease progression, its potential benefit in conjunction with other prophylactic and therapeutic interventions could be substantial [15–18].

The correlate of immune protection against HIV re-

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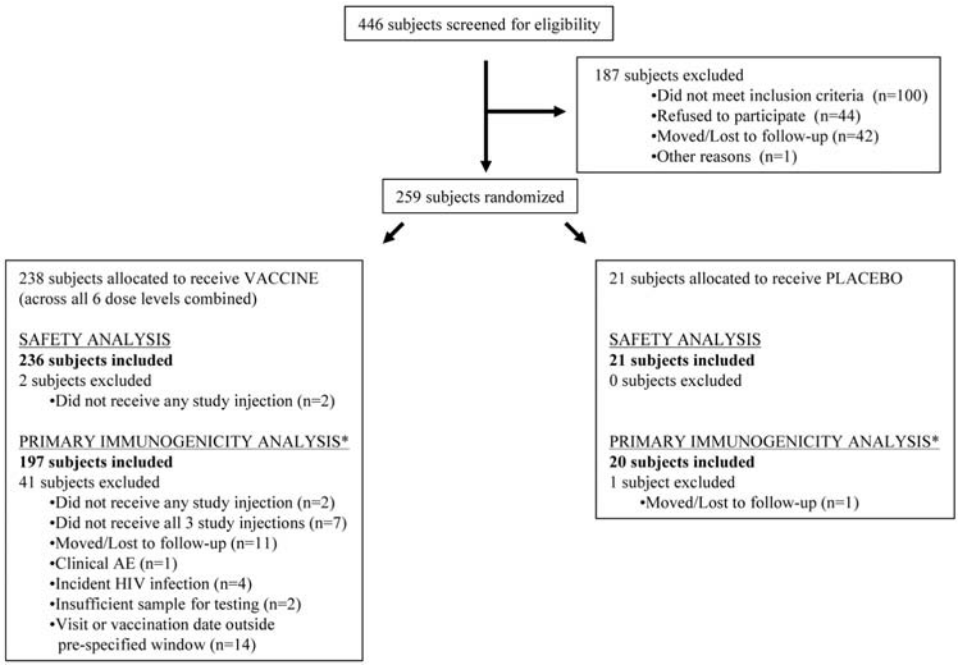

Figure 1. Randomization of study participants. *Primary immunogenicity analysis performed at week 30 (4 weeks after the third injection).

mains unknown [13, 19–24]. Virus-specific $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ helper T lymphocytes appear to be critical for control of simian immunodeficiency virus and HIV infections [24–26]. Vaccines eliciting cell-mediated immunity to conserved HIV peptides may be effective in both the prevention and control of natural infection [20, 22, 23, 25, 27–44]. Vaccination with attenuated adenovirus type 5 (Ad5) vectors expressing HIV-1 *gag* has variably elicited strong cytotoxic T lymphocyte responses in primate models [45–50]. Broad cell-mediated immunity responses against multiple viral determinants may be more efficacious in preventing or slowing HIV infection [12, 14, 28, 51].

We conducted a dose-ranging study of an HIV-1 vaccine, using a replication-incompetent MRKAd5 vector encoding HIV-1 *gag, pol,* and *nef* genes on the basis of near-consensus clade B sequences. Because immunity to Ad5 varies among populations and could affect vaccine responses [52], safety and immunogenicity were evaluated in participants with and without preexisting Ad5 immunity. During preparation of this report, enrollment in a proof-of-concept trial with use of this vaccine to prevent or modulate HIV-1 infection was suspended because of lack of efficacy.

METHODS

Vaccine Composition

The MRKAd5 HIV-1 clade B *gag/pol/nef* vaccine consists of equal parts of 3 recombinant Ad5 vectors: MRKAd5gag [53], MRKAd5pol, and MRKAd5nef. The E1 region of the Ad5 vector was deleted, which rendered the virus incapable of growing in human cells, and was replaced with a transgene construct that consisted of the human cytomegalovirus promoter [54]; the HIV-1 clade B *gag, pol,* or *nef* open reading frame; and the bovine growth hormone poly A [55]. The open reading frames encode Gag from CAM-1, Pol (including only the reverse transcriptase and integrase gene products) from IIIB, and Nef from JRFL strains [56]. Gene sequences were codon optimized to enhance expression in mammalian cells [57]. The *pol* transgene segment was inactivated by substituting alanine codons for amino acids at enzymatically active sites [58–64], and the *nef* transgene segment was inactivated through substitutions, which prevents attachment to the cytoplasmic membrane and retrotrafficking into endosomes [51]. The vaccine was formulated in 10 mmol TRIS buffer with 10 mmol histidine, 5% sucrose, 75 mmol sodium chloride, 1 mmol magnesium chloride, 0.1 mmol EDTA, 0.5% ethanol, and 0.02% polysorbate 80 (pH 7.4). Placebo consisted of an identical vehicle.

Objectives

The primary objectives of the study were to assess the safety, tolerability, and immunogenicity of a 3-dose regimen of the vaccine administered on day 1, at week 4, and at week 26 at doses of 3×10^6, 3×10^7, 3×10^8, 3×10^9, 3×10^{10}, and 1×10^{11} total viral particles. Study participants were to be followed up for adverse events for 29 days after each dose. The primary immunogenicity end point was the proportion of study participants with a positive unfractionated Gag-, Pol-, or Nef-

Table 1. Baseline characteristics by treatment group.

Variable	Placebo recipients (n = 21)	Dose of HIV-1 MRKAd5 *gag/pol/nef* vaccine						
		3×10^6 (n = 42)	3×10^7 (n = 42)	3×10^8 (n = 42)	3×10^9 (n = 42)	3×10^{10} (n = 39)	1×10^{11} (n = 31)	Total (N = 259)
Sex								
Female	15 (71.4)	21 (50.0)	14 (33.3)	17 (40.5)	21 (50.0)	12 (30.8)	14 (45.2)	114 (44.0)
Male	6 (28.6)	21 (50.0)	28 (66.7)	25 (59.5)	21 (50.0)	27 (69.2)	17 (54.8)	145 (56.0)
Age, median years (range)	30.0 (20–48)	32.5 (19–50)	31.5 (19–50)	33.5 (18–50)	33.5 (18–50)	40.0 (19–50)	35.0 (19–50)	34.0 (18–50)
Ethnicity								
White	17 (81.0)	36 (85.7)	30 (71.4)	32 (76.2)	28 (66.7)	30 (76.9)	27 (87.1)	200 (77.2)
Black	3 (14.3)	4 (9.5)	6 (14.3)	7 (16.7)	7 (16.7)	5 (12.8)	3 (9.7)	35 (13.5)
Hispanic	0 (0.0)	1 (2.4)	5 (11.9)	2 (4.8)	3 (7.1)	2 (5.1)	0 (0.0)	13 (5.0)
Other	1 (4.8)	1 (2.4)	1 (2.4)	1 (2.4)	4 (9.5)	2 (5.1)	1 (3.2)	11 (4.3)
Baseline anti-Ad5 antibody titer								
≤200	14 (66.7)	26 (61.9)	23 (54.8)	25 (59.5)	25 (59.5)	24 (61.5)	19 (61.3)	156 (60.2)
>200	7 (33.3)	16 (38.1)	19 (45.2)	17 (40.5)	17 (40.5)	15 (38.5)	12 (38.7)	103 (39.8)

NOTE. Data are no. (%) of patients, unless otherwise indicated. Dose units are viral particles per dose. *n*, No. of randomized individuals.

specific IFN-γ enzyme-linked immunosorbent spot (ELISPOT) response to 15mer HIV-1 clade B peptides with use of PBMCs obtained at week 30 (4 weeks after administration of the last dose).

Study Design

This study was a multicenter, blind, randomized, dose-escalating, placebo-controlled trial of a homologous prime-boost 3-dose regimen in HIV-seronegative adults who would be followed up for up to 78 weeks for immunogenicity and 260 weeks for safety. Healthy HIV-seronegative study participants 18–50 years old were assessed for eligibility at 25 sites in the United States. Individuals were excluded if they were considered to be at high behavioral risk of acquiring HIV infection during the study on the basis of risk factor assessment. Individuals with chronic medical conditions, including chronic hepatitis B or C infection, were also excluded. The protocol was approved by review boards at participating centers. Written informed consent was obtained from participants. Ongoing risk assessments and preventive counseling were offered to participants during the trial.

Enrolled study participants were randomized to receive 1.0-mL injections of placebo or vaccine into the deltoid muscle. Allocation schedules were computer generated. Investigators, study participants, clinical monitors, and laboratory personnel performing the biological assays were blind to treatment as-

Table 2. Summary of pertinent data for individuals who received a diagnosis of HIV infection during the study.

HIV-infected patient	Doses received, viral particles per dose × n	Baseline anti-Ad5 titer	Study week of diagnosis[a]	Plasma HIV RNA level at diagnosis[b]	ELISPOT reactivity[c]	Gag-ELISPOT responses[c,d]
1	$3 \times 10^{10} \times 3$	18	42	>75,000	Positive	1676
2	$3 \times 10^7 \times 3$	48	78	25,000	Negative	51
3	$3 \times 10^7 \times 3$	1239	78	>75,000	Negative	35
4	$3 \times 10^{10} \times 3$	599	52	>75,000	Negative	60
5	$3 \times 10^7 \times 3$	381	104	7600	Negative	24
6	$3 \times 10^9 \times 1$	18	156	49,000	Positive	83

NOTE. ELISPOT, enzyme-linked immunosorbent spot.

[a] Diagnosis was based on positive EIA and reflexive Western-blot results confirmed by PCR demonstration of viremia. Per protocol, EIA was to be performed at screening and week 78 but could also be performed at the investigator's discretion. Time of infection cannot be precisely determined because EIA was not periodically repeated during the study (and because of the expected but variable delay between infection and seroconversion).

[b] Ultrasensitive HIV RNA levels were measured by the HIV-1 Amplicor Monitor test (Roche Diagnostics) and are reported as copies per milliliter. The dynamic range for this assay is 50–75,000 copies/mL. This study was not designed to assess virologic end points. Because serologic and PCR testing was not performed systematically throughout the study and treatment histories were unavailable, the HIV RNA levels at the time of diagnosis may not reflect the steady-state viral set point.

[c] ELISPOT results are presented for the primary week 30 immunogenicity time point. A positive ELISPOT result required both ≥55 spot-forming cells per 10^6 PBMCs and ≥4-fold increase over the mock control. Negative reactivity indicates that responses to Gag, Pol, and Nef were all negative.

[d] Responses are reported as spot-forming cells per 10^6 PBMCs.

Table 3. Percentage of vaccination recipients with common injection site and systemic adverse events during the 29 days after any dose.

	Percentage of patients											
	Baseline Ad5 Titer ≤1:200				Baseline Ad5 Titer >1:200				Combined[a]			
Adverse event	Placebo (n = 14)	3×10^9 (n = 24)	3×10^{10} (n = 24)	1×10^{11} (n = 19)	Placebo (n = 7)	3×10^9 (n = 17)	3×10^{10} (n = 15)	1×10^{11} (n = 12)	Placebo (n = 21)	3×10^9 (n = 41)	3×10^{10} (n = 39)	1×10^{11} (n = 31)
Injection site												
Pain	14	29	50	79	0	18	60	75	9	25	**54**[b]	**77**[b]
Erythema	14	0	8	16	0	18	13	42	9	7	10	**26**[b]
Swelling	7	0	8	16	0	12	7	17	4	5	8	**16**[b]
Pruritus	0	0	0	0	14	0	0	17	6	0	0	7
Headache	36	29	46	58	29	12	13	17	33	22	33	42[b]
Temperature ≥37.8°C	0	4	13	47	0	6	20	17	0	5[b]	**16**	**35**[b]
Chills	7	4	17	42	0	6	0	8	4	5	10	**29**[b]
Fatigue	7	25	29	21	0	0	0	25	4	15	18	**23**[b]
Diarrhea	0	13	13	21	14	12	0	8	6	12	8	16
Myalgia	0	4	8	21	0	6	7	8	0	5	8	**16**[b]
Nausea	0	8	13	11	0	0	13	8	0	5	**13**[b]	**10**[b]
Nasopharyngitis	0	4	4	5	0	6	7	8	0	5	5	7
Pain	0	0	8	5	0	12	7	8	0	5	8[b]	**7**[b]
Arthralgia	7	13	4	11	0	0	0	0	4	8	3	6
Back pain	0	4	4	11	0	0	0	0	0	3	3	6
Muscle spasms	0	0	0	11	0	0	0	0	0	0	0	6
Neck pain	7	0	0	11	0	0	0	0	4	0	0	6
Pharyngolaryngeal pain	14	17	8	11	29	6	13	0	20	12	10	6
Rash	0	8	0	0	0	0	0	8	0	5	0	3
Cough	7	8	0	5	0	0	0	0	4	5	0	3
Diaphoresis	0	0	8	5	0	0	0	0	0	0	5	3
Pain in extremity	0	8	0	5	0	0	0	0	0	5	0	3
Upper respiratory tract infection	7	4	4	5	14	6	7	0	10	5	5	3
Insomnia	0	0	8	0	0	0	7	0	0	0	8	0
Pharyngitis	0	0	8	0	14	6	0	0	6	2	5	0
Vomiting	0	8	0	0	0	0	7	0	0	5	3	0

NOTE. Adverse events with combined incidence ≥5% for at least 1 of the active dose levels are displayed. Ad5, adenovirus type 5; dose units, viral particles per dose; *n,* no. of study participants with follow-up.

[a] Combined across baseline Ad5 strata with use of a weighted average that was based on observed stratum sizes in the trial (60.2% had Ad5 titers ≤200; 39.8% had Ad5 titers >200).

[b] One-tailed $P < .025$ for given dose versus placebo was based on a Cochran-Armitage trend test. Combined incidence is shown in bold if the P value remained <.025 after applying a multiplicity adjustment for the given dose level. Statistical comparisons were not performed within Ad5 strata because of small sample sizes.

signments. Study participants were initially enrolled in successive dose-escalating stages, allowing safety data from 6–8 participants at each dose level to be reviewed by an independent safety evaluation committee before participants were enrolled in the next higher dose group. Study participants were randomized to receive vaccine or placebo. Randomization with use of permuted blocks in the subsequent open-enrollment stage was stratified by baseline neutralizing anti-Ad5 antibody titers, to ensure adequate representation of study participants with low (≤200) and high (>200) titers across groups.

For 5 days after each dose, study participants were to record the largest diameter of induration or erythema at the injection site. A vaccine report card was used to track daily temperatures and physical complaints for 29 days after each dose. Fever was defined as a temperature of ≥37.8°C. Surveillance for shedding MRKAd5 was to be performed during the 2-week postdose period by collecting pharyngeal samples for culture from study participants developing symptoms compatible with a viral respiratory tract infection and by collecting urine samples for culture from study participants experiencing symptoms of urinary tract infection or with asymptomatic hematuria or pyuria [65].

Immunologic and Virologic Assays

Unfractionated IFN-γ ELISPOT assays with 15mer HIV-1 peptide pools were performed to detect HIV-specific T cell responses [66, 67]. Clade B peptides homologous to the vaccine strain were used, except where specified. A positive ELISPOT response was defined as ≥55 spot-forming cells per 10^6 PBMCs

Table 4. ELISPOT response rates at week 30 by antigen pool and dose level.

Antigen and dose level, viral particles per dose	Baseline Ad5 titer ≤200			Baseline Ad5 titer >200			Combined[a]	
	n	Responders, %	Geometric mean ELISPOT, spot-forming cells per 10^6 PBMCs	*n*	Responders, %	Geometric mean ELISPOT, spot-forming cells per 10^6 PBMCs	Responders, %	Geometric mean ELISPOT, spot-forming cells per 10^6 PBMCs
Gag								
Placebo	14	0.0	26	6	0.0	18	0.0	22
3×10^6	25	32.0	81	10	0.0	21	19.3[b]	47[b]
3×10^7	15	60.0	115	18	0.0	26	36.1[b]	64[b]
3×10^8	23	47.8	176	15	6.7	44	31.4[b]	101[b]
3×10^9	17	70.6	224	15	40.0	77	58.4[b]	147
3×10^{10}	22	72.7	144	10	70.0	179	71.6[b]	157[b]
1×10^{11}	16	68.8	331	11	45.5	106	59.5[b]	210[b]
Pol								
Placebo	14	0.0	57	6	0.0	34	0.0	46
3×10^6	25	0.0	78	10	0.0	43	0.0	62
3×10^7	15	20.0	98	18	0.0	54	12.0[b]	77
3×10^8	23	43.5	189	15	0.0	71	26.2[b]	128[b]
3×10^9	17	47.1	241	15	20.0	87	36.3[b]	160[b]
3×10^{10}	22	59.1	225	10	40.0	155	51.5[b]	194[b]
1×10^{11}	16	87.5	462	11	45.5	189	70.7[b]	323[b]
Nef								
Placebo	14	0.0	21	6	0.0	16	0.0	18
3×10^6	25	16.0	47	10	0.0	24	9.6	36[b]
3×10^7	15	13.3	55	18	0.0	20	8.0	37[b]
3×10^8	23	39.1	125	15	0.0	37	23.5[b]	77[b]
3×10^9	17	58.8	143	15	26.7	60	46.0[b]	101[b]
3×10^{10}	22	68.2	122	10	60.0	150	64.9[b]	132[b]
1×10^{11}	16	62.5	226	11	45.5	70	55.7[b]	142[b]

NOTE. A parallel modified intention-to-treat analysis including all participants who received ≥1 dose yielded similar results. ELISPOT, enzyme-linked immunosorbent spot; *n,* no. of study participants who were HIV seronegative through week 78, received all 3 doses, did not commit any major protocol violations, and had evaluable immunogenicity data at week 30.

[a] Combined across baseline adenovirus type 5 (Ad5) strata using a weighted average based on observed stratum sizes in the trial (60.2% had Ad5 titers ≤200; 39.8% had Ad5 titers >200).

[b] One-tailed $P < .025$ for given dose versus placebo was based on a Cochran-Armitage trend test. *P* values for geometric mean ELISPOT responses were computed using an analogous trend test. ELISPOT responder is defined as ≥55 spot-forming cells per 10^6 PBMC and ≥4-fold increase over the nonantigen control; Pol responder is defined as responder to either Pol-1 or Pol-2 peptide pool. Overall, 173 (80%) of the 217 study participants included in the per-protocol immunogenicity analysis at week 30 had PBMCs processed under less than optimal conditions [66]. Statistical comparisons were not performed within Ad5 strata because of small sample sizes.

and a ≥4-fold increase over responses with use of the nonantigen control [67, 68]. Unless otherwise indicated, reported geometric mean ELISPOT responses include all study participants tested, regardless of whether their ELISPOT result was categorized as positive or negative. The T cell phenotype underlying the ELISPOT response was identified as CD4 and/or CD8 by intracellular cytokine staining [69]. Neutralizing antibody titers against Ad5 were measured using serial dilutions of serum obtained ≤45 days before the first injection and were measured periodically thereafter [70].

HIV-1/2 EIAs (Abbott Laboratories) were to be performed at baseline, at week 78, and at the discretion of the investigator. Western blots were reflexively performed after positive EIA results. Plasma from study participants with indeterminate or positive immunoblots was tested for HIV by PCR (Amplicor 1.5; Roche Diagnostics).

Statistical Analyses

Safety. All study participants receiving ≥1 dose were included in the safety assessment. The proportions of study participants with adverse events ≤29 days after each dose were summarized by treatment group for each baseline anti-Ad5 antibody stratum. For the combined strata, summary statistics were calculated using a weighted average of the observed stratum-specific percentages, with weights proportional to the overall stratum sizes. Frequencies of specific adverse events in the vaccine and placebo groups were compared by the Cochran-Armitage trend

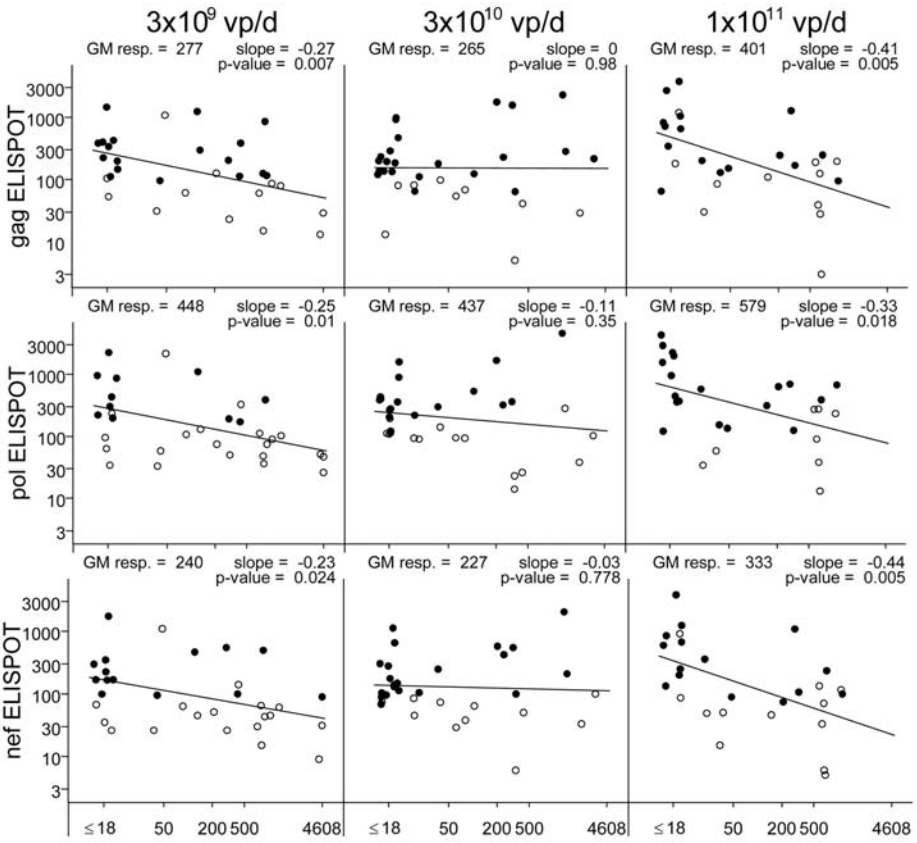

Figure 2. Quantitative enzyme-linked immunosorbent spot (ELISPOT) response (spot-forming cells per 10^6 PBMCs) at study week 30 versus baseline anti–adenovirus type 5 (Ad5) antibody titer by antigen and dose level. A positive ELISPOT response is defined as ⩾55 spot-forming cells per 10^6 PBMCs and a ⩾4-fold increase over the nonantigen control. *Y*-axis, ELISPOT responses presented as spot-forming cells per 10^6 PBMCs. GM resp, geometric mean spot-forming cells per 10^6 PBMCs of the positive ELISPOT responders only; vp/d, viral particles per dose.

test, starting with all the vaccine groups and sequentially excluding the highest remaining vaccine dose group as long as the difference versus placebo yielded a 1-tailed *P* value <.025. To gauge which adverse events were likely to represent true signals rather than type 1 errors, *P* values were deemed to be statistically significant only if they remained <.025 after a multiplicity adjustment [71].

Immunogenicity. A per-protocol rather than intention-to-treat approach was prespecified as the primary analysis, so that immune responses could be assessed under idealized conditions. Study participants without major protocol violations were included in the immunogenicity analyses. The proportion of ELISPOT responders was summarized by treatment arm at each time point [72]. Study week 30 was prespecified as the primary time point, because it was 4 weeks after the last vaccination. Differences in the frequencies of week 30 ELISPOT responders between a given vaccine dose group and the placebo group were analyzed using the Cochran-Armitage trend test. A 1-tailed *P* value <.025 was considered to be statistically significant; a multiplicity adjustment was not required, because a given dose was formally compared with placebo only if results of the trend tests for each higher dose were statistically significant [73].

RESULTS

From 1 May 2003 through 29 June 2004, 446 study participants were screened for eligibility (figure 1). Of 259 randomized study participants, 257 received ⩾1 dose of vaccine or placebo. Two study participants randomized to vaccine groups did not receive any doses. All treated study participants were included in the safety analysis. The 212 vaccinated study participants randomized in the open-enrollment stage were stratified by baseline anti-Ad5 titers at entry. The placebo and vaccine groups were generally comparable in age, ethnicity, and baseline anti-Ad5 titer, although the placebo group had a higher proportion of women (table 1). Overall, 40% of study participants had baseline anti-Ad5 titers >200.

Before week 30, 12 vaccine recipients and 1 placebo recipient discontinued participation in the study because of loss to follow-up (12 study participants) or an adverse clinical event (fatal head trauma in 1 vaccine recipient). No individuals discontin-

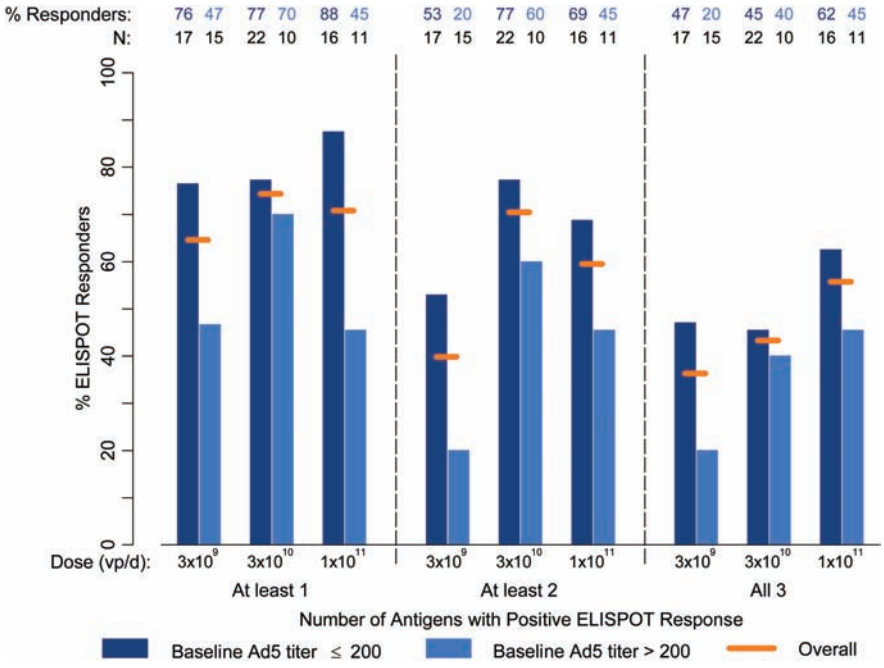

Figure 3. Frequency of positive enzyme-linked immunosorbent spot (ELISPOT) responders to ⩾1, ⩾2, and all 3 protein pools encoded by the 3 vaccine transgenes at week 30. A positive ELISPOT response is defined as ⩾55 spot-forming cells per 10^6 PBMCs and a ⩾4-fold increase over the nonantigen control. The data for the "Overall" entry were combined across baseline adenovirus type 5 (Ad5) strata with use of a weighted average that was based on observed stratum sizes in the trial (60.2% with anti-Ad5 titers ⩽200; 39.8% with anti-Ad5 titers >200). *N*, number of study participants with evaluable immunogenicity data; vp/d, viral particles per dose.

ued participation because of vaccine-related adverse events. Of 259 randomized subjects, ELISPOT results were available for 244 study participants (94%) at some point after randomization, including 217 study participants (84%) with data at the primary week 30 immunogenicity time point. A total of 229 study participants (88%) completed 78 weeks of the study and entered the long-term safety follow-up phase.

Six vaccine recipients received a diagnosis of HIV infection during the study (table 2). Four of these study participants were excluded from the immunogenicity analysis, but the 2 subjects who received a diagnosis of HIV infection after week 78 were included in the analysis. In retrospect, all 6 study participants reported high-risk behavior during the study period.

Safety and Tolerability

No vaccine-related serious adverse events occurred in any group (95% CI, 0%-1.6%). The frequencies of injection-site reactions and systemic adverse events in the 3×10^6, 3×10^7, and 3×10^8 viral particles vaccine groups were similar to the corresponding rates in the placebo group. Injection-site reactions that consisted primarily of local pain, erythema, and swelling occurred more frequently in vaccine recipients of the 2 highest doses than in placebo recipients (table 3). Most study participants who received doses of 3×10^{10} viral particles and 1×10^{11} viral particles experienced injection-site discomfort. Fever, headache, and fatigue occurred more commonly among recipients of the higher vaccine doses than among those who received the placebo, especially in the 1×10^{11} viral particles dose group. Fever and nausea were more common among recipients of the 3×10^{10} and 1×10^{11} viral particles doses than among those who received the placebo. Most documented temperatures in these dose groups (in 35 of 38 subjects [92%]) were <38.9°C. The proportions of study participants with local reactions remained fairly constant after each injection, whereas the incidence of systemic adverse events generally decreased with subsequent doses. Preexisting Ad5 immunity did not appear to affect the frequency of injection-site reactions. Participants with low baseline anti-Ad5 titers had numerically higher rates of systemic adverse events than did participants with high baseline anti-Ad5 titers.

Abnormal laboratory results in vaccine recipients at all doses developed at similar rates as in placebo recipients. From 305 adenoviral culture specimens obtained from 155 study participants, 1 urine specimen from a vaccine recipient in the 3×10^9 viral particles dose group yielded Ad5; DNA was insufficient to determine whether the isolate was a vaccine strain.

Four pregnancies were reported among vaccine recipients during the first 78 weeks of the study. Two resulted in the delivery of healthy full-term newborns at study weeks 104 and

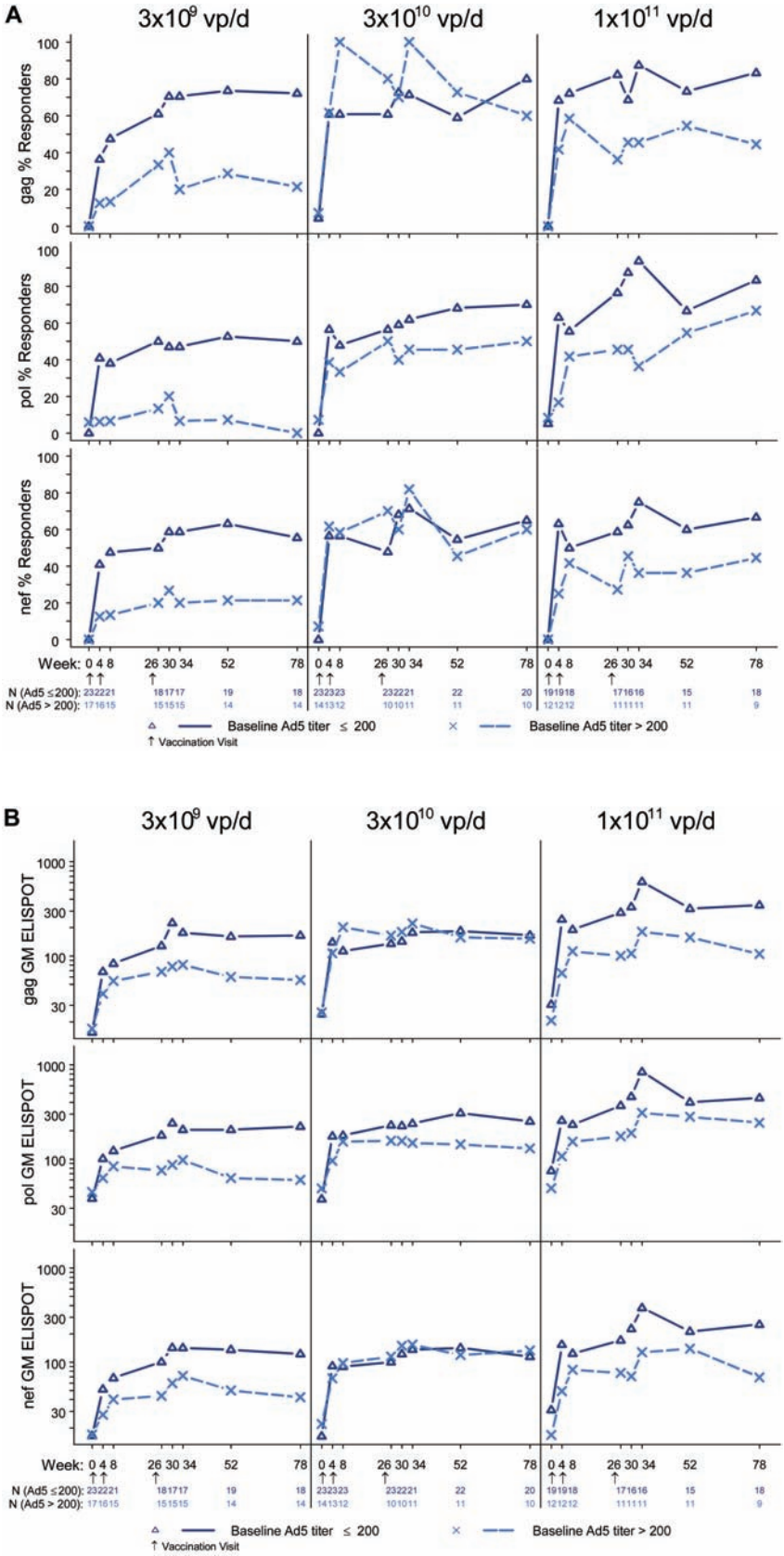

Figure 4. *A,* Frequency of positive enzyme-linked immunosorbent spot (ELISPOT) responders by dose level, study week, and baseline antiadenovirus type 5 (Ad5) antibody strata. *B,* Geometric mean ELISPOT responses (spot-forming cells per 10^6 PBMCs) by dose level, study week, and baseline anti-Ad5 antibody strata. *Y*-axis, ELISPOT responses presented as spot-forming cells per 10^6 PBMCs. A positive ELISPOT response is defined as ⩾55 spot-forming cells per 10^6 PBMCs and a ⩾4-fold increase over the nonantigen control. GM, geometric mean ELISPOT (spot-forming cells per 10^6 PBMCs) responses; vp/d, viral particles per dose.

118, 1 resulted in an elective abortion at 8 weeks of gestation at study week 33, and 1 resulted in a spontaneous abortion at 10 weeks of gestation at study week 32.

Immunogenicity

Vaccine induced ELISPOT responses against proteins encoded by all 3 transgenes in a dose-dependent fashion (table 4). In the highest 3 dose groups at the primary immunogenicity time point, the frequencies of positive ELISPOT responders and the ELISPOT geometric mean spot-forming cells per 10^6 PBMCs combined across baseline anti-Ad5 strata were significantly higher than in the placebo group for each peptide pool. In the 3×10^{10} viral particles dose group, 23 (72%) of 32 subjects had positive ELISPOT responses to $\geq$2 peptide pools at the primary immunogenicity time point 4 weeks after the third vaccination, with 14 subjects (44%) responding to all 3 transgene products. The 1×10^{11} viral particles dose did not increase the frequency of ELISPOT responders compared with the 3×10^{10} viral particles dose. Among ELISPOT responders, geometric mean responses had a range of 200–579 spot-forming cells per 10^6 PBMCs in a dose-dependent manner. T cell responses differentiated by intracellular cytokine staining identified both $CD8^+$ and $CD4^+$ cells, with the former cell type predominating. In a convenience sample of 57 study participants with a positive week 30 clade B Gag-ELISPOT tested for cross-clade Gag reactivity, 35 (61%) had positive clade A responses, and 38 (67%) had positive clade C responses.

ELISPOT immune responses were attenuated in study participants with high versus low baseline anti-Ad5 antibody titers (figure 2). The effect of preexisting Ad5 immunity appeared to be partially overcome with higher vaccine doses (figure 3). Statistically significant ($P < .05$) inverse associations were found between Gag-ELISPOT responses and baseline Ad5 titers in the 3×10^9 and 1×10^{11} dose groups ($P < .05$) but not in the 3×10^{10} dose group. Similar trends were seen for Pol and Nef responses. Although preexisting Ad5 immunity attenuated responses to the vaccine in the higher 1×10^{11} viral particles dose group but not in the lower 3×10^{10} viral particles dose group, geometric mean baseline anti-Ad5 titers were essentially identical in both dose groups. Postvaccination anti-Ad5 titers increased to similarly high levels in vaccine recipients who received 3 doses of $\geq 3 \times 10^8$ viral particles per dose.

ELISPOT responses were generally durable, persisting near levels achieved at week 30 for at least 78 weeks, regardless of baseline anti-Ad5 status (figure 4). The second and third immunizations resulted in increased geometric mean ELISPOT responses, but responses after the third dose subsequently decreased to levels comparable to levels before this dose.

Postvaccination EIA results were available by week 78 in 207 (88%) of 236 vaccine recipients. Overall, 55 (27%) of the 207 evaluable study participants had positive EIA results but negative PCR results. The frequencies of EIA positivity were directly related to vaccine dose and were inversely related to baseline anti-Ad5 immunity. For example, among recipients of the 3×10^{10} viral particles dose, 20 (91%) of 22 versus 3 (23%) of 13 had positive EIA results in the low versus high baseline Ad5 immunity strata. Indeterminate results were seen in 54 (98%) of 55 EIA-positive vaccine recipients with available Western-blot interpretations; the most commonly observed bands were p24, p40, and p55. Equivocally positive Western-blot results occurred in 1 study participant, who had an indeterminate result when retested 3 weeks later.

DISCUSSION

In this phase 1 trial, the MRKAd5 trivalent vaccine was generally well tolerated. The frequencies of adverse events were dose related. The vaccine was immunogenic in healthy HIV-seronegative adults. In general, higher doses of vaccine induced immune responses to multiple HIV-1 peptides. Response rates to Gag proteins elicited by the trivalent vaccine were similar to historical results with the monovalent Ad5 vaccine, indicating that addition of *pol* and *nef* transgene vectors did not interfere with response to the *gag* vector [46]. The proportion of study participants with ELISPOT responses to the *gag* transgene 4 weeks after the third injection was greater than placebo at doses as low as 3×10^6 viral particles, but consistent responses to the *pol* and *nef* transgenes were not observed with low-dose vaccine. Quantitative ELISPOT responses elicited by all vaccine doses were stable from week 30 to at least week 78.

Positive ELISPOT responses in vaccine recipients with high preimmunization Ad5 titers were more common with higher doses. At the 3×10^{10} viral particles dose, ELISPOT responses were nearly equivalent in the low and high baseline anti-Ad5 strata. High preexisting Ad5 immunity did not dampen responses to the 3×10^{10} viral particles dose to the same degree as to the higher 1×10^{11} viral particles dose, possibly reflecting biological or assay variability versus a ceiling on the optimum dose of Ad5 vector. Because baseline and postvaccination Ad5 titers were comparable in study participants receiving the 3×10^{10} and 1×10^{11} viral particles doses, it is unlikely that antibodies to the MRKAd5 vector boosted by the 1×10^{11} viral particles dose accounted for the difference in immunogenicity between the 3×10^{10} and 1×10^{11} viral particles dose groups with high preexisting Ad5 immunity.

Positive EIA results were common in vaccine recipients. Vaccine dose and baseline Ad5 immunity were major determinants of vaccine-induced EIA positivity. In EIA-positive uninfected study participants, Western blots were typically indeterminate, with a characteristic band pattern directed at *gag*-encoded proteins. Although enrollment was restricted to adults with presumably low-risk behavior, 6 vaccine recipients received a diagnosis of HIV-1 infection during the study to date, under-

scoring the difficulty of identifying a low-risk population for vaccine trials. Although no cases of HIV infection were recognized in placebo recipients, the composite vaccine group was ~10 times larger than the placebo group. Of the 6 vaccine recipients infected during the time of the study, 3 study participants (50%) had high preexisting anti-Ad5 titers, 2 study participants (33%) received a full series of high-dose vaccine, and 5 study participants (83%) had negative or weakly positive ELISPOT responses. The predictive value of ELISPOT reactivity for clinical efficacy has not been established.

The trivalent vaccine induced broad cell-mediated immunity against HIV-1 clade B consensus peptides, with a predominant CD8-cell phenotype. Additional data are necessary to confirm whether this vaccine consistently elicits cell-mediated immunity to non–clade B peptides in different populations [74–77]. On the basis of its balance of tolerability and immunogenicity, the 3×10^{10} viral particles dose was carried forward into phase 2 trials evaluating whether the trivalent vaccine would be effective in the prevention and/or control of HIV-1 infection.

Despite the favorable immunogenicity of the vaccine candidate, enrollment in the proof-of-concept STEP trial that used this exact vaccine was recently stopped after a preplanned interim review by the Data and Safety Monitoring Board, because a futility analysis indicated that the study was unlikely to yield evidence of efficacy [78]. The STEP study was being conducted in areas of the world where HIV-1 infection caused by clade B predominates. The vaccine reduced neither the incidence of HIV acquisition nor the viral load set point in those who became infected. The disappointing results of the phase 2 trial, juxtaposed with our promising phase 1 data, highlight the challenges of developing an effective HIV-1 vaccine that targets cell-mediated immunity, especially the lack of reliable correlation between primate models versus human study participants and the unsolved challenge of identifying immune correlates of protection against HIV infection [48–50, 79–82]. The discordance between the observed immunologic responses in our study and the preliminary efficacy findings from STEP indicate that the specific T cell responses elicited by vaccination were inadequate to prevent or modulate infection. Vaccine-induced activation of Ad5-primed T cells could theoretically increase susceptibility to HIV infection [81]. Strategies building on Ad5-vectored HIV-1 vaccines, such as heterologous prime-boost regimens or combination vaccines eliciting both cell-meditated and neutralizing humoral responses, may yet prove rewarding as the field of HIV vaccinology moves forward [83]. We hope that the ongoing analyses of the STEP results will provide instructive answers to many critical questions [84].

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Potential conflicts of interest. Current (indicated in the affiliations footnote) and former employees (J.K. and P.K.) of Merck may own stock or stock options in the company. F.P. is employed by the International AIDS Vaccine Initiative and has previously received fees from Merck for serving on safety advisory committees for phase 1 HIV-1 vaccine trials. M.M. and M.L. have received grant support from Merck related to its HIV-1 vaccine program; M.L. has also received honoraria from Merck for lectures about other Merck vaccines.

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