Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN®-Filo Ebola vaccine regimen: a phase 2 randomised clinical study in Europe (EBOVAC2)

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Summary

Background To address the unmet medical need for an effective prophylactic vaccine against Ebola virus we assessed three regimens of heterologous vaccination with Ad26.ZEBOV, a replication-incompetent adenovirus 26 expressing Zaire Ebola glycoprotein (GP), and MVA-BN-Filo, a non-replicating, recombinant modified vaccinia Ankara virus encoding GPs from Zaire, Sudan, Marburg and nucleoprotein from Taï Forest viruses.

Methods In this observer-blind, placebo-controlled phase 2 trial in the UK and France participants were healthy 18–65 years-olds with no history of Ebola vaccination. After a two-step randomisation, participants received intramuscular injections of Ad26.ZEBOV (or saline) on Day 1 then MVA-BN-Filo (or saline) 28, 56 or 84 days later. The primary outcome was safety and tolerability assessed as participant-reported solicited local and systemic adverse events (AEs) for 7 days post-vaccinations, unsolicited AEs until 42 days after MVA-BN-Filo and serious adverse events (SAEs) throughout the study. Secondary outcome was humoral immunogenicity measured as EBOV GP binding antibodies 21 days after MVA-BN-Filo.

Findings We enrolled 423 participants from 23 June, 2015; 421 (99·5%) received at least one study injection. A temporary clinical hold following two neurological SAEs, one considered possibly related to vaccination, disrupted per protocol vaccination in some participants. Vaccinations were generally well-tolerated. Mild/moderate local reactions, mainly pain, were reported after 206 (62·0%) of 332 Ad26.ZEBOV doses, 136 (57·6%) of 236 MVA-BN-Filo doses, and 11 (15·3%) of 72 placebo injections. Systemic AEs after 255 (76·8%) of 332 Ad26.ZEBOV doses, 116 (49·2%) of 236 MVA-BN-Filo doses, and 33 (45·8%) of 72 placebo injections, were mostly mild/moderate fatigue, headache or myalgia. Unsolicited AEs occurred after 34·6%, 34·3% and 33·3% of Ad26.ZEBOV, MVA-BN-Filo and placebo injections, respectively. After MVA-BN-Filo geometric mean concentrations of EBOV GP

binding antibodies were 4627 EL/mL (95% CI: 3649–5867), 10131 EL/mL (8554–11999), and 11312 EL/mL (9072–14106) in 28-, 56- and 84-day interval groups, respectively, which persisted at levels of 1149–1205 EL/mL up to Day 365. Cellular responses against GP detected after Ad26.ZEBOV were enhanced by MVA-BN-Filo.

Interpretation The two-dose Ad26.ZEBOV, MVA-BN-Filo vaccination regimen was safe, well-tolerated and immunogenic, with humoral and cellular immune responses persisting for one year after vaccination.

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INTRODUCTION

The Ebola virus was recognised over 40 years ago as the cause of an outbreak of haemorrhagic fever in the northern part of the Democratic Republic of Congo.[1] Ebola disease is caused by a family of Ebola viruses (Zaire, Sudan, Taï Forest, and Bundibugyo) with an animal reservoir. Patients typically present with non-specific viral symptoms from 2 to 21 days after exposure to the body fluids of an index case and infection has a case fatality rate of up to 88% in humans.[2] Since the first documented outbreaks in 1976 numerous short-lived explosive outbreaks (involving 1 to 425 documented cases) have been controlled by enhanced infection control precautions, including isolation.[3]

At the time this study was done no vaccine had been licensed to control this high mortality filovirus infection.[4] Despite encouraging protection data from development studies in animals, vaccines had not been taken into human clinical trials until a large and complicated outbreak in West Africa in 2014–16 resulting in 11,000 deaths.[3] This led to a rapid increase in global vaccine research and development with 13 vaccine candidates now tested in a series of clinical trials.[5] A recombinant vesicular stomatitis virus (rVSV) vaccine which expresses the glycoprotein (GP) of the Kikwit strain of Zaire Ebola had a high efficacy in a ring-vaccination clinical trial in Guinea during the 2014–16 outbreak.[6] The WHO Strategic Advisory Group of Experts on Immunization (SAGE) recommended implementation of rVSV as reactive use vaccine for outbreak control and for those at high risk of Ebola exposure such as case contacts and healthcare workers,[7] and conditional marketing authorisation was recently granted in Europe.

In a second large outbreak from August 2018 in the Democratic Republic of Congo (DRC) there have been 2194 deaths reported up to November 2019,[8] including three exported to Uganda after probable infection in the DRC.[9] Although the rVSV ring vaccination approach has been proven to be highly effective in the recent outbreak in DRC, the complexities of the

political situation in the region and lack of trust of the local population during the outbreak highlight the importance of licensing and deploying effective prophylactic vaccines in times of stability to protect all those at future risk of exposure to Ebola virus, rather than rely on reactive use of the rVSV vaccine. Indeed, the WHO SAGE recommendations include implementation of a prophylactic vaccination regimen in low-risk populations in the DRC.[7]

To respond to this urgent need, Janssen Vaccines initiated an accelerated vaccination development programme using their recombinant replication-incompetent adenovirus type 26 viral vector platform. In a heterologous two-dose strategy the Ad26.ZEBOV vaccine encoding Mayinga Ebola glycoprotein was paired with the readily available recombinant non-replicating modified vaccinia Ankara viral vector encoding glycoproteins from Ebola Mayinga, Sudan Gulu and Marburg Musoke viruses and nucleoprotein from Taï Forest virus (MVA-BN-Filo) supplied by Bavarian Nordic (Hellerup, Denmark). In non-human primate (NHPs) studies this heterologous Ad26.ZEBOV, MVA-BN-Filo regimen induced protection against lethal Ebola virus challenge with doses given four to eight weeks apart.[10] Phase 1 studies of this vaccination strategy in the UK, US, Kenya, Uganda, and Tanzania have demonstrated an excellent safety profile and strong humoral and cellular immune responses in human volunteers,[11–13] that persisted at least 1 year after vaccination.[14]

These data encouraged further clinical development to develop this promising strategy for disease control and prevention. We present the safety and immunogenicity from a phase 2 vaccine study using the heterologous two-dose Ad26.ZEBOV, MVA-BN-Filo immunisation strategy at various intervals in adult volunteers in the UK and France.

METHODS

Study design and participants

This was a randomised, observer-blind, placebo-controlled, phase 2 study in healthy adults to evaluate the safety, tolerability and immunogenicity of a heterologous two-dose Ebola vaccination regimen using Ad26.ZEBOV and MVA-BN-Filo vaccines with three different dosing intervals. We did the study in seven sites in France (Creteil, Lyon, Paris, Rennes, Saint-Etienne, Strasbourg, Tours) and two sites in the UK (London, Oxford). The protocol was approved in France by the French national Ethics Committee "CPP Ile de France III" (recorded under No. 3287) and the French Medicine Agency (ANSM) (recorded under No. 150646A-61) and in the UK by the Medicines and Healthcare Products Regulatory Agency (MHRA) and National Research Ethics Service (Reference South Central – Oxford A 15/SC/0211). The study was done according to the current Declaration of Helsinki and Good Clinical Practice guidelines. All participants supplied written informed consent before enrolment.

The primary outcome was an assessment of safety and tolerability of the three vaccination schedules with Ad26.ZEBOV administered on Day 1 and MVA-BN-Filo 28, 56 or 84 days later. The secondary outcome was assessment of anti-EBOV glycoprotein (GP) total IgG binding antibody concentrations measured by ELISA at 21 days after the second vaccination. Exploratory outcomes included assessment of binding antibody concentrations at other time points, EBOV GP-specific virus neutralising antibody titres measured by pseudovirion neutralisation assay (psVNA), and EBOV GP-specific CD4+ and CD8+ T cell responses at selected timepoints. In addition, exploratory Ad26-vector-shedding was investigated on Days 1, 2, and 8 after vaccination.

Participants were adults aged 18–65 years who were healthy in the investigators' judgement based on a physical examination, ECG, laboratory assessments and clinical history at screening. All women had a negative pregnancy test prior to enrollment and each vaccination and were required to use approved effective methods of contraception during the study. Other exclusion criteria included prior exposure to Ebola virus (including travel to West Africa less than one month prior to screening) or previous diagnosis of Ebola virus disease, previous receipt of any Ebola vaccine candidate or any known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or study vaccine constituents (including egg, egg products and aminoglycosides). In case of acute illness or temperature ≥ 38.0ºC on Day 1, vaccination of the volunteer was delayed until recovery.

Vaccines

The two vaccines were Ad26.ZEBOV and MVA-BN®-Filo used in a heterologous two-dose regimen.[10–12] Ad26.ZEBOV is a monovalent, recombinant, replication-incompetent adenovirus 26-vectored vaccine expressing Ebola virus Mayinga variant GP. MVA-BN-Filo (Bavarian Nordic) is a recombinant, non-replicating, modified vaccinia Ankara-vectored vaccine expressing Mayinga, Sudan virus Gulu and Marburg virus Musoke variant GPs as well as Taï Forest virus nucleoprotein. The regimen consisted of a first vaccination with 5 x 10^{10} viral particles of Ad26.ZEBOV, followed by a second vaccination with 1 x 10^{8} infectious units of MVA-BN-Filo. Both vaccines and placebo (0·9% saline) were administered by intramuscular injection (0·5 mL) in the deltoid, the second injection being administered in the opposite arm from the first.

Procedures

After screening, four cohorts (I, II, III and IV) of volunteers were enrolled. Cohorts I, II and III were randomised (1:1:1) using sponsor-supplied randomisation codes into three parallel

groups (1, 2 and 3) to receive their two study injections with 28-, 56- or 84-day intervals, respectively (**Figure 1**).

All Cohort I participants were enrolled at the Oxford site and randomised to three groups (block size 3) to receive one dose each of Ad26.ZEBOV and MVA-BN-Filo in an open-label fashion at 28-, 56- or 84-day intervals to provide data on safety and timing of anti-EBOV GP antibody-secreting cell responses. Cohort I participants were not included in further safety and immunogenicity analyses.

Participants enrolled into Cohorts II and III were randomly allocated to one of the three groups with different vaccination intervals (block size 3) stratified by country and by age (≤50 years and >50 years) and further randomised 14:1 in Cohort II (block size 15), 10:3 in Cohort III (block size 13) to receive active vaccines or placebo. Participants from Cohort II had additional blood sampling for exploratory immunological assessments. Finally, the fourth cohort (Cohort IV) comprising 18 volunteers was to be enrolled in France and randomised (5:1, block size 6) to receive Ad26.ZEBOV or placebo, for Ad26 vector shedding assessments in urine samples and mid-turbinate swabs by quantitative polymerase chain reaction (qPCR).

Safety and tolerability assessments

Participants were assessed at 30 and 60 minutes after each vaccination for any immediate adverse events (AEs). They were supplied with diary cards to report solicited local and systemic AEs and daily body temperature for 7 days after each vaccination, which were graded as mild (Grade 1), moderate (Grade 2) or severe (Grade 3). After 7 days, participants continued to record any other AEs as "unsolicited AEs" until 42 days after the second vaccination in Cohorts I–III and 28 days post vaccination for Cohort IV. Serious AEs (SAEs) were to be reported to the investigators at any time during the study. An independent data

monitoring committee (IDMC) was established to assess the safety data on a regular basis during the study.

Immunogenicity assessments

Four serum samples for assessment of immune responses were obtained from all participants in Cohorts II and III: immediately before the first vaccination on Day 1, before the second vaccination on Days 29, 57 or 85, and then 21 days after the second vaccination, and at Day 365 in all groups. EBOV GP-specific total IgG binding antibody concentrations were measured using an EBOV GP Filovirus Animal Non-Clinical Group (FANG) ELISA at Q2 Solutions (San Juan Capistrano, CA, USA).[15] EBOV GP-specific neutralising antibody (psVNA) titres were measured using a pseudovirion neutralisation assay (**Supplementary materials**) at Monogram (San Francisco, CA, USA).

Peripheral blood mononuclear cells (PBMC) were collected from a subset of participants and frozen for later analysis to determine the percentage of CD4+ and CD8+ T cells producing interferon (IFN)-γ and/or interleukin (IL)-2 by intracellular cytokine staining (ICS) at the Inserm U955 unit laboratory/VRI. Defrosted PBMC were stimulated by two peptide pools (GP1 and GP2) covering the GP from Mayinga variant of Ebola virus (**Supplementary materials**).

Statistical Analyses

This was originally designed as a prospective study with no formal hypothesis testing, for which the planned sample size was 612 participants (pooled across cohorts) to include 492 participants randomised to receive Ad26.ZEBOV and MVA-BN-Filo (Cohorts II and III), 30 participants in Cohort I to receive Ad26.ZEBOV and MVA-BN-Filo in an open-label fashion, and 90 placebo recipients. Following the occurrence of two SAEs (described in

Results) there was a clinical hiatus until the study was restarted following an amended protocol with the agreement of the IDMC and relevant competent authorities.

The descriptive safety analysis is presented on the full analysis set consisting of all participants who were randomised and received at least one administration of vaccine or placebo irrespective of protocol deviations. The immunogenicity analysis, based on the per protocol set, includes all randomised and vaccinated participants who were administered both vaccinations within the protocol-defined window (± 1 day), had at least one evaluable immunogenicity sample post-vaccination, and no major protocol violations influencing the immune response. Binding antibodies were expressed as group geometric mean concentrations (GMC) of arbitrary ELISA units with 95% confidence intervals (CI) at each timepoint, and responder rates, i.e. the percentage of each group with post vaccination concentrations >2·5-fold the Lower Limit of Quantitation (LLOQ), i.e. 36·11 ELISA units/mL, in baseline seronegative individuals, or >2·5-fold the baseline value in initially seropositive participants. psVNA titres are expressed as geometric mean titres (GMT) with 95% confidence intervals (CI) and group proportions of responders at each timepoint. psVNA responders were those negative at baseline and positive post vaccination with a titre two-fold higher than LLOQ (120 IC_{50} titre), or positive at baseline with greater than two-fold increase post vaccination. Spearman correlation was calculated for EBOV GP-specific binding antibody concentrations (FANG ELISA) and psVNA titres at selected timepoints.

For cell-mediated immune responses assessed by ICS, the frequency of responders, calculated on IFN-γ and/or IL-2 separately for the sum of the two EBOV GP peptide pools, was determined at each timepoint. Responders were either: i) negative at baseline and positive post-baseline with a value >2-fold the LLOQ (0·05%, CD4+ IFN-γ; 0·08%,CD4+ IL-2; 0·06%, CD8+ IFN-γ; 0·19%, CD8+ IL-2), or ii) positive at baseline with > 2-fold increase in background adjusted percentage of the combined peptide pools.

All statistical analyses were performed by the sponsor with SAS (version 9·2, SAS Institute, Cary, NC) with independent validation completed by Inserm. The study protocol was registered on ClinicalTrials.gov (NCT02416453) and EudraCT (2015-000596-27).

Role of the funding source

As full-time employees of the legal sponsor, Janssen Vaccines and Prevention, NG, CR, AG, VB, ML, KL and MD took part in the conception, design, and preparation of the protocol, and did some of the laboratory and statistical analyses. Other authors contributed to the design and did the study with no financial interest. All authors had full access to the data, contributed to the manuscript over which academic contributors had full editorial control, and all authors agreed with the decision to submit.

RESULTS

The study began in July 2015 but was paused from 27 April 2016 to 9 May 2016 to investigate an SAE and was stopped for a second time by the sponsor on May 20, 2016 until 27 Sep 2016 following a second event. After reviewing both cases, including by an independent panel of expert neurologists, the MHRA approved resumption of the study on 27 September 2016. Screening for groups 1 and 2 in Cohorts II and III was restarted in the UK but not in France. Randomisation and collection of one-year data for group 3 (84-day regimen) was halted. Following reconsent, participants already vaccinated with dose 1 were offered the second dose outside of the protocol window (**Figure 1**).

Baseline demographics

Of 423 participants enrolled, 421 received at least one dose of study vaccine, including: 30 participants in Cohort I, 376 participants in pooled Cohorts II and III (194 in the UK and 182 in France) and 15 participants in Cohort IV. Participant demographic data and assignment of pooled Cohorts II and III to groups 1, 2 and 3 are shown in **Table 1**. In total, 363 (86·2%) participants completed the study: 23 (76·7%) of 30 in Cohort I, 325 (86·4%) of 376 in pooled Cohorts II and III, and all 15 (100%) in group 4 (**Figure 1**).

Safety

Solicited local AEs were reported after 206 (62·0%) of 332 Ad26.ZEBOV and 136 (57·6%) of 236 MVA-BN-Filo vaccinations, compared with 11 (15·3%) of 72 placebo injections (**Table 2**). The most frequent local AE was pain at the injection site, reported after 191 (57·5%) of 332 Ad26.ZEBOV and 126 (53·4%) of 236 MVA-BN-Filo vaccinations, compared with 9 (12·5%) of 72 placebo injections (**Table 2**). Solicited local AEs were generally described as mild to moderate; six cases (three of pain and two of swelling after Ad26.ZEBOV, and one case of erythema after MVA-BN-Filo) were graded as severe.

Median durations of local AEs were two and three days after Ad26.ZEBOV and MVA-BN-Filo vaccinations, respectively, and one day after placebo. One participant reported injection site swelling for 19 days following MVA-BN-Filo vaccination.

Solicited systemic AEs were reported after 255 (76·8%) of 332 Ad26.ZEBOV, 116 (49·2%) of 236 MVA-BN-Filo and 33 (45·8%) of 72 placebo administrations. The most frequent systemic AEs were fatigue, headache, myalgia and chills (**Table 2**). Severe solicited systemic AEs were reported after 32 (9·6%) of 332 Ad26.ZEBOV administrations, but lower proportions of MVA-BN-Filo (4 [1·7%] of 236) or placebo (3 [4·2%] of 72) injections. Fever (temperature ≥ 38°C) was more frequent after receipt of Ad26.ZEBOV (38 [11·5%] of 331) than MVA-BN-Filo (2 [0·9%] of 232) or placebo (3 [4·2%] of 72). There were eight cases of temperature ≥ 39°C: six after Ad26.ZEBOV, one after MVA-BN-Filo and one after placebo. The median duration of any solicited systemic adverse event was no longer than 3 days.

Rates of unsolicited AEs were similar after Ad26.ZEBOV (115 [34·6%] of 332), MVA-BN-Filo (81 [34·3%] of 236), and placebo (24 [33·3%] of 72) and mainly consisted of mild to moderate headache, upper respiratory tract infections and rhinitis (**Table 2**). No clinical laboratory safety signals were observed. The most frequent laboratory abnormalities were decreased haemoglobin from baseline and low segmented neutrophil counts. Both types of abnormality were observed at comparable rates after Ad26.ZEBOV, MVA-BN-Filo and placebo administration. The majority of low segmented neutrophil values were grade 1 or grade 2 in severity; a grade 3 value ($<1{\cdot}0 \times 10^9$/L) was reported in 2 participants (**Supplementary Tables 1 and 2**).

Thirteen SAEs were reported by 11 (3·3%) of 332 participants who received active vaccines and 2 (4·5%) of 44 participants after a total of 72 doses of placebo (**Table 2**). Two neurological SAEs were initially identified as related to study vaccination and declared as suspected unexpected serious adverse reactions (SUSAR), leading to the study pause. One

participant who experienced double vision, pain on moving the eye, and difficulty with balance while walking, was diagnosed with Miller Fisher syndrome. Symptoms onset was about a week after an upper respiratory tract infection (URTI) and one month after MVA-BN-Filo vaccination, and they resolved before the end of the study. After extensive evaluation, including a second neurological evaluation, the investigator and sponsor considered the event was not related to study vaccine and most likely related to the prior URTI. A second participant experienced intermittent episodes of paraesthesia of the palms and soles two weeks after vaccination with Ad26.ZEBOV. The investigator reported the event as an SAE as the participant was unable to continue their work activity. The diagnosis of possible small fibre neuropathy was retained although a definitive diagnosis was not possible as the participant refused further specific diagnostic exploration and no histological examination was performed. The expert panel, investigator and sponsor considered this event, which had not resolved before the end of the study, to be possibly related.

Humoral immunogenicity

The immunogenicity analysis is based on the 223 (59·3%) of 376 participants in Cohorts II and III who received both vaccines according to protocol; 36 participants (29 MVA-BN-Filo and 7 placebo) received their second injections outside of the planned window and 116 did not receive the second injection. Ad26.ZEBOV vaccination on Day 1 induced binding antibody responses (**Figure 2**), with 92·2%, 95·7% and 100% responding to the Ad26.ZEBOV vaccination in the 28-, 56- and 84-day groups, respectively. The magnitude of the response increased with increasing interval; GMCs were 452 EU/mL after 28 days, 880 EU/mL after 56 days, and 1156 EU/mL after 84 days (**Table 3**).

GMCs increased further 21 days after MVA-BN-Filo vaccination, by 10·4-, 11·6- and 10·0-fold over post Ad26.ZEBOV levels in the 28-, 56- and 84-day groups, when GMCs were 4627 EU/mL, 10131 EU/mL, and 11312 EU/mL, respectively. All participants responded to

the 2-dose vaccine regimen except for one participant vaccinated with the 28-day interval (**Table 3**). In the 28- and 56-day groups antibodies persisted up to Day 365 when 56 (98·2%) of 57 and all 50 (100%) of these group participants were still responders; the 84-day group was not tested at Day 365. GMCs at Day 365 were similar in both 28- and 56-day groups; 1149 EU/mL and 1205 EU/mL, respectively (**Figure 2A**). No placebo recipient demonstrated an increase in antibodies at any point during the study. In a sensitivity analysis of responses to MVA-BN-Filo vaccination administered out of the protocol window, responses were consistent with the per protocol analyses. Increasing the interval >98 days had no negative impact on the response (**Supplementary Figure 1**).

The GMT (IC_{50} titre) of neutralising antibodies 21 days after receiving the vaccinations 28 days apart was 988 (95% CI 712–1370), increasing to 4046 (3091–5297) when the interval between vaccinations was 56 days (**Figure 2B** and **Supplementary table 3**). Day 365 GMTs were 354 (257–448) and 341 (268–435), respectively. In the 28-day group responder rates were 43 (87·8%) of 49 participants at Day 50, and 31 (63·3%) of 49 at Day 365. All 52 (100%) participants in the 56-day group were responders at Day 78, which persisted in 27 (51·9%) of 52 participants at Day 365. There were strong correlations between binding antibody concentrations and neutralising antibody titres measured 21 days after MVA-BN-Filo (Spearman coefficient, r = 0·825) and at Day 365 (Spearman coefficient, r = 0·861, **Supplementary Figure 2**). No virus neutralising activity was detected in any placebo recipient.

Cell-mediated immune responses

EBOV GP-specific cellular immune responses were assessed in 76 participants from Cohorts II and III (**Figure 3, Supplementary Figure 3, and Tables 4 and 5**). EBOV GP-specific CD4+ and CD8+ T-cell responses were low after Ad26.ZEBOV dose 1, but EBOV GP-specific CD4+ and CD8+ T-cell responses were observed in substantial proportions of

participants 21 days after MVA-BN-Filo vaccination (37–58% for CD4+ T cells, 55–67% for CD8+ T cells). Median CD4+ T-cell responses at 21 days post Dose 2 were similar across the 28-, 56-, and 84-day vaccination intervals (median range: 0·12–0·15%). There was a trend for median CD8+ T-cell responses to be higher with the 28-day interval (median 0·29%) compared with the 56- and 84-day intervals (median 0·12% and 0·20%, respectively). No EBOV GP-specific cellular response was detected in placebo recipients (**Supplementary Figure 3**). At 21 days post dose 2, across vaccination schedules, the majority of the CD4+ T cell responses were polyfunctional, expressing both IFN-γ and IL-2, while the majority of the CD8+ T cell responses expressed only IFN-γ (**Supplementary Figure 4**).

Ad26.ZEBOV shedding

Shedding of Ad26.ZEBOV was assessed in the 15 participants of group 4 (13 vaccinees and 2 placebo) using urine samples and nasal swabs taken on Days 1, 2 and 8 after vaccination. No evidence of shedding was observed in any sample (not shown).

DISCUSSION

These first phase 2 results of the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccination regimen in 421 healthy adults confirm the safety and immunogenicity profiles of this regimen previously observed in phase 1 studies.[11–14] The frequency and severity of solicited local reactions, mainly mild to moderate injection site pain, were comparable to those reported in phase 1 studies performed in Oxford,[11] and in African participants.[12,13] The systemic reactogenicity profile comprising mainly headache, myalgia and fatigue was consistent with the phase 1 studies,[11–13] although there were slightly fewer solicited systemic adverse events and laboratory events especially with regards to neutrophil levels than reported in previous studies. More generally, observed local and systemic reactions were also reported with other vectors such as the recombinant adenovirus type-5,[17] the replication defective recombinant chimpanzee adenovirus type 3-vectored ebolavirus vaccine (cAd3-EBO)[18] or the replication-competent, recombinant vesicular stomatitis virus (rVSV).[19] As expected with replication-incompetent vectors, no vector replication was observed following Ad26.ZEBOV when checked in a specific cohort.

The study was paused following two neurological SAEs that occurred within weeks of each other. Both events occurred at different phases of the two-dose regimen, a case of Miller Fisher syndrome which most commonly occurs after a recent infection was reported one week after an URTI and one month after receiving MVA-BN-Filo, and the second, which did not have a definitive diagnosis, occurred one month after Ad26.ZEBOV vaccination. The occurrence of these two unsolicited SAEs led to a concurrent and careful assessment of the neurological events, including by an external panel of expert neurologists, but did not raise any specific safety signals. Regulatory authorities in the UK and France confirmed that the development of the Ad26.ZEBOV and MVA-BN-Filo vaccination regimen could continue.

Robust EBOV GP-specific binding antibody responses were observed in 99–100% of the participants 21 days after the second dose, and these persisted in 98–100% of the participants tested after 1 year after dose 1. Antibody responses were lower 21 days after the second vaccination when the interval was 28 days rather than 56 or 84 days but levels at one year were similar in 28- and 56-day groups. There was a high degree of correlation between binding and neutralising antibody responses. EBOV-specific CD4+ and CD8+ T cell responses were observed in a substantial portion of participants analysed. CD4+ T cell responses were similar in the three intervals, whereas CD8+ T cell responses tended to be higher in the 28-day interval group. CD4+ and CD8+ T cell responses persisted at least 1 year in 11–16% and 42–56% of the participants, respectively.

We have shown that a heterologous two-dose vaccination regimen with intervals from 28 to 84 days between doses elicits robust humoral and cellular responses. Boosting with MVA vectors has previously been reported after ChAd3 prime immunisation as reported in macaques[20] and humans.[21,22] The Ad26.ZEBOV, MVA-BN-Filo strategy with a 56-day interval provided protection against lethal EBOV challenge in non-human primates (NHP) model,[10] as well as robust humoral and cellular immune responses in humans with binding antibodies induced as early as 14 days after the first vaccination.[11]

In outbreak conditions the single-dose rVSV vaccine had 100% clinical efficacy from 10 days after vaccination.[6] In lethal challenge studies in mice[23] and NHP[24] ZEBOV-GP–specific IgG antibodies were found to play a critical role in rVSV-induced protection from 10 days post-vaccination onwards. The specific mechanism of action of Ad26.ZEBOV, MVA-BN-Filo is currently unknown and may differ from that of rVSV, but NHP studies have demonstrated that EBOV GP-specific IgG binding antibodies represent the immune parameter that correlates best with survival for the Ad26.ZEBOV, MVA-BN-Filo regimen.[10] Although a mechanistic correlate of protection has not been identified yet, the binding

antibody GMCs observed 21 days after the second dose in the two-dose regimens ranged from 4627 to 11312 EU/mL depending on the interval. A GMC of 1262 EU/mL was reported 28 days post-rVSV-ZEBOV vaccination of North American and Spanish volunteers using the same assay in the same laboratory.[25] In the present study, and consistent with previous reports,[11] levels above 1000 EU/mL were maintained up to Day 365, our furthest time-point from vaccination.[25] Recent modelling work based on phase 1 data predicts a sustained response over time, which is currently being evaluated in the EBOVAC3 consortium.[26]

One limitation of this study was that some participants received their MVA-BN-Filo vaccination outside the planned window due to the study pause. Although the number of such individuals was limited, it did provide data on a delayed interval, as may occur in real-life settings. Strong immune responses were observed which were at least as high as those observed in the per protocol population, providing support for flexibility in the timing of dosing that may be needed in real-world use, when dose intervals are not strictly respected. Another limitation was that this phase 2 study was done in European volunteers who, except for healthcare and aid workers involved in outbreak response, are unlikely to be targets for vaccination.[27] Similar studies assessing different vaccination regimens in different populations of healthy adults and children, and also HIV-infected individuals are ongoing in African populations in Burkina Faso, Cote d'Ivoire, Kenya and Uganda (ClinicalTrials.gov NCT02564523), and Sierra Leone (ClinicalTrials.gov NCT02509494). The ongoing PREVAC study in Sierra Leone and Guinea includes an assessment of the impact of malaria on the immune response (ClinicalTrials.gov NCT02876328).

In conclusion, the safety, including the absence of Ad26.ZEBOV shedding, and immunogenicity of the heterologous Ad26.ZEBOV, MVA-BN-Filo regimen with intervals from 28 to 84 days has been demonstrated in this European phase 2 study. Ancillary immunological analyses performed in this study should provide more insight into the immune

response established while other phase 2 and 3 studies performed by EBOVAC1 and EBOVAC2 African consortia and PREVAC will provide additional information in target populations at risk of Ebola infection in Africa.

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Conflicts of interest

AJP chairs the UK Department of Health's (DoH) Joint Committee on Vaccination and Immunisation (JCVI) and the European Medicines Agency Scientific Advisory Group on

Vaccines and is a member of the World Health Organization's (WHO) Strategic Advisory Group of Experts. The views expressed in this manuscript are those of the authors and do not necessarily reflect the views of the JCVI, the DoH, or the WHO.

Author contributions

VB, MD, AG, MG, NG, CL, JDL, YL, KL, A, LR, CR, MS, RT participated in the conception and design of the work being described in the publication.

CB, MG, NG, SG, CL, ML, AP, RT, AW participated in the acquisition/collection of data.

CB, VB, EC, MD, AG, NG, CL, OL, JDL, ML, KL, AP, LR, CR, MS, RT, AW participated in the analysis or interpretation of data.

CB, VB, EC, MD, AG, MG, SG, NG, CL, OL, JDL, YL, ML, KL, AP, LR, CR, MS, RT, AW drafted or critically revised and reviewed the manuscript for important intellectual content.

All authors approve this version of the manuscript and agree to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Sharing Statement

Janssen has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for evaluation of requests for CSRs and participant level data from investigators and physicians for scientific research that will advance medical knowledge and public health. Data will be made available following publication and approval by YODA of any formal requests with a defined analysis plan. For more information on this process or to make a request, please visit The Yoda Project site at http://yoda.yale.edu. The data sharing

policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency

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Table 1. Demographic characteristics of the study population in all four Cohorts.

	Cohort I	**Cohorts II and III**						**Cohort IV**	
	Open label	**28-day Interval**		**56-day Interval**		**84-day Interval**		Ad26 shedding	
	Active vaccines	Active vaccines	Placebo	Active vaccines	Placebo	Active vaccines	Placebo	Ad26	Placebo
Full analysis set, N =	30	112	13	114	13	106	18	13	2
Male, n (%)	13 (43·3)	55 (49·1)	7 (53·8)	52 (45·6)	5 (38·5)	53 (50·0)	8 (44·4)	10 (76·9)	1 (50·0)
Mean age ± SD, years	40·1 ± 15·1	41·0 ± 15·0	39·1 ± 13·9	41·0 ± 14·0	38·2 ± 13·7	38·3 ± 14·3	41·1 ± 15·1	37·9 ± 11·4	47·0 ± 4·2
(range)	(18, 63)	(18, 65)	(22, 60)	(18, 64)	(23, 62)	(18, 65)	(20, 61)	(21, 55)	(44, 50)
Mean BMI ± SD, kg/m^2	25·6 ± 5·4	25·2 ± 4·0	23·1 ± 2·7	25·1 ± 4·7	25·5 ± 5·3	24·7 ± 4·0	24·1 ± 2·9	23·8 ± 2·8	22·3 ± 4·2
Country, n (%)									
France	-	54 (48·2)	7 (53·8)	55 (48·2)	7 (53·8)	50 (47·2)	9 (50·0)	13 (100)	2 (100)
UK	30 (100)	58 (51·8)	6 (46·2)	59 (51·8)	6 (46·2)	56 (52·8)	9 (50·0)	-	-
Per Protocol set, n (%) =	-	80 (71·4)	8 (61·5)	70 (61·4)	7 (53·8)	52 (49·1)	6 (33·3)	-	-
Initially seropositive for EBOV GP, n (%) =	-	6 (7·5)	1 (12·5)	6 (8·6)	0 (0)	4 (7·8)	0 (0)	-	-

Table 2. Incidence rates of the most frequent solicited local and systemic adverse events within 7 days of vaccination or placebo injection, and the most frequent unsolicited adverse events (AEs) and serious adverse events (SAEs) throughout the study in Cohorts II and III.

		Incidence, reports following n doses (%)		
		Ad26.ZEBOV	**MVA-BN-Filo**	**All Placebo**
	Doses	N = 332	N = 236	N = 72
Local AEs	**Any**	206 (62·0)	136 (57·6)	11 (15·3)
	Severe	*4 (1·2)*	*1 (0·4)*	*0*
Pain	**Any**	191 (57·5)	126 (53·4)	9 (12·5)
	Severe	*3 (0·9)*	*0*	*0*
Swelling	**Any**	36 (10·8)	28 (11·9)	0
	Severe	*2 (0·6)*	*0*	*0*
Pruritis	**Any**	19 (5·7)	7 (3·0)	3 (4·2)
	Severe	*0*	*0*	*0*
Erythema	**Any**	5 (1·5)	3 (1·3)	0
	Severe	*0*	*1 (0.4)*	*0*
Systemic AEs	**Any**	255 (76·8)	116 (49·2)	33 (45·8)
	Severe	*32 (9·6)*	*4 (1.7)*	*3 (4.2)*
Fatigue	**Any**	203 (61·1)	84 (35·6)	21 (29·2)
	Severe	*18 (5·4)*	*3 (1.3)*	*2 (2.8)*
Headache	**Any**	162 (48·8)	53 (22·5)	16 (22·2)
	Severe	*9 (2·7)*	*2 (0·8)*	*1 (1·4)*
Myalgia	**Any**	156 (47·0)	52 (22·0)	15(20·8)
	Severe	*8 (2·4)*	*1 (0·4)*	*0*
Chills	**Any**	123 (37·0)	23 (9·7)	6 (8·3)
	Severe	*14 (4·2)*	*3 (1·3)*	*2 (2·8)*
Arthralgia	**Any**	76 (22·9)	22 (9·3)	5 (6·9)
	Severe	*4 (1·2)*	*1 (0.4)*	*0*
Nausea	**Any**	45 (13·6)	14 (5·9)	4 (5·6)
	Severe	*3 (0·9)*	*1 (0·4)*	*0*
Fever	≥ 38.0°C	38 (11·5) [a]	2 (0·9) [b]	3 (4·2)
	≥ 39.0°C	*6 (1·8)*	*1 (0·4)*	*1 (1·4)*
Any Unsolicited AE		115 (34·6)	81 (34·3)	24 (33·3)
MEDRA classes of main reported AEs				
Infections and infestations		30 (9·0)	28 (11·9)	7 (9·7)
Upper respiratory tract Infection		4 (1·2)	12 (5·1)	3 (4·2)
Rhinitis		10 (3·0)	7 (3·0)	0
Nervous system disorders		17 (5·1)	12 (5·1)	3 (4·2)
Headache		5 (1·5)	7 (3·0)	2 (2·8)
Investigations		16 (4·8)	13 (5·5)	2 (2·8)
Respiratory, thoracic and mediastinal disorders		17 (5·1)	7 (3·0)	2 (2·8)
Gastrointestinal disorders		9 (2·7)	8 (3·4)	5 (6·9)
Musculoskeletal and connective tissue disorders		12 (3·6)	6 (2·5)	3 (4·2)
General disorders and administration site conditions		10 (3·0)	5 (2·1)	3 (4·2)
SAEs throughout study		**Ad26.ZEBOV & MVA-BN-Filo**		**All placebo**
		N = 332		N = 44
Any reported SAE		11 (3·3)		2 (4·5)
SAE related to vaccination		0		0

[a] n = 331, [b] n = 232.

Table 3. Geometric mean concentrations (GMC) of EBOV GP binding antibodies and responder rates in Cohorts II and III throughout the study.

		28-day interval		56-day interval		84-day interval	
Day		**Ad26, MVA**	**Placebo**	**Ad26, MVA**	**Placebo**	**Ad26, MVA**	**Placebo**
1	N GMC [95% CI]	80 <LLOQ [<LLOQ; <LLOQ]	8 <LLOQ [<LLOQ; 58]	70 <LLOQ [<LLOQ; <LLOQ]	7 <LLOQ [<LLOQ; <LLOQ]	51 <LLOQ [<LLOQ; <LLOQ]	6 <LLOQ [<LLOQ; <LLOQ]
29*	N GMC [95% CI] Responders, n (**%**)	77 452 [351–581] 71 (**92·2**)	8 <LLOQ [<LLOQ; 61] 0 (**0**)	-	-	-	-
50**	N GMC [95% CI] Responders, n (**%**)	77 4627 [3649–5867] 76 (**98·7**)	7 <LLOQ [<LLOQ; 55] 0 (**0**)	-	-	-	-
57*	N GMC [95% CI] Responders, n (**%**)	-	-	69 880 [709–1093] 66 (**95·7**)	7 <LLOQ [<LLOQ; <LLOQ] 0 (**0**)	-	-
78**	N GMC [95% CI] Responders, n (**%**)	-	-	69 10131 [8554–11999] 69 (**100**)	7 <LLOQ [<LLOQ; <LLOQ] 0 (**0**)	-	-
85*	N GMC [95% CI] Responders, n (**%**)	-	-	-	-	52 1156 [923–1448] 51/51[#] (**100**)	6 <LLOQ [<LLOQ; <LLOQ] 0 (**0**)
106**	N GMC [95% CI]	-	-	-	-	48 11312 [9072–14106]	6

	Responders, n (**%**)					47/47[#] (**100**)	<LLOQ [<LLOQ; <LLOQ] 0 (**0**)
365	N GMC [95% CI] Responders, n (**%**)	57 1149 [859–1537] 56 (**98·2**)	6 <LLOQ [<LLOQ; <LLOQ] 0 (**0**)	50 1205 [971–1497] 50/50 (**100**)	5 <LLOQ [<LLOQ; <LLOQ] 0 (**0**)	-	-

* Pre-dose 2, **21 days post-dose 2. <LLOQ = below the Lower Limit of Quantitation. [#] Responder rates for those with Day 1 data.

Figure 1. Study flow chart.

Figure 2. Anti-EBOV GP geometric mean binding antibody concentrations (ELISA Units/mL) (A) and virus neutralising geometric mean IC_{50} titres (B) in the pooled Cohorts II and III (per protocol analysis set).

Figure 3. EBOV GP-specific T cell responses in participants assigned to receive active vaccine as assessed by Intracellular Cytokine Staining.