Preliminary Communication

Safety and Immunogenicity of Novel Adenovirus Type 26– and Modified Vaccinia Ankara–Vectored Ebola Vaccines
A Randomized Clinical Trial

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IMPORTANCE Developing effective vaccines against Ebola virus is a global priority.

OBJECTIVE To evaluate an adenovirus type 26 vector vaccine encoding Ebola glycoprotein (Ad26.ZEBOV) and a modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein (MVA-BN-Filo).

DESIGN, SETTING, AND PARTICIPANTS Single-center, randomized, placebo-controlled, observer-blind, phase 1 trial performed in Oxford, United Kingdom, enrolling healthy 18- to 50-year-olds from December 2014; 8-month follow-up was completed October 2015.

INTERVENTIONS Participants were randomized into 4 groups, within which they were simultaneously randomized 5:1 to receive study vaccines or placebo. Those receiving active vaccines were primed with Ad26.ZEBOV (5×10^{10} viral particles) or MVA-BN-Filo (1×10^{8} median tissue culture infective dose) and boosted with the alternative vaccine 28 or 56 days later. A fifth, open-label group received Ad26.ZEBOV boosted by MVA-BN-Filo 14 days later.

MAIN OUTCOMES AND MEASURES The primary outcomes were safety and tolerability. All adverse events were recorded until 21 days after each immunization; serious adverse events were recorded throughout the trial. Secondary outcomes were humoral and cellular immune responses to immunization, as assessed by enzyme-linked immunosorbent assay and enzyme-linked immunospot performed at baseline and from 7 days after each immunization until 8 months after priming immunizations.

RESULTS Among 87 study participants (median age, 38.5 years; 66.7% female), 72 were randomized into 4 groups of 18, and 15 were included in the open-label group. Four participants did not receive a booster dose; 67 of 75 study vaccine recipients were followed up at 8 months. No vaccine-related serious adverse events occurred. No participant became febrile after MVA-BN-Filo, compared with 3 of 60 participants (5%; 95% CI, 1%-14%) receiving Ad26.ZEBOV in the randomized groups. In the open-label group, 4 of 15 Ad26.ZEBOV recipients (27%; 95% CI, 8%-55%) experienced fever. In the randomized groups, 28 of 29 Ad26.ZEBOV recipients (97%; 95% CI, 82%- 99.9%) and 7 of 30 MVA-BN-Filo recipients (23%; 95% CI, 10%-42%) had detectable Ebola glycoprotein-specific IgG 28 days after primary immunization. All vaccine recipients had specific IgG detectable 21 days postboost and at 8-month follow-up. Within randomized groups, at 7 days postboost, at least 86% of vaccine recipients showed Ebola-specific T-cell responses.

CONCLUSIONS AND RELEVANCE In this phase 1 study of healthy volunteers, immunization with Ad26.ZEBOV or MVA-BN-Filo did not result in any vaccine-related serious adverse events. An immune response was observed after primary immunization with Ad26.ZEBOV; boosting by MVA-BN-Filo resulted in sustained elevation of specific immunity. These vaccines are being further assessed in phase 2 and 3 studies.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT02313077

JAMA. 2016;315(15):1610-1623. doi:10.1001/jama.2016.4218

Supplemental content at jama.com

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The recent outbreak of Ebola virus disease in West Africa has caused in excess of 28 600 cases and 11 300 deaths since the first cases were identified in December 2013 in Guinea.[1] The international response has included the accelerated clinical development of several candidate Ebola vaccines, which have largely converged on the stratagem of vector vaccine technology to induce Ebola-specific immune responses.[2-7] Vaccines using adenovirus serotype 26 (Ad26) as a vector for delivery of viral proteins have been shown to induce robust humoral and cellular immune responses in preclinical studies.[8-10] Clinical trials of an Ad26-vectored candidate human immunodeficiency virus (HIV) vaccine have demonstrated that this vaccine platform is immunogenic and well tolerated in healthy adults.[11,12] In nonhuman primates, an Ad26-vectored vaccine was able to generate up to 75% protection from Ebola challenge,[13] and increased durability of Ebola protection is achieved in adenovirus-vector vaccine primed macaques that receive a booster dose of a modified vaccinia Ankara (MVA)-based vaccine.[14] Further Ebola challenge studies demonstrated 100% efficacy for nonhuman primates receiving a priming dose of either Ad26- or MVA-vectored vaccines with subsequent boosting by the alternative vector vaccine encoding the same Ebola glycoprotein (heterologous prime/boost).[15]

Ad26.ZEBOV *adenovirus type 26 vector vaccine encoding Ebola glycoprotein*

ELISA *enzyme-linked immunosorbent assay*

ELISpot *enzyme-linked immunospot*

MVA-BN-Filo *modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein*

VSV *vesicular stomatitis virus*

This study evaluated the reactogenicity and immunogenicity of immunization schedules using 2 novel candidate Ebola vaccines—an adenovirus type 26 vector vaccine encoding Ebola glycoprotein (Ad26.ZEBOV) and a modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein (MVA-BN-Filo)—administered to healthy adult volunteers in the United Kingdom at intervals between 2 and 8 weeks (see the trial protocol in Supplement 1).

Methods

Study Design and Participants

This single-center, randomized, observer-blind, placebo-controlled, first-in-human, phase 1 trial took place in Oxford, United Kingdom. Eligible participants were healthy men and women aged 18 to 50 years who provided formal, written consent and reported no prior immunization with a candidate Ebola vaccine or any MVA- or Ad26-vectored vaccine (see the inclusion/exclusion criteria in eAppendix 1 in Supplement 2). Baseline demographic characteristics were recorded for all participants; given this phase 1 study was conducted in a population geographically and ethnically distinct from those experiencing the Ebola virus outbreak, these included self-defined ethnicity, classified using protocol-defined options. Participants were randomized equally into 4 vaccination schedules in the original study design, and within each schedule, they were simultaneously randomized to receive active vaccine or placebo in a 5:1 ratio (**Figure 1** and eTable 1 in Supplement 2). The intervention groups included 2 with MVA-BN-Filo as prime vaccine on day 1 boosted by Ad26.ZEBOV on day 29 or day 57 and 2 with a priming dose of Ad26.ZEBOV boosted by MVA-BN-Filo on day 29 or day 57. In response to a need to urgently obtain data on shorter vaccine schedules that might be of additional benefit in an outbreak setting, an additional, nonrandomized, open-label group receiving Ad26.ZEBOV prime and MVA-BN-Filo on day 15 was subsequently added by protocol amendment (see the trial protocol in Supplement 1). The protocol and study documents were approved by the National Research Ethics Service.

Randomization and Masking

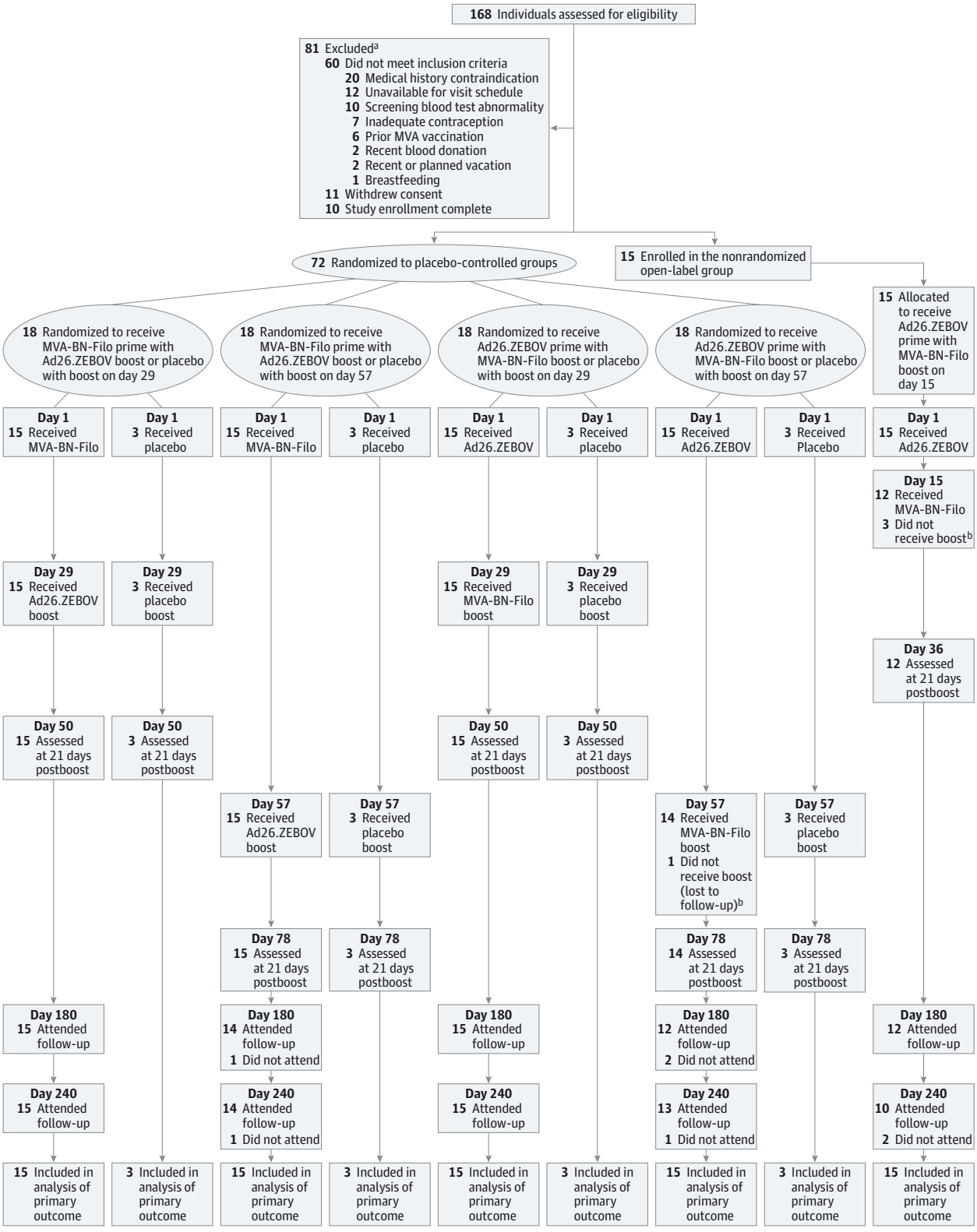
Participants in the randomized component of the study were centrally randomized to 1 of 4 groups based on a computer-generated block randomization schedule with randomly permuted blocks and an interactive web response system (eAppendix 1 in Supplement 2). Within groups, participants were simultaneously randomized to active vaccine or placebo at a ratio of 5:1. Participants and study team members were blinded to active/placebo vaccine allocation until all participants reached 21 days after boost, except for a team of unblinded study personnel with the primary responsibility for study vaccine preparation and administration. The unblinded team had no other involvement in study procedures or assessments. Staff undertaking laboratory analyses remained blinded throughout the study.

Participants enrolled into the group receiving vaccines at a 14-day interval were not randomized or blinded.

Procedures

Ad26.ZEBOV (Crucell Holland) is a monovalent, recombinant, E1/E3-deleted, replication-defective, Ad26-vectored vaccine expressing Ebola virus Mayinga variant glycoprotein, produced in PER.C6 human cells and provided in sterile, single-dose vials at a concentration of 1×10^{11} viral particles/mL. MVA-BN-Filo (Bavarian Nordic) is a recombinant, replication-defective, modified vaccinia Ankara-vectored vaccine expressing Mayinga variant glycoprotein as well as Sudan virus Gulu variant glycoprotein, Marburg virus Musoke variant glycoprotein, and Tai Forest virus nucleoprotein. This multivalent vaccine was manufactured in chicken embryo fibroblasts and provided in sterile, 2-mL vials at a concentration of 2×10^8 median tissue culture infective dose ($TCID_{50}$)/mL. Both vaccines were manufactured in accordance with current good manufacturing practices. All vaccines were administered into the deltoid muscle at a dose of 5×10^{10} viral particles for Ad26.ZEBOV, 1×10^8 $TCID_{50}$ for MVA-BN-Filo, and 0.5-mL 0.9% sodium chloride solution for the placebo.

Figure 1. Participant Flow Diagram



Numbers with blood samples at the 1-month visit after each vaccination event are shown. Follow-up at days 180 and 240 was not planned for those who received placebo. Ad26.ZEBOV indicates adenovirus-type 26 vector vaccine encoding Ebola glycoprotein; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein.

[a] Participants could be excluded for >1 reason.

[b] Participants who did not receive boosts are described in the Results section.

Adverse Events Monitoring

Participants were observed for 1 hour after vaccination to record any immediate adverse event. For the day of each immunization and 7 subsequent days, participants recorded solicited symptoms that were local (eg, injection site pain) and systemic (eg, myalgia). Safety blood tests were performed at 3 and 7 days after each prime and boost immunization and a 12-lead electrocardiogram (ECG) trace performed at 3 days postimmunization. All adverse events were recorded until 21 days after the boost immunization, and serious adverse events were actively monitored throughout the trial by direct questioning at each study visit. With the exception of the 15 participants receiving vaccines at a 14-day interval, staff performing safety evaluations were blinded to receipt of vaccine or placebo. All adverse events were graded as mild (grade 1), moderate (grade 2), or severe (grade 3) on a scale adapted from the US National Institute of Allergy and Infectious Diseases Division of Microbiology and Infectious Diseases toxicity table for use in trials enrolling healthy adults.[16]

Immunogenicity Measurements

Immunogenicity assessments were performed on blood samples taken immediately before primary and boost immunizations, 7 days after primary and boost immunizations, and 21 days after boost immunizations. Those receiving vaccines at a 56-day interval had an additional blood test 28 days after primary immunization (eTable 1 in Supplement 2). After unblinding of vaccine allocation, participants who received investigational vaccines had further blood samples taken at day 180 and day 240; a further visit at day 360 is planned and not reported here.

Total IgG responses against Ebola glycoprotein were assessed by enzyme-linked immunosorbent assay (ELISA) at Battelle Biomedical Research Center. Purified recombinant glycoprotein from the Ebola virus Kikwit variant provided by the Joint Vaccine Acquisition Program was immobilized on a 96-well microtiter plate. Test samples were serially diluted and Ebola-specific antibodies were detected with antihuman IgG antibodies conjugated with horseradish peroxidase followed by a colorimetric reaction.

Frozen peripheral blood mononuclear cells were analyzed by enzyme-linked immunospot (ELISpot) and intracellular cytokine staining at the HIV Vaccine Trials Network laboratory as previously described.[17]

Further details of the ELISA and T-cell assays are provided in eAppendix 1 in Supplement 2, as are details of the Ad26 neutralization assay used to evaluate seropositivity to adenovirus 26 prior to immunization.

Outcomes

The primary outcome was the number of participants with documented adverse events. Secondary outcomes were the percentage of vaccine responders and the magnitude of the humoral and cellular immune response as assessed by ELISA and ELISpot. Exploratory outcomes included the number of Ebola-specific CD4+ and CD8+ T cells and the proportion of polyfunctional cytokine-secreting T cells as assessed by intracellular cytokine staining.

Statistical Analysis

We analyzed safety and immunogenicity data using summary statistics. No formal statistical testing of these outcomes was planned nor performed. The study provided preliminary safety and immunogenicity assessments, and thus the sample size was not based on formal hypothesis testing considerations but is within the range of participants as recommended by the International Conference on Harmonisation for first-in-human studies. With a sample size of 15 vaccine recipients in each group, it was determined that if no participants experienced a significant reaction, this would be associated with an upper limit of the 1-sided 97.5% confidence interval, which excludes a true rate of 22% of such reactions.

Geometric mean concentrations (GMCs) with 95% confidence intervals were determined for Ebola-specific antibody responses measured by ELISA and also for end point titers (the reciprocal of the most dilute titer at which specific IgG remained detectable) to facilitate cross-study comparisons. Median and interquartile ranges for background subtracted T-cell interferon-γ (IFN-γ) from ELISpot and cytokine responses from intracellular cytokine staining assays were determined. All values below the lower limit of quantification were substituted with half the lower limit of quantification for analysis (18.3 ELISA units/mL, 25 spot-forming units [SFUs] for ELISpot, and 0.02% for intracellular cytokine staining results); see eAppendix 1 in Supplement 2 for further information.

At each time point after baseline, a participant was defined as a responder for ELISA, ELISpot,[18] or intracellular cytokine staining if negative at baseline and positive after baseline or positive at baseline with at least 3-fold increase from baseline.

The sponsor conducted all data analyses using SAS version 9.2 (SAS Institute). Independent validation of all results from raw data was completed at the University of Oxford using SAS version 9.3.

Results

Participants were enrolled from December 30, 2014, to February 18, 2015, and the last participant attended the day 240 assessment on October 29, 2015. All 87 participants were aged between 18 and 50 years (median, 38.5 years); 66.7% were female and 33.3% male (**Table 1**). Seventy-two participants were randomized to 4 groups of 18 (15 to active vaccine and 3 to placebo). An additional 15 participants were included in the open-label group. Preexisting immunity to Ad26 was observed in 3 of 87 participants (3.4%) at baseline. Baseline characteristics were largely similar across groups.

Four participants did not receive a booster immunization and were excluded from postboost safety and immunogenicity analyses (Figure 1). In the open-label group, 1 participant voluntarily withdrew after prime vaccination with Ad26.ZEBOV, having completed follow-up to 35 days after prime immunization with no safety concerns. Two participants in the open-label group experienced a decrease in

Table 1. Baseline Characteristics

	MVA-BN-Filo With Ad26.ZEBOV Boost on Day 29 (n = 15)	Ad26.ZEBOV With MVA-BN-Filo Boost on Day 29 (n = 15)	MVA-BN-Filo With Ad26.ZEBOV Boost on Day 57 (n = 15)	Ad26.ZEBOV With MVA-BN-Filo Boost on Day 57 (n = 15)	Ad26.ZEBOV With MVA-BN-Filo Boost on Day 15 (n = 15)	Placebo (n = 12)
Sex, No. (%)						
Female	8 (53.3)	10 (66.7)	10 (66.7)	10 (66.7)	12 (80.0)	8 (66.7)
Male	7 (46.7)	5 (33.3)	5 (33.3)	5 (33.3)	3 (20.0)	4 (33.3)
Age median (range), y	39.0 (20-50)	39.0 (20-46)	39.0 (18-49)	42.0 (22-48)	34.0 (19-50)	30.0 (20-50)
White ethnic origin, No. (%)[a]	15 (100)	15 (100)	14 (93.3)	13 (86.7)	14 (93.3)	12 (100)
BMI, mean (SD)[b]	25.11 (3.9)	24.38 (4.1)	27.19 (5.0)	26.67 (3.8)	23.53 (3.3)	23.79 (2.6)

Abbreviations: Ad26.ZEBOV, adenovirus type 26 vector vaccine encoding Ebola glycoprotein; BMI, body mass index; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein.

[a] Self-designated using options provided by the sponsor.

[b] Calculated as weight in kilograms divided by height in meters squared.

neutrophil count (<1.0 × 10^9/L) after prime vaccination and were excluded from receiving booster immunization. In the group receiving AD26.ZEBOV prime and a MVA-BN-Filo boost at day 57, 1 participant was lost to follow-up after prime vaccination, having completed an observation period of 7 days after prime immunization and last making contact with the study team at 34 days after prime immunization with no safety concerns.

Reactogenicity and Adverse Events

Solicited local and systemic reactions are displayed in **Table 2**. Fever occurred after 3 of 60 (5%; 95% CI, 1%-14%) Ad26.ZEBOV immunization episodes, 0 of 59 MVA-BN-Filo immunization episodes, and 1 of 24 (4.2%; 95% CI, 0.1%-21%) placebo immunization episodes in blinded participants. In the open-label component, fever occurred in 4 of 15 participants (26.7%; 95% CI, 8%-55%) after Ad26.ZEBOV prime and 0 of 12 participants after MVA-BN-Filo boost. All episodes of fever resolved within 24 to 48 hours. Three participants reported a grade 3 local reaction (injection site erythema [n = 2], injection site pain [n = 1], and swelling [n = 1]) after Ad26.ZEBOV immunization. Five participants (2/60 in the blinded groups, 3/15 in the open-label group) reported grade 3 solicited systemic reactions after receiving Ad26.ZEBOV (headache [n = 4], myalgia [n = 1], nausea [n = 3], fatigue [n = 3], chills [n = 3], fever [n = 1]), and 1 participant reported grade 3 fatigue after receiving placebo. No grade 3 local or systemic solicited reactions were reported after MVA-BN-Filo administration.

Unsolicited adverse events were observed in blinded groups after 45 of 60 (75.0%; 95% CI, 62%-85%) Ad26.ZEBOV immunizations, 40 of 59 (67.8%; 95% CI, 54%-79%) MVA-BN-Filo immunizations, and 20 of 24 (83.3%; 95% CI, 63%-95%) placebo immunizations. For the open-label group, unsolicited adverse events were observed in 14 of 15 participants (93.3%; 95% CI, 68%-99.9%) after the Ad26.ZEBOV prime and 8 of 12 participants (66.7%; 95% CI, 35%-90%) after the MVA-BN-Filo boost immunizations. Grade 3 unsolicited adverse events were observed in 4 vaccine recipients (2/60 in blinded groups, 2/15 in open-label group). Of these, 2 had a decrease in neutrophil count to below 1.0 × 10^9/L after the Ad26.ZEBOV prime vaccination; 1 reported an influenza-like illness at 42 days after the Ad26.ZEBOV prime; and 1 individual sustained an unrelated, scalding injury 5 days after the Ad26.ZEBOV booster. Grade 3 unsolicited adverse events were also observed in 3 placebo recipients (1 with grade 3 tonsillitis and grade 3 gastritis and 2 with serum potassium <3.0 mmol/L). A transient decrease in neutrophil count was reported after 24 of 75 (32.0%; 95% CI, 22%-44%) Ad26.ZEBOV immunization episodes and 5 of 71 (7.0%; 95% CI, 2%-16%) MVA-BN-Filo immunization episodes, which was evident at 3 days postimmunization and was resolved or resolving in all cases by 7 days postimmunization. A decreased neutrophil count of 1.5 to 2.0 × 10^9/L was reported after 5 of 24 (20.8%; 95% CI, 7%-42%) placebo immunization episodes. No abnormalities in ECG recordings occurred postimmunization. Four serious adverse events occurred (details in eAppendix 2 in Supplement 2); none of these were considered related to the study vaccines.

Antibody Response

Ebola glycoprotein-specific responses are shown in **Table 3**, **Figure 2**, and eFigure 1 in Supplement 2. At 14 days after the Ad26.ZEBOV prime immunization, vaccine-induced antibody responses were detected by ELISA in 11 of 14 (79%; 95% CI, 49%-95%) Ad26.ZEBOV-primed recipients in the nonrandomized group. At 28 days after Ad26.ZEBOV in the randomized groups, these proportions were 14 of 15 (93%; 95% CI, 68%-99.9%) for those boosted at a 28-day interval and 14 of 14 (100%) for those boosted at a 56-day interval. By contrast, at 28 days after MVA-BN-Filo prime, vaccine-induced antibodies were observed in 6 of 15 (40%; 95% CI, 16%-68%) of those boosted at a 28-day interval and in 1 of 15 (6.7%; 95% CI, 0.2%-32%) of those boosted at a 56-day interval.

At 21 days postboost, all vaccine recipients had Ebola-specific IgG responses, with GMCs being highest in those primed and boosted at a 56-day interval (7553 [95% CI, 5114-11 156] for Ad26.ZEBOV prime recipients and 18 474 [95% CI, 12 418-27 483] for MVA-BN-Filo prime recipients).

The IgG responses were also analyzed as end point titers as shown in eFigure 2 in Supplement 2. At 21 days after an Ad26.ZEBOV prime and MVA-BN-Filo boost schedule given at a 28-day interval, these titers were 8098.9 (95% CI,

Table 2. Solicited Local and Systemic Adverse Reactions[a]

	Randomized Groups						Open-Label Group	
	Prime			Boost on Day 29 or Day 57			Prime	Boost on Day 15
	MVA-BN-Filo	Ad26.ZEBOV	Placebo	Ad26.ZEBOV	MVA-BN-Filo	Placebo	Ad26.ZEBOV	MVA-BN-Filo
All participants with reactogenicity data, No.	30	30	12	30	29	12	15	12
Any solicited event, No. (%)								
Any	22 (73.3)	29 (96.7)	8 (66.7)	28 (93.3)	19 (65.5)	5 (41.7)	13 (86.7)	12 (100.0)
Grade 1	21 (70.0)	19 (63.3)	8 (66.7)	10 (33.3)	15 (51.7)	4 (33.3)	5 (33.3)	9 (75.0)
Grade 2	1 (3.3)	9 (30.0)	0	15 (50.0)	4 (13.8)	0	5 (33.3)	3 (25.0)
Grade 3	0	1 (3.3)	0	3 (10.0)	0	1 (8.3)	3 (20.0)	0
Local Events, No. (%)[b]								
Any local event								
Any	18 (60.0)	26 (86.7)	2 (16.7)	28 (93.3)	17 (58.6)	2 (16.7)	12 (80.0)	10 (83.3)
Grade 1	18 (60.0)	20 (66.7)	2 (16.7)	15 (50.0)	15 (51.7)	2 (16.7)	11 (73.3)	7 (58.3)
Grade 2	0	5 (16.7)	0	11 (36.7)	2 (6.9)	0	1 (6.7)	3 (25.0)
Grade 3	0	1 (3.3)	0	2 (6.7)	0	0	0	0
Injection site pain								
Any	17 (56.7)	26 (86.7)	2 (16.7)	26 (86.7)	16 (55.2)	1 (8.3)	11 (73.3)	8 (66.7)
Grade 1	17 (56.7)	21 (70.0)	2 (16.7)	17 (56.7)	14 (48.3)	1 (8.3)	10 (66.7)	5 (41.7)
Grade 2	0	4 (13.3)	0	9 (30.0)	2 (6.9)	0	1 (6.7)	3 (25.0)
Grade 3	0	1 (3.3)	0	0	0	0	0	0
Injection site warmth								
Any	1 (3.3)	3 (10.0)	0	11 (36.7)	4 (13.8)	1 (8.3)	7 (46.7)	4 (33.3)
Grade 1	1 (3.3)	2 (6.7)	0	10 (33.3)	4 (13.8)	1 (8.3)	7 (46.7)	4 (33.3)
Grade 2	0	1 (3.3)	0	1 (3.3)	0	0	0	0
Injection site erythema								
Any	0	0	0	5 (16.7)	0	0	0	0
Grade 1	0	0	0	1 (3.3)	0	0	0	0
Grade 2	0	0	0	2 (6.7)	0	0	0	0
Grade 3	0	0	0	2 (6.7)	0	0	0	0
Injection site pruritus								
Any	0	2 (6.7)	0	2 (6.7)	0	0	0	1 (8.3)
Grade 1	0	2 (6.7)	0	1 (3.3)	0	0	0	1 (8.3)
Grade 2	0	0	0	1 (3.3)	0	0	0	0
Injection site swelling								
Any	0	1 (3.3)	0	3 (10.0)	1 (3.4)	0	0	0
Grade 1	0	1 (3.3)	0	0	1 (3.4)	0	0	0
Grade 2	0	0	0	2 (6.7)	0	0	0	0
Grade 3	0	0	0	1 (3.3)	0	0	0	0
Injection site induration								
Any	0	1 (3.3)	0	0	1 (3.4)	0	0	1 (8.3)
Grade 1	0	0	0	0	1 (3.4)	0	0	1 (8.3)
Grade 2	0	1 (3.3)	0	0	0	0	0	0
Systemic Events, No. (%)[c]								
Any systemic event								
Any	21 (70.0)	27 (90.0)	7 (58.3)	27 (90.0)	7 (24.1)	4 (33.3)	11 (73.3)	8 (66.7)
Grade 1	20 (66.7)	19 (63.3)	7 (58.3)	14 (46.7)	5 (17.2)	3 (25.0)	3 (20.0)	7 (58.3)
Grade 2	1 (3.3)	7 (23.3)	0	12 (40.0)	2 (6.9)	0	5 (33.3)	1 (8.3)
Grade 3	0	1 (3.3)	0	1 (3.3)	0	1 (8.3)	3 (20.0)	0
Fatigue								
Any	13 (43.3)	18 (60.0)	4 (33.3)	18 (60.0)	6 (20.7)	3 (25.0)	9 (60.0)	3 (25.0)
Grade 1	12 (40.0)	14 (46.7)	4 (33.3)	12 (40.0)	4 (13.8)	2 (16.7)	2 (13.3)	2 (16.7)
Grade 2	1 (3.3)	4 (13.3)	0	6 (20.0)	2 (6.9)	0	4 (26.7)	1 (8.3)
Grade 3	0	0	0	0	0	1 (8.3)	3 (20.0)	0

(continued)

Table 2. Solicited Local and Systemic Adverse Reactions[a] (continued)

	Randomized Groups						Open-Label Group	
	Prime			Boost on Day 29 or Day 57			Prime	Boost on Day 15
	MVA-BN-Filo	Ad26.ZEBOV	Placebo	Ad26.ZEBOV	MVA-BN-Filo	Placebo	Ad26.ZEBOV	MVA-BN-Filo
Headache								
Any	10 (33.3)	17 (56.7)	5 (41.7)	16 (53.3)	3 (10.3)	2 (16.7)	8 (53.3)	4 (33.3)
Grade 1	10 (33.3)	11 (36.7)	5 (41.7)	9 (30.0)	3 (10.3)	2 (16.7)	2 (13.3)	3 (25.0)
Grade 2	0	5 (16.7)	0	6 (20.0)	0	0	4 (26.7)	1 (8.3)
Grade 3	0	1 (3.3)	0	1 (3.3)	0	0	2 (13.3)	0
Myalgia								
Any	9 (30.0)	14 (46.7)	2 (16.7)	16 (53.3)	4 (13.8)	1 (8.3)	9 (60.0)	4 (33.3)
Grade 1	9 (30.0)	8 (26.7)	2 (16.7)	8 (26.7)	4 (13.8)	1 (8.3)	7 (46.7)	4 (33.3)
Grade 2	0	5 (16.7)	0	8 (26.7)	0	0	2 (13.3)	0
Grade 3	0	1 (3.3)	0	0	0	0	0	0
Chills								
Any	0	10 (33.3)	2 (16.7)	10 (33.3)	2 (6.9)	1 (8.3)	9 (60.0)	1 (8.3)
Grade 1	0	8 (26.7)	2 (16.7)	8 (26.7)	2 (6.9)	1 (8.3)	2 (13.3)	1 (8.3)
Grade 2	0	2 (6.7)	0	2 (6.7)	0	0	4 (26.7)	0
Nausea								
Any	3 (10.0)	6 (20.0)	2 (16.7)	8 (26.7)	1 (3.4)	1 (8.3)	4 (26.7)	4 (33.3)
Grade 1	3 (10.0)	4 (13.3)	2 (16.7)	6 (20.0)	0	1 (8.3)	2 (13.3)	4 (33.3)
Grade 2	0	1 (3.3)	0	1 (3.3)	1 (3.4)	0	1 (6.7)	0
Grade 3	0	1 (3.3)	0	1 (3.3)	0	0	1 (6.7)	0
Arthralgia								
Any	2 (6.7)	4 (13.3)	1 (8.3)	6 (20.0)	2 (6.9)	1 (8.3)	6 (40.0)	0
Grade 1	2 (6.7)	1 (3.3)	1 (8.3)	3 (10.0)	2 (6.9)	1 (8.3)	3 (20.0)	0
Grade 2	0	3 (10.0)	0	3 (10.0)	0	0	3 (20.0)	0
Pyrexia								
Any	0	0	0	3 (10.0)	0	1 (8.3)	4 (26.7)	0
Grade 1	0	0	0	2 (6.7)	0	1 (8.3)	2 (13.3)	0
Grade 2	0	0	0	1 (3.3)	0	0	1 (6.7)	0
Pruritus generalized								
Any	1 (3.3)	1 (3.3)	0	0	0	0	0	1 (8.3)
Grade 1	1 (3.3)	1 (3.3)	0	0	0	0	0	1 (8.3)
Rash								
Any	1 (3.3)	0	0	0	0	0	0	0
Grade 1	1 (3.3)	0	0	0	0	0	0	0
Vomiting								
Any	0	0	1 (8.3)	0	0	0	0	0
Grade 1	0	0	1 (8.3)	0	0	0	0	0

[a] Solicited adverse events with unknown severity are not taken into account in this table.

[b] Grading scale used for solicited local adverse events: 0 = none: absent; 1 = mild: does not interfere with activity or discomfort only to touch; 2 = moderate: repeated use of nonnarcotic pain reliever >24 hours, interferes with activity, or discomfort with movement; 3 = severe: any use of narcotic pain reliever, prevents daily activity, or significant discomfort at rest.

[c] Grading scale used for solicited systemic adverse events: 0 = none: absent; 1 = mild: does not interfere with activity; 2 = moderate: repeated use of nonnarcotic pain reliever >24 hours or interferes with activity; 3 = severe: any use of narcotic pain reliever or prevents daily activity.

4575.3-14 336.1), compared with 17 428.6 (95% CI, 10 303.4-29 481.3) when given in reverse order.

T-Cell Response

Ebola glycoprotein-specific T-cell responses, as assessed by IFN-γ ELISpot, are displayed in **Table 4** and eFigure 3 in Supplement 2. Vaccine-specific responses were observed 14 days after Ad26.ZEBOV prime immunization in 11 of 14 participants (79%; 95% CI, 49%-95%) boosted at day 15 and at 28 days after prime immunization in 9 of 15 (60%; 95% CI, 32%-84%) and 7 of 14 (50%; 95% CI, 23%-77%) recipients (in those boosted at 28- and 56-day intervals, respectively). This response was only observed in 1 of 29 (3%; 95% CI, 0%-18%) primary MVA-BN-Filo recipients.

After booster immunizations, the percentage of T-cell responders was 92%, 79%, and 100% in those receiving Ad26.ZEBOV with MVA-BN-Filo booster at 14-, 28-, and 56-day intervals, respectively, compared with 73% and 87% of those receiving MVA-BN-Filo with Ad26.ZEBOV booster at 28- and 56-day intervals, respectively.

Table 3. Ebola Glycoprotein-Specific Antibody Responses Detected by ELISA[a]

	Prime on Day 1, Boost on Day 29			Prime on Day 1, Boost on Day 57			Prime on Day 1, Boost on Day 15: Ad26.ZEBOV, Then MVA-BN-Filo
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	
Baseline							
No. of participants	15	15	6	15	15	6	15
Geometric mean concentration (95% CI), ELISA units/mL	18.3 (18.3-18.3)	18.3 (18.3-18.3)	18.3 (18.3-18.3)	20.8 (15.8-27.4)	22.0 (14.8-32.7)	18.3 (18.3-18.3)	18.3 (18.3-18.3)
Day 8							
No. of participants	15	15	6	15	15	6	15
Geometric mean concentration (95% CI), ELISA units/mL	18.3 (18.3-18.3)	18.3 (18.3-18.3)	23.4 (12.4-44.0)	21.1 (15.5-28.7)	22.0 (14.8-32.7)	18.3 (18.3-18.3)	21.7 (16.9-27.9)
Responders, No. (%)[b]	0	0	1 (16.7)	0	0	0	2 (13.3)
95% CI	0-21.8	0-21.8	0.4-64.1	0-21.8	0-21.8	0-45.93	1.66-40.46
Day 15							
No. of participants							14
Geometric mean concentration (95% CI), ELISA units/mL							164.4 (68.0-397.8)
Responders, No. (%)[b]							11 (78.6)
95% CI							49.2-95.3
Day 22							
No. of participants							12
Geometric mean concentration (95% CI), ELISA units/mL							298.1 (110.5-803.7)
Responders, No. (%)[b]							10 (83.3)
95% CI							51.6-97.9
Day 29							
No. of participants	15	15	6	15	14	6	
Geometric mean concentration (95% CI), ELISA units/mL	36.0 (21.6-59.8)	532.9 (243.0-1168.8)	18.3 (18.3-18.3)	22.3 (16.5-30.1)	581.1 (363.2-930.1)	18.3 (18.3-18.3)	
Responders, No. (%)[b]	6 (40)	14 (93.3)	0	1 (6.7)	14 (100)	0	
95% CI	16.3-67.7	68.1-99.8	0-45.9	0.2-32.0	76.8-100	0-45.9	
Day 36							
No. of participants	15	15	6				12
Geometric mean concentration (95% CI), ELISA units/mL	269.1 (96.7-748.8)	945.6 (466.0-1919.1)	18.3 (18.3-18.3)				914.7 (432.2-1935.8)
Responders, No. (%)[b]	12 (80)	15 (100)	0				12 (100)
95% CI	51.9-95.7	78.2-100	0-45.9				73.5-100
Day 50							
No. of participants	15	15	6				
Geometric mean concentration (95% CI), ELISA units/mL	10 573.1 (6451.8-17 326.8)	4274.0 (2349.6-7774.7)	23.3 (12.5-43.1)				
Responders, No. (%)[b]	15 (100)	15 (100)	1 (16.7)				
95% CI	78.2-100	78.2-100	0.4-64.1				
Day 57							
No. of participants				15	14	6	
Geometric mean concentration (95% CI), ELISA units/mL				23.6 (16.3-34.2)	854.3 (556.2-1312.2)	18.3 (18.3-18.3)	
Responders, No. (%)[b]				1 (6.7)	14 (100)	0	
95% CI				0.2-32.0	76.8-100	0-45.9	

(continued)

Table 3. Ebola Glycoprotein-Specific Antibody Responses Detected by ELISA[a] (continued)

	Prime on Day 1, Boost on Day 29			Prime on Day 1, Boost on Day 57			Prime on Day 1, Boost on Day 15: Ad26.ZEBOV, Then MVA-BN-Filo
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	
Day 64							
No. of participants				15	14	6	
Geometric mean concentration (95% CI), ELISA units/mL				568.5 (216.4-1493.5)	1554.4 (922.0-2620.6)	18.3 (18.3-18.3)	
Responders, No. (%)[b]				15 (100)	14 (100)	0	
95% CI				78.2-100	76.84-100	0-45.93	
Day 78							
No. of participants				15	14	6	
Geometric mean concentration (95% CI), ELISA units/mL				18 473.5 (12 417.6-27 482.7)	7553.1 (5113.8-11 155.8)	18.3 (18.3-18.3)	
Responders, No. (%)[b]				15 (100)	14 (100)	0	
95% CI				78.2-100	76.84-100	0-45.93	
Day 180							
No. of participants	14	15		14	12		12
Geometric mean concentration (95% CI), ELISA units/mL	3765.2 (2473.0-5732.9)	2114.8 (1244.3-3594.5)		4445.2 (2982.3-6625.5)	2931.6 (2149.3-3998.7)		2082.8 (1197.3-3623.1)
Responders, No. (%)[b]	14 (100)	15 (100)		14 (100)	12 (100)		12 (100)
95% CI	76.8-100	78.2-100		76.8-100	73.5-100		73.5-100
Day 240							
No. of participants	14	15		14	13		10
Geometric mean concentration (95% CI), ELISA units/mL	3739.8 (2511.4-5569.3)	2443.0 (1344.1-4440.4)		3038.1 (1958.3-4713.2)	2240.8 (1555.5-3228.1)		1811.3 (1082.5-3030.7)
Responders, No. (%)[b]	14 (100)	15 (100)		14 (100)	13 (100)		10 (100)
95% CI	76.8-100	78.2-100		76.8-100	75.3-100		69.2-100

Abbreviations: Ad26.ZEBOV, adenovirus type 26 vector vaccine encoding Ebola glycoprotein; CI, exact Clopper-Pearson confidence interval; ELISA, enzyme-linked immunosorbent assay; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein.

[a] Follow-up time points differed across groups; therefore, empty cells represent time points where no follow-up visit was scheduled. Individual values for participants are displayed separately for each group along with geometric mean concentration and confidence intervals in eFigure 1 in Supplement 2. Geometric mean concentrations and confidence intervals for randomized vaccine groups only are presented together in Figure 2.

[b] Responders were those participants whose antibody responses were negative at baseline and positive after baseline or positive at baseline with at least 3-fold increase from baseline.

Exploratory Outcomes

Ebola-specific CD8+ T-cell cytokine expression was observed following primary immunization in 29% and 57% of Ad26.ZEBOV recipients at 14 and 28 days after immunization, respectively, but no MVA-BN-Filo recipients (eFigure 4 and eTable 2 in Supplement 2). At 21 days postboost, the responder rates were 50% to 79% in the Ad26.ZEBOV prime groups and 47% to 53% in MVA-BN-Filo prime groups. All schedules induced highly polyfunctional T-cell responses that were maintained after boost (eFigure 6 in Supplement 2). CD4+ T-cell responses were observed in 57% to 67% of vaccine recipients at 21 days after boosting (eFigure 5 and eTable 3 in Supplement 2).

Immunogenicity Persistence

Sixty-seven of 75 active vaccine recipients attended follow-up at day 240, at which time the durability of immune responses was analyzed. One-hundred percent of these individuals maintained Ebola-specific IgG responses (Table 3). Vaccine-induced T-cell responses persisted in 77% to 80% of randomized Ad26.ZEBOV-prime-with-MVA-BN-Filo-boost participants, compared with 79% to 100% of those receiving the reverse schedules (Table 4). Persistence of the CD8+ response is shown in eFigure 4 and eTable 2 and the CD4+ response in eFigure 5 and eTable 3 in Supplement 2.

Discussion

To our knowledge, this is the first report on the safety and immunogenicity of the Ad26.ZEBOV vaccine and its heterologous combination with MVA-BN-Filo. This phase 1 study demonstrated an acceptable safety profile in recipients of Ad26.ZEBOV and MVA-BN-Filo, albeit in a small sample size. More than 90% of healthy adults generated Ebola glycoprotein-specific IgG 4 weeks after a priming dose of Ad26.ZEBOV, and 55% (95% CI, 35%-74%) developed spe-

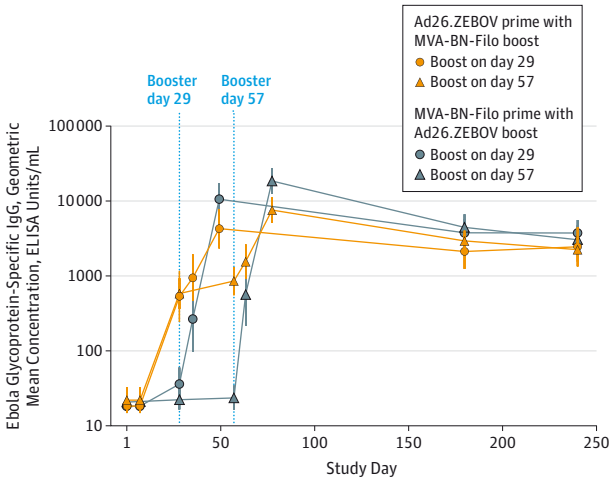
cific T cells. These responses were enhanced by administration of an MVA-BN-Filo booster dose and were sustained at 8 months after the prime vaccination.

The data reported here add to the growing literature on the clinical application of candidate Ebola vaccines. This field includes replication-defective adenovirus 5 and chimpanzee adenovirus 3-vectored vaccines.[2,3,6] The latter has been reported as a single-dose regimen, with persistence of immune responses to 6 months after immunization.[19] Priming with this vaccine and subsequent MVA-BN-Filo boosting resulted in further elevation in humoral and cellular immunity.[7] Interim efficacy data from a ring vaccination trial using a live vesicular stomatitis virus (VSV) vector vaccine in Guinea provided the first indication that protective vaccine-derived immunity against Ebola may be attainable in humans via Ebola glycoprotein expressed by a vectored vaccine.[20] As a live vaccine, the VSV vaccine may be relatively reactogenic, and there were reports of vaccine-induced arthritis and dermatitis in phase 1 studies.[4,5] At lower doses of VSV vaccine, there was reduced immunogenicity and reduced early-onset reactogenicity, but vaccine-induced arthritis and dermatitis were still observed.[21] It is highly desirable that multiple options for immunization against Ebola are available in the event of a resurgence in the incidence of Ebola, either in West Africa or elsewhere, thus reducing any risks associated with potential safety signals or supply problems due to a single vaccine manufacturer.

In our study, injection-site pain was the most commonly reported adverse event, which was generally of mild to moderate severity. Hematological measures were notable for a transient decrease in neutrophil count, observed after 7% of MVA-BN-Filo immunizations and 32% of Ad26.ZEBOV immunizations. Of note, decreased neutrophil count was also observed after 21% of placebo immunizations. Transient neutropenia has been described following immunization with other adenoviral-vectored vaccines, including the chimpanzee adenovirus-vectored Ebola vaccine,[3] and is not perceived to be of any clinical significance.

There is, as yet, no known correlate of protection against Ebola disease. Ebola glycoprotein-specific IgG does appear to have an important role in immunity.[22,23] In addition, data from nonhuman primate models provide evidence of a role for cellular immunity, particularly CD8+ T cells producing TNF-α and IFN-γ (+/− IL-2),[14,24] as detected in this study. No data are published on the cellular immune response to the VSV vaccine shown to be protective in humans; however, comparisons between the ELISA end point titers observed following VSV immunization and the heterologous Ad26.ZEBOV and MVA-BN-Filo immunization schedule can be made, as both studies conducted ELISA analyses according to the Joint Vaccine Acquisition Program protocol. Within the limits of comparisons between different clinical laboratories and studies, the end point titers observed at 21 days after the booster dose in the 4 randomized groups in our study were higher than was reported 28 days after a single dose of the VSV vaccine at a dose of 2×10^7 plaque-forming units.[4,5]

Figure 2. Ebola Glycoprotein-Specific Antibody Responses for Vaccine Recipients in Randomized Groups



These data, along with those for recipients of Ad26.ZEBOV prime and MVA-BN-Filo boost at 14-day interval and for placebo recipients, are in Table 3 and eFigure 1 in Supplement 2. Day 1 is baseline, the day of first vaccination. For numbers of participants included at each time point, see Table 3. Error bars indicate 95% confidence intervals; Ad26.ZEBOV, adenovirus-type 26 vector vaccine encoding Ebola glycoprotein; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein.

The increased immunogenicity seen after heterologous prime-boost in this study and the persistence of cellular and humoral immune responses to at least 8 months after priming immunization might provide an advantage over a single-dose strategy, particularly given the dwindling Ebola epidemic when durability of protection may become more important than the speed with which complete protection is achieved. Epidemiological modeling provides evidence that prophylactic immunization of health care workers in Ebola epidemic risk areas using vaccines offering durable protection would offer far greater benefit, in terms of limiting the effect of any future outbreak, when compared with a reactive immunization strategy such as ring vaccination.[25] Furthermore, the evidence of persistence of Ebola virus in bodily fluids,[26,27] as well as the potential for onward sexual transmission by convalescent Ebola survivors,[28] reinforce the importance of generating a durable immune response.

Our data showed that, in contrast to MVA-BN-Filo, Ad26.ZEBOV priming generated an initial immune response, and there is evidence for protection from this vaccine given alone in nonhuman primate models.[13] Therefore, this priming dose would be expected to generate at least partial protection against Ebola; for this reason, Ad26.ZEBOV prime schedules with MVA-BN-Filo boost are currently being further evaluated in phase 1, 2, and 3 studies.

The relatively low response to MVA-BN-Filo prime might limit the use of the MVA-BN-Filo prime schedules in an Ebola outbreak situation. However, the postboost antibody and T-cell responses appear robust in these groups,

Table 4. Ebola Glycoprotein-Specific T-Cell Responses as Assessed by Interferon-γ ELISpot[a]

	Prime on Day 1, Boost on Day 29			Prime on Day 1, Boost on Day 57			Prime on Day 1, Boost on Day 15: Ad26.ZEBOV, Then MVA-BN-Filo
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	
Baseline							
No. of participants	15	15	6	15	15	6	15
Median (IQR), SFUs/million cells	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)
Day 8							
No. of participants	15	15	6	15	15	6	15
Median (IQR), SFUs/million cells	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-25)	25 (25-56.7)
Responders, No. (%)[b]	0	1 (6.7)	0	0	1 (6.7)	0	2 (13.3)
95% CI	0-21.8	0.17-32.0	0-45.9	0-21.8	0.2-32.0	0-45.9	1.7-40.5
Day 15							
No. of participants							14
Median (IQR), SFUs/million cells							112.5 (70-156.7)
Responders, No. (%)[b]							11 (78.6)
95% CI							49.2-95.3
Day 22							
No. of participants							12
Median (IQR), SFUs/million cells							354.2 (211.7-405.8)
Responders, No. (%)[b]							9 (75)
95% CI							42.8-94.5
Day 29							
No. of participants	15	15	6	15	14	6	
Median (IQR), SFUs/million cells	25 (25-81.7)	103.3 (61.7-173.3)	25 (25-25)	25 (25-25)	57.5 (25-278.3)	25 (25-25)	
Responders, No. (%)[b]	1 (6.7)	9 (60)	0	0	7 (50)	0	
95% CI	0.2-31.95	32.3-83.7	0-45.9	0-21.8	23.0-77.0	0-45.9	
Day 36							
No. of participants	15	15	6				12
Median (IQR), SFUs/million cells	881.7 (430-2360)	463.3 (228.3-781.7)	25 (25-25)				202.5 (112.5-299.2)
Responders, No. (%)[b]	14 (93.3)	13 (86.7)	0				11 (91.7)
95% CI	68.1-99.8	59.5-98.3	0-45.9				61.52-99.79
Day 50							
No. of participants	15	14	5				
Median (IQR), SFUs/million cells	455 (146.7-940)	390 (265-540)	25 (25-25)				
Responders, No. (%)[b]	11 (73.3)	11 (78.6)	0				
95% CI	44.9-92.2	49.2-95.3	0-52.2				
Day 57							
No. of participants				15	14	6	
Median (IQR), SFUs/million cells				25 (25-25)	282.5 (196.7-521.7)	25 (25-25)	
Responders, No. (%)[b]				0	12 (85.7)	0	
95% CI				0-21.8	57.19-98.2	0-45.9	
Day 64							
No. of participants				15	14	6	
Median (IQR), SFUs/million cells				440 (313.3-883.3)	648.3 (223.3-998.3)	25 (25-25)	
Responders, No. (%)[b]				15 (100)	12 (85.7)	0	
95% CI				78.2-100	57.2-98.2	0-45.9	

(continued)

Table 4. Ebola Glycoprotein-Specific T-Cell Responses as Assessed by Interferon-γ ELISpot[a] (continued)

	Prime on Day 1, Boost on Day 29			Prime on Day 1, Boost on Day 57			Prime on Day 1, Boost on Day 15: Ad26.ZEBOV, Then MVA-BN-Filo
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Placebo	
Day 78							
No. of participants				15	14	6	
Median (IQR), SFUs/million cells				238.3 (118.3-416.7)	464.2 (161.7-673.3)	25 (25-25)	
Responders, No. (%)[b]				13 (86.7)	14 (100)	0	
95% CI				59.5-98.3	76.8-100	0-45.9	
Day 180							
No. of participants	14	15		14	12		10
Median (IQR), SFUs/million cells	785 (166.7-1255)	325 (181.7-563.3)		421.7 (298.3-575)	370.8 (200-1033.3)		353.3 (140-510)
Responders, No. (%)[b]	10 (71.4)	12 (80)		13 (92.9)	10 (83.3)		8 (80)
95% CI	41.9-91.6	51.9-95.7		66.1-99.8	51.6-97.9		44.4-97.5
Day 240							
No. of participants	14	15		14	13		10
Median (IQR), SFUs/million cells	485 (95-810)	266.7 (110-395)		419.2 (281.7-461.7)	216.7 (100-585)		353.3 (140-510)
Responders, No. (%)[b]	11 (78.6)	12 (80)		14 (100)	10 (76.9)		8 (80)
95% CI	49.2-95.3	51.9-95.7		76.8-100	46.2-95.0		44.4-97.5

Abbreviations: Ad26.ZEBOV, adenovirus type 26 vector vaccine encoding Ebola glycoprotein; CI, exact Clopper-Pearson confidence interval; ELISpot, enzyme-linked immunospot on frozen peripheral blood mononuclear cells shown as number of spot-forming units (SFUs) per million cells; IQR, interquartile range; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein; No., number of participants with data and for responders, number of participants with data at baseline and at postbaseline time point.

[a] Follow-up time points differed across groups; therefore, empty cells represent time points where no follow-up visit was scheduled.

[b] Responders were those participants whose antibody responses were negative at baseline and positive after baseline or positive at baseline with at least 3-fold increase from baseline.

and these schedules merit further study. Additional immunization schedules using priming with the MVA-BN-Filo vaccine are being evaluated in a separate phase 1 study and will further inform the potential role for this approach.[29] These data may also have relevance to other investigational vaccines using MVA-based vaccines in heterologous prime-boost immunization schedules, such as those against malaria.[30]

This study has limitations. Although the data presented here suggest the potential for sustained elevation of specific immunity, this was a phase 1 study designed to determine safety and immunogenicity in a population unconfounded by intercurrent Ebola infections. As such, it was conducted in a population unlikely to be affected by Ebola. This may be of relevance when considering the importance of baseline immunity against the Ad26 vector in potentially impairing the immune response to the target antigen, as was seen for an Ad5-vectored Ebola vaccine.[6] Seroepidemiological studies reveal that high neutralizing antibody titers to the Ad26 serotype are uncommon in the populations of Europe, North America, and sub-Saharan Africa,[31-33] with titers above 1000 seen in 0% to 6.3% of adults from sub-Saharan Africa.[32] Approximately 40% to 60% of tested sera from sub-Saharan Africa did display low to moderate (18-1000) neutralizing antibody titers against Ad26.[33] While the lack of interference observed in Ad26-seropositive rhesus monkeys immunized with an Ad26-vectored HIV vaccine provides some reassurance that such titers will not interfere with the vaccine's immunogenicity,[33] no human data are yet available to address this issue. The very low proportion of individuals with baseline Ad26-neutralizing antibody observed in our study population (3.4%) precludes any conclusion regarding the effect of preexisting vector immunity on vaccine immunogenicity. This issue will be evaluated in ongoing phase 1 and phase 2 studies. Furthermore, analyses of the immune response to the additional filovirus antigens incorporated in the MVA-BN-Filo vaccine are planned but not available as yet. Another limitation is the pragmatic addition of the open-label, nonrandomized group to provide additional, expedited, data; the rates of solicited symptoms in the open-label group therefore need to be interpreted with caution.

Conclusions

Immunization with Ad26.ZEBOV or MVA-BN-Filo demonstrated no serious vaccine-related adverse events. An immune response was observed after primary immunization with Ad26.ZEBOV; boosting by MVA-BN-Filo resulted in sustained elevation of Ebola glycoprotein-specific immunity. The immunogenicity and safety of these vaccines are being further assessed in phase 2 and 3 studies.

ARTICLE INFORMATION

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Obtained funding: Pau, Douoguih, Pollard, Snape.
Administrative, technical, or material support: Gibani, Sewell, Clutterbuck, Plested, Nuthall, Silva-Reyes, McElrath, Frahm, Cohen, Splinter, Ewer, Angus, Snape.
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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Milligan, Dr Gibani, Mr Sewell, Dr Clutterbuck, Dr Campbell, Ms Plested, Dr Nuthall, Ms Voysey, Dr Silva-Reyes, Dr Ewer, Dr Angus, Dr Pollard, and Dr Snape are all employed by the University of Oxford, which received a grant from Crucell Holland for the conduct of aspects of this study (14-day interval regimen). Mr Sewell reported having received grants from Global Clinical Operations (a division of Janssen-Cilag). Dr Nuthall reported having received grants from Novartis, GlaxoSmithKline, and Pfizer. The Department of Paediatrics at the University of Oxford has received unrestricted educational grants for scientific meetings from vaccine manufacturers. Dr Snape has participated in advisory boards for vaccine manufacturers, has presented at industry-sponsored symposia, and has had assistance from vaccine manufacturers to attend conferences. Payments for these activities are made to the University of Oxford and Dr Snape has not received any personal financial benefit. Drs Pollard and Snape reported having received grants from GlaxoSmithKline. Dr McElrath reported having received support from Merck Sharp and Dohme. Drs De Rosa and Frahm reported having received support from Janssen. Drs Shukarev, Orzabal, van Duijnhoven, Truyers, Bachmayer, Splinter, Pau, Schuitemaker, Luhn, Callendret, Van Hoof, and Douoguih are all employees of Janssen, Pharmaceutical Companies of Johnson & Johnson, of which Crucell Holland is a subsidiary. Drs van Duijnhoven, Bachmayer, Pau, Schuitemaker, Luhn, Callendret, Van Hoof, and Douoguih reported having received grants or other support from the National Institutes of Health. Dr Orzabal, Pau, Callendret, and Douoguih reported having patents pending related to the work in the study. Dr Samy is an employee of Bavarian Nordic. No other disclosures were reported.

Funding/Support: This trial was funded by the European Union's Innovative Medicines Initiative, as part of the EBOVAC1 consortium, under grant agreement 115854. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and the European Federation of Pharmaceutical Industries and Associations. The study sponsor was Crucell Holland, who also provided funding for the nonrandomized group immunized at a 14-day interval.

Role of the Funder/Sponsor: The Innovative Medicines Initiative, the National Institutes of Health (NIH), and the National Institute of Allergy and Infectious Diseases (NIAID) had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The study was designed as a collaboration between the sponsor (Crucell Holland) and the investigators. Data were collected by investigators at the Oxford Vaccine Group. Data analysis was performed by employees of the sponsor and independently verified by a statistician at the University of Oxford.

Additional Contributions: MVA-BN Filo was produced under NIAID/Fisher BioServices contract FBS-004-009 and NIH contract HHSN272200800044C. The Janssen filovirus project has been funded in part with direct federal funds from the NIAID, part of NIH, under contract HHSN272200800056C. The Joint Vaccine Acquisition Program, part of the US Joint Program Executive Office for Chemical and Biological Defense, provided recombinant Ebola virus glycoprotein for the ELISA reported here. Professor Pollard and Dr Snape are Jenner Investigators. Professor Pollard and Drs Snape and Ewer acknowledge the support of the NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS trust. We thank our partners in EBOVAC1, the London School of Hygiene and Tropical Medicine, and the French National Institute for Health and Medical Research (INSERM) for their important contribution to the clinical development of these vaccines; the Medicines and Healthcare Products Regulatory Agency and the South Central–Oxford A Research Ethics Committee for their rapid review and approval of the study; and all the volunteers who took part in the study and the research staff who contributed to the successful completion of the trial.

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