The Journal of Infectious Diseases

MAJOR ARTICLE

Safety and Immunogenicity of a 2-Dose Heterologous Vaccine Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Nairobi, Kenya

Gaudensia Mutua,[1] Omu Anzala,[1] Kerstin Luhn,[2] Cynthia Robinson,[2] Viki Bockstal,[2] Dickson Anumendem,[2] and Macaya Douoguih[2]

[1]Kenya AIDS Vaccine Initiative Institute of Clinical Research, College of Health Sciences, University of Nairobi, Kenya; and [2]Janssen Vaccines and Prevention, Leiden, the Netherlands

(See the Major Article by Anywaine et al on pages 46–56.)

Background. During the 2014 West African Ebola outbreak, Ebola vaccine development was accelerated. The phase 1 VAC52150EBL1003 study was performed to investigate 2-dose heterologous vaccination with Ad26.ZEBOV and MVA-BN-Filo in an African population located in a high-altitude setting in Nairobi, Kenya.

Methods. Healthy adult volunteers were randomized to receive one of four 2-dose vaccination schedules. The first vaccination was administered at baseline (Ad26.ZEBOV or MVA-BN-Filo), followed by the second vaccination with the alternate vaccine after either 28 or 56 days. Each schedule had a placebo comparator group. The primary objective was to assess the safety and tolerability of these regimens.

Results. Seventy-two volunteers were randomized into 4 groups of 18 (15 received vaccine, and 3 received placebo). The most frequent solicited systemic adverse event was headache (frequency, 50%, 61%, and 42% per dose for MVA-BN-Filo, Ad26.ZEBOV, and placebo, respectively). The most frequent solicited local AE was injection site pain (frequency, 78%, 63%, and 33% per dose for MVA-BN-Filo, Ad26.ZEBOV, and placebo, respectively). No differences in adverse events were observed among the different vaccine regimens. High levels of binding and neutralizing anti–Ebola virus glycoprotein antibodies were induced by all regimens and sustained to day 360 after the first dose.

Conclusions. Two-dose heterologous vaccination with Ad26.ZEBOV and MVA-BN-Filo was well tolerated and highly immunogenic against Ebola virus glycoprotein.

Clinical trials registration. NCT02376426

Keywords. Ebola vaccine; heterologous 2-dose; Ad26.ZEBOV; MVA-BN-Filo; safety and immunogenicity.

The 2014 Ebola virus (EBOV) outbreak in West Africa caused 11 300 deaths and major socioeconomic disruption [1]. In response to this international public health emergency, the global community hastened the clinical development of several candidate Ebola vaccines [2]. Emerging data from various vaccine regimens provide encouraging evidence that vaccination to prevent EBOV disease is feasible. A 2-dose heterologous filovirus vaccine regimen has been under development at Janssen Vaccines and Prevention, in collaboration with other partners, including the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases, National Institutes of Health. In 2014, the development of a monovalent Ebola vaccine was accelerated. For this approach, which is described here, 2 vaccine candidates are used. The first vaccine is a recombinant, live, nonreplicating adenovirus serotype 26 vector (Ad26) expressing the EBOV glycoprotein (Ad26.ZEBOV). The second vaccine is a multivalent, replication-deficient modified vaccinia Ankara (MVA) vector expressing EBOV, Sudan virus, and Marburg virus glycoproteins and Tai Forest virus nucleoprotein (MVA-BN-Filo; Bavarian Nordic, Kvisgaard, Denmark). Both vaccines are being investigated in various phase 2/3 clinical studies as a 2-dose heterologous strategy.

The clinical program for Ad26.ZEBOV and MVA-BN-Filo heterologous 2-dose vaccination comprises four phase 1 trials (completed) and 7 ongoing phase 2/3 studies. Safety, tolerability, and immunogenicity of the vaccine are being comprehensively evaluated in a broad range of populations, including healthy adults, adults infected with human immunodeficiency virus (HIV), adolescents, and children ≥1 year of age. In the first-in-human study

Received 21 September 2018; editorial decision 31 January 2019; accepted 20 February 2019; published online February 25, 2019.

Correspondence: C. Robinson, Janssen Vaccines and Prevention, Archimedesweg 4–6, Leiden, 2333CN, the Netherlands (crobins6@its.jnj.com).

The Journal of Infectious Diseases® 2019;220:57–67

DOI: 10.1093/infdis/jiz071

based in the United Kingdom, no vaccine-related serious adverse events (AEs) were reported. EBOV glycoprotein–specific immunoglobulin G responses were detected in 80% of participants (healthy volunteers) as early as 14 days after the first vaccination. Response levels increased further, with all participants exhibiting a binding antibody response 21 days after dose 2. Vaccine-induced immune responses persisted to 1 year [3, 4].

Here we present results of the phase 1 VAC52150EBL1003 study (clinical trials registration NCT02376426), which was performed in an African population in urban Nairobi, Kenya, to characterize the safety, tolerability, and immunogenicity of heterologous, 2-dose regimens involving Ad26.ZEBOV- and MVA-BN-Filo–based vaccines. Findings at dosing intervals of 28 and 56 days were compared, and the durability of the immune responses over 12 months was assessed. This study was conducted as a precursor to the VAC52150EBL1004 study, which was performed in 2 mid-level–altitude, malaria-endemic settings (described by Anywaine et al [5]).

METHODS

Study Population

Healthy volunteers aged 18–50 years and living in Kenya were eligible to participate. Recruited participants were local to the study center, living in the relatively high-altitude, urban setting of Nairobi, which generally has a lower incidence of malaria than the rest of Kenya.

Exclusion criteria included (but were not limited to): prior vaccination with a candidate Ebola vaccine or any other Ad26.ZEBOV- or MVA-BN-Filo–based vaccine; diagnosis of EBOV disease or exposure to EBOV, including travel to West Africa during the preceding 12 months; history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products; chronic medical conditions that required medication or were not adequately controlled and significant acute or chronic infective conditions (eg, infection with HIV, hepatitis B virus, or hepatitis C virus). Women who were pregnant or breastfeeding were also excluded. Eligible participants had safety laboratory parameters (determined by chemistry analysis, hematologic analysis, and urinalysis) within institutional normal ranges.

Study Design

This phase 1, randomized, placebo-controlled, observer-blinded, single-center study (clinical trials registration NCT02376426) was conducted at the Kenya AIDS Vaccine Initiative Institute of Clinical Research, University of Nairobi, from 1 April 2015 to 21 June 2016. The Nairobi study site is an urban area of Kenya with no endemic malaria, located at an altitude of around 1700 m. The protocol and study documents were reviewed and approved by the local ethics committee and the Kenyan regulatory authority. The trial was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki. All participants gave formal, written consent before undergoing any trial-related procedure. The study used stringent eligibility criteria, assessed by travel and medical history, to check whether subjects were pre-exposed to EBOV. However, participants were not specifically tested for previous exposure to EBOV. Following a ≤28-day screening period for eligible subjects, participants were randomized to receive one of 4 vaccination schedules (Figure 1). First vaccination (dose 1) was administered at baseline (day 1; Ad26.ZEBOV or MVA-BN-Filo), followed by second vaccination (dose 2) with the alternate vaccine after either 28 or 56 days. Each vaccination schedule had a placebo comparator group. All vaccines were administered intramuscularly into the deltoid muscle. Ad26.ZEBOV was given in doses of 0.5 mL, each containing 5×10^{10} viral particles. Each dose of MVA-BN-Filo (0.5 mL) comprised 1×10^{8} 50% tissue culture infectious doses.

Randomization and Masking

Participants were randomized centrally, using a computer-generated block randomization schedule with randomly permuted blocks and an interactive web response system. Participants and most study personnel were blinded to active/placebo vaccine allocation until the last participant attended the visit on day 21 after the second vaccination or discontinued participation in the study. A small team of unblinded study personnel was responsible for study vaccine preparation but had no other involvement in study procedures or assessments.

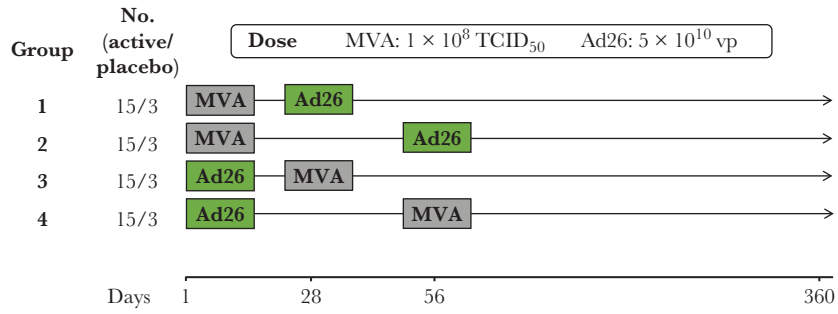

Figure 1. VAC52150EBL1003 study design. Ad26, Ad26.ZEBOV; MVA, MVA-BN-Filo; $TCID_{50}$, 50% tissue culture infectious doses; vp, virus particle.

Objectives

The primary objective was to assess safety and tolerability as expressed by the number of participants with AEs. The main secondary outcomes were EBOV glycoprotein–specific humoral and cellular immune responses induced by the vaccine regimens.

Safety and Tolerability Assessments

Participants were observed for 1 hour after vaccination, and any immediate AEs were recorded. Subsequent local and systemic solicited AEs were recorded by diary card for 7 days following each vaccination. Unsolicited AEs were collected for 28 days following each vaccination. All AEs were graded according to the DMID toxicity table for use in trials enrolling healthy adults [6]. Blood safety parameters were measured before study vaccination and 7 days after each vaccination; the troponin I level was additionally assessed 3 days after each vaccination. Abnormal laboratory findings that were clinically significant or classified as grade 3, according to Food and Drug Administration laboratory toxicity grading, were reported as AEs. Twelve-lead electrocardiography was performed at screening and 3 days after each vaccination. AEs of special interest were as follows: any cardiac sign or symptom, clinically significant electrocardiogram changes, or increased troponin I levels (ie, ≥0.06 μg/L). Troponin I levels were of interest because of concerns with early generation MVA-BN–based vaccines.

Immunogenicity Assessments

Immune responses to the study vaccine regimens were measured using serum samples collected before each vaccination, 7 days after each vaccination, and 21 days after the second vaccination. Participants who received vaccines with a 56-day interval had an additional blood specimen collected 28 days after the first vaccination. Long-term follow-up samples were collected in all groups at days 180, 240, and 360.

Total immunoglobulin G responses against EBOV glycoprotein were analyzed using the EBOV Glycoprotein (Kikwit) FANG Enzyme-Linked Immunosorbent Assay (ELISA; Q2 Solutions) as described previously [3]. The neutralizing activity of vaccine-induced antibody responses was assessed using the EBOV glycoprotein (Makona) Pseudovirus Neutralizing Antibody Assay (Monogram Biosciences; San Francisco, CA). This assay is an adaptation of the Monogram PhenoSense HIV Neutralizing Antibody Assay [7, 8]. Briefly, EBOV glycoprotein and a luciferase reporter gene expressing pseudovirions are mixed with a serially diluted serum sample. After incubation, the sample is transferred to a HEK293 cell monolayer. Luciferase expression is used as a measure of pseudovirion infection.

Frozen peripheral blood mononuclear cell samples were analyzed with an intracellular cytokine staining (ICS) assay at the HIV Vaccine Trials Network Laboratory (Seattle, WA) [3, 9].

Data Analysis and Statistics

This phase 1 study was conducted to provide a preliminary safety and immunogenicity assessment, with no formal sample size calculation. The primary analysis set for safety (ie, the full analysis set) included all participants who were randomized and received at least 1 dose of study vaccine, regardless of protocol deviations. The primary analysis set for immunogenicity included all randomized and vaccinated participants with immunogenicity data at baseline and at least 1 postvaccination immunogenicity measurement.

Safety and immunogenicity data were analyzed using descriptive statistics for continuous variables and were tabulated for discrete variables. The frequencies of local and systemic solicited AEs and unsolicited AEs were reported as the percentage per dose of active vaccine or placebo.

Immunogenicity data are presented in the same way as in a previous phase 1 study [3, 4]. Antigen-specific binding immunoglobulin G responses and neutralizing antibody activity are shown as geometric mean concentrations (GMCs) and geometric mean titers, respectively, with 95% confidence intervals (CIs). Humoral immune response values were $\log_{10}$ transformed, and these values were used throughout the analyses. Medians and interquartile ranges, with background subtracted, are reported for the total $CD4^+$ and $CD8^+$ T-cell responses from ICS assays, expressed as the percentage of T-cell subsets ($CD4^+$ and $CD8^+$) that produce any of 3 cytokines (interferon γ, tumor necrosis factor α, and interleukin 2). ICS was interpreted as positive if the probability of expressing cytokines was statistically different (by the Fisher exact test) between the antigen and the pooled negative controls for at least 1 antigen (peptide pools 1 or 2). All values below the lower limit of quantification (LLOQ) were substituted with half the LLOQ (ie, 13.11 ELISA units [EU]/mL for ELISA, a 50% inhibitory concentration (IC_{50}) of 60 for VNA, and 0.02% of T-cell subsets for background-subtracted ICS results).

A participant was defined as a responder, based on ELISA, VNA, or ICS findings, at each time point after baseline if the test result was negative at baseline and positive after baseline or if a test result that was positive at baseline was followed by a result that increased by at least a 3-fold. Responses in placebo recipients were low or not quantifiable and are therefore not described in Results.

All statistical analysis was done using SAS, version 9.2. Given the small sample sizes in each vaccination group and minimal evidence available regarding statistical hypothesis testing, no formal statistical testing of safety data or immune responses was planned or performed.

RESULTS

The study was initiated in March 2015 and completed in September 2016. Seventy-two healthy adult volunteers were recruited and randomized among 4 groups of 18 (15 receiving vaccine and 3 receiving placebo). Participants' baseline characteristics are shown in Table 1. In the overall population, there were 23 females (31.9%), the median age was 25 years (range,

Table 1. Baseline Characteristics of the Full Analysis Set

Characteristic	Dose 2 at Day 28			Dose 2 at Day 56		
	MVA/Ad26 (n = 15)	Ad26/MVA (n = 15)	Placebo (n = 6)	MVA/Ad26 (n = 15)	Ad26/MVA (n = 15)	Placebo (n = 6)
Sex						
Female	6 (40)	3 (20)	3 (50)	4 (26.7)	4 (26.7)	3 (50)
Male	9 (60)	12 (80)	3 (50)	11 (73.3)	11 (73.3)	3 (50)
Age, y	27 (18–38)	25 (18–41)	32.5 (23–45)	25 (18–34)	24 (20–29)	23.5 (18–28)
Black or African American race	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)
Body mass index[a]	22.7 (18.7–30.9)	23.8 (19.0–29.0)	22.7 (19.9–31.6)	21.9 (16.5–29.9)	21.8 (17.4–34.0)	24.1 (18.4–31.8)

Data are no. (%) of participants or median value (range).

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

18–45 years), and the median body mass index (calculated as the weight in kilograms divided by the height in meters squared) was 22.6 (range, 16.5–34.0).

Safety and Tolerability

Solicited local and systemic AEs are shown in Tables 2 and 3, respectively, for placebo, MVA-BN-Filo, and Ad26.ZEBOV (whether administered as the first or second vaccination). Solicited AEs following MVA-BN-Filo and Ad26.ZEBOV were generally mild to moderate in severity. No differences in AE patterns were seen with the different vaccine sequences or intervals. The most frequently reported solicited local AE was injection-site pain (Table 2). No cases of injection-site erythema, induration, or swelling were reported. No grade 3 solicited local AEs were reported with MVA-BN-Filo, while 1 vaccination with Ad26.ZEBOV elicited grade 3 injection site pain, pruritus, and warmth (the participant was receiving treatment for concurrent clinical malaria). The same participant reported grade 3 solicited systemic AEs of fatigue, headache, and chills. The most frequently reported solicited systemic AEs were headache, fatigue and myalgia (Table 3). Solicited local and systemic AEs were generally short-lived, with median durations between 1 and 3 days. The median time to onset was 1–2 days.

Unsolicited AEs 28 days after vaccination were reported by 87% of volunteers when MVA-BN-Filo was administered first, by 67% of volunteers when Ad26.ZEBOV was administered as the first vaccination, and by 67% after receipt of placebo. The most frequent unsolicited AE was upper respiratory tract infection, regardless of the regimen or study vaccine. Grade 3 abnormal laboratory findings, reported as unsolicited AEs, were reported by 4 participants after receiving Ad26.ZEBOV as the first vaccination (3 had a lower hemoglobin level than at baseline, and 1 had a lower platelet count than at baseline), by 1 after receiving MVA-BN-Filo as the second vaccination (a lower neutrophil count than at baseline), and by 1 after receiving a second vaccination with placebo (a lower hemoglobin level than at baseline). Only the grade 3 decrease in the neutrophil count was considered to be related to the study vaccine.

AEs of special interest were reported as follows. One volunteer had an asymptomatic grade 1 electrocardiographic T-wave inversion following MVA-BN-Filo dose 2. This was considered possibly related to the vaccine and resolved without intervention. Two volunteers who received Ad26.ZEBOV as the first dose experienced an increased troponin I level, to 0.07 and 0.08 μg/L (normal value, <0.03 μg/L), but no associated clinical manifestations were observed.

No vaccine-related serious AEs were reported. In the MVA-BN-Filo, Ad2.ZEBOV 0, 56 regimen, 1 participant discontinued participation after first vaccination, owing to an AE (wheezing, a grade 1 unsolicited AE). Four participants had malaria during

Table 2. Solicited Local Adverse Events (AEs) Following First and Second Dose Vaccination With Standard Doses of Ad26.ZEBOV (Ad26) and MVA-BN-Filo (MVA)

AE, Severity	MVA (n = 60)	Ad26 (n = 59)	Placebo (n = 24)
Any			
Any	48 (80)	38 (64)	11 (46)
Grade 1	40 (67)	32 (54)	10 (42)
Grade 2	8 (13)	5 (9)	1 (4)
Grade 3	0	1 (2)	0
Injection site pain			
Any	47 (78)	37 (63)	8 (33)
Grade 1	39 (65)	32 (54)	8 (33)
Grade 2	8 (13)	4 (7)	0
Grade 3	0	1 (2)	0
Injection site pruritus[a]			
Any	10 (17)	10 (17)	2 (8)
Grade 1	10 (17)	9 (15)	2 (8)
Grade 2	0	1 (2)	0
Injection site warmth			
Any	13 (22)	18 (31)	6 (25)
Grade 1	13 (22)	15 (25)	5 (21)
Grade 2	0	2 (3)	1 (4)
Grade 3	0	1 (2)	0

Data are no. (%) of doses and reflect pooled first and second dose vaccination data from all 4 vaccination regimens.

[a]No grade 3 AEs were reported.

Table 3. Solicited Systemic Adverse Events (AEs) Following First and Second Dose Vaccination With Standard Doses of Ad26.ZEBOV (Ad26) and MVA-BN-Filo (MVA)

AE, Severity	MVA (n = 60)	Ad26 (n = 59)	Placebo (n = 24)
Any			
Any	41 (68)	44 (75)	14 (58)
Grade 1	28 (47)	30 (51)	13 (54)
Grade 2	13 (22)	13 (22)	1 (4)
Grade 3	0	1 (2)	0
Headache			
Any	30 (50)	36 (61)	10 (42)
Grade 1	22 (37)	26 (44)	9 (38)
Grade 2	8 (13)	9 (15)	1 (4)
Grade 3	0	1 (2)	0
Fatigue			
Any	26 (43)	27 (46)	8 (33)
Grade 1	23 (38)	21 (36)	7 (29)
Grade 2	3 (5)	5 (9)	1 (4)
Grade 3	0	1 (2)	0
Myalgia[a]			
Any	19 (32)	17 (29)	4 (17)
Grade 1	16 (27)	11 (19)	4 (17)
Grade 2	3 (5)	6 (10)	0
Arthralgia[b]			
Any	13 (22)	18 (31)	4 (17)
Grade 1	12 (20)	15 (25)	4 (17)
Grade 2	1 (2)	3 (5)	0
Chills[a]			
Any	6 (10)	16 (27)	1 (4)
Grade 1	6 (10)	13 (22)	1 (4)
Grade 2	0	2 (3)	0
Grade 3	0	1 (2)	0
Nausea[a]			
Any	5 (8)	10 (17)	4 (17)
Grade 1	5 (8)	8 (14)	4 (17)
Grade 2	0	2 (3)	0
Pruritus (generalized)[a]			
Any	5 (8)	7 (12)	1 (4)
Grade 1	4 (7)	5 (9)	1 (4)
Grade 2	1 (2)	2 (3)	0
Vomiting[a]			
Any	5 (8)	5 (9)	1 (4)
Grade 1	5 (8)	4 (7)	1 (4)
Grade 2	0	1 (2)	0
Pyrexia[a,c]			
Any	3 (5)	7 (12)	0
Grade 1	0	5 (9)	0
Grade 2	3 (5)	2 (3)	0
Rash[a]			
Any	3 (5)	1 (2)	1 (4)
Grade 1	3 (5)	0	1 (4)
Grade 2	0	1 (2)	0

Data are no. (%) of doses and reflect pooled first and second dose vaccination data from all 4 vaccination regimens.

[a]No grade 3 AEs were reported.

[b]No grade 2 or 3 AEs were reported.

[c]Grade 1 pyrexia, ≥37°C; grade 2, ≥38.5°C; and grade 3, ≥40.0°C.

the study (all were in the Ad26.ZEBOV dose 1 groups; 1 was in the group with a 28-day interval, and 3 were in the group with a 56-day interval).

Immunogenicity

Binding-Antibody Responses

In the Ad26.ZEBOV, MVA-BN-Filo groups, the percentage of participants with an antigen-specific binding-antibody response reached 93% (in the group with a 28-day interval) and 100% (in the group with a 56-day interval) at the time of the second vaccination. Twenty-one days following the second vaccination with MVA-BN-Filo, 100% of participants in both interval groups were responders, with GMCs increasing to 5156 and 16 341 EU/mL for the 28- and 56-day regimens, respectively (Figure 2A).

For the MVA-BN-Filo, Ad26.ZEBOV groups, 40% (in the group with a 28-day interval) and 60% (in the group with a 56-day interval) of participants generated binding-antibody responses at the time of the second vaccination. At 21 days after Ad26.ZEBOV dose 2 vaccination, the percentage of responders increased to 100% with the 28-day interval and to 93% with the 56-day interval. GMCs increased over time to 8613 and 15 308 EU/mL for the 28-day and 56-day intervals, respectively (Figure 2A).

In all vaccine regimens, a decline in antibody concentrations was observed between their peak after the second vaccination and the 6-month period after dose 1, reaching a stable level that was sustained to day 360 after dose 1 for all regimens (Figure 3A). At day 360, responder rates ranged from 93% to 100% across all regimens, with GMCs ranging from 403 to 613 EU/mL.

Virus-Neutralizing Antibody Responses

In the Ad26.ZEBOV, MVA-BN-Filo groups, 93% (for the 28-day interval group) and 100% (for the 56-day interval group) of participants demonstrated neutralizing antibody responses 21 days after dose 2, with a geometric mean IC_{50} of 6555 in the 56-day regimen (Figure 2B).

In the MVA-BN-Filo, Ad26.ZEBOV groups, neutralizing antibody responses were observed 21 days after dose 2 in 93% of participants. Geometric mean IC_{50} values reached 669 and 3169 twenty-one days after vaccination for the 28-day and 56-day interval groups, respectively (Figure 2B).

With all regimens, the magnitude of the responses declined by day 180 after dose 1 but remained stable thereafter to day 360 (Figure 3B).

$CD8^+$ T-Cell Responses

The frequency of participants with a $CD8^+$ T-cell response (as measured by ICS) tended to be low, with the highest frequency, (responder rate) 27%, detected in both Ad26.ZEBOV,

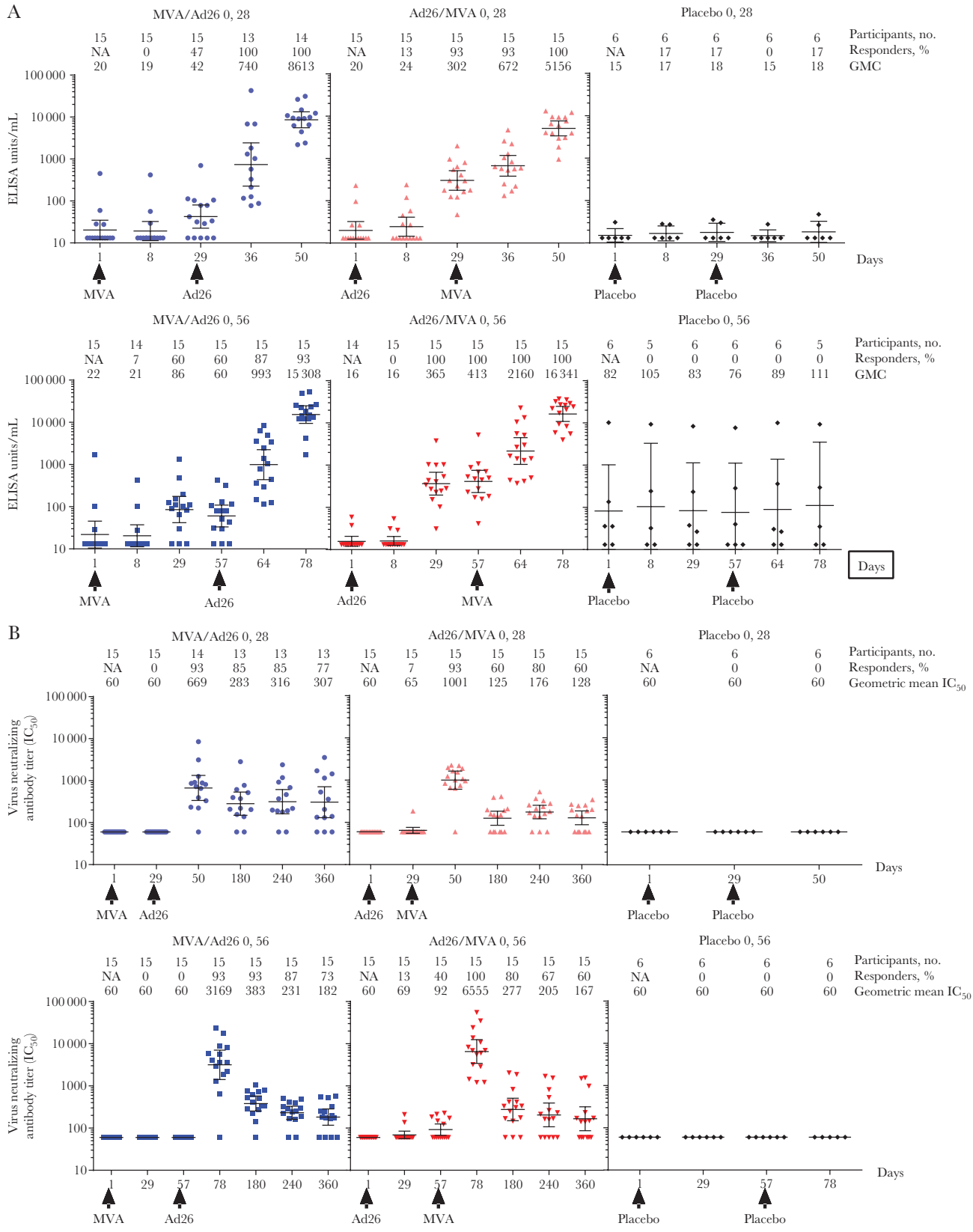

Figure 2. Anti–Ebola virus glycoprotein immunoglobulin G binding antibody responses (detected by enzyme-linked immunosorbent assay [ELISA]) binding antibody responses (*A*) and virus neutralizing antibody (VNA) responses (*B*) following dose 1 vaccination with Ad26.ZEBOV (Ad26) or MVA-BN-Filo (MVA) and heterologous dose 2 vaccination with MVA or Ad26 on day 29 or day 57, 21 days after dose 2. Data are geometric mean concentration (GMC), for ELISA, and geometric mean 50% inhibitory concentration (IC_{50}), for VNA analysis. Error bars represent 95% confidence intervals. NA, not applicable.

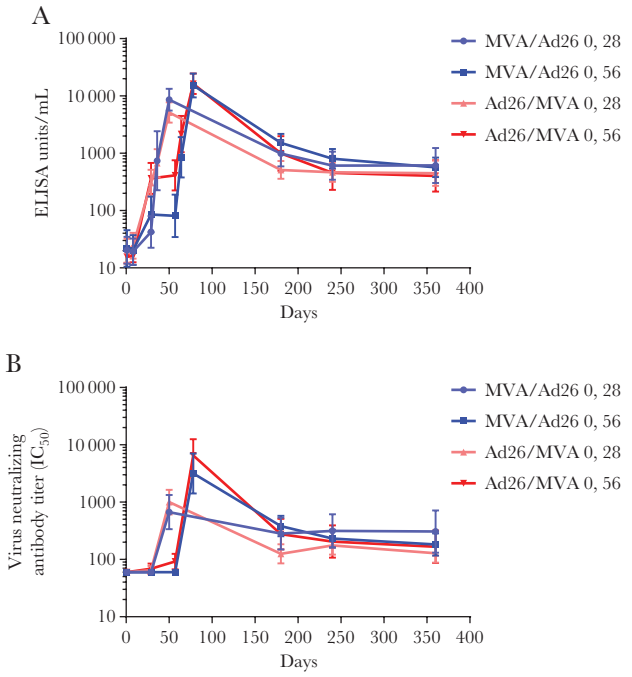

Figure 3. Durability of anti–Ebola virus glycoprotein immunoglobulin G binding (*A*) and neutralizing (*B*) antibody responses following dose 1 vaccination with Ad26.ZEBOV (Ad26) or MVA-BN-Filo (MVA) and heterologous dose 2 vaccination with MVA or Ad26 on day 29 or day 57. Data are geometric mean values; error bars represent 95% confidence intervals. ELISA, enzyme-linked immunosorbent assay; IC_{50}, 50% inhibitory concentration.

MVA-BN-Filo groups after MVA-BN-Filo vaccination (Figure 4A). For those in the Ad26.ZEBOV, MVA-BN-Filo groups who responded, response levels were robust, with individual values ranging from 0.084% to 0.29% in the 28-day interval group and 0.16%–1.46% in the 56-day interval group. Of the 8 individuals in the Ad26.ZEBOV, MVA-BN-Filo groups with a $CD8^+$ T-cell response 21 days after dose 2, 6 remained responders at day 240 after dose 1 (of whom, 3 continued to have a response until day 360 after dose 1).

$CD4^+$ T-Cell Responses

Twenty-one days after the second vaccination, robust $CD4^+$ T-cell responses (as measured by ICS) were observed for the Ad26.ZEBOV, MVA-BN-Filo groups, with median responses peaking at 0.16% for the 56-day interval group and 0.14% for the 28-day interval group. At this time point, response frequencies among participants in both Ad26.ZEBOV, MVA-BN-Filo groups were 60% (Figure 4B). $CD4^+$ T-cell responses declined but remained detectable until days 180 and 360 in the Ad26.ZEBOV, MVa-BN-Filo 28-day and 56-day interval groups, respectively.

In the MVA-BN-Filo, Ad26.ZEBOV groups, $CD4^+$ T-cell responses were observed in 31% and 40% of participants 21 days after dose 2 for the 28-day and 56-day regimens, respectively (Figure 4B). $CD4^+$ T-cell responses declined but remained detectable for the 28-day and 56-day interval groups until days 240 and 180, respectively. In both MVA-BN-Filo, Ad26.ZEBOV groups, the highest median response was approximately half the value observed in the Ad26.ZEBOV, MVA-BN-Filo groups.

The majority of $CD4^+$ and $CD8^+$ T-cell responses were polyfunctional, with most T cells producing 2 or 3 of the investigated cytokines (interferon γ, interleukin 2, and/or tumor necrosis factor α).

DISCUSSION

The severity of the outbreak in 2014 highlighted an urgent need for protection against EBOV and triggered accelerated development of a heterologous 2-dose vaccination regimen that used AdVac and MVA-BN technologies based on proof-of-concept data in nonhuman primates [10, 11]. This phase 1, randomized study has demonstrated that heterologous Ad26.ZEBOV- and MVA-BN-Filo–vectored Ebola vaccination regimens are well tolerated and highly immunogenic in healthy Kenyan adult volunteers, regardless of the vaccination interval or sequence. A long-term humoral immune response was shown by high levels of binding and neutralizing anti-EBOV glycoprotein antibodies and persisted to day 360 after all vaccination regimens.

The favorable safety and tolerability data are consistent with previously published results with Ad26.ZEBOV and MVA-BN-Filo 2-dose vaccination [3, 12]. Phase 1 studies performed in the United Kingdom and United States also reported a favorable safety profile with the Ad26.ZEBOV and MVA-BN-Filo 2-dose approach [3, 12]. Solicited local AEs were mostly mild to moderate in severity and of short duration, and the most frequently reported solicited local AE was injection site pain. The most commonly observed solicited systemic AEs observed in this trial were headache, fatigue, and myalgia (which were of grade 1 or 2 in severity and transient), as observed previously [3], and fever, a common symptom of EBOV, was not a prevalent solicited systemic AE. No vaccine-related serious AEs were reported in this African study or in the United Kingdom and US studies [3, 12].

The present study demonstrated that heterologous vaccine regimens based on Ad26.ZEBOV and MVA-BN-Filo induced robust binding and neutralizing antibody responses against EBOV glycoprotein. Responses persisted at least up to 1 year after the first vaccination, regardless of sequence or the dosing interval (28 vs 56 days) in urban Nairobi. Efficacy data with Ad26.ZEBOV and MVA-BN-Filo are not yet available in humans, but nonhuman primate EBOV challenge studies have shown a strong correlation between binding antibody responses and survival after challenge with EBOV [13]. Cellular immune responses in the current study were variable, with the Ad26.ZEBOV, MVA-BN-Filo regimens producing more-robust responses than the reversed sequence.

The finding that Ad26.ZEBOV first dose vaccination induced more-robust antibody and T-cell responses prior to the second

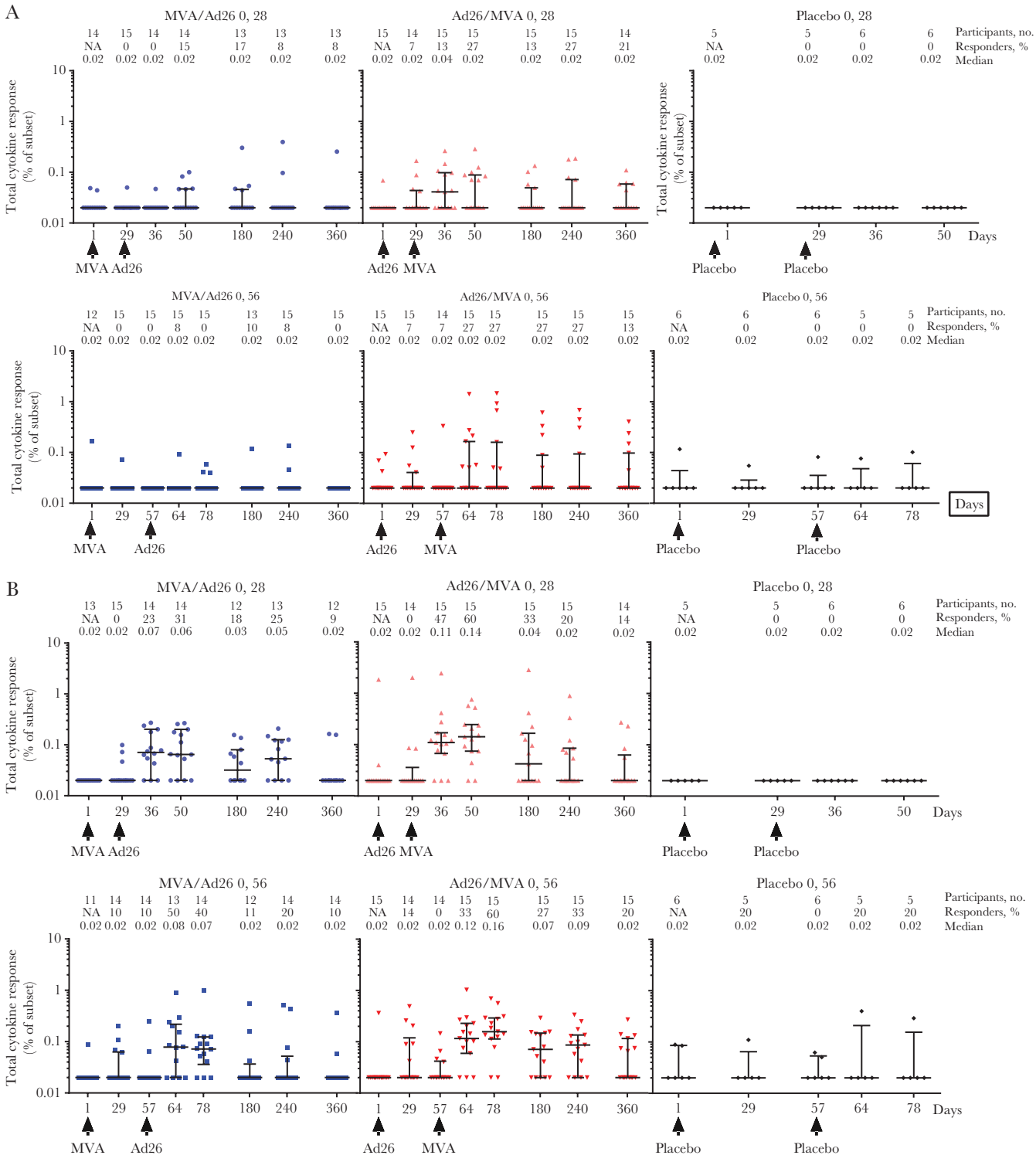

Figure 4. Median CD8$^+$ T-cell responses (*A*) and CD4$^+$ T-cell responses (*B*) following first dose vaccination with Ad26.ZEBOV (Ad26) or MVA-BN-Filo (MVA) and heterologous second dose vaccination with MVA or Ad26 on day 29 or day 57.

vaccination than MVA-BN-Filo first dose vaccination is consistent with previous phase 1 data [3]. The immunogenicity results of this study demonstrated that the second vaccination elicited a robust and sustained effect, in line with previous observations [3].

The CD8$^+$ T-cell responses in this African population differ from those in the United States and European populations, but because of the small number of subjects in these studies it is not possible to draw firm conclusions. Such observations have

been made with vaccines against other infectious diseases and may be related to differences in the pathogens to which individuals are exposed during everyday life [14–18]. In contrast to this, $CD4^+$ T-cell responses detected in this African study were comparable to the responses determined in the FIH study in the United Kingdom [3].

EBOV outbreaks in 2017 and 2018 in the Democratic Republic of the Congo highlighted the necessity for preparedness for future outbreaks [19, 20]. Vaccination could be a key element of future protection against the virus [2, 21, 22]. The features of an ideal vaccine depends on the identity of the virus is currently circulating in the population and with respect to the characteristics of the target population [23], and >1 vaccine may be required. A ring vaccination strategy, with a single-dose vaccine and rapid onset, can be deployed in an outbreak setting in an effort to contain the outbreak [19, 24–27]. The recombinant vesicular stomatitis virus–based Ebola vaccine (rVSV-ZEBOV) has now been recommended for use in emergency settings [28]. However, a broader preventive vaccination campaign that could be based on a heterologous 2-dose strategy may potentially confer long-lasting immunity [23, 26, 29] and as such may be used in response to an outbreak, as well as in a prophylactic strategy. In addition to protecting individuals at risk of exposure to the virus, there is evidence that vaccination may prevent sexual and vertical transmission of EBOV [30, 31]. Population-wide vaccination or vaccination of high-risk populations is generally considered preferable to more narrowly focused approaches because it reduces the risk of an outbreak being reignited and because survivors are less likely to be stigmatized, owing to a reduced fear of infection in the general population [23, 32, 33].

Strengths of this study included the long follow-up period and characterization of the vaccine-induced immune responses at both the humoral and cellular levels. The main limitation was the small number of participants in each study arm.

In conclusion, this study shows that 2-dose heterologous vaccination with Ad26.ZEBOV and MVA-BN-Filo was well tolerated and conferred durable immune responses to EBOV in healthy African volunteers for up to 360 days. Ad26.ZEBOV and MVA-BN-Filo vaccine regimens are being assessed further in larger studies and could potentially play a role in the containment or prevention of future EBOV outbreaks.

Supplementary Data

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

Notes

Acknowledgments. We thank our partners in the EBOVAC1 trial, the EBOVAC2 trial, the London School of Hygiene and Tropical Medicine, the University of Oxford, and the French National Institute for Health and Medical Research, for their important contributions to the clinical development of these vaccines; the local ethics committee, for its rapid review and approval of the study; all of the volunteers who took part in the study; the research staff, who contributed to the successful completion of the trial; all members of the clinical team at the Kenya AIDS Vaccine Initiative Institute of Clinical Research, for their work on the study; and all members of the Clinical Operations Group at Janssen, who contributed to the successful completion of the trial. Medical writing support was provided by Kaedy Bryson and Morgan McKenzie of Zoetic Science, an Ashfield Company. All authors had full access to the study data, contributed to the manuscript development, and approved the final version of the manuscript. The corresponding author had final responsibility for the decision to submit for publication.

Financial support. This work was supported by the Innovative Medicines Initiative 2 Joint Undertaking (grants 115854 [to the EBOVAC 1 trial], 115861 [to the EBOVAC 2 trial], 115850 [to the EBOMAN Project via the Janssen Ebola Vaccine Program], and 115847 [to the EBODAC Project via the Janssen Ebola Vaccine Program]), Janssen Vaccines and Prevention, the European Union's Horizon 2020 Research and Innovation Programme (to the Innovative Medicines Initiative 2 Joint Undertaking), the European Federation of Pharmaceutical Industries and Association (to the Innovative Medicines Initiative 2 Joint Undertaking), and the National Institute of Allergy and Infectious Diseases, National Institutes of Health (contract HHSN272200800056C to the Janssen Filovirus Project).

Potential conflicts of interest. K. L., C. R., V. B., M. D., and D. A. are employees of Janssen Vaccines and Prevention (the sponsor of the trial that generated the data reported herein) or of an affiliate thereof. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Centers for Disease Control and Prevention (CDC). 2014 Ebola outbreak in West Africa—case counts. **2016**. https://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html. Accessed April 2018.
2. Lambe T, Bowyer G, Ewer KJ. A review of phase I trials of Ebola virus vaccines: what can we learn from the race to develop novel vaccines? Philos Trans R Soc Lond B Biol Sci **2017**; 372:20160295.
3. Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia ankara-vectored Ebola vaccines: a randomized clinical trial. JAMA **2016**; 315:1610–23.

4. Winslow RL, Milligan ID, Voysey M, et al. Immune responses to novel adenovirus type 26 and modified vaccinia virus ankara-vectored Ebola vaccines at 1 year. JAMA **2017**; 317:1075–7.
5. Anywaine Z, Whitworth H, Kaleebu P, et al. Randomized clinical trial examining safety and immunogenicity of heterologous prime-boost Ebola vaccines, Ad26.ZEBOV and MVA-BN-Filo: 12-month data from Uganda and Tanzania. J Infect Dis **2019. In press.**
6. Division of Microbiology and Infectious Diseases). Adult toxicity table (draft). **2007**. https://www.niaid.nih.gov/sites/default/files/dmidadulttox.pdf. Accessed April 2018.
7. Parkin NT, Hellmann NS, Whitcomb JM, Kiss L, Chappey C, Petropoulos CJ. Natural variation of drug susceptibility in wild-type human immunodeficiency virus type 1. Antimicrob Agents Chemother **2004**; 48:437–43.
8. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci U S A **2003**; 100:4144–9.
9. Frahm N, DeCamp AC, Friedrich DP, et al. Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. J Clin Invest **2012**; 122:359–67.
10. Food and Drug Administration (FDA). FDA briefing document: vaccines and related biological products advisory committee meeting—May 12. **2015**. https://www.pharmamedtechbi.com/~/media/Supporting%20Documents/The%20Pink%20Sheet%20DAILY/2015/November/Pregnancy_vaccines_AC_FDA_brfg_revised_11102015.pdf. Accessed April 2018.
11. Zahn R, Gillisen G, Roos A, et al. Ad35 and Ad26 vaccine vectors induce potent and cross-reactive antibody and T-cell responses to multiple filovirus species. PLoS One **2012**; 7:e44115.
12. Douoguih M, Goldstein N, Bart S, et al. Safety and immunogenicity of heterologous Ad26.ZEBOV.ZEBOV and MVA-BN®-Filo prime-boost Ebola vaccines: 6-month follow-up of a phase I randomized trial in healthy adults. Presented at: 2016 International Symposium on HIV and Emerging Infectious Diseases, Marseille, France, 25–27 May **2016**.
13. Callendret B, Vellinga J, Wunderlich K, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. PLoS One **2018**; 13:e0192312.
14. Colditz GA, Brewer TF, Berkey CS, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA **1994**; 271:698–702.
15. Labeaud AD, Malhotra I, King MJ, King CL, King CH. Do antenatal parasite infections devalue childhood vaccination? PLoS Negl Trop Dis **2009**; 3:e442.
16. Mirza NB, Wamola IA, Estambale BA, Mbithi E, Poillet M. Typhim Vi vaccine against typhoid fever: a clinical trial in Kenya. East Afr Med J **1995**; 72:162–4.
17. Onoja AL, Adu FD, Tomori O. Evaluation of measles vaccination programme conducted in two separate health centres. Vaccine **1992**; 10:49–52.
18. Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis **1991**; 13:926–39.
19. Gulland A. WHO ready to deploy Ebola vaccine in disease outbreak. BMJ **2017**; 357:j2454.
20. World Health Organization (WHO). EBOLA virus disease Democratic Republic of Congo: external situation report 1. **2018**. http://apps.who.int/iris/bitstream/handle/10665/272509/SITREP-EVD-DRC-20180511.pdf?ua=1. Accessed July 2018.
21. World Health Organization (WHO). WHO coordinating vaccination of contacts to contain Ebola flare-up in Guinea. **2016**. http://www.who.int/features/2016/ebola-contacts-vaccination/en/. Accessed April 2018.
22. Wolfe DN, Zarrabian AG, Disbrow GL, Espeland EM. Progress towards a vaccine against Ebola to meet emergency medical countermeasure needs. Vaccine **2017**; S0264-410X(17):31661–4.
23. Coltart CE, Johnson AM, Whitty CJ. Role of healthcare workers in early epidemic spread of Ebola: policy implications of prophylactic compared to reactive vaccination policy in outbreak prevention and control. BMC Med **2015**; 13:271.
24. Gsell PS, Camacho A, Kucharski AJ, et al. Ring vaccination with rVSV-ZEBOV under expanded access in response to an outbreak of Ebola virus disease in Guinea, 2016: an operational and vaccine safety report. Lancet Infect Dis **2017**; 17:1276–84.
25. Henao-Restrepo AM, Camacho A, Longini IM, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet **2017**; 389:505–18.
26. Osterholm M, Moore K, Ostrowsky J, Kimball-Baker K, Farrar J; Wellcome Trust-CIDRAP Ebola Vaccine Team B. The Ebola Vaccine Team B: a model for promoting the rapid development of medical countermeasures for emerging infectious disease threats. Lancet Infect Dis **2016**; 16:e1–9.
27. Wells C, Yamin D, Ndeffo-Mbah ML, et al. Harnessing case isolation and ring vaccination to control Ebola. PLoS Negl Trop Dis **2015**; 9:e0003794.
28. Nature News. Ebola vaccine approved for use in ongoing outbreak. **2017**. https://www.nature.com/news/ebola-vaccine-approved-for-use-in-ongoing-outbreak-1.22024. Accessed 5 July 2018.

29. Tapia MD, Sow SO, Lyke KE, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. Lancet Infect Dis **2016**; 16:31–42.
30. Borchert M, Muyembe-Tamfum JJ, Colebunders R, Libande M, Sabue M, Van Der Stuyft P. Short communication: a cluster of Marburg virus disease involving an infant. Trop Med Int Health **2002**; 7:902–6.
31. Christie A, Davies-Wayne GJ, Cordier-Lassalle T, et al.; Centers for Disease Control and Prevention (CDC). Possible sexual transmission of Ebola virus - Liberia, 2015. MMWR Morb Mortal Wkly Rep **2015**; 64:479–81.
32. Blackley DJ, Wiley MR, Ladner JT, et al. Reduced evolutionary rate in reemerged Ebola virus transmission chains. Sci Adv **2016**; 2:e1600378.
33. Kucharski AJ, Eggo RM, Watson CH, Camacho A, Funk S, Edmunds WJ. Effectiveness of ring vaccination as control strategy for Ebola virus disease. Emerg Infect Dis **2016**; 22:105–8.