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Safety and immunogenicity of an Ad26.ZEBOV booster vaccine in Human Immunodeficiency Virus positive (HIV+) adults previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen against Ebola: A single-arm, open-label Phase II clinical trial in Kenya and Uganda

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ABSTRACT

Background: People living with HIV constitute an important part of the population in regions at risk of Ebola virus disease outbreaks. The two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen induces strong immune responses in HIV-positive (HIV+) adults but the durability of this response is unknown. It is also unclear whether this regimen can establish immune memory to enable an anamnestic response upon re-exposure to antigen.
Methods: This paper describes an open-label, phase 2 trial, conducted in Kenya and Uganda, of Ad26.ZEBOV booster vaccination in HIV+ participants who had previously received the Ad26.ZEBOV, MVA-BN-Filo primary regimen. HIV+ adults with well-controlled infection and on highly active antiretroviral therapy were enrolled, vaccinated with booster, and followed for 28 days. The primary objectives were to assess Ad26.ZEBOV booster

Abbreviations: Ad26.ZEBOV, adenovirus serotype 26 encoding the Ebola virus Mayinga glycoprotein; AE, adverse event; β-hCG, beta human chorionic gonadotropin; CI, confidence intervals; DAIDS, United States National Institutes of Health, Division of AIDS; EBOV, Ebola virus; ELISA, enzyme-linked immunosorbent assay; EMA, European Medicines Agency; EU, ELISA Units; EVD, Ebola virus disease; FANG, Filovirus Animal Non-Clinical Group; FDA, United States Food and Drug Administration; GMC, geometric mean concentration; GP, glycoprotein; HAART, highly active antiretroviral therapy; HIV, Human Immunodeficiency Virus; IMI, Innovative Medicines Initiative; Inf U, infectious units; LLOQ, lower limit of quantification; MVA-BN-Filo, Modified Vaccinia Ankara Bavarian Nordic vector encoding multiple filovirus proteins; NHP, non-human primates; SAE, Serious Adverse Events; SD, standard deviation; ULOQ, upper limit of quantification.

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safety and antibody responses against the Ebola virus glycoprotein using the Filovirus Animal Non-Clinical Group ELISA.

Results: The Ad26.ZEBOV booster was well-tolerated in HIV+ adults with mostly mild to moderate symptoms. No major safety concerns or serious adverse events were reported. Four and a half years after the primary regimen, 24/26 (92 %) participants were still classified as responders, with a pre-booster antibody geometric mean concentration (GMC) of 726 ELISA units (EU)/mL (95 %CI 447–1179). Seven days after the booster, the GMC increased 54-fold to 38,965 EU/mL (95 %CI 23532–64522). Twenty-one days after the booster, the GMC increased 176-fold to 127,959 EU/mL (95 %CI 93872–174422). The responder rate at both post-booster time points was 100 %.

Conclusions: The Ad26.ZEBOV booster is safe and highly immunogenic in HIV+ adults with well-controlled infection. The Ad26.ZEBOV, MVA-BN-Filo regimen can generate long-term immune memory persisting for at least 4·5 years, resulting in a robust anamnestic response.

Trial Registration: Pan African Clinical Trial Registry (PACTR202102747294430). Clinicaltrials.gov (NCT05064956).

1. Introduction

Ebola virus disease (EVD), formally known as Ebola haemorrhagic fever, is caused by an *Ebolavirus* of the Filoviridae family [1,2]. The species Zaire *ebolavirus,* simply called the Ebola virus (EBOV), is responsible for the majority of the highly fatal epidemics, including the 2014–2016 outbreak that affected multiple countries in West Africa [3]. The risk of future EVD epidemics is real and increasing as a result of heightened interactions between humans and the suspected intermediate hosts and/or the natural reservoirs of Ebolavirus [4]. There is a second pool of Ebola virus, which persists in EVD survivors and can initiate new outbreaks. Therefore, there is an urgency to develop effective pharmaceutical and public health measures against EVD, including safe and effective vaccines.

To date, one Ebola vaccine, rVSV-ZEBOV-GP (Ervebo®) has been approved by the United States Food and Drugs Administration (FDA) for use in individuals aged 1 year and above who are at an increased risk of contracting EVD during epidemics [5,6]. However, the safety and efficacy of this replicating live virus-vectored vaccine in vulnerable populations like people with immunosuppression are not well-understood. A second Ebola vaccine, the two-dose regimen of adenovirus serotype 26 encoding the Ebola virus Mayinga glycoprotein (Ad26.ZEBOV; Zabdeno®) followed by Modified Vaccinia Ankara Bavarian Nordic vector encoding multiple filovirus proteins (MVA-BN-Filo; Mvabea®) given eight weeks later, was granted marketing authorisation under exceptional circumstances in 2020 by the European Medicines Agency (EMA). The authorization also included a recommendation to administer an Ad26.ZEBOV booster dose to populations at risk of EVD transmission who received the primary regimen more than four months earlier [7].

Ad26.ZEBOV is a replication-incompetent, adenovirus-vectored vaccine, encoding the full-length Ebola virus glycoprotein (EBOV GP). It constitutes the first dose of the Ad26.ZEBOV, MVA-BN-Filo primary regimen and has also been evaluated as a booster dose in two Phase II trials, EBL3001 in Sierra Leone and EBL2002 in Burkina Faso, Côte d'Ivoire, Kenya and Uganda. When Ad26.ZEBOV was given as a booster dose to healthy adults one or two years after they received the primary regimen, there was no exacerbated reactogenicity and it induced strong anamnestic humoral responses [8,9].

HIV-infected individuals form an important population in areas prone to Ebola outbreaks. As part of the EBL2002 parent trial, the Ad26.ZEBOV, MVA-BN-Filo regimen was assessed in adults with HIV infection well-controlled by highly active antiretroviral therapy (HAART). Compared to healthy adults, these HIV+ participants experienced higher rate of fever post-dose 1, lower rate of solicited local adverse events post-dose 2, and mounted binding antibody responses that were comparable in kinetics and magnitude up to one year [9]. However, there was no data on the durability of vaccine-immune response in HIV+ adults beyond one year. It was unknown whether the primary regimen has established immune memory that can be rapidly reactivated upon antigen re-exposure. In addition, it was unclear whether an Ad26.ZEBOV booster dose is safe and immunogenic among people living with HIV.

In this paper, we present data on the safety and immunogenicity of an Ad26.ZEBOV booster dose in HIV-infected adults in Kenya and Uganda at approximately 4·5 years following the primary regimen.

2. Methods

2.1. Study design

The EBL2010 study is a single-arm, open-label, phase 2 trial conducted in Kenya and Uganda. HIV+ adults previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo primary regimen against EVD were given a booster dose of Ad26.ZEBOV. The primary objectives were to evaluate the safety, tolerability, and immunogenicity of the booster dose in HIV+ adults.

The trial was sponsored by the London School of Hygiene & Tropical Medicine (LSHTM) in the UK and approved by the LSHTM Research Ethics Committee. In Kenya, it received approvals from the Kenyatta National Hospital-University of Nairobi Ethics & Research Committee, the Pharmacy and Poison Board, and the National Commission for Science, Technology & Innovation. In Uganda, it received approvals from the Uganda Virus Research Institute Research and Ethics Committee, Uganda National Council for Science and Technology, and the National Drug Authority.

This study has been registered with the Pan African Clinical Trial Registry (PACTR202102747294430) and with Clinicaltrials.gov (NCT05064956).

2.2. Study participants

In Kenya, participants were recruited at the KAVI – Institute of Clinical Research, Kenyatta National Hospital, Nairobi. In Uganda, participants were recruited at the MRC/UVRI & LSHTM Uganda Research Unit, Masaka Field Station. Only HIV+ individuals who had previously participated in the EBL2002 parent trial, who were aged ≥18 to ≤50 years at the time of randomization in that trial, and who had completed the two-dose Ebola vaccine regimen were invited to join this study. To be eligible for the parent trial, all participants must have had a well-controlled and documented HIV infection for at least 6 months and must have been on a stable regimen of HAART. All participants had previously received Ad26.ZEBOV (5×10^{10} viral particles) as dose 1 and MVA-BN-Filo (1×10^{8} infectious units [Inf U]) as dose 2, with a 28-day or 56-day interval between doses [9].

During the screening visit for this study, potential participants were given a full physical examination and safety laboratory assessments (HIV viral load, CD4 T cell count and full blood count) after providing written consent. To be eligible for this study, participants had to be healthy (based on physical examination, medical history, and clinical judgment), virologically suppressed (HIV viral load <50 copies/mL),

and immunologically controlled (CD4+ T cell count ≥350 cells/mL). All participants also must have been on a stable regimen of HAART (defined as a combination of two or more antiretroviral agents) for at least four consecutive weeks prior to screening and be willing to continue their HAART during the course of the study. Female participants of childbearing potential had to have a negative urine beta human chorionic gonadotropin (β-hCG) pregnancy test result at screening and to use adequate birth control measures throughout the study. The full lists of inclusion and exclusion criteria can be found in the study protocol (Supplementary material).

2.3. Booster vaccination

Ad26.ZEBOV is a replication-incompetent, adenovirus serotype26-vectored vaccine, encoding the full-length glycoprotein from Ebola virus Mayinga (EBOV GP). All eligible participants received Ad26.ZEBOV as an intramuscular injection of a 0·5 mL liquid suspension containing $5x10^{10}$ virus particles into the anterolateral deltoid muscle, using a sterile, single use, 25G hypodermic needle. Ad26.ZEBOV does not contain any adjuvant. The Ad26.ZEBOV booster dose is the same vaccine as dose 1 of the Ad26.ZEBOV, MVA-BN-Filo two-dose regimen given in the EBL2002 parent study.

2.4. Study outcomes

The primary safety and reactogenicity outcomes included the occurrence of solicited adverse events (AEs), unsolicited AEs, and serious adverse events (SAEs) after booster vaccination. The primary immunogenicity outcomes consisted of EBOV GP-specific IgG binding antibody concentrations and vaccine responder status at pre-booster, seven days, and 21 days post-booster.

2.5. Safety evaluations

After vaccination, participants were observed at the study site for a minimum of 30 min for any acute reaction and solicited AEs. Each participant received a participant identification card with an investigator's phone number to call in case of medical issues at any time. They were also given a thermometer, a ruler, and a paper symptoms diary to record any solicited AE daily for the subsequent seven days. The solicited local AEs included tenderness/pain, redness (erythema), swelling, and pruritus (itching) at the injection site; the solicited systemic AEs included body temperature (pyrexia), fatigue, chills, headache, nausea/vomiting, muscle pain (myalgia), and joint pain (arthralgia). Unsolicited AEs were recorded from the day of the booster vaccination for 28 days. Severity of AEs was graded from 1 to 3 using a modified version of the United States National Institutes of Health, Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (Version 2.1, July 2017) [10]. SAEs related to study procedures were reported from the signing of the informed consent form to the end of the study. All other SAEs were reported from vaccination to the end of the study.

2.6. Immunogenicity evaluations

Venous blood samples were collected for immunogenicity evaluation during participant visits immediately before booster administration, seven days post-booster, and 21 days post-booster. IgG binding antibodies against EBOV GP were measured using the FDA-approved Filovirus Animal Non-Clinical Group (FANG) enzyme-linked immunosorbent assay (ELISA) by Q^2 Solutions (San Juan Capistrano, CA, USA). Therefore, this study used the same assay performed by the same laboratory as the EBL2002 parent study.

2.7. Statistical analysis

This study originally planned to enrol approximately 50 participants. The sample size was based on the expected number of eligible individuals from the EBL2002 parent trial, without any formal hypothesis testing consideration. The primary analysis was conducted when all participants had completed the 28-days post booster visit, including all available data up to this time point.

All safety analyses were performed on the full analysis set, which included all participants with documented booster administration. No formal statistical testing of safety data was planned and safety data were analysed descriptively.

The primary analysis for immunogenicity was performed on the per protocol immunogenicity set which included all vaccinated participants for whom immunogenicity data were available and excluded those with major protocol deviations that could affect immunogenicity outcomes. No formal hypothesis on immunogenicity was planned. Descriptive statistics (on observed values and fold-changes following booster vaccination) were calculated for continuous immunologic parameters at each time point.

In line with the parent study, participants were considered vaccine responders if they had a negative antibody ELISA result at baseline (pre-dose 1 in EBL2002) and a positive post-vaccination value of more than 2·5-times the lower limit of quantification (LLOQ; 36·11 ELISA Unit [EU]/mL), or a positive ELISA result at pre-dose 1 baseline and post-vaccination value that was 2·5-times higher than the pre-dose 1 baseline value. The binding antibody concentration of the cohort at each time point was summarised as a geometric mean concentration (GMC) with 95 % confidence intervals (CI). All values below the LLOQ were imputed as half of the LLOQ value (18·055 EU/mL). All values over the upper limit of quantification (ULOQ) were imputed as the ULOQ value (194938·88 EU/mL).

All statistical analyses were performed using STATA version 17 (StataCorp, TX USA).

2.8. Protocol deviations

There were two protocol deviations in this study. One major protocol deviation concerned two female participants, a 44 year old with amenorrhoea for over 2 years and a 54 year old with amenorrhoea for 18 months. They were vaccinated prior to laboratory confirmation that they were not of childbearing potential. Nonetheless, their subsequent FSH tests showed that they were indeed not of childbearing potential. A second minor protocol deviation involved eight female participants of childbearing potential who were vaccinated less than 14 days after screening. All of them were on contraception for over 28 days prior to vaccination. These deviations were not expected to impact on the immunogenicity outcomes and therefore these participants were included in the immunogenicity data analysis.

3. Results

This study began screening participants on 6 October 2021 and the first eligible participant was vaccinated on 14 October 2021. The study was completed when last participant last visit took place on 17 November 2021. Overall, 48 individuals were screened across the two sites and 26/48 (54 %) eligible participants were enrolled. In Kenya, 15 people were screened, 14 failed screening, and one participant was enrolled. In Uganda, 33 people were screened, eight failed screening, and 25 participants were enrolled. Among the 22 ineligible individuals who were excluded, a HIV viral load of ≥50 copies/mL was present in 20, abnormal blood counts in four, pneumonia in one, and two were not available for study visits. Four of these individuals failed multiple eligibility criteria. All 26 participants received an Ad26.ZEBOV booster vaccine with 28 days of safety follow-up and completed the study. The study participant flow is described in Fig. 1.

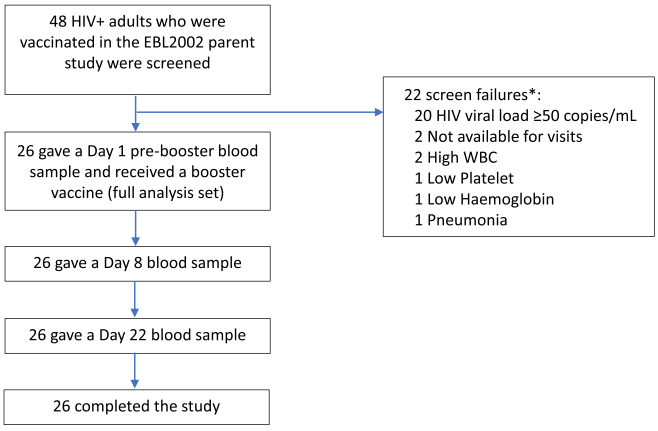

Fig. 1. Study participant flow. *Four potential participants failed to meet more than one eligibility criteria. White blood cells (WBC).

The participant demographic and baseline characteristics are summarised in Table 1. The median age of the participants was 47·5 years (IQR 41–51) at screening for this study. The majority, 19/26 (73 %), were female, similar to the original HIV+ cohort in the EBL2002 parent trial. Half of the participants, 13/26 (50 %), had received the primary Ad26.ZEBOV, MVA-BN-Filo regimen with a 28-day interval between doses, and the other half, 13/26 (50 %), received the regimen with a 56-day interval between doses. On average, study participants were given the Ad26.ZEBOV booster 1655 days (range 1649–1659 days) or 4·5 years (range 4·5-4·6 years) after receiving dose 1 in the EBL2002 parent trial.

Baseline results of the HIV and haematological assessments of the 26 eligible participants are detailed in Table S1. The average CD4+ T cell count was 808.8 cells/μL (SD 175.3), and the HIV viral load was not detectable in 13/26 (50 %) participants, was <40 copies/mL in 12/26 (46 %) participants and was 45 copies/mL in 1/26 (4 %) participants.

3.1. Vaccine safety

All 26 participants in the full analysis set were included in the safety analysis. The booster vaccine was well-tolerated with no safety concerns. Solicited and unsolicited AEs are summarised in Table 2. During the first seven days after vaccination, 20/26 (77 %) participants reported at least one solicited local AE, including 8/26 (31 %) individuals who experienced an event of grade 3 severity. The most common solicited local AEs were injection site pain, pruritus, and

Table 1
Participant demographic and baseline characteristics.

Full analysis set	Total (N = 26)
Sex, n (%)	
Male	7 (27)
Female	19 (73)
Ethnicity	
Black or African	26 (100)
Age at booster dose, years	
Mean (SD)	45.7 (6.3)
Median (range)	47.5 (34–54)
Weight at booster dose, kg	
Mean (SD)	68.3 (15.2)
Median (range)	69.5 (33–86.5)
Body Mass Index at booster dose, kg/m^2	
Mean (SD)	27.5 (6.2)
Median (range)	27.7 (16–39.9)
Interval between dose 1 and dose 2, n(%)*	
0, 28 days	13 (50)
0, 56 days	13 (50)
Duration since dose 1, days*	
Mean (SD)	1655 (10.7)
Median (range)	1652 (1649–1659)
Duration since dose 1, years*	
Mean (SD)	4.5 (0.03)
Median (range)	4.5 (4.5–4.6)

Standard deviation (SD).
* Data from the EBL2002 parent study.

Table 2
Adverse Events after Ad26.ZEBOV booster vaccination. Solicited adverse events (AEs) were collected daily on a diary for 7 days after booster vaccination. Unsolicited AEs were reported until 28 days after booster vaccination.

Full analysis set		Total (N = 26) n (%)[a]
Summary of AEs		
Solicited AE	**Any**	**22 (85)**
	Grade 3[b]	**8 (31)**
Solicited local AE	**Any**	**20 (77)**
	Grade 3	8 (31)
Solicited systemic AE	**Any**	**17(65)**
	Grade 3	1 (4)
Unsolicited AE	**Any**	**12 (46)**
	Grade 3	0
Solicited local AEs		
Number of participants with at least 1 local AE		n (%)
Any solicited local AE, n (%)	**Any**	**20 (77)**
	Grade 1	11 (42)
	Grade 2	1 (4)
	Grade 3	8 (31)
Injection site erythema	**Any**	**6 (23)**
	Grade 2	2 (8)
	Grade 3	4 (15)
Injection site pain	**Any**	**20 (77)**
	Grade 1	17 (65)
	Grade 2	2 (8)
	Grade 3	1 (4)
Injection site pruritus	**Any**	**12 (46)**
	Grade 1	11 (42)
	Grade 2	1 (4)
Injection site swelling	**Any**	**9 (35)**
	Grade 2	1 (4)
	Grade 3	8 (31)
Solicited systemic AEs		
Number of participants with at least 1 systemic AE		n (%)
Any solicited systemic AE, n (%)	**Any**	**17(65)**
	Grade 1	12 (46)
	Grade 2	4 (15)
	Grade 3	1 (4)
Arthralgia, n (%)	**Any**	**10 (38)**
	Grade 1	7 (27)
	Grade 2	3 (11)
Chills, n (%)	**Any**	**12 (46)**
	Grade 1	10 (38)
	Grade 2	2 (8)
Fatigue, n (%)	**Any**	**14 (54)**
	Grade 1	10 (38)
	Grade 2	4 (15)
Headache, n (%)	**Any**	**13 (50)**
	Grade 1	10 (28)
	Grade 2	2 (8)
	Grade 3	1 (4)
Myalgia, n (%)	**Any**	**8 (31)**
	Grade 1	6 (23)
	Grade 2	2 (8)
Nausea, n (%)	**Any**	**3 (12)**
	Grade 1	2 (8)
	Grade 2	1 (4)
Pyrexia, n (%)	**Any**	**2 (8)**
	Grade 1	1 (4)
	Grade 3	1 (4)

[a] n (%): number (percentage) of participants reporting one or more events. AEs were coded using MedDRA version 23.1.
[b] Severity Grade 3 AEs involved symptoms that caused inability to perform usual social and functional activities.

swelling, reported by 20/26 (77 %), 12/26 (46 %), and 9/26 (35 %) participants, respectively. All local solicited adverse event (AE) were considered related to the study vaccine. In the same period, 17/26 (65 %) participants reported at least one solicited systemic AE, including 1/26 (4 %) individuals who experienced a grade 3 headache and grade 3 pyrexia. The most common solicited systemic AEs were fatigue, headache, and chills, reported by 14/26 (54 %), 13/26 (50 %), and 12/26 (46 %) participants, respectively. All local and systemic solicited AEs were temporary and resolved within 7 days. Solicited local AEs are described in Fig. 2A and Table 2; solicited systemic AEs are described in Fig. 2B and Table 2.

During the 28 days after booster administration, 12/26 (46 %) participants reported at least one unsolicited AE. A total of 19 unsolicited AEs were reported in this study, with the most common unsolicited AE being upper respiratory tract infection, urinary tract infection, headache, and low back pain, reported by 3/26 (12 %), 2/26 (8 %), 2/26 (8 %), and 2/26 (8 %) participants, respectively. None of these events were grade 3 or higher in severity and none were considered related to the study vaccine. A list of unsolicited AEs collected in this study are shown in Table S2. There were no SAEs related to study procedures between consent and vaccination. No SAEs or pregnancies were reported in the study period.

3.2. Vaccine immunogenicity

For immunogenicity analysis, all 26 participants who received the Ad26.ZEBOV booster dose were included in the per protocol immunogenicity set. All participants provided samples with analysable results. The EBOV GP binding antibody GMCs are summarised in Table 3.

At an average of 4·5 years after receiving the Ad26.ZEBOV, MVA-BN-Filo primary regimen and prior to booster administration, 24/26 (92 %) participants still had binding antibodies in circulation and were considered responders when compared to the pre-dose 1 baseline. The pre-booster antibody GMC was 726 EU/mL (95 %CI 447–1179). Seven days after booster vaccination, the GMC was 38,965 EU/mL (95 %CI 23532–64522), representing a 54-fold increase over the pre-booster GMC. Twenty-one days after booster vaccination, the GMC was 127,959 EU/mL (95 %CI 93872–174422), representing a 176-fold increase over the pre-booster GMC. At both of these post-booster time points, all 26/26 (100 %) participants had binding antibodies against EBOV GP and were considered responders when compared to the pre-dose 1 baseline.

Table 3
Binding Antibody Responses against Ebola Virus Glycoprotein.

Full analysis set	Total (N = 26)
Day 1 (Pre-Booster)	
N	26
GMC (95 % CI)	726 (447; 1 179)
Responder*	24/26 (92; 75–99)
Day 8 (7 days post-booster)	
N	26
GMC (95 % CI)	38 965 (23 532; 64 522)
Responder [a]	26/26 (100; 87–100)
Day 22 (21 days post-booster)	
N	26
GMC (95 % CI)	127 959 (93 872; 174 422)
Responder [a]	26/26 (100; 87–100)

Confidence interval (CI); Geometric mean concentration (GMC).

* Expressed as n/N (%; 95 % CI), where n is the number of responders at that time point and N is the total number of participants with data at dose 1 vaccination in the EBL2002 parent study and at the indicated time point in the current study.

[a] Expressed as n/N (%; 95 % CI), where n is the number of responders at that time point and N is the total number of participants with data at dose 1 vaccination in the EBL2002 parent study and at the indicated time point in the current study. A one-sided, 97.5 % confidence interval was calculated because all (100 %) 26 participants were responders.

4. Discussion

This is the first clinical trial of an Ad26.ZEBOV booster in HIV-infected adults, about 4·5 years after they received the Ad26.ZEBOV, MVA-BN-Filo primary vaccine regimen against Ebola. The most common solicited local adverse events (AEs) were injection site pain, pruritus, and swelling, while the most common solicited systemic AEs were fatigue, headache, and chills. The AEs reported in this study were mostly mild to moderate in intensity and transient in nature (resolved within 7 days). No SAEs were observed during the study period and there were no major safety concerns.

Because this study does not have a HIV-negative comparator group, there are no data on Ad26.ZEBOV booster vaccination in HIV-negative adults with a similar timelapse after the primary regimen. However, a subset of healthy adults in the EBL2002 parent trial was boosted one year after receiving the primary regimen [9]. Compared to the reactogenicity observed in these HIV-negative adults, higher proportions of HIV+ adults in this study experienced solicited local AEs (20/26, 77 % vs 34/73, 47 %), Grade 3 local AEs (8/26, 31 % vs 0/73, 0) and solicited systemic AEs (17/26, 65 % vs 35/74, 48 %) after booster vaccination.

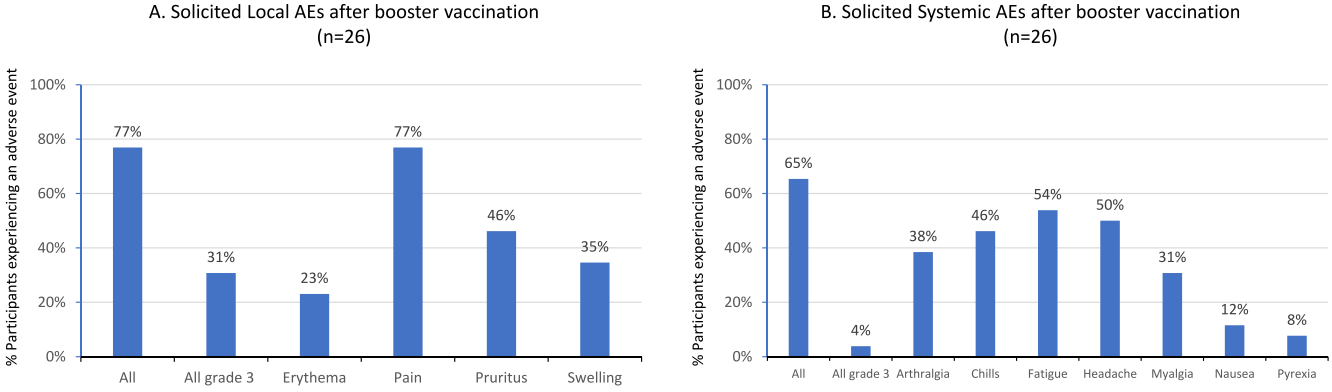

Fig. 2. Solicited adverse events reported after booster vaccination. Panel A: Solicited local AEs after booster vaccination. *Panel B*: Solicited systemic AEs after booster vaccination. Solicited adverse events (AEs) were collected by participants on a diary, from the evening of booster administration, daily for 7 days. Solicited local AEs included erythema, pain, pruritus, and swelling at the injection site (A). Solicited systemic AEs included arthralgia, chills, fatigue, headache, myalgia, nausea, and pyrexia (B). Severity of AEs were graded from 1 to 3 using a modified version of the United States National Institutes of Health, Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (Version 2.1, July 2017). Grade 3 AEs involved symptoms that caused inability to perform usual social and functional activities.

Compared to the reactogenicity of Ad26.ZEBOV given as dose 1 of the primary regimen to HIV+ adults in the parent trial, higher proportions of HIV+ booster recipients in this study experienced solicited local AEs (20/26, 77 % vs 69/118, 59 %) and Grade 3 local AEs (8/26, 31 % vs 0/118). When administered as dose 1 in the parent trial, Ad26.ZEBOV was found to induce higher rate of fever among HIV+ adults (13/118, 11 %) than healthy adults (25/559, 4.5 %). The rate of fever observed in this study after Ad26.ZEBOV booster vaccination of HIV+ adults (2/26, 8 %) therefore falls within the range previously recorded for Ad26.ZEBOV when administered as dose 1.

Given that EBOV GP binding antibodies were measured by the same laboratory using the same assay in this study and in the EBL2002 parent study, we were able to combine all GP binding antibody results for the 26 HIV+ adult participants who received the primary regimen in the parent study and the booster dose in this study. Fig. 3 provides a longitudinal plot of binding antibody GMC before and after dose 1 (Ad26.ZEBOV) and dose 2 (MVA-BN-Filo), administered either 28 or 56 days apart (in the parent study), and before and after a booster dose (Ad26.ZEBOV) 4·5 years later (in this study). Among these 26 participants, the GMC was 33 EU/mL (95 %CI 21 – 52) at pre-dose 1 baseline, 447 EU/mL (95 %CI 318 – 628) at seven days post-dose 2 and 4182 EU/mL (95 %CI 2946 – 5936) at 21 days post-dose 2. Approximately one year later, the GMC of this cohort had declined to 489 EU/mL (95 %CI 310 – 772) at 365 days post-dose 1. The antibody concentrations remained stable for the following 3·5 years, as the GMC at 4·5 years post-dose 1 was 726 EU/mL (95 %CI 447 – 1179). Within three weeks after Ad26.ZEBOV booster vaccination, the antibody concentrations rose to a level 31-times higher than the GMC achieved three weeks after the primary regimen.

These vaccine immunogenicity results can be interpreted through the two arms of humoral immune memory, the maintenance of long-term antibodies and the induction of B cell memory [11]. At approximately 4·5 years after receiving the primary regimen, almost all the HIV+ participants in this study (24/26; 92 %) still had antibodies in circulation and were considered responders prior to booster administration. This indicates that the primary regimen is capable of producing long-term antibodies in HIV+ adults with well-controlled infection [12]. This extends previous findings from the EBL3001 study in Sierra Leone, where 21/31 (68 %) healthy adult participants (Stage 1) were responders almost 2 years after receiving the primary regimen [8]. Our results also confirm the results of modelling of the persistence of EBOV-specific antibodies up to 5 years [13]. In addition, this paper shows that the Ad26.ZEBOV booster is highly immunogenic in previously vaccinated HIV+ adults with well-controlled infection, increasing the antibody GMC by 176-fold within three weeks. This finding is consistent with the primary regimen establishing B cell memory capable of mounting a strong and rapid anamnestic antibody response upon antigen re-exposure in the form of a booster dose in these HIV+ participants.

HIV infection can impair antibody responses to infections and to vaccines. In influenza vaccine studies, virologically suppressed HIV+ individuals had lower antibody titre and fewer responders than HIV- individuals following vaccination [14]. It is therefore encouraging to see that among our virologically suppressed and immunologically controlled HIV+ adult participants, the 21-day post-booster antibody GMC (127 959 EU/mL (95 %CI 93 872 – 174 422); boosted 4.5 years after the primary regimen) was not diminished compared to the post-booster GMCs observed in HIV-negative adults in the parent study (41 643 EU/mL (95 % CI 32 045 – 54 116); boosted one year after the primary regimen) and in Stage 1 adults in the EBL3001 study in Sierra

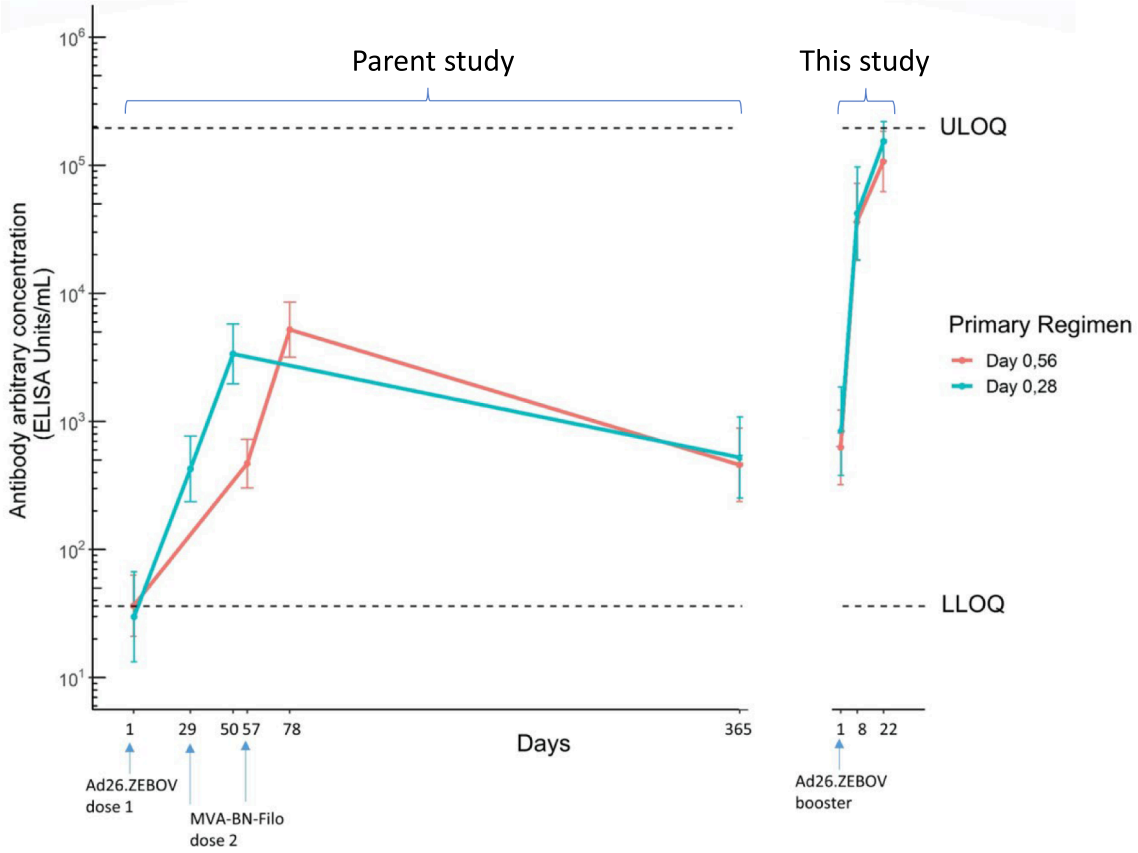

Fig. 3. Long-term antibody persistence and anamnestic responses against Ebola in HIV+ **adults.** Twenty-six participants who previously received the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen as part of the EBL2002 parent study were given a booster dose of Ad26.ZEBOV 4.5 years after the first dose. EBOV GP IgG binding antibody GMCs of HIV+ participants who received the primary regimen in day 0,28 or day 0,56 intervals are plotted against time since the first dose. Ebola virus (EBOV); geometric mean concentration (GMC); glycoprotein (GP); lower limit of quantification (LLOQ); upper limit of quantification (ULOQ).

Leone (30 411 EU/mL (95 % CI 21 972 – 42 091); boosted two years after the primary regimen) [8,9]. This is consistent with the original findings of the parent study that healthy adults and HIV+ adults with well-controlled infection mounted comparable binding antibody responses upon vaccination with the Ad26.ZEBOV, MVA-BN-Filo primary regimen [9].

In non-human primate (NHP) studies, the Ad26.ZEBOV, MVA-BN-Filo primary regimen fully protected NHPs against lethal EBOV challenge. GP-specific binding antibody concentration is strongly associated with neutralising antibody concentration as well as survival, allowing the protective effect of the primary regimen in humans to be inferred using immunobridging analysis [15]. This study shows that within one week of re-exposure to the EBOV GP antigen, in the form of an Ad26.ZEBOV booster, EBOV GP binding antibody concentrations are substantially higher than those observed after the primary regimen. Therefore, it is likely that the Ad26.ZEBOV booster vaccination would also confer protection. Taken together, our results support the deployment of Ad26.ZEBOV booster vaccines to HIV+ adults with well-controlled infection in at-risk populations who have received Ad26.ZEBOV and MVA-BN-Filo up to 4·5 years previously.

This study has several limitations. The responder rate was first characterised during the Phase I trials as one of the parameters for cross-comparison of different regimens of Ad26.ZEBOV and MVA-BN-Filo vaccines across different studies. Although the majority (92 %) of the study participants who received the Ad26.ZEBOV, MVA-BN-Filo primary regimen 4·5 years before are still considered responders, it is unclear if they are still protected and when an Ad26.ZEBOV booster dose is necessary to sustain protection against Ebola infection. In line with the EMA recommendation, we suggest that previously vaccinated individuals should be given a booster dose if there is an active EVD outbreak in the area where they reside. The sample size of this study is small. This is partly due to a very short recruitment window that limited the number of participants the sites could enroll and partly due to the stringent HIV viral load threshold for participant inclusion, resulting in 20/48 (42 %) screening failures. By restricting enrolment to HIV+ individuals with viral load that is less than 50 copies/mL or undetectable, there might be selection bias towards HIV+ individuals with better self-care or higher compliance with HAART. HIV infection may be undetected or poorly controlled in some populations at risk of EVD. Further studies of the primary vaccine regimen and booster doses in HIV+ adults and children across a spectrum of HIV suppression, including those with viral load over 50 copies/mL, are therefore recommended. The study cohort had a sex imbalance (19/26 females, 73 %) but this is representative of the parent HIV+ cohort in the EBL2002 study (99/142 females, 70 %) and reflects the sex imbalance in HIV care often seen in sub-Saharan Africa. The 28-days post-booster safety follow-up period was limited. However, we believe this was sufficient because previous studies of the same booster did not observe any vaccine-related SAE even after one year of follow-up [8,9].

In conclusion, the Ad26.ZEBOV booster dose is safe and highly immunogenic in HIV+ adults with well-controlled infection. The safety profile of the booster in these participants is characterised by mild to moderate adverse events. There were no serious adverse events and no major safety concerns. Vaccination with the Ad26.ZEBOV, MVA-BN-Filo primary regimen produced long-term antibodies and B cell memory for at least 4·5 years. The Ad26.ZEBOV booster induced strong anamnestic antibody responses in previously immunized HIV+ adults. These results extend previous demonstration of long-term vaccine-induced responses among healthy adults, which were available up to two years, confirm modelling of Ebola antibody persistence up to 5 years.

5. Data sharing

The rights of study subjects and partners, the sharing of data between partners and the transfer of data to external third party will be governed by the Data Sharing Agreement. Deidentified participant-level data collected in this trial will be disseminated through a FAIR-compliant data repository, such as the LSHTM Data Compass (https://datacompass.lshtm.ac.uk/), from 6 to 60 months after the publication of the main trial results. Other study documents (e.g. full protocol, statistical codes, statistical analytical plan, medical review plan) will be available on request to Edward Choi (corresponding author, ORCID: 0000-0002-8148-120X), Philip Ayieko (study statistician, ORCID: 0000-0002-7147-7354) or Deborah Watson-Jones (Chief Investigator, ORCID: 0000-0001-6247-1746).

Role of the funding source

This study received funding from the Innovative Medicines Initiative (IMI). IMI had no role in the study design, data collection, or publication of the results. LSHTM, the study sponsor, was responsible for the study protocol, oversight of study implementation, sample analysis, and data interpretation. All authors had full access to all the data in the study and the corresponding author had the final responsibility for manuscript submission.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Janssen Vaccines & Prevention B.V. was the vaccine manufacturer and donated the vaccine for this study. BKe, AG, CM, KL, and CR were full-time employees of Janssen, Pharmaceutical Companies of Johnson & Johnson at the time of the study. AG, CM, KL, and CR, declared ownership of shares in Janssen, Pharmaceutical Companies of Johnson & Johnson. All other authors declare funding from the Innovative Medicines Initiative 2 Joint Undertaking. GAM reports having received travel grant from the vaccine manufacturer to attend scientific meetings and present, after the study has completed.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.10.055.

References

[1] Kuhn JH, Amarasinghe GK, Basler CF, Bavari S, Bukreyev A, Chandran K, et al. ICTV virus taxonomy profile: filoviridae. J Gen Virol 2019;100(6):911–2.
[2] Jacob ST, Crozier I, Fischer 2nd WA, Hewlett A, Kraft CS, Vega MA, et al. Ebola virus disease. Nat Rev Dis Primers 2020;6(1):13.
[3] World Health Organization. Ebola virus disease [updated 2021 Feb 2Available from: https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease.

[4] Anywaine ZM, G.A. African Cities and Urban Health. Handbook of Global Urban Health: Routledge; 2019.
[5] First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response [press release]. 2019 Dec 19 2019.
[6] Piszczatoski CRG, Ervebo JG. (Ebola Zaire Vaccine, Live/rVSVΔG-ZEBOV-GP): The First Licensed Vaccine for the Prevention of Ebola Virus Disease. Journal of Pharmacy Technology. 2020;36(6).
[7] New vaccine for prevention of Ebola virus disease recommended for approval in the European Union [press release]. https://www.ema.europa.eu/en/news/new-vaccine-prevention-ebola-virus-disease-recommended-approval-european-union: European Medicines Agency, 29 May 2020 2020.
[8] Ishola D, Manno D, Afolabi MO, Keshinro B, Bockstal V, Rogers B, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. Lancet Infect Dis 2022;22(1):97–109.
[9] Barry H, Mutua G, Kibuuka H, Anywaine Z, Sirima SB, Meda N, et al. Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in healthy and HIV-infected adults: A randomised, placebo-controlled Phase II clinical trial in Africa. PLoS Med 2021;18(10).
[10] National Institute of Allergy and Infectious Diseases NIoH. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. In: Services UDoHaH, editor. Corrected Version 2.1 edJuly 2017.
[11] Akkaya M, Kwak K, Pierce SK. B cell memory: building two walls of protection against pathogens. Nat Rev Immunol 2020;20(4):229–38.
[12] Robinson MJ, Webster RH, Tarlinton DM. How intrinsic and extrinsic regulators of plasma cell survival might intersect for durable humoral immunity. Immunol Rev 2020;296(1):87–103.
[13] Pasin C, Balelli I, Van Effelterre T, Bockstal V, Solforosi L, Prague M, et al. Dynamics of the humoral immune response to a prime-boost Ebola vaccine: quantification and sources of variation. J Virol 2019;93(18).
[14] Pallikkuth S, de Armas L, Rinaldi S, Pahwa S. T follicular helper cells and B cell dysfunction in aging and HIV-1 infection. Front Immunol 2017;8:1380.
[15] Bockstal V, Leyssen M, Heerwegh D, Spiessens B, Robinson C, Stoop JN, et al. Non-human primate to human immunobridging demonstrates a protective effect of Ad26.ZEBOV, MVA-BN-Filo vaccine against Ebola. npj Vaccines 2022;7(1):156.