28 May 2020
EMA/323670/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zabdeno

Common name: ebola vaccine (rDNA, replication-incompetent)

Procedure No. EMEA/H/C/005337/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	ZABDENO
Applicant:	Janssen-Cilag International N.V. Turnhoutseweg 30 B-2340 Beerse BELGIUM
Active substance:	Recombinant Adenovirus type 26 (Ad26) encoding the glycoprotein (GP) of the Ebola virus Zaire (ZEBOV) Mayinga strain
Common Name:	Ebola vaccine (Ad26.ZEBOV-GP [recombinant])
Pharmaco-therapeutic group (ATC Code):	J07BX02 Ebola vaccines
Therapeutic indication(s):	Active immunization for prevention of disease caused by Ebola virus (Zaire ebolavirus species) in individuals $\geqslant$ 1 year of age
Pharmaceutical form(s):	Suspension for injection
Strength(s):	not less than 8.75 $\log_{10}$ infectious units (Inf.U) in 0.5 mL
Route(s) of administration:	Intramuscular use
Packaging:	vial (glass)
Package size(s):	20 vials

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List of abbreviations

Ad26	adenovirus type 26
AE	adverse event
AESI	adverse events of special interest
AS	active substance
BMI	body mass index
BN	Bavarian Nordic
CI	confidence interval
CMA	critical material attributes
CMV	cytomegalovirus
CPPs	critical process parameters
CRT	controlled room temperature
CSR	clinical study report
d	days
DCS	differential centrifugation sedimentation
DLS	dynamic light scattering
DoE	design of experiment
DRC	Democratic Republic of the Congo
DRM	development reference material
EBOV	Zaïre ebolavirus
ECG	electrocardiogram
EDTA	ethylenediaminetetra acetic acid
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot (assay)
EMA	European Medicines Agency
EOPC	end of production cells
EU/mL	ELISA units/mL
EVD	Ebola virus disease
FANG	Filovirus Animal Nonclinical Group
FAS	Full Analysis Set
FDA	Food and Drug Administration
FP	finished product
FU	follow-up
GMC	geometric mean concentration(s)
GMI	geometric mean fold increase(s)
GMP	Good Manufacturing Practice
GMT	geometric mean titer(s)
GP	glycoprotein
HAART	Highly Active Antiretroviral Therapy
HBsAg	hepatitis B surface antigen
HCP	host cell protein
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IC50	50% inhibitory concentration
IC90	90% inhibitory concentration
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

ICS	intracellular cytokine staining
IFN-γ	interferon-gamma
IG	Immunogenicity Analysis (Set)
IM	intramuscular(ly)
Inf.U	infectious units
IPCs	in-process controls
LLOQ	lower limit of quantification
LVHD	large volume high density
MAA	Marketing Authorisation Application
MARV	Marburg virus
MCB	master cell bank
MOI	multiplicity of infection
MOR	maximum operating ranges
MVA	Modified Vaccinia Ankara
MVS	Master Virus Seed
N	number of participants (with data)
NA	not applicable
NHP	nonhuman primate(s)
PACMP	post-approval change management protocol
PAR	proven acceptance ranges
PBMC	peripheral blood mononuclear cell(s)
PCR	polymerase chain reaction
pfu	plaque forming unit(s)
Ph.Eur.	European Pharmacopoeia
PHEIC	Public Health Emergency of International Concern
PP	Per Protocol Analysis (Set)
PPQ	process performance qualification
PRM	Primary Reference Material
PRNT	plaque reduction neutralization test
psVNA	pseudovirion neutralization assay
PV	process verification
QC	quality control
QPA	quantitative potency assay
qPCR	quantitative PCR
QTPP	quality target product profile
RCA	replication competent adenovirus
RP-HPLC	reversed-phase high-performance liquid chromatography
SD	standard deviation
SE	standard error
SFU	spot forming unit(s)
SI-VP-qPCR	stability indicating virus particle quantitative polymerase chain reaction
SUDV	Sudan ebolavirus
TAFV	Tai Forest ebolavirus
TCID50	50% tissue culture infective dose
TGE	transgene expression
TNF-α	tumour necrosis factor-alpha
TSE/BSE	Transmissible Spongiform Encephalopathy/Bovine Spongiform Encephalopathy
VNA	virus neutralization assay
vp	virus particles
WCB	working cell bank

WHO	World Health Organization
WRM	working reference material
WVS	Working Virus Seed
ZEBOV	Zaire ebolavirus

1. Background information on the procedure

1.1 Submission of the dossier

The applicant Janssen-Cilag International N.V. submitted on 6 November 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Zabdeno, through the centralised procedure falling within the Article 3(1) of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 28 March 2019.

The applicant applied for the following indication: *"Zabdeno, as part of the Zabdeno, Mvabea vaccine regimen, is indicated for active immunisation for prevention of disease caused by Ebola virus (Zaire ebolavirus species) in individuals ⩾1 year of age. The use of the vaccine regimen should be in accordance with official recommendations".*

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0116/2019 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-002307-PIP01 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's requests for consideration

Marketing authorisation under exceptional circumstances

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

New active Substance status

The applicant requested the active substance Ebola vaccine (Ad26.ZEBOV-GP [recombinant]) contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Scientific advice

The applicant received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
22 January 2015	EMEA/H/SA/3018/1/2014/III	*Dr Walter Janssens, Dr Mair Powell*
9 March 2015	EMEA/H/SA/3018/1/FU/1/2015/II	*Dr Walter Janssens (Rapid Advice)*
24 September 2015	EMEA/H/SA/3018/1/FU/2/2015/III	*Dr Walter Janssens (Rapid Advice)*
22 June 2017	EMEA/H/SA/3018/1/FU/3/2017/III	*Dr Mair Powell, Dr Filip Josephson*
22 June 2017	EMEA/H/SA/3018/2/2017/PED/II	*Dr Mair Powell, Dr Filip Josephson*
28 March 2019	EMEA/H/SA/3018/1/FU/4/2019/III	*Dr Walter Janssens, Dr Hans Ovelgönne*
12 June 2019	EMEA/H/SA/3018/1/FU/4/2019/III	*Dr Walter Janssens, Dr Hans Ovelgönne*

The Scientific advice pertained to the following *quality, non-clinical, and clinical* aspects:

- *Analytical comparability to support changes in manufacturing*
- *Planned process validation approach*
- *Release tests for drug substance and drug product*
- *The data requirements for process verification at MAA*
- *The ERA*
- *The timing and design of the combined pre- and postnatal developmental toxicity study*
- *The adequacy of nonclinical biodistribution and toxicology studies for MAA*
- *The design of the phase II safety and immunogenicity studies in EU and Africa*
- *The design of an open-label, cluster-randomized controlled Phase 3 study to evaluate the efficacy, immunogenicity, and safety of a heterologous prime-boost regimen*
- *The strategy regarding demonstration of clinical Lot to Lot consistency*
- *The strategy to use the NHP challenge data and immunobridgingto human in the absence of the possibility to generate evidence of vaccine efficacy or effectiveness in humans*
- *The design of the Ebola challenge study in Cynomolgus macaques (NHP)*
- *The statistical methodology and assays for immunobridging from the NHP model to human*
- *The paediatric development strategy*

- *The indication statement*
- *The regulatory procedures, timing, approval mechanism and evidence base needed for deploying the vaccine for "rapid access"*
- *The proposal for informal rolling data submission in order the Agency to be able to react promptly if needed*
- *Dossier requirements for the separate MAAs for each of the 2 vaccines included in the single heterologous vaccine regimen*

1.2 Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Johann Lodewijk Hillege Co-Rapporteur: Jean-Michel Race

Accelerated Assessment procedure was agreed-upon by CHMP on	19 September 2019
The application was received by the EMA on	6 November 2019
The procedure started on	28 November 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	29 January 2020
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	28 January 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	5 February 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 February 2020
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 February 2020
The applicant submitted the responses to the CHMP consolidated List of Questions on	26 March 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	16 April 2020
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	28 April 2020
The applicant submitted the responses to the CHMP List of Outstanding Issues on	04 May 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	14 May 2020
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zabdeno on	28 May 2020

2. Scientific discussion

2.1 Problem statement

2.1.1 Disease or condition

Zabdeno, as part of the Zabdeno, Mvabea vaccine regimen, is indicated for active immunization for prevention of disease caused by Ebola virus (Zaire ebolavirus species) in individuals ≥1 year of age.

The use of the vaccine regimen should be in accordance with official recommendations.

Ebola virus disease (EVD) is an acute systemic febrile syndrome caused by Ebola viruses. Zaire Ebola virus is a member of the Filoviridae family; the virus is transmitted through human-to-human contact. Ebola virus disease affects both adults and children with most cases in people aged 20 to 50 years. EVD has a case fatality ranging from 30% to 90% and an incubation period of 2 to 21 days. The pathogenesis of EVD is characterized by an intense inflammatory process, impaired haemostasis and capillary leak, with mortality resulting from septic shock and multi organ system failure.

2.1.2 Epidemiology

The first Ebola virus disease (EVD) outbreaks were reported back in 1976. Since then more than 30 outbreaks have occurred in Africa, mostly in Sudan, Uganda, Democratic Republic of Congo, and Gabon, with more than 30.000 people affected by the disease and almost 15.000 deaths. The first outbreaks occurred in remote villages in Central Africa, near tropical rainforests. The 2014–2016 outbreak in West Africa involved major urban areas as well as rural ones and was declared a public health emergency of international concern (PHEIC) by WHO. Three EVD outbreaks have since been declared in the DRC. The most recent one, which started in August 2018, is still ongoing and is the second largest outbreak after the 2014-2016 epidemic. As of 29 September 2019, a total of 3,191 cases and 2,133 deaths have been reported with an overall case fatality rate of 67%. This Ebola outbreak was declared a PHEIC on 17 July 2019.

So far Ebola virus disease outbreaks have been restricted to African countries. However, there is a risk that the disease could spread to other continents due to the ease of international travel and secondary infection from patients immigrating from African countries has been reported in Spain and the US. The risk of a global spread is judged limited and the risk of introduction and spread within Europe is considered very low.

Beyond the direct morbidity and mortality due to Ebola, the disease has indirect effects on population health based on the diversion of resources from programmes aimed at controlling other diseases of major importance.

2.1.3 Aetiology and pathogenesis

EVD is a zoonosis, with probable reservoir in bats, that is transmitted by direct contact with body fluids or tissues of an infected individual

The causative agent of the disease is the Ebola virus, a negative-stranded RNA virus belonging to the filoviridae family. All members of this order possess a non-segmented, negative-sense RNA genome of 19 kb with seven open reading frames that is encapsidated by the viral nucleoprotein (NP). The NP–

RNA complex acts as the template for genome replication and assembles into a helical nucleocapsid (NC) along with accessory proteins.

Since the discovery of Ebola virus in 1976, 6 species of Ebola virus have been identified: Zaïre ebolavirus (EBOV), Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV), Taï Forest ebolavirus (TAFV), Reston ebolavirus (RESTV), and the newly discovered (2018) Bombali ebolavirus (BOMV). The Zaire ebolavirus, EBOV, is the main causative agent of human EVD outbreaks. EBOV persists in the environment in a still unidentified animal reservoir, most likely fruit bats, which maintains the virus in an enzootic cycle. Human infection represents a sporadic event. Transmission is mainly due to the contact with blood or body fluids from infected humans or animals.

2.1.4 Clinical presentation and diagnosis

Clinical presentation:

EVD is a viral haemorrhagic fever affecting humans and other primates that is caused by the infection with ebolaviruses. Following an incubation period of 2–21 days, Ebola disease typically starts as a non-specific viral syndrome with abrupt onset. At this stage the most frequent symptoms are high fever, malaise, fatigue, and body aches. These symptoms usually develop after a few days into gastrointestinal symptoms including nausea, vomiting, and diarrhoea. These manifestations can range from mild-to-severe, with body fluid loss of up to 5–10 L/day. Other, rarer, symptoms are cough and dyspnoea, conjunctival injection, hiccups, or localised pain of chest, abdomen, muscles, or joints.

Some of the patients may recover from this stage, others however will enter into deterioration of symptoms finally going into shock, possibly due to hypovolaemia and a systemic inflammatory response. Around this time, patients can present with haemorrhagic events, such as conjunctival bleeding, petechiae, gastrointestinal bleeding, and mucosal haemorrhage. Neurological events are rare and include confusion, delirium, and convulsions. Cases of Ebola disease-related encephalitis have been reported. Other late symptoms include dysphagia, throat pain, and oral ulcers. A maculopapular rash has been described. Exceptionally, sudden death can occur in recovering patients, possibly due to cardiac arrhythmias. If patients survive the stage of shock, gradual recovery can occur.

Laboratory features include variable degrees of anaemia and thrombocytopenia as well as changes in number and type of white blood cells. Renal dysfunction (in up to 50% of case) and substantial increases in liver enzymes are common. Likewise, creatine phosphokinase and amylase concentrations can be increased. Electrolyte abnormalities are common, especially hypokalaemia, hyponatraemia, and hypocalcaemia. Clotting tests can indicate a varying degree of intravascular coagulation. Metabolic acidosis can occur, particularly in cases of shock and renal failure.

High viral loads, combined with severe muscle breakdown and renal impairment, have consistently been predictive of death. Differences in severity of clinical events and outcome might exist between young children, young adults, and older people. Pregnant women face higher mortality and risk of miscarriage and stillbirth. Clinical presentation can be aggravated by concurrent comorbidities and infections, such as malaria and bacterial sepsis. Clinical signs and symptoms have varied across the different Ebola outbreaks reported during the last decades. This variation is at least partly due to the specific outbreak context and the ebolavirus species involved. For example, haemorrhagic events were highly prevalent in the 1976 outbreak in Yambuku, but less so in many other outbreaks, including the West African one. Death occurs due to blood loss and/or coagulation. In patients with fatal outcome, death occurs within 6 to 16 days of onset of disease. The average EVD case fatality rate is around 50% but rates have varied from 25% to 90% in past outbreaks depending on the causative virus species.

Diagnosis:

Diagnosis of EVD on the basis of clinical symptoms can be difficult as clinical manifestations are similar to those of other infectious diseases such as malaria, typhoid fever and meningitis. Confirmation that symptoms are caused by EBOV infection are made using diagnostic laboratory methods:

- antibody-capture enzyme-linked immunosorbent assay (ELISA)
- antigen-capture detection tests
- serum neutralization test
- reverse transcriptase polymerase chain reaction (RT-PCR) assay
- electron microscopy
- virus isolation by cell culture

Real-time RT-PCR tests were the cornerstone of the laboratory response during the 2013–16 West African outbreak. However, for many years the main methods for detection of filoviruses has been virus isolation in cell culture from patient specimens.

Viral load peaks 3–7 days after the onset of symptoms. In fatal cases, viraemia is usually 10–100 fold higher than in survivors. IgG and IgM humoral responses develop in survivors but not in all fatal cases thus, diagnosing of EVD using serology is only possible in a fraction of symptomatic patients and requires seroconversion or a substantial increase in antibody titre in paired serum samples. However, serology is the method of choice to diagnose asymptomatic Ebola virus infections characterised by extremely low viraemia and development of IgG and IgM about 3 weeks after infection.

2.1.5 Management

Therapeutics:

No registered curative therapy exists to date. Standard treatment is mainly supportive and consists of provision of fluids and electrolytes, maintaining blood pressure and oxygen status, and managing fever and pain.

Four investigational treatments have been evaluated in a randomized controlled study in the DRC under coordination of the World Health Organization (WHO) (the PALM study). These include 3 treatments based on monoclonal antibodies (mAbs) all targeting the EBOV glycoprotein (GP) (Zmapp, mAb114, and REGN-EB3) and an inhibitor of viral RNA synthesis (remdesivir). Zmapp is composed of 3 chimeric mAbs, mAb114 is based on an isolate from a survivor of the 1995 outbreak of EVD in Kikwit, the DRC, REGN-EB3 is a cocktail of 3 humanized mAbs, and remdesivir is a small-molecule nucleotide prodrug.

From August 2019, based on advice from an independent data safety monitoring board, all patients were assigned to MAb114 and REGN-EB3 as both MAb114 and REGN-EB3 were superior to ZMapp in preventing death (Mulangu et al NEJM 2019; 381:2293-2303). The clinical study will continue comparing these REGN-EB3 and mAb114 in Ebola treatment centres in the DRC.

Vaccines:

Three Ebola recombinant viral-vectored vaccines that encode for the EBOV GP, have previously been submitted for marketing approval or have been approved. Two vaccine regimens (Ad5.EBOV single-dose; and rVSV-ZEBOV followed by rAd5-EBOV), developed from the circulating strain of the 2014/2016 EVD outbreak, are approved for human use in China (approved in 2017 for emergency use

in case of an outbreak) and Russia, respectively, based on preclinical efficacy and clinical Phase 1-2 immunogenicity and safety data. A conjugate vaccine based on 2 peptide antigens conjugated to a protein carrier (undefined) is also approved for use in Russia.

Ebola vaccine rVSV-ZEBOV-GP was recently authorised in the EU and in the US. This vaccine proved protective against the deadly virus in Guinea in 2015 as well as in the current outbreak in the DRC for vaccination of contacts of cases and of contacts of contacts of cases (ring vaccination) and frontline health workers. The duration of protection afforded by the rVSV vaccine is not known.

An outbreak of Zaire ebolavirus is still ongoing since August 2018 in the DRC (North Kivu and Ituri provinces). As of January 2020, 3390 cases have been reported with 2233 confirmed deaths (source: WHO, https://www.who.int/emergencies/diseases/ebola/drc-2019). The setting is very complex and despite ongoing efforts the outbreak is not under control. The number of health care workers affected is estimated at 6% of total cases. Since November 2019 the Ad26.ZEBOV/MVA-BN-Filo vaccine regimen has been used to try to prevent spread of the virus outside of the outbreak zones.

In Europe, an Ebola vaccine may be used to immunize healthcare workers who will potentially travel to outbreak areas to participate in outbreak response, military personnel that may be involved in affected regions, healthcare workers that will potentially take care of imported Ebola cases in reference hospitals in Europe, and laboratory personnel with risk of exposure to Ebola virus.

There remains a recognised unmet medical need for effective Ebola vaccines.

About the product

Ad26.ZEBOV is to be given as part of a vaccine regimen consisting of a single dose of Ad26.ZEBOV followed by a single dose of MVA-BN-Filo 8 weeks after the first dose. This can be followed by a booster dose of Ad26.ZEBOV.

Induction of protective immunity by the Ad26.ZEBOV, MVA-BN-Filo regimen is thought to occur mainly through antibody responses against the GP along with involvement of collaborative cellular immunity components. The EBOV GP encoded by Ad26.ZEBOV has 100% homology to the one encoded by MVA-BN-Filo.

Adenoviruses are nonenveloped viruses composed of an icosahedral nucleocapsid and a single double-stranded linear DNA genome. The genome contains 4 so-called "early region" (E) genes of which the encoding proteins have major roles in early adenovirus infection events. The E1 region encodes proteins that are involved in transactivation of viral and cellular genes, induction of cellular DNA synthesis, and mitosis. Proteins encoded by the E3 region are involved in modulating the host immune response and promote persistence within the host cell. The E4 region is essential for viral growth.

Ad26.ZEBOV is a monovalent recombinant Ad26-based vector that was rendered replication-incompetent by deletion of the E1 region. A large portion of the E3 region, which promotes persistence within the host cell, has also been removed to create sufficient space in the genome for the transgene encoding the full-length EBOV Mayinga GP, which was inserted in the E1 region. Ad vectors that have been engineered in this manner can gain entrance into a normal host cell and cause the cell to make viral proteins but are unable to spread into new host cells in contrast to a typical infection. The adenoviral genome remains epichromosomal, thus avoiding the risk of integration of the viral DNA into the host genome following cell infection.

EBOV GP is encoded but not expressed by the Ad26.ZEBOV vaccine particle. Host cell DNA-dependent RNA polymerase transcribes the transgene encoding EBOV GP into mRNA, which is then translated to

produce the GP. The GP is then presented on the cell membrane to the host immune system. Following administration, the EBOV GP is expressed locally and stimulates an immune response.

The vaccine regimen consisting of 1 dose of Ad26.ZEBOV vaccine corresponding with 5×10^{10} vp (this has been translated into "not less than 8.75 $\log_{10}$ infectious units (Inf. U.) in 0.5 mL" in the SmPC) followed by 1 dose of MVA-BN-Filo vaccine (1×10^8 Inf.U) is intended for prophylactic vaccination. In the following report viral particles (vp) are used to express the strength as per development program.

The proposed indication is:

For active immunization for prevention of disease caused by Ebola virus (Zaïre ebolavirus species) in individuals ≥1 year of age.

The proposed posology indicates that:

The prophylactic 2-dose heterologous Ebola vaccine regimen consists of vaccination with Ad26.ZEBOV followed by a second vaccination with MVA-BN-Filo given approximately 8 weeks later.

In addition, a later dose of Ad26.ZEBOV induces a booster response. The proposed posology indicates that:

Individuals who have previously completed the 2-dose vaccine regimen can receive a booster dose of Ad26.ZEBOV vaccine. It is particularly recommended for individuals at risk of imminent exposure to Ebola virus, for example those living in or visiting areas affected by Ebola virus disease outbreaks. It may also be recommended for individuals living in close contact with patients with Ebola virus disease and those with an occupational risk of exposure to Ebola virus.

Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the potential of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, if it is able to prevent EVD, to address (part of) the unmet medical need in context of the ongoing Public Health Emergency of International Concerns as declared by the WHO.

The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation based on the inability to provide comprehensive clinical data on the efficacy under normal conditions of use.

Given the unmet and ongoing medical need and in the absence of clinical efficacy data, the Applicant assessed the likelihood of the vaccine regimen to induce clinical protection against Ebola Virus Disease through the bridging of clinical immunogenicity results from pivotal Phase 2 and 3 studies to efficacy and immunogenicity data obtained in non-human primates (agreed with EMA Scientific Advice procedure EMEA/H/SA/3018 /1/FU/3/2017/III).

The Applicant claimed that conducting a randomised (placebo) controlled efficacy study is not feasible for ethical reasons considering the high mortality of EVD, due to the security situation in the current DRC outbreak and due to operational difficulties of conducting such a study during an ongoing Ebola outbreak. Although an observational study is underway to estimate effectiveness of the vaccine regimen, it is far from certain that this study will be able to accrue sufficient cases to provide a reliable estimate of effectiveness.

The Applicant claimed that there is no certainty as to the possibility of generating effectiveness data within a reasonable time frame post-approval, something that would be expected in the context of the Conditional Approval pathway. The impossibility to generate efficacy data and the uncertainty regarding the confirmation of effectiveness motivated the Applicant to seek approval under exceptional

circumstances. This was accepted by CHMP, who additionally noted that Zabdeno, as part of the Zabdeno, Mvabea vaccine regimen, is not in principle intended to be used in the context of an outbreak, contrary to the use of the previously authorized Ebola vaccine, which received a Conditional Marketing Authorization.

2.2 Quality aspects

2.2.1 Introduction

The Ad26.ZEBOV-GP (Adenovirus 26 virus encoding the Zaire ebolavirus glycoprotein) finished product (FP) is supplied as a sterile, liquid suspension for injection. One 0.5 mL dose contains not less than 8.75 log 10 Infectious Units (Inf.U.) of Ad26.ZEBOV-GP as active substance. This product is not described as 'live' in the product information since the virus is non-replicating in human cells other than those specifically engineered to permit growth e.g. PER.C6 cells (see general information section).

Other ingredients are: sodium chloride, sucrose, polysorbate-80, ethylenediaminetetraacetic acid (EDTA) disodium dihydrate salt, L-histidine monohydrochloride monohydrate, ethanol and water for injection. Sodium hydroxide is used as a pH adjuster.

The product is available in a single dose Type I glass vial with a rubber stopper (fluoropolymer coated chlorobutyl), aluminium crimp and red plastic cap.

2.2.2 Active Substance

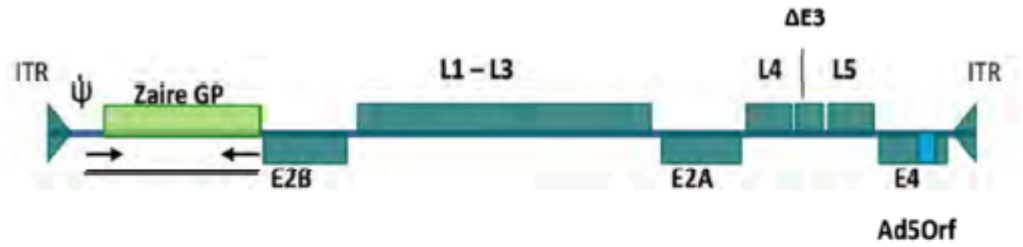
General information

Ad26.ZEBOV-GP (also referred to as Ad26.ZEBOV in this report) encodes the full-length glycoprotein of the Zaire ebolavirus.

Adenovirus Serotype 26 (Ad26) consists of non-enveloped virions, each containing a single linear molecule of double stranded DNA (dsDNA) of approximately 35 kilobase pairs (kbp) which encode the adenoviral proteins. The dsDNA molecule is encapsulated by an icosahedral protein structure consisting of the structural proteins II (hexon), III (penton), IV (fiber), VI, VIII, IX, and IIIa. Core proteins V, VII, and X and the terminal protein are directly associated with the DNA molecule.

The recombinant Ad26 vector Ad26.ZEBOV is replication incompetent due to deletions in E1 (Δ E1A/E1B). To ensure that recombinant Ad26 vectors can also be propagated efficiently in E1 complementing cell lines such as PER.C6, the orf6/7 in the E4 region is replaced by the same region from Ad5. A large part of the E3 region, which promotes persistence within the host cell, has also been removed to create sufficient space in the viral genome for insertion of foreign antigens (Δ E3). The vector contains the full-length Zaire ebolavirus glycoprotein sequence. The Zaire ebolavirus glycoprotein antigen is a naturally occurring sequence. This vaccine is produced in the human cell line PER.C6.

A schematic representation of the Ad26 vector is shown in Figure 1.



Ad5Orf = Open reading frame
E2-E4 = Early regions
E = Early genes
ITR = Inverted terminal repeat
L = Late genes
Zaire GP = Zaire Ebolavirus glycoprotein

Figure 1: Schematic representation of the Ad26 vector

Manufacture, characterisation and process controls

The active substance (AS) is manufactured by Cilag GmbH International, Bern, Switzerland. All relevant active substance sites have valid manufacturing authorisations or valid GMP certificates as appropriate.

Description of manufacturing process and process controls

The AS manufacturing process consists of ten stages: 1) cell expansion; 2) cell production; 3) virus production;4) lysis; 5) DNA precipitation; 6) sedimentation and clarification; 7) AEX chromatography; 8) diafiltration; 9) formulation and filling and, finally, 10) freezing of the AS. The batch size of the Ad26.ZEBOV AS batch is defined starting from one single PER.C6 large volume high density (LVHD) cell bank bag. The minimum and maximum batch size are defined by the technical limits of the downstream process. Reprocessing is not applicable for the AS.

Manufacturing is initiated by thawing of a PER.C6 LVHD cell bank bag. The culture is expanded until the viable cell density required for inoculation of the virus production bioreactor is obtained. Virus is produced through infection of the cell culture with working virus seed (WVS) at a target multiplicity of infection (MOI). Recombinant replication defective adenovirus is released from the PER.C6 cells by chemical lysis of the cells by the addition of, lysis agent in the bioreactors.

Levels of residual host cell DNA are reduced by precipitation followed by depth filtration.. The harvest is further purified by chromatography and then formulated by diafiltration and dilution. Impurity levels of e.g. host cell DNA, host-cell protein, process additives and media components are further reduced. The resulting diluted bulk is further processed with a 0.2 µm filtration step and filled in specified single-use flexible bags (see container-closure section). This step results in the final Ad26.ZEBOV active substance (AS). The AS is blast frozen and then stored.

As regards the control strategy, the manufacturing process is controlled using process parameters and in-process controls. Critical process parameters (CPPs) are described for all steps with proven acceptance ranges (PARs). In-process controls (IPCs) are described, the acceptance criteria or predefined instructions for each IPC are detailed. The different holding times in the downstream processes are well specified.

Control of materials

Cell banks

The PER.C6 cell line is used for virus production. The PER.C6 cell line was derived from human embryonal retina (HER) cells, which were rendered immortal by transfection with the linearised pIG.E1A.E1B plasmid. A three-tiered cell banking system is used. The tiers consist of the suspension master cell bank (MCB(susp)), suspension working cell banks (WCB(susp)) and suspension large volume high density (LVHD(susp)) cell banks. Information on the MCB and WCB adherence cell banks and MCB, WCB and large volume high density cell banks (LVHD) suspension cell banks is in line with ICH Q5D and ICH Q5A (R1). Cell banks are stored frozen.

Sufficient information has been provided on the history and establishment of the cell line and cell bank characterisation, including sequencing of the integrated plasmid (pIG.E1A.E1B) containing the E1 genes. Purity and identity, genetic stability, origin of the cell line, evaluation of prion genes and prion proteins and tumorigenicity and oncogenicity have been investigated.

Testing of cell banks for viral and non-viral adventitious agents and screening for retroviruses has been sufficiently described and is acceptable. The tumorgenicity and oncogenicity studies did not raise any significant safety concerns. The generation of the replication incompetent Ad26.ZEBOV virus is detailed. None of the mutations associated with an increased predisposition to prion disease were detected. The genetic cell bank stability of WCB and LVHD cell bank was demonstrated. The investigated end of production cells (EOPC) were representative of the full-scale commercial process.

Information on storage and stability testing of the WCB and the LVHD suspension cell bank is available. The provided protocols for the establishment of a new WCB and LVHD suspension cell bank are considered sufficient.

Virus seed

The pre-master virus seed (preMVS) was generated by transfecting PER.C6 cells from adherent working cell bank B127-006 with the pAdApt26 plasmid and a cosmid containing the remaining sections of the Ad26 genome. The cloning strategy for the generation of the pAdApt26 plasmid containing the full-length ZEBOV antigen encoding DNA and for the generation of the cosmid with the restriction enzymes used is well described. The origin and the synthesis of the gene encoding for the Ebola Zaire glycoprotein are sufficiently detailed.

A two-tiered viral seed bank system is used with a master viral seed bank (which is expected to last for the life of the product) and working viral seed banks (primary and backup WVS).

The preparation of the MVS and the WVS, their characterisation (including appropriate tests for identity, safety and viral particle quantity), storage and shipment were well described. The genetic stability of the MVS and the WVS, after undergoing several passages beyond the level at which the final vaccine will be produced, was demonstrated. Furthermore, testing for absence of replication competent adenoviruses is performed at AS release. An acceptance criterion for the quantity by virus particles determination will be defined in the testing plan of future WVS and infectivity will be added as a release test. The protocol for the establishment of future working viral seed banks and the associated testing plan will be updated accordingly. In view of the frozen storage, stability monitoring was performed for MVS and the primary WVS. A stability testing plan for future WVS with associated acceptance criteria will be provided post approval (see recommendation 2). A stability indicating viral particle quantitative PCR (VP-qPCR) method should also be validated and data generated in order to define suitable acceptance criteria for virus particles in release and stability of any future viral seeds (master virus seed and working virus seed) (see recommendation 5).

Raw materials

A list of raw materials used in the entire AS manufacturing process, is provided. Raw materials are tested according to European pharmacopoeia (where available), or according to in-house monographs. Specifications for non-compendial raw materials are detailed.

None of the raw materials used in the manufacture of the MVS, WVS, AS or FP were identified as being of animal or human origin or as involving animal-derived products in their production process. Some raw materials of animal origin were used for the generation of pre-MVS. TSE and viral safety risk with respect to these materials has been evaluated. For the commercial PER.C6 cell banks, several materials of animal origin are used for which TSE and viral safety risk has been assessed (see adventitious virus section).

The overview of the culture and cryopreservation media used for production of the cell-banks is sufficiently informative. The components of the less common culture media used are indicated. The composition of the PERMEXCIS production media used for manufacturing LVHD and during the upstream process, are described in detail. The information concerning PERMEXCIS media is complemented with the storage conditions (temperature and duration of storage after preparation and duration of hold in the bioreactor). The PERMEXIS medium is controlled by the supplier.

Overviews of the compendial raw materials and non-compendial buffers used for downstream production are provided. Identity tests of non-compendial components are briefly described.

The formulation buffer (used in AS manufacturing stage 8 and stage 9) is purchased ready for use from an approved supplier and consists of compendial components only. The controls performed by the applicant are included in the CTD S.2.3. This formulation buffer will constitute approximately half of the FP formulation.

A general description regarding preparation, compounding and sterilisation methods applied for preparation of the buffers and media is provided. Following medium or buffer preparation, the liquid is passed through a 0.2-µm filter. Each 0.2-µm filter is tested for integrity after use. Overall, the information is sufficient for the manufacturing of the active substance.

The filters and bags are specified and the manufacturing steps where they are used during AS manufacturing are indicated. The nature of the filters and AEX membrane characteristics has been specified. Overall the information regarding media, buffers, filters and storage bags is satisfactory.

Control of critical steps and intermediates

The control program was established based on the control of AS critical quality attributes (CQAs) at critical steps and intermediates to ensure product quality and consistency during the AS manufacturing process. These include IPC tests and release tests. IPCs with acceptance criteria were defined for the control of contaminants (adventitious agents, mycoplasma, mycobacteria, bacterial and fungal contamination during the upstream process and bioburden during the purification steps). IPCs with predefined instructions were also defined during the upstream process and during the purification steps. The complete list of IPCs and their acceptance and/or action limits has been provided. The action to be taken in case of IPC with a "predefined instruction" is described in sufficient detail.

Critical process parameters and the associated ranges for the manufacturing process are described.

The CPP, critical material attributes (CMA) and their PAR per stage are included in 3.2.S.2.2 of the dossier. Normal Operating Ranges (NORs) are not separately defined for each parameter, but in general the observed ranges (presented in 3.2.S.2.6 of the dossier) are considered as NORs. Analytical procedures for the IPC, process monitoring and release testing together with their validation are

provided. Sufficiently detailed descriptions of the analytical procedures for IPC testing and concise information on their validation are provided.

Process validation

Process validation has a lifecycle, starting with process development followed by process verification/process performance qualification (PV/PPQ) runs and then continues in the form of ongoing process verification throughout routine production. To expedite the MAA filing, the applicant's approach has been to demonstrate process consistency on a large number of active substances batches whilst PV/PPQ was on-going at the time of MAA filing. Full PV/PPQ data and study reports were not available at start of the procedure and therefore, as agreed, interim data were accepted during the procedure in line with the proposed PACMP.

Process validation /PPQ is conducted in three stages: process characterisation and evaluation, PV and continued process verification for commercial batches.

Prior to PV, the applicant acquired extensive manufacturing experience by manufacture of a large number of AS batches at commercial scale to support the Ebola development program as well as from additional large-scale process development runs. Seven process variants have been described. These data are considered important because PV studies were still ongoing at the time of submission of this MAA.

The release data generated from historical batches were generally found to be consistent. IPC with acceptance criteria met their specifications for all historical batches. As regards CPP and CMA, all data points are within the PARs or any deviations outside the PARs had no impact on the process performance and the AS met the release specifications. Several assays were conducted as part of AS characterisation of the historical lots (protein composition, adenovirus protein degradation products, biophysical characterisation and product-related impurities, characterisation of the virus DNA, characterisation of biological activity). All these assays demonstrate batch-to-batch consistency.

The maximum hold times for intermediates have been supported by data. Shipping qualification studies were conducted for the transport of AS.

It is agreed that based on the process evaluation, the AS manufacturing process is considered suitable for reproducibly manufacturing Ad26.ZEBOV AS that meets its CQAs. Data show that the AS manufacturing process is well controlled and that it results in a consistent AS of suitable quality to support approval, even with submission of confirmatory AS PV data post-authorisation (see also manufacturing process development section). The interim data of the PV/PPQ batches (AS release data, IPC results, manufacturing control and characterisation results for three PV/PPQ batches) are consistent with the results obtained for historical batches and support that the commercial manufacturing process is well controlled and results in a consistent AS.

Manufacturing process development

Extensive information has been provided about the manufacturing process development and the information justifies the proposed commercial AS production process and the overall control strategy. A control strategy has been developed to verify that the process performs as intended and that active substance and intermediates comply with pre-defined acceptance criteria.

Control of AS CQA is based on the integrated control of the following elements: parametric controls (control of manufacturing (C)PPs); material controls (control of attributes for materials including raw materials, culture media, buffers, consumables, and product storage containers); IPC tests; release tests; stability tests; process and product characterisation (deeper scientific understanding of process and product facilitated special testing and product characterisation studies), process validation; procedural controls (SOPs, batch records, validated computer systems); and process monitoring tests

performed at selected points in the AS manufacturing process. As such, the design of the overall control strategy meets current standards for the control of biological active substance production.

CQAs have been identified based on criticality assessment and ranked according to the severity of their impact to patient safety and finished product efficacy, and the degree of knowledge uncertainty. The critical process parameters (CPP) have also been identified. During the criticality assessment, each PP is evaluated for its potential effect on each CQA.

Reduced scale models were used to support the PARs. Upon request during the MA procedure, examples have been provided and were considered satisfactory.

Information about the quality management system is provided and includes information about the change management system. It describes the handling of future changes to the MA (including changes to the control strategy). This information has not been scrutinised in detail as it is considered to be covered by the ICH Q12 Guideline and therefore elements which are not currently in place in the EU legislative framework are not considered approved in this MA. According to the dossier, changes will be assessed and managed through the internal change control system, and (if applicable, based on the assessment) reported to regulatory authorities in accordance with regional regulations and guidance. It is expected that changes to the control strategy as registered in the approved MA will be filed according to current EU variation regulations.

All AS batches were produced at the commercial manufacturing scale. In total, seven versions of the AS process were applied during development (DS1-DS7).

In 2014, a research batch was manufactured in the development labs at Janssen Leiden (DS1, non GMP batch) and used for non-clinical toxicity studies and phase 1 clinical studies. The process was subsequently transferred to a GMP manufacturing facility (DS2-DS5), then to a another GMP manufacturing facility (DS7) and finally to the commercial manufacturing facility at Janssen Vaccines, Branch of Cilag GmbH International, Bern, Switzerland (DS7). In total 22 AS batches were released from the three GMP facilities. Process DS2 was used for phase 1 clinical trials, process DS3 was used for phase 2 clinical trials and processes DS3 to DS6 were used for phase 3 clinical trials. Process DS7 was never used in a clinical trial. The major process changes were: the change from WCB to LVHD and change from MVS to WVS for initiation of the manufacturing process. The manufacturing site changed from DS5 to DS6 and from DS6 to DS7. No scale-up took place from process DS2 to DS7. All batches were manufactured at the commercial manufacturing scale. The intended commercial process is essentially identical to DS7 and will be manufactured in the same facility.

To support changes between the clinical campaigns and to support the commercial transfer, three sets of comparability exercises were performed according to ICH Q5E i.e., by analytical testing with a focus on release results, characterisation tests, and in-process tests, e.g., process impurities. Although stability data was not specifically considered to conclude on analytical comparability in the three studies mentioned above, stability data for representative batches of each AS and FP process were obtained. Study 1 focused on pre-clinical and phase 1 AS and FP comparability. Comparability study 2 was designed to assess the impact of a series of consecutive changes introduced in the 2015 emergency supply campaign. Comparability study 3 was designed to assess the impact of the AS process transfer to the commercial facility at the Bern site. All batches met release criteria.

Process verification occurs in the commercial facility in Bern. The intended commercial process is essentially identical to process DS7 and labelled as such. The changes made after the most recent AS batch (DS7) reported in the initial submission and the commercial manufacturing process are considered minor and mainly pertain to tightening of the maximum operating ranges (MORs) to ensure manufacturing within PAR. MORs/PARs are defined for the commercial process if not previously defined (indicated as ‘not applicable’) for DS7. Target values are not changed. In addition IPCs for bioburden

are implemented at stage 6 (sedimentation and clarification), stage 7 (AEX chromatography) and stage 8 (diafiltration). For stage 1-3 the IPCs with their pre-defined instructions are listed. These IPCs pertain to control of overall population doubling time and osmolality, viable cell density, viability, and pH.

These changes described above are further supported by means of a PACMP in the current MAA and data will be submitted via a post-authorisation variation (see also process validation section).

Characterisation

Characterisation studies were conducted using several AS batches, FPP batches, lot of research material used for proof-of-concept studies and the reference material, investigating capsid composition, particle heterogeneity, virus DNA, biological activity and structure-function relationships.

- Capsid composition: Identified capsid and core proteins of Ad26.ZEBOV are: II (hexon), III (penton), IIIa, IVa2, V, 52.55K truncated, VI protease cleaved, VII, VIII protease cleaved and IX.

- Particle heterogeneity: All batches (AS and FP) showed an identical size distribution, containing mainly complete, monomeric adenovirus particles and a small fraction of incomplete/empty particles of around 12%.

- Virus DNA: The complete genome of MVS and the first AS produced with each WVS batch was sequenced by Sanger sequencing. The sequences showed a 100% sequence identity compared to the reference sequence. The genetic stability of the virus DNA is assessed in the dossier section 3.2.S.2.3. The presence of the correct transgene and flanking region is confirmed. The genetic stability of the transgene region was characterised for each Ad26.ZEBOV AS batch.

- Biological activity: The infectivity by the quantitative potency assay (QPA) met the acceptance criteria at release for AS and FP. All characterised AS and FP batches show consistent relative transgene expression levels. In addition, transgene expression, as determined by transgene expression (TGE) ELISA, and infectivity as determined by QPA, show very good correlation in AS and FP batches. All characterised FP batches induce expression of the ZEBOV transgene at the cell surface in a conformation that is expected to elicit a potentially protective immune response.

It was shown across all manufacturing stages that AS consistently has the expected capsid composition, the expected molecular size, the expected virus DNA sequence, the ability to infect, the ability to express the transgene and the ability to express the transgene in the right conformation.

From the AS manufacturing process, 77 process-related impurities were identified (from cell culture media components and additives, PER.C6 host cell constituents and downstream buffer components).

A criticality assessment was performed for each process-related impurity and two process-related impurities were identified as critical: HCP and host cell DNA. These impurities are controlled in the AS process through the integrated control strategy.

The other process-related impurities were classified as non-critical since a margin of safety (direct comparison of the safe level of an impurity per single vaccine dose to the theoretical exposure level) ≥ 3log10 and a low or medium uncertainty were found for those. Spiking studies were performed under conditions that are relevant for the performance of the commercial process for specified reagents used during manufacturing. The results of these spiking studies have been submitted upon request and are found satisfactory.

Potential product-related impurities include empty or incomplete adenovirus particles adenovirus aggregates and adenovirus proteins free in solution and have been investigated. The level of incomplete/empty particles present in AS batches is consistent. The particle size distribution is sufficiently consistent between the AS batches and with FP batches.

Temperature induced and other degradation pathways are appropriately investigated and discussed. All identified impurities have been present in the product used in clinical trials. There is sufficient reassurance that similar levels are expected to be present in the commercial process product. So far, interim data of three PV/PPQ lots, submitted with the response to CHMP Day 90 LoQ, are consistent with results obtained for historical batches. Full confirmatory data from the PV/PPQ lots are expected post-authorisation.

Specification

The specification of Ad26.ZEBOV AS consists of the following tests: identity by ID-PCR and virus protein fingerprinting by reversed phase high performance liquid chromatography (RP-HPLC); impurities (HCP by ELISA and host cell DNA by qPCR); potency (transgene expression by Western Blot, infectious units by QPA, ratio virus particles/infectious units); quantity (virus particles by VP-qPCR), safety (bacterial and fungal, endotoxins, replication competent adenovirus) and general tests (pH and polysorbate 80 concentration).

Upon request during the procedure, the applicant has included a release test for aggregates based on dynamic light scattering (DLS), and a temporary action limit has been set. The applicant has committed to set acceptance criteria in line with Ph Eur, once the DLS method is validated (see recommendation 10). The acceptance criteria for HCP and host cell DNA have been tightened in line with manufacturing experience.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

The procedures for HCP, host cell DNA as well as replication competent adenovirus were shown to be suitable for use in release testing of DS. The verification of the compendial tests for "bacterial and fungal" and endotoxin were satisfactory.

The specifications include tests for both viral particle (VP) concentration and infectious units (Inf.U) to measure potency.

The quantitative PCR (qPCR) based potency assay (QPA) utilises PCR to measure newly synthesised viral DNA. The infectivity of test articles is then determined using a calibration curve prepared using a suitable reference material. The ratio of VP to infectious units Inf.U is a calculation and validation is not required. Points raised for clarification on validation of assays have been addressed sufficiently for MA. However, the QPA, HCP ELISA test and DNA qPCR method will be revalidated in due time taking into account some points raised during the procedure (see recommendations 3, 6, 7). The applicant has also committed to validate the stability indicating virus particle quantitative polymerase chain reaction ((SI)-VP-qPCR) method and intends to then introduce the stability SI VP-qPCR method for both release and stability testing of AS and FP (see recommendations 4, 9).

Batch analysis

Twenty-two AS batches (representative for DS2-DS7, manufactured at the commercial manufacturing scale) were tested for the specifications proposed for commercial manufacturing and the reported levels of infectious units, viral particles ratio VP/ Inf.U, pH and polysorbate 80 concentration are consistent. Host cell protein, host cell DNA and bacterial endotoxin and replication competent adenovirus are also appropriately controlled.

Reference materials

See finished product reference materials section.

Container closure system

Ad26.ZEBOV AS is stored in single-use flexible bags, the contact layer of which is in compliance with the Ph. Eur. requirements. The container also complies with the European requirements on leachables and extractables outlined in CPMP/QWP/4359/03.

Stability

A shelf life of active substance (AS) when the AS is stored frozen was proposed by the applicant.

Long-term (-60±10°C) and accelerated (5±3°C) stability studies are ongoing for 8 AS batches (1 from DS2 batch, 1DS3 batch, 1 DS4 batch, 2 DS5 batches, 2 DS6 batches and 1 DS7 batch). These batches are manufactured at the commercial scale and the AS stability studies were conducted in a primary packaging representative of the actual packaging of commercial batches. Up to 36 months data are available for 5 batches at long-term conditions and for 24 months for accelerated storage conditions (24 months data available for the DS7 batch at long-term and accelerated conditions). In general, the stability studies have been performed in line with relevant ICH guidelines (ICH Q5C) as regards stability studies at recommended and accelerated conditions and time points for analyses of quality attributes.

Appropriate stability-indicating tests are used to monitor AS stability. All batches are tested for pH, Infectious Units, viral particle ratio VP/ Inf.U and transgene expression at all timepoints. Overall the current data support the proposed shelf life of active substance (when stored frozen at the recommended storage condition). The company has committed to test for VP at all time points of the stability protocol. However, in order to confirm that VP is stable throughout long term storage, the company has committed to develop a stability indicating VP-qPCR (see recommendation 9).

The applicant commits to continuing the ongoing stability studies until completion. Any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA. Although sufficient data are provided to support the proposed shelf life, the applicant should provide confirmatory stability results from the PPQ batches, post authorisation. Six months stability data at -60°C and 5°C for three PV/PPQ batches were submitted on request during the procedure.

An appropriate shelf-life of the AS and storage conditions were agreed.

Comparability

The FP manufacturing process was developed in parallel with the AS manufacturing process. AS and FP process changes were introduced simultaneously and therefore FP comparability is also discussed in this section. Three comparability studies have been conducted.

In comparability study 1, the materials used for pre-clinical toxicity study and Phase 1 clinical studies were compared. The presented pre-clinical DP1 batch was prepared from the DS1 batch, while the DP2 and DP3 batches (associated with phase 1 clinical studies) were prepared from one DS2 batch.

Comparability study 2 was designed to assess the impact of a series of consecutive changes introduced in the 2015 emergency supply campaign, during which 17 AS batches, using 4 different AS processes (DS3 to DS6), and 17 FP batches, using 2 different FP processes (DP4 and DP5) were manufactured. Of each process, at least two representative batches were selected. Changes included: site changes for AS and FP manufacturing; the introduction of a working virus seed (WVS); other changes in the

upstream process; and changes to reduce the downstream process time from 11 days to 5 days. In addition, a new, larger primary packaging for the AS and a blast freezing step were introduced. For FP, the manufacturing scale was increased and in a next FP process version, adaptations to accommodate the new AS primary packaging were introduced. The batches included are representative of clinical batches.

Comparability study 3 was designed to assess the impact of the AS process transfer to the commercial facility at the Bern site. At the same time, several process improvements were implemented for DS7.

The comparability studies showed that the release test results met the acceptance criteria of the corresponding product specifications applicable for the respective AS/FP production stage. Also in additional characterisation studies, no significant differences in quality attributes have been observed between any of the production stages. Overall it can be concluded that the introduced manufacturing differences are not expected to have adverse impact on safety, efficacy and immunogenicity.

As such, considering that the commercial AS and FP process is identical to the late stage process, it can be inferred that the product produced by the commercial process is comparable to the product that is used in most of the non-clinical toxicity study and clinical studies. Overall sufficient information regarding the FP and underlying AS batches used in NHP efficacy/ immunogenicity studies is provided. Results of release and characterisation testing of all four batches used in non-human primate efficacy/immunogenicity studies are provided and show that those batches are, in essence, comparable to AS/FP used in clinical trials.

2.2.3 Finished Medicinal Product

Description of the product and pharmaceutical development

The Ad26.ZEBOV finished product (FP) is supplied as a sterile liquid suspension for injection. Each dose contains not less than 8.75 log10 Infectious Units (Inf.U) and not less than 2.5 x 10^{10} VP. The container closure system used for the finished product (FP) is a 2R Type I glass vial closed with a fluoropolymer coated 13 mm chlorobutyl stopper and a 13 mm aluminium seal with red flip-off cap. The composition of the finished product is identical as the composition of the active substance, except for the concentration of viral particles, which is lower. The FP is manufactured with an excess fill volume to ensure an extractable volume of at least 0.5 mL/vial. The Ad26.ZEBOV finished product (FP) target formulation titre is above the lower limit of the release specification and has been adopted to take into account, any potential process loss during formulation and filling and the variability of the analytical methods used to analyse active substance and the finished product. This adopted formulation target titre is aimed to meet the minimal release titre.

The composition of Ad26.ZEBOV is presented in Table 1 below. Sodium hydroxide is used as a pH adjuster in the formulation buffer.

All excipients are tested according to compendial methods and conform to Ph. Eur. Requirements. No excipients of human or animal origin and no novel excipients are used. The function of each excipient is detailed.

Table 1 Complete composition of Ad26.ZEBOV

Ingredient	**Amount per Dose (0.5 mL)**	**Function**
Ad26.ZEBOV	NLT 2.5 × 10^{10} VP[c]	Active
	NLT 8.75 log10 Inf.U	
Sodium chloride		Tonicity agent

Sucrose		Stabiliser and tonicity agent
Polysorbate-80		Stabiliser
Ethylenediaminetetra acetic acid (EDTA)[a]		Stabiliser
L-Histidine monohydrochloride monohydrate		Buffering agent
Ethanol		Stabiliser
Sodium hydroxide[b]		pH adjuster
Water for injections		Solvent

[a]disodium dihydrate salt EDTA

[b]Sodium hydroxide used as pH adjuster in the formulation buffer

[c] minimum dose or concentration

Excipients comply with the European pharmacopoeia

Inf.U= Infectious Units

NLT = not less than

q.s. = sufficient quantity

VP = Virus Particle

The quality target product profile (QTPP) of the FP is presented, as well as the quality attributes based on it. For each quality attribute, a criticality score was calculated based on a criticality assessment. The non-AS-related are sterility, endotoxin, mycoplasma, mycobacteria, content of residual HCP, content of residual DNA, adventitious virus, appearance (colour of solution, turbidity, particulate matter), visible particles, extractable volume, osmolality, pH; sucrose, NaCl, L-histidine monohydrochloride monohydrate, EDTA, ethanol and polysorbate 80 concentrations. The AS-related are genome sequence, identity of adenovirus proteins, transgene identity, transgene expression level, infectivity, vector particle concentration, viral protein degradation products, presence and content of virus particle aggregates and replication competent adenovirus.

The suitability of the selected formulation was demonstrated in formulation confirmation and long-term (AS and FP) stability studies. In the formulation confirmation studies, the effect of thermal stress, agitation stress, temperature cycling (including freeze/thaw) and photostability have been investigated. The formulation is sufficiently robust to undergo multiple cycles of freeze/thaw and to face incidental temperature excursions to elevated temperatures (up to 40°C), even after prolonged frozen or refrigerated storage. Photostability studies show that exposure of Ad26.ZEBOV FP to light stress according to ICH Q1B Option 1 led to minor potency loss within the acceptance criteria. Light-induced degradation occurred mostly in response to UV light exposure and at higher temperature (40°C), especially in unlabelled vials. However, no light protective measures are required during manufacturing. To reduce the risk of the impact of light during storage and shipment, Ad26.ZEBOV FP is to be kept in its secondary packaging until use. Acceptable ranges were defined for the different excipients by a robustness design of experiment (DoE) study.

The data gathered from the manufacturing of FP development and clinical batches was used to define the commercial process, the control strategy and the process performance qualification plan. The future commercial process is identical to the manufacturing process that was used, except for some changes to proven acceptable ranges (PARs).

GMP batches were produced at commercial scale for possible clinical studies, readiness in case of outbreaks and potential stockpiling. During the pharmaceutical development, five process variants were used: DP1 (preclinical process, manufactured at Janssen Vaccines Leiden with DS1 process variant), DP2 (small scale, manufactured at IDT Rockville with DS2 process variant, used in clinical

phase 1), DP3 (small scale, manufactured at Vibalogics, used in clinical phase 1 with DS2 process variant), DP4 and DP5 (commercial scale, manufactured at IDT Dessau DP4 was used in clinical phases 2 and 3 with DS3 process variant and DP5 was used in clinical phase 3 with either DS5 or DS6 process variants). Some process adjustments were made during the pharmaceutical development: change of DS storage container from DP4 to DP5 and therefore change of thawing temperature (from 2-8°C to RT), change of target FP titre from DP3 to DP4, definition of stirring parameters (time and speed) for the final formulated bulk compounding, change of filling volume DP1 to DP3 to DP4 and DP5.

The comparability between those process variants has been assessed. Two small scale, pre-change FP batches (DP2 and DP3) were compared to the two post-change full scale FP batches (DP4 and DP5). The results generated from this study indicated that the biophysical and biological properties of the FP batches were comparable and not impacted by manufacturing site or scale.

Seventeen batches were manufactured at commercial scale of which three were according to DP4 and fourteen according to DP5. The intended commercial process can be concluded to be essentially identical to process DP5.

The analytical development history has been described in sufficient detail. For the quantitative assays QPA and VP-QPCR, which rely on a calibration curve, it has been assured that no drift in assigned quantitative values occurs through repetitive bridging activities of new calibration curve material to the former one.

A criticality assessment was performed to define critical process parameters. PARs are defined based on process characterisation studies. Several hold times were assessed: prior to pooling (optional hold time after thawing), cumulative hold time in formulation (from start of AS addition to the end of pre-filtration), time before the sterile filtration, time before filling, cumulative hold time in storage vessel at 2-8°C from the start of pre-filtration to the end of sterile filtration, cumulative time of FP vials at controlled room temperature and at 2-8°C.

The container closure system used for FP is a 2R Type I glass vial closed with a fluoropolymer film coated stopper and an aluminium seal with flip off cap. To demonstrate compatibility of the FP with the designated vial and stopper, stability testing of the FP was performed. The extractable and leachables studies did not give rise to concerns. Data indicate that the container closure system is suitable for use and comply with Ph. Eur requirements. Compatibility of the FP with the polycarbonate syringe/stainless steel needle combination and with the polypropylene syringe/needle combination has been sufficiently demonstrated. Compatibility has been verified.

Manufacture of the product and process controls

The FP is manufactured at IDT Biologika GmbH (Dessau-Rosslau, Germany). All sites (including QC sites) hold appropriate GMP authorisation. Batch certification is performed at Janssen Biologics BV (Leiden, the Netherlands). The batch size is defined by the volume of active substance (AS) available with minimum and maximum batch sizes defined respectively.

The manufacturing process consists of thawing of bags of AS, pooling, dilution with formulation buffer and homogenisation, followed by a pre-filtration step. Next, homogenisation is performed followed by sterile filtration prior to filling. Target values and proven acceptable ranges (PARs) are provided for each critical process parameter. IPCs consist of bioburden (membrane filtration conform Ph. Eur. 2.6.12, Microbiological examination of nonsterile products (microbial enumeration tests), filter integrity (by bubble point test) and fill weight (100% Control of fill weight takes place for each run throughout the filling process). The test for bioburden is verified. The acceptance criteria for bioburden are acceptable. The description of the manufacturing process is acceptable**.**

Process verification/process performance qualification (PV/PPQ) studies are ongoing. The applicant intends to submit the results and conclusions of those studies in a post-approval variation. The post approval change management protocol is provided in section 3.2.R of the dossier and is acceptable. Interim data for the PV/PPQ batches for Ad26.ZEBOV FP have been provided and compared with historical data.

Process validation of Ad26.ZEBOV FP to support the MAA is based on: consistency of finished product (evaluation of historical data of 17 batches from DP1 to DP5 against the predefined acceptance criteria as set for PV/PPQ); media fill tests and sterilisation and shipping qualification.

Assurance that the intended commercial manufacturing process is capable of producing a consistent FP of high enough quality is based on the data gathered for the historical batches. In 2014 a manually filled development batch for toxicity testing was produced (manufacturing process DP1), followed by manufacturing of four small scale GMP batches intended for phase 1 clinical trials, filled at two different GMP facilities (manufacturing process DP2 and DP3). Thereafter, the process was scaled up and 17 batches were manufactured with the intended commercial process at the commercial scale (three with manufacturing process DP4 and 14 with DP5). The consistency assessment is focused mainly on the seventeen commercial scale batches manufactured at IDT Dessau, Germany. Results for release parameters, IPC and CPP are provided as well as process characterisation data and stability data. Data show that the FP manufacturing process is well controlled and that the manufacturing process results in a consistent FP of constant quality. Two series of media fill tests are presented validating the filling time. Validation data for depyrogenation/sterilisation of the vials and sterilisation of the stoppers and the caps were provided and are satisfactory. Filter validation data have been submitted. Qualification of shipping included a shipping route verification study by means of a mock shipment.

Comparability studies have been described in the AS section of this report. Sufficient evidence for process and product consistency is presented, based on the applicant's manufacturing experience. The applicant has indeed demonstrated comparability between all batches. Furthermore, the applicant has provided data for the process parameters, in process controls, QC release test results and product characterisation data. The data demonstrate that the manufacturing process is in control and that it results in a consistent product. The applicant indicated that the clinical batches are representative for the future commercial lots. Differences were introduced in some PARs but these are small and are not expected to affect quality attributes of the FP. Furthermore, the effect of extended holding times at controlled room temperature (CRT) and 2-8°C will be verified through the PPQ batches, which is acceptable. Finally, the interim data of the PV/PPQ batches (FP release data, IPC results and characterisation results for three PV/PPQ batches) confirm that the commercial manufacturing process is well controlled and results in a consistent finished product of good quality. This will be considered upon evaluation of the full study results via a post-approval variation.

Product specification

Release and stability specifications as well as description of the analytical procedures were provided. The parameters tested are appearance (degree of coloration, turbidity, and visible particles), identity by identity (ID)-PCR and virus protein fingerprinting by RP-HPLC, potency with transgene expression by Western Blot and infectious units by QPA, quantity by VP-qCR, safety tests (sterility, bacterial endotoxins, container closure integrity by dye ingress) and general tests (pH, osmolality, extractable volume, and polysorbate concentration).

The shelf life specification for Infectious units has been raised as a result of the multidisciplinary MO. For Ad26.ZEBOV, for immunogenicity and clinical efficacy, the shelf life limit for potency, as expressed in Infectious Units (Inf.U), is relevant, whereas for safety, the dosing, as expressed in viral particles

(VP) is important. The applicant has therefore increased the shelf life limit for Inf.U. Furthermore, also the shelf life of the Ad26.ZEBOV vaccine has been tightened in response to quality MO3 raised for MVA-BN-Filo, which will also reduce overall potential degradation. From a quality point of view, it is agreed that the shelf life limit for Inf.U can be increased without affecting the release limit of the finished product.

Since for immunogenicity and clinical efficacy the potency as expressed in Infectious units is relevant, the potency of the vaccine should be expressed on the labelling in line with other viral vaccines. Hence, it was proposed to change labelling to "not less than 8.75 $\log_{10}$ Inf.U/dose", instead of "not less than 2.5×10^{10} VP/dose". In the responses to the D129 LoI, the applicant agreed to express the labelling in Inf.U (see 'discussion' section).

Identity, quantity, bacterial endotoxins, osmolality, extractable volume and polysorbate 80 concentration are not tested during stability studies. A test for replication-competent adenovirus (RCA) is performed on the AS only. Testing for aggregates is currently a characterisation test.

The applicant has already committed to implement aggregate testing as a release and shelf life test. Results will be reported as average hydrodynamic radius, as established by dynamic light scattering (DLS). Furthermore, the polydispersity index will be reported (see recommendation 10). The validation of the DLS method should be submitted by Q3, 2021. In the meantime, an action limit of average hydrodynamic radius will be used.

The ratio infectious units/viral particles is established at AS level and a similar specification has also been established for FP release.

Stability specifications are in place for infectious units, viral particles, pH, sterility, container closure integrity (CCI), transgene expression and appearance. The applicant commits to implementing a stability specification for VP with a stability indicating VP-qPCR. As the method has not been validated yet, transitional specifications using the VP-qPCR will be used. The applicant will consider implementing the stability indicating VP-qPCR at release also (see specific recommendations- 4 for FP,9 for AS).

The impurity profile and degradation pathways are comparable for active substance and finished product. No new impurity arises in the FP. In conclusion, specifications have been adequately justified to support MA.

It is recommended that the applicant provides a risk assessment and control of elemental impurities following the principles of ICH guideline Q3D (R1) on elemental impurities (see recommendation 8).

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines Most tests (including for virus particles and infectious units) are the same as for AS.

Batch analysis

Batch analysis data from 17 GMP batches of Ad26.ZEBOV finished product manufactured in 2015, 2016 and 2017 are presented. These batches have been manufactured at the intended manufacturing site at commercial scale (three with manufacturing process DP4 and 14 with DP5). Results are sufficiently consistent.

Reference materials

Reference material is relevant for the Virus Protein Fingerprinting by RP-HPLC, the QPA and VP-qPCR. Three batches of development reference material (DRM) were used so far. The primary reference material (PRM) and the first working reference material (WRM) will be prepared from 2 different FP

batches manufactured during PV/PPQ using the commercial manufacturing process. PRM will be qualified through extensive testing of their quality attributes in line with finished product specifications and, in comparison with the current DRM batch; WRM will be qualified against the PRM through extensive testing of their quality attributes in line with finished product specifications and additional characterisation testing. Prior to use, the qualification results for any new reference material are evaluated against the previous reference material. In addition, a process has been established to re-qualify the RM on an annual basis to demonstrate stability and monitor any drift or trends in the generated results. This includes evaluation of potency data generated during the re-qualification.

Calibration curve material for QPA and VP-qPCR are also included in the description of Reference Materials. Assurance that no drifting has occurred when establishing the new calibration curves has been provided. The qualification and requalification of the PRM, WRM and future WRMs in relation to the control on drifting has been clarified upon request.

Stability of the product

The proposed shelf life of the Ad26.ZEBOV FP is 4 years between -85 and -55°C (long term storage).

The FP must be stored in the original packaging in order to protect it from light.

The SmPC states:

Transport frozen at -25°C to -15°C. Upon receipt, the product can be stored as indicated below:

Store in a freezer at -85°C to -55°C at the distributor in case of stockpiling. The expiry date for storage at -85°C to -55°C is printed on the vial and outer carton (EXP).

The vaccine can also be stored by the distributor or end user in a freezer at -25°C to -15°C for a single period of up to 20 months. Upon removal from the -85°C to -55°C freezer, the new expiry date must be written by the distributor or end user on the outer carton and the vaccine should be used or discarded at the end of the 20 months. This new expiry date should not exceed the original expiry date. The original expiry date should be made unreadable.

The vaccine can also be stored by the distributor or end user in a refrigerator at 2°C to 8°C for a single period of up to 8 months. Upon moving the product to 2°C to 8°C storage, the discard date must be written by the distributor or end user on the outer carton and the vaccine should be used or discarded at the end of the 8 month period. This discard date should not exceed the original expiry date, or the new expiry date assigned for the -25°C to -15°C storage condition. The original expiry date and/or the new expiry date assigned for the -25°C to -15°C storage condition should be made unreadable.

Once thawed, the vaccine cannot be refrozen.

The vial must be kept in the original package in order to protect from light and to track the expiry or discard date for the different storage conditions.

Stability studies in line with ICH were performed on 11 FP batches manufactured at commercial scale (3 according to DP4 and 8 according to DP5) at the commercial site. Four of these batches were used in clinical studies. The FP batches were stored at -60 ± 10°C, at -20 ± 5°C, at 5 ± 3°C (upright and inverted), and at the accelerated storage condition of 25 ± 2°C. Appropriate stability-indicating tests were used to monitor stability. The batches were tested for appearance, pH, infectious units, VP/ Inf.U ratio (calculated using the VP concentration obtained at release), transgene expression, CCI and sterility. Initially, stability studies supporting the recommended long-term storage condition were performed at a storage temperature of -80 ± 10°C. Later on, the storage condition for the stability study samples was changed to -60 ± 10°C.

So far, data for four batches on the 48 months storage at -60°C and 48 months at -20°C have been provided, 24 months at 5°C (upright) and 12 months at 5°C (inverted) and 25°C. The FP is stable at -60°C and a small decline in infectious units is seen at -20°C and 5°C. The applicant commits to continue the stability studies on 6 FP batches up to 60 months at -60°C, and up to 48 months at -20°C. Stability data for three PV/PPQ batches have been provided for 3 months at -60°C, -20°C, 5°C (upright and inverted) and 25°C.

As response to a MO3 for the MVABEA vaccine on shelf life, also the shelf life for Ad26.ZEBOV has been adapted. The proposed shelf life of the Ad26.ZEBOV FP is 4 years between -85 and -55°C (long term storage). Within this 4 year period the product can be stored for up to 20 months at -25 to -15°C, and the product is then marked with the new expiry date. The product can also be moved to and stored for 8 months at 2 to 8°C for short term storage at local sites, also with a new expiry date being marked on the packaging.

In order to support the revised claims using the tightened specifications, the applicant outlined a statistical analysis using 95% confidence intervals, taking into account a margin for packaging and labelling after initial freezing and storage of vials and the slopes of the storage at -60°C , -20°C and 5°C with the associated standard errors. As such the outlined approach is considered suitable. Details on the statistical model and the calculation demonstrating that the lower one-sided 95 percent confidence limit of the titre at end of shelf life meets the proposed acceptance criterion have been provided.

The applicant commits to continue the stability studies on 3 Ad26.ZEBOV finished product (FP) clinical batches and 3 FP GMP batches up to 60 months at -60 ±10°C and up to 48 months at -20 ±5°C to confirm the stability profiles of these batches in line with the stability monitoring programs for these batches. In accordance with EU GMP guidelines[1], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA. See manufacturing process development for photostability information.

In conclusion, the FP shelf-life is adequately supported by the submitted data. A 4 year shelf life for the vaccine when stored between -85°C and -55°C (long-term storage) and the additional storage conditions described in section 6.4 (Special precautions for storage) of the SmPC are accepted.

Post approval change management protocol(s)

A Post Approval Change Management Protocol (PACMP) is provided to support the post-approval process verification (PV/PPQ) component of the Ad26.ZEBOV FP and to confirm process consistency and capability of the overall control strategy to ensure that the intended commercial manufacturing process is capable of consistently yielding a product that meets its critical quality attributes.

The intention of the PACMP is in this case not primarily to support a foreseen change/variation in terms of marketing authorisation but rather a regulatory tool to submit the outstanding data as foreseen at the time of the MAA. The intended commercial process, for which interim data are presented in the PV/PPQ studies, is almost identical to the process used for manufacture of the AS and FP batches. Small differences have been justified.

The AS and FP PACMP outlines the post-approval data in support of the process verification but also includes information about the additional characterisation studies, monitoring testing and stability studies as well as the commitment to update the PACMP if there are changes that fundamentally impact the basis of the presented PACMP. Finally, the PACMP summarises the information that will be

[1] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal products in the European Union

included in the Type IB variation submission. PACMPs has been updated in line with the outcome of the assessment of the initial MAA.

Adventitious agents

Adventitious agents safety for Ad26.ZEBOV is assured through the design and control of the manufacturing process; i.e. controlled selection and appropriate specifications for raw materials and excipients, specifications, in-process controls and release testing for starting materials, the active substances and finished product, minimise the risk of contamination by adventitious agents. No risks regarding TSE are identified.

As regards non-viral adventitious agents, adequate controls and specifications for starting materials, raw materials and excipients, appropriate specifications, in-process controls, release tests and validation of the relevant steps demonstrate that the AS and FP are prepared under conditions designed to minimise the risk of microbial contamination.

None of the raw materials used in the manufacture of the MVS, WVS, AS or FP was identified as being of animal or human origin or as involving animal-derived products in its production process. Some raw materials of animal origin were used for the generation of pre-MVS. The PER.C6 cell line is of human origin and some raw materials of animal origin were used during the generation of MCB and WCB.

As the product is a non-enveloped virus vector, it is accepted that the manufacturing process does not include any steps that are claimed to be capable to effectively remove adventitious virus. Nevertheless some steps of the production process have clearly a potential in reducing some viruses (as demonstrated by exploratory studies).

TSE, viral and microbial safety has been suitably assured.

GMO

Please see the ERA.

2.2.4 Discussion on chemical, pharmaceutical and biological aspects

The provided information shows that the manufacturing process is well controlled and that the manufacturing process results in a consistent AS and FP of constant quality. In total seven variants of the AS manufacturing process and five variants of the FP manufacturing process have been used during development.

The following issues have been addressed during the procedure:

Comparability and PACMP

Comparability of the different AS and FP process stages throughout development has been demonstrated. Considering that the commercial AS and FP process is identical to the late stage process; it is assured that the product produced by the commercial process is comparable to the product that is used in most of the non-clinical toxicity study and clinical studies. The PV/PPQ studies are ongoing at the time of submission of this MAA and a PACMP is provided for both AS and FP. The PACMP has been updated in line with outcome of the assessment of the MAA. The results and conclusions of these studies will be provided in a post-approval variation. The submitted stability data of the PV/PPQ batches (6 months data for AS and 3 months data for FP) support the stability of the AS and FP.

MVS and WVS

The control of the MVS and WVS have been discussed. The applicant committed that an acceptance criterion for the 'quantity by virus particles determination' will be defined in the testing plan of future WVS and infectivity will be added as an additional release specification (Recommendation-REC 1). A stability testing plan for future WVS with associated acceptance criteria will be provided post approval. (REC 2).

Control strategy

The design of the overall control strategy meets current standards for the control of biological active substance and finished product production. The strategy to determine the "proven acceptable ranges" (PARs) has been sufficiently justified. All questions regarding the control strategy have been solved. It is recommended that the applicant performs a risk assessment for the presence of elemental impurities (as per Ph. Eur. monograph on pharmaceutical preparations (2619)) and controls the levels of elemental impurities using the principles of risk management according to ICH Q3D (REC 8)

Specifications and test validation

The remaining other concerns regarding the release testing and (shelf life) specifications of AS and FP FP have been sufficiently addressed and commitments have been provided where appropriate. These issues pertained to the following:

A test for aggregates by Dynamic Light Scattering (DLS) has been added as release (AS and FP) and shelf life (FP) test. As the test has not been validated yet a temporary action limit for average hydrodynamic radius has been set. The applicant has committed to validate the DLS test post approval and set acceptance criteria in line with Ph. Eur. (REC 10).

A shelf life specification for Viral Particles has been added as requested. The applicant has committed to validating a stability indicating VP-qPCR ((SI)-VP-qPCR) method and intends to use the stability indication VP-qPCR method for both release (AS and FP) and stability testing (FP). (REC 4 and 9).

Furthermore the applicant has provided assurance that the Quantitative Potency assay (QPA) indeed quantitates newly synthesised viral DNA and no additional development or validation is required for MA. It was noted that a number of assays, although overall suitable for their respective purpose, could be further improved. Taking into account comments raised during assessment, the applicant committed to revalidate the QPA, Host Cell Protein (HCP) ELISA test and Host Cell DNA qPCR method in due time. Regarding the QPA test it was recommended to perform an additional validation to extend the range so as to cover the batch data taking into account tightened acceptance criteria for intermediate precision and accuracy (REC 3). Although the impurity levels of the AS detected with the current assays are well within specifications and are not questioned as such, the validated range of the current host cell protein (HCP) ELISA and host Cell DNA qPCR should be extended to cover the full range of the specifications. The applicant is encouraged to optimise these methods to minimise the matrix effect for HCP and the underestimation for the host cell DNA (REC 6 and REC 7).

Shelf life limits for Inf.U and VP

For immunogenicity and clinical efficacy the shelf life limit for potency, as expressed in Infectious Units- Inf.U, should be considered, whereas for safety, the dose, as expressed in viral particles (VP) is important. As a result of Multidisciplinary MO1, the applicant has increased the shelf life limit for Inf.U. Furthermore, also the shelf life of the Ad26.ZEBOV vaccine has been tightened (in response to quality MO3 raised for MVA-BN-Filo-Mvabea). From a quality point of view, it is agreed with the applicant that the shelf life limit for Inf.U can be increased without affecting the release limit of the finished product.

Shelf life:

The statistical model to calculate the FP shelf life is similar for Ad26.ZEBOV and MVA-BN-Filo. Upon request, details were provided for Ad26.ZEBOV regarding the statistical model and the calculation demonstrating that the lower one-sided 95 percent confidence limit of the titre at end of shelf life meets the proposed acceptance criterion. In addition, data justifying the slopes and standard errors used for storage at -60 °C, -20°C and 5 °C have been provided.

Labelling/SmPC:

From the response to multidisciplinary MO1 it was clear that the clinical justification of the specifications and shelf-life is (mainly) based on Infectious Units (Inf.U). Taking into account that labelling of viral vaccine doses are usually expressed as Inf.U, release and shelf life specifications for infectious units have been set for Ad26.ZEBOV and clinical justification of Ad26.ZEBOV is mainly based on Inf.U, it was considered appropriate to include Inf.U in the labelling of Ad26.ZEBOV. Hence, the labelling was requested to state "not less than 8.75 $\log_{10}$ Inf.U/dose", instead of "not less than 2.5×10^{10} VP/dose". However, in their responses, the applicant did not concur with the Rapporteur to amend the labelling "not less than 8.75 $\log_{10}$ Inf.U/dose", instead of "not less than 2.5×10^{10} VP/dose. The applicant wished to express the Ad26.ZEBOV dose in VP instead of Inf.U, to comply with relevant EU guidance, specifically, Section 5.1 of EMA/CHMP/VWP/141697/2009 "*Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines"* which states: "The requirements contained within the Ph. Eur. general chapter on Vaccines for Human Use (Ph. Eur. Section 6.3) should be followed". Further, the company stated that although these vaccines are not gene therapy products as defined by Regulation (EC) No 1394/2007], where the specific vectors addressed in Ph. Eur. general chapter 5.14 on Gene Transfer Medicinal Products for Human Use are used in a viral vectored vaccine, adherence to the guidance provided is recommended. In Ph. Eur. 5.14, it is stated that labelling for Adeno viral vectors should be expressed in vector particle concentration."

In the view of the CHMP, Ph. Eur 5.14 is in principle not applicable, as Zabdeno is a vaccine and according to the legislation, gene transfer products do not include any products for infectious diseases, although relevant principles of the chapter might apply. Furthermore, as Ph. Eur 5.14 is a general chapter it is not legally binding. In their responses, the applicant agreed to express the labelling in Inf.U instead of VP.

All issues are considered solved and from quality point of view the MAA for Zabdeno is acceptable.

2.2.5 Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6 Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

Area	Number	Description	Classification*
Quality	1	It is recommended that an acceptance criterion for the 'quantity by virus particles determination' will be defined	REC

		in the testing plan of future WVS and infectivity will be added as an additional release specification.	
Quality	2	A stability testing plan for future WVS with associated acceptance criteria should be provided post approval.	REC
Quality	3	An additional validation of the Quantitative Potency assay (applied for AS and FP) should be performed to extend the range to cover obtained batch data, with tightened acceptance criteria for intermediate precision and accuracy. The applicant has already indicated that this will be available by July 2021	REC
Quality	4	A stability indicating viral particle quantitative PCR (VP-qPCR) method should be validated and data will be generated in order to define suitable acceptance criteria for virus particles (VP) in release and stability of FP. The proposed accuracy criteria should be tightened as were considered to be too wide. The applicant has already indicated that this will be submitted in Q4 2020	REC
Quality	5	A stability indicating viral particle quantitative PCR (VP-qPCR) method should be validated and data will be generated in order to define suitable acceptance criteria for virus particles (VP) in release and stability of any future viral seeds (master virus seed and working virus seed). The proposed accuracy criteria should be tightened as were considered to be too wide. The validated range will also cover the specifications of the MVS and WVS.	REC
Quality	6	A new validation of the host cell protein (HCP) assay for AS should be performed in a specific range). The applicant is encouraged to further optimise the method in order to minimise the matrix effect. The applicant has already indicated that this will be available in Q3 2021.	REC
Quality	7	It is recommended that a new host cell (HC) DNA qPCR method validation is performed with a specific reduced range. During this new validation, further adjustment will be performed to minimise the underestimation. The applicant has already indicated that this will be available in Q3 2021	REC
Quality	8	It is recommended that the applicant performs a risk assessment for the presence of elemental impurities (as per Ph. Eur. monograph on pharmaceutical preparations (2619)) and controls the levels of elemental impurities using the principles of risk management according to ICH Q3D. The previously notified anticipated date of submission is Q1 2021.	REC
Quality	9	It is recommended that the applicant considers implementing the stability indicating viral particle	REC

		quantitative PCR (SI-VP-qPCR) method as a AS release test (once the test is established for FP).	
Quality	10	It is recommended that the dynamic light scattering (DLS) method used for aggregate analysis of AS and FP is validated such that the action limit for the average hydrodynamic radius is reassessed and replaced with acceptance criteria. In addition, it is recommended that acceptance criteria for the % polydispersity test should be set. The applicant has already indicated that this will be submitted in Q3 2021.	REC

*REC- Recommendation

2.3 Non-clinical aspects

2.3.1 Introduction

2.3.2 Pharmacology

The vaccine consists of two components, the Ad26.ZEBOV and the MVA-BN-Filo component. The Ad26.ZEBOV component is based on adenovirus type 26, containing the transgene of the Zaire Ebola virus Mayinga glycoprotein. The glycoprotein is produced and presented on the cell membrane to the host immune system, but the viral genome is not integrated in the host genome. The regions of the viral genome coding for proteins involved in viral replication and persistence within the host cell, have been removed. The MVA-BN-Filo component is based on Modified Vaccinia Ankara (MVA), a strongly attenuated poxvirus. MVA, containing glycoproteins of EBOV Mayinga, Sudan ebolavirus, Marburg virus and nucleoprotein of the Tai Forest ebolavirus, enters human cells, predominantly antigen-presenting cells, in which the virally-coded genes are expressed. MVA-BN has been shown not to replicate in human cells.

Immunogenicity and efficacy testing was performed in cynomolgus macaques, infected with the Zaire Ebola virus of the Kikwit strain. The data in cynomolgus monkeys were used to infer a protective effect in humans. The monkeys develop hemorrhagic fever as in humans, with shorter time from infection to symptoms (5.4 vs 6.2-9.7 days) and more rapid disease progression (1.4 vs 5.8-14.4 days) than in humans. Ebola virus disease is at least as lethal in cynomolgus monkeys as it is in humans.

First, four proof of concept studies with lethal challenge were conducted, demonstrating the protective efficacy (ie, survival) of vaccine regimens with Ad26.ZEBOV and MVA-BN-Filo using a lethal IM EBOV challenge model. The length of the dosing interval was investigated in these studies. Three additional challenge studies were performed in which the Ad26.ZEBOV, MVA-BN-Filo regimen was tested in the 56-day interval. In these studies, the relationship between EBOV GP-binding antibody levels and survival in NHP was confirmed and refined and also lower doses were tested. A logistic regression model was constructed using data from all vaccine regimens combined (7 NHP studies), or the Ad26.ZEBOV, MVA-BN-Filo regimen in the 56-day interval (4 out of the 7 NHP studies). Using the same validated EBOV GP-binding antibody ELISA (FANG ELISA), human EBOV GP-binding antibody levels are compared to the NHP logistic regression model to infer a vaccine protective effect in humans. Finally, three additional NHP studies were conducted to assess the kinetics of vaccine-elicited immune responses, including persisting immune response (induction of immune memory).

The highest protective efficacy was obtained with a dosing interval of 56 days (100% survival among 10 animals that received the clinical dose). Shorter dosing intervals gave less protection (80% at 42 days and 50-57% at 28 days). Survival was not completely 100% among monkeys (5/6 = 83%) receiving a slightly less than the clinical dose for Ad26.ZEBOV ($4x10^{10}$ instead of $5x10^{10}$ vp) and slightly more for MVA-BN-Filo ($5x10^{8}$ instead of $1x10^{8}$ Inf.U), with a dosing interval of 56 days. In one of the studies, at a dosing interval of 28 days, protection was lower with MVA-BN-Filo as first dose than with Ad26.ZEBOV as first dose (25% vs 75% survival). At a dosing interval of 56 days and in the other study at 28 days, there was no difference in protective efficacy between MVA-BN-Filo and Ad26.ZEBOV as first dose. Among regimens with different dosages (Ad26.ZEBOV dosed down to $2x10^{9}$ vp combined with $1x10^{8}$ Inf.U MVA-BN-Filo) and a 56-day dosing interval, survival of 100% was obtained. Combinations with lower doses of MVA-BN-Filo were not protective (see table below).

Table 2: Overview of protective efficacy of the Ad26.ZEBOV, MVA-BN-Filo regimen in the 56-day interval, using different dosing regimens

Ad26.ZEBOV (vp)	MVA-BN-Filo (Inf U)	Survival/N (%)
$1x10^{11}$	$5x10^{8}$	2/2 (100%)
$5x10^{10}$	$5x10^{8}$	4/4 (100%)
$5x10^{10}$	$1x10^{8}$	10/10 (100%)
$2x10^{10}$	$1x10^{8}$	3/3 (100%)
$5x10^{9}$	$1x10^{8}$	10/10 (100%)
$2x10^{9}$	$1x10^{8}$	2/2 (100%)
$5x10^{9}$	$1x10^{6}$	1/9 (11%)
$5x10^{9}$	$1x10^{5}$	0/8 (0%)
$5x10^{9}$	$1x10^{4}$	1/12 (8%)
$5x10^{8}$	$1x10^{4}$	0/4 (0%)
$5x10^{7}$	$1x10^{4}$	0/4 (0%)

The Ad26.ZEBOV, MVA-BN-Filo regimen in the 56-day interval was tested in studies C29#1, C29#8, TO14#1 and TO14#2. The survival ordered by dose level is shown, dose levels above the dashed line gave full protection from 100 pfu EBOV Kikwit challenge. The heterologous 2-dose vaccine regimen intended for regulatory approval is highlighted in a box.

Among non-survivors receiving lower doses, time to death tended to be dose-related delayed compared to the negative controls. The difference was maximally 2-3 days (compared to time to death of 6-7 days for negative controls).

Different dose levels were tested across the study program and some dosing regimens sometimes induced a higher immunogenicity. Dose selection was a multistep process which begun in 2015. Beyond data in NHP, in clinical study EBL1002, albeit in a limited number of participants (N=15), a higher dose of MVA-BN-Filo ($4.4x10^{8}$ $TCID_{50}$) in the Ad26.ZEBOV, MVA-BN-Filo 14-day interval regimen and a higher dose of both Ad26.ZEBOV (Ad26.ZEBOV $1x10^{11}$ vp) and MVA-BN-Filo ($4.4x10^{8}$ $TCID_{50}$) in the 28-day interval only had a moderate positive impact on the humoral and cellular immune responses compared to the selected dose levels.

Immunogenicity was evaluated by assessing glycoprotein-binding antibodies, glycoprotein neutralizing antibodies, and glycoprotein-reactive T cells. After the second vaccination (according to the clinical regimen), glycoprotein-binding antibody concentrations reached a peak at 3 weeks after the second dose, after which they declined and reached a gradually declining plateau around 196 days after dose 1, which remained up to around 500 days after dose 1. After 500 days, antibody levels were about 10-fold lower than those obtained in the acute phase post-immunization, i.e. 3 weeks post-dose 2. The

neutralizing antibodies showed the same pattern. Ad26.ZEBOV as dose 1 appeared to induce higher antibody concentrations than MVA-BN-Filo as dose 1. T-cell response was higher after an 8-week interval than after a 4-week interval. Both CD4 and CD8 T cell responses were observed. Also the neutralizing antibody response seemed higher after the 6-week interval than after the 4-week interval.

After a booster immunization at day 196, antibody concentrations showed an anamnestic response by 7 days but not by 3 days after the booster. Nevertheless, 4 out of 5 monkeys which were given a third vaccination ($4x10^{10}$ vp Ad26.ZEBOV) one and a half year after the second vaccination and challenged 3 days later, survived. The fifth animal died 3 days after challenge, due to unrelated causes and was excluded from the study. Nine other monkeys which were given the third vaccination one and a half year after the second vaccination and challenged 7 days later, all survived. Due to the fact that the booster dose and the viral challenge were very close in time, it was not possible to determine if only the viral challenge could have induced the reactivation of the memory response. The kinetics of the observed anamnestic response are in line with the observed kinetics of an anamnestic response after a booster dose of Ad26.ZEBOV, though exposure to the virus may have contributed. Six monkeys treated with the clinical regimen and challenged 70 weeks after the second vaccination, did not survive. Antibody concentrations showed that the monkeys succumbed before an effective anamnestic response could be mounted. Although this may not be representative for the situation in humans, because the disease progression is extremely fast in this model in cynomolgus macaques.

In several studies, some NHP were vaccinated, non-surviving, with a non-detectable viral load. In these cases, the viral load results with the qRT-PCR test may have been false negative, because the plaque assay was positive. The Applicant generally excluded viral load values taken at EOP (end of project) time arguing that it was taken by cardiac puncture and not venous blood sample and was considered to reflect remaining viral genome copies in the tissue. Due to the limited time of 28 days post-challenge per protocol, some vaccinated, surviving, animals may have a detectable virus load at the end of the study. Persistence of virus in some vaccinated people cannot thus formally be excluded based on these data. it is known that human Ebola survivors maintain infectious virus in immuno-privileged sites like testes and the eyes.

One monkey that died had relatively high binding antibody levels. There is however an overlap between protective and non-protective levels of immunogenicity. In this particular case, the fact that the monkey received a regimen with a 28-day dosing interval may have played a role, because the highest protective efficacy was obtained with a dosing interval of 56 days.

A sustainable duration of protective efficacy after the booster dose cannot be investigated non-clinically in the current model, because of the rapid disease course in NHP. The animals succumb to infection before they can build up a response from their immunological memory. It has been however shown that the disease course is longer in the human than in the NHP. This could allow time for an anamnestic response to be mounted in the human. The Applicant has outlined several possibilities of developing an animal model with a closer resemblance to human disease course of Ebola, including different administration routes and lower challenge doses. They intend to seek further scientific advice at a later stage, which is welcomed.

Glycoprotein-binding antibodies was chosen as the parameter for the immunobridging. All investigated immune response parameters correlated significantly with survival, but the correlation was stronger for glycoprotein-binding antibodies and neutralizing antibodies. Considering the more robust assay for glycoprotein-binding antibodies compared to neutralizing antibodies, it is endorsed that this is chosen as the parameter for immunobridging. Binding and neutralizing antibody responses were too strongly correlated to be used together in a logistic model (multicollinearity), meaning that binding antibody responses are reflective of neutralizing antibody responses. T cell responses have been shown to have a limited contribution to the discriminatory capacity of the binding antibody levels in a dual-covariate

model. Two logistic regression models were used for the immunobridging to the efficacy in humans. One model used data from all vaccine regimens combined and the other model was based only on data from the Ad26.ZEBOV, MVA-BN-Filo regimen in the 56-day interval (N=66).

No separate safety pharmacology studies were performed since data from the toxicology studies did not suggest that the vaccine regimen may affect physiological functions (e.g. central nervous system, respiratory, cardiovascular, and renal functions) other than those of the immune system.

2.3.3 Pharmacokinetics

Immunogenicity was investigated by measuring EBOV GP binding total IgG antibody levels, neutralizing antibody activity and by measuring the cellular immune response. Glycoprotein-binding antibodies were analysed by ELISA. The analyses of the samples used for the immunobridging were performed with the FANG ELISA assay. This assay was sufficiently validated. Neutralizing antibodies were analysed by the pseudovirion Neutralizing Antibody Assay. T-cell response was investigated using the NHP enzyme-linked immunospot assay (ELISpot) and by performing intracellular cytokine staining (ICS). The ELISpot assay was sufficiently validated. The ICS assay was previously validated, but at another location than where the analyses for the current application were performed. As ICS data is at most considered supportive in this application, this is not an issue.

Biodistribution studies were performed in rabbits. Biodistribution of the Ad26 vector was tested using two Ad26-based vaccines encoding other antigens than the Ebola glycoprotein. The MVA-BN vector was tested without the presence of a specific antigen transgene. This approach was agreed in a scientific advice (Scientific Advice Clarification Letter of Procedure EMEA/H/SA/3018/1/FU/4/2019/III, dated 12 June 2019) and is endorsed. Distribution of the Ad26 vector in rabbits was limited to the injection site, the spleen and local lymph nodes. From these tissues, Ad26 DNA diminished slowly, with a small amount remaining in iliac lymph node of 1 animal at 180 days. Considering the removal of regions in the genome necessary for replication, its limited distribution and the low integration frequency of adenoviruses, it is considered unlikely that Ad26 will replicate in human tissues. MVA-BN mainly distributed to the injection site, with also small amounts in blood, spleen, lung, liver, and pooled lymph nodes (popliteal, inguinal and iliac nodes). MVA-BN was cleared rapidly; at 7 days after administration, only injection site was weakly positive in a few rabbits. MVA-BN is known not to replicate in human cells. It is therefore considered unlikely that MVA-BN will replicate or persist in human tissues. Dissemination of Ad26.ZEBOV and MVA-BN-Filo to breast milk or to/across the placenta has not been specifically assessed in these non-clinical biodistribution studies. Even if a small quantity would be excreted via the milk or disseminated across the placenta, it would not be considered a risk, as Ad26.ZEBOV and MVA-BN-Filo are non-replicating vaccines and do not encode a complete Ebola virus.

Studies on absorption, metabolism and excretion were not performed, which is in accordance with the WHO Guidelines on the Nonclinical Evaluation of Vaccines.

2.3.4 Toxicology

The preclinical safety profile of various 2-dose vaccine regimens of Ad26.ZEBOV and MVA-BN-Filo vaccine was assessed in a pivotal general toxicity study, including local tolerance, as well as in a combined embryo-fetal and pre- and postnatal developmental study in rabbits. Two additional general toxicity studies evaluated the nonclinical safety profile of either MVA-BN-Filo alone, or regimens of the trivalent Ad26.Filo vaccine (which includes Ad26.ZEBOV) and MVA-BN-Filo in the rabbit and are supportive for the nonclinical safety of the Ebola vaccine regimen.

Following treatment of rabbits with different regimens among which Ad26.ZEBOV – MVA-BN-Filo, or Ad26.Filo – MVA-BN-Filo – Ad26.Filo, at dosages at or close to the clinical dose, with a dosing interval of 14 days, findings such as inflammatory changes at the injection site and increased cellularity in iliac lymph node and spleen were as expected following vaccination. Furthermore, slight decreases in red blood cell parameters were observed. Also, a statistically significant lower number of neutrophils was noted in females in all treated groups. This was however at least partly due to higher values in the control animals and of transient nature. In the supportive, non-pivotal toxicology study with Ad26.Filo, some cysts were observed in oviducts of treated females at terminal necropsy; some were also found at recovery euthanasia in ovaries and oviducts. These are however considered spontaneous lesions that are frequently observed in this species and are unrelated to treatment.

Neurovirulence testing was not done as both Ad26.ZEBOV and MVA-BN-Filo do not replicate in human cells and distribution into the brain was not seen. It is agreed that the results from the repeated dose studies do not indicate towards damage of the nervous system. There was only cell infiltration around the sciatic nerves which may be associated with the vaccinations itself (which is plausible since vaccinations were administered into the thigh). In brain there were no findings except for minimal ventricular dilatation in one animal and minimal cell infiltration in another animal (among animals given the clinically most relevant regimen in the supportive, non-pivotal study with Ad26.Filo) which may have been an artefact and/or an incident.

In accordance with the WHO Guidelines on Nonclinical Evaluation of Vaccines no genotoxicity and carcinogenicity studies were performed for Ad26.ZEBOV or MVA-BN-Filo. As regard to Ad26 vector, and its integration ability: in nature, wild type adenoviruses do not integrate their genomes into the host cell chromosomes. With a few exceptions they replicate as linear, extra-chromosomal DNA (episomic) elements in the nucleus. The GUIDELINE ON NON-CLINICAL TESTING FOR INADVERTENT GERMLINE TRANSMISSION OF GENE TRANSFER VECTORS EMEA/273974/2005 indicates that adenoviruses have traditionally been regarded as non-integrating. Regarding the MVA-BN-Filo vector, due to their replication in the cytoplasm of host cells, they do not have a potential for genomic integration into the nucleus of infected cells. Compared to other DNA viruses, the possibility for integration of their genetic material into the host chromosome is therefore extremely low. Consequently, the risk for insertional mutagenesis is not a concern for these vectors. On the basis of above justifications, and the absence of potential integration inside host genome, absence of genotoxicity/carcinogenicity studies for both vectors is endorsed.

In a combined embryo-fetal and pre- and postnatal development study in the rabbit, Ad26.ZEBOV was administered at 8 days prior to mating, followed by either MVA-BN-Filo or Ad26.ZEBOV at gestation day 6. Another group of rabbits was treated with MVA-BN-Filo at 8 days prior to mating followed by Ad26.ZEBOV at gestation day 6No significant treatment-related effects were observed on reproduction or on F0 or F1 animals. All investigated regimens were immunogenic. Fetal antibody levels on GD29 were similar to maternal levels. Antibody levels in the kits on LD28 were lower than maternal levels. No juvenile toxicity studies were performed. This is agreed, because no target organs of toxicity have been identified. The study report did not give information on the possible transfer of maternal antibodies in breast milk. The number of antibodies transferred to the milk is however expected to be very low.

Local tolerance was evaluated as part of the repeated dose toxicity studies. Very slight erythema was observed at the injection sites. Histopathologically, minimal to moderate inflammatory changes were observed at the injection sites, sometimes associated with minimal to slight focal necrosis. Minimal to slight sciatic nerve mixed cell infiltration was also attributed to the inflammatory changes at the injection site. There were no severe findings.

Impurities and extractables/leachables in Ad26-ZEBOV drug substance and MVA-BN drug substance and in both drug products were either below acceptable limits or specified at a level below acceptable limits and do not raise a concern for safety.

2.3.5 Ecotoxicity/environmental risk assessment

The Ad26.ZEBOV component is based on adenovirus type 26, containing the transgene of the Zaire Ebola virus Mayinga glycoprotein. The glycoprotein is produced and presented on the cell membrane to the host immune system, but the viral genome is not integrated in the host genome. The regions of the viral genome coding for proteins involved in viral replication and persistence within the host cell have been removed.

Shedding

The genetically modified vector Ad26.ZEBOV is derived from Ad26. The replacing and deletions done in the viral genome have led to a replication deficient viral vector with reduced persistence and invasiveness and more proneness to host immune responses. Besides induction of immune responses against the Ad26 vector and EBOV GP no adverse effects are expected. The consequences of shedding of vector particles are negligible.

After administration, Ad26.ZEBOV might be able to enter the environment for a limited time by shedding. Shedding of Ad26.ZEBOV upon IM administration of $5x10^{10}$ VP was not detected in urine and nasal swab samples at day 2 and day 8 after administration. Studies with another Ad26-based vaccineAd26.RSV.preF showed limited quantities of vector DNA at the infection site up to 4 days post administration. No shedding of vector DNA was observed through urine and only infrequent and very low levels of vector DNA were present in other secretions. Note that the presence of vector DNA does not necessarily mean that infectious Ad26.ZEBOV particles are present. Additionally, the amount of vector particles that non-target individuals might be exposed to via shedding is much lower compared to the dose given to the vaccinated individuals. In the unlikely event that a possible infection of non-target individuals occurs, this effect will extinguish due to the replication defective nature of the viral vector. The likelihood of further spreading of the GMO from these non-target individuals is negligible since the GMO is replication deficient.

Shedding from vaccinated individuals will be limited as Ad26.ZEBOV is administered IM and the viral vector does not replicate in humans. If shedding does occur, the concentration of the GMO that might end up in the environment will be many times lower than the administered dose.

Overall, the risk to human health of shedding of viral particles is negligible.

Potential hazards to animal health

In case of shedding of Ad26.ZEBOV, animals might theoretically be exposed to Ad26.ZEBOV. However, Ad26.ZEBOV particle numbers involved in such an incident would be much smaller than the numbers contained in the vaccine. Unintentional exposure of animals to Ad26.ZEBOV may result in expression of EBOV GP and induction of an immune response against the Ad26 vector and EBOV GP. Besides induction of immune responses against the Ad26 vector and EBOV GP no adverse effects are expected.

Most adenovirus species have a narrow host range restricted to a single or just a few very closely related animal species. The tropism of Ad26.ZEBOV is identical to wildtype Ad26, as such, it is expected that besides humans, only primates might be infected by the Ad26.ZEBOV particles. Contact between vaccinated individuals and primates is expected to be limited in Europe. Overall, the risk to animal health is negligible.

Risk management strategies

Even though the overall risk of Ad26.ZEBOV is deemed negligible, measures have been taken by the Applicant to minimize the likelihood of spread in the environment or to non-target. As a precautionary measure the vaccination with Ad26.ZEBOV should be avoided during pregnancy and breast feeding unless it is considered that the benefit of preventing Ebola virus disease outweighs the risk.

If Ad26.ZEBOV must be given at the same time as another injectable vaccine(s), then the vaccine(s) should always be administered at different injection sites. Ad26.ZEBOV should not be mixed with any other vaccine in the same syringe or vial.

The SmPC gives some guidance in relation to protection of personnel during handling and administration, including disinfection of accidental spills in section 6.6: "*Potential spills should be disinfected with agents with viricidal activity against adenovirus*".

Since the environmental risks of Ad26.ZEBOV are negligible, the inclusion of additional risk management strategies for reasons of environmental safety and safety of non-target individuals is not necessary.

The overall risk for human health and the environment under the proposed conditions of release of Zabdeno and Mvabea is negligible.

2.3.6 Discussion on non-clinical aspects

The approach of the Applicant to focus on animal efficacy is in accordance with scientific advice and is acceptable. The animal model used for studying efficacy can be considered adequate because Ebola virus disease is at least as lethal in cynomolgus monkeys as it is in humans. The animal model appears to be more stringent relative to human disease, which allows for proof-of-concept testing of the vaccine.

Challenge studies were performed in cynomolgus monkeys. Four studies were conducted demonstrating protecting efficacy and testing the length of the dosing interval. Three additional studies were performed using the 56-day dosing interval. Three other studies were performed to assess the kinetics of the immune responses and long-term immunogenicity. When challenged around or shortly after the peak in antibody levels, survival was nearly 100%. Thereafter, antibody levels decreased to approximately 10-fold lower levels. It is questionable whether there are sufficient data to allow adequate characterization of the efficacy if the challenge is made more than one month after the last immunization.

The highest protective efficacy was obtained with a dosing interval of 56 days. Among monkeys treated with the clinical regimen and challenged approximately 4 weeks after the second dose, survival was nearly 100%. A few monkeys treated with the clinical regimen or only slightly less, did not survive. Furthermore, one monkey that died had relatively high binding antibody levels. There is however an overlap between protective and non-protective levels of immunogenicity. In this particular case, the fact that the monkey received a regimen with a 28-day dosing interval may have played a role, because the highest protective efficacy was obtained with a dosing interval of 56 days.

Different dose levels were tested across the study program and some dosing regimens sometimes induced a higher immunogenicity. Dose selection was a multistep process which begun on 2015. Beyond data in NHP, in clinical study EBL1002, higher doses of Ad26.ZEBOV at $1x10^{11}$ vp and MVA-BN-Filo at $4.4x10^{8}$ $TCID_{50}$ in the 28-day interval, only had a moderate positive impact on the humoral and cellular immune responses compared to the selected dose levels. Protective efficacy (survival) in NHP has been assessed after challenge realized 4 weeks after the last immunization. This time point for

viral challenge appears as an early point after the last immunization, occurring at the time of the development of an acute immune response. NHP data support the choice of regimen for clinical development, however, the NHP model is not suitable for investigating the memory response after a challenge at later time points, because of the rapid disease course in NHP. The NHP succumb to infection before an anamnestic response can be mounted. To address this issue, an NHP model with a comparable disease course to human EVD would be needed to evaluate the potential contribution of an anamnestic response to protection. The Applicant intends to explore the feasibility of other models (i.e. lower dose IM challenge and intranasal challenge), which is supported.

In several studies, some NHP were vaccinated, non-surviving, with a non-detectable viral load. In these cases the viral load results with the qRT-PCR test may have been false negative, because the plaque assay was positive. The Applicant generally excluded viral load values taken at EOP (end of project) time, arguing that it was taken by cardiac puncture and not venous blood sample and was considered to reflect remaining viral genome copies in the tissue. Due to the limited time of 28 days post-challenge per protocol, some vaccinated, surviving, animals may have a detectable virus load at the end of the study. Persistence of virus in some vaccinated people cannot thus formally be excluded based on these data.

Biodistribution of the Ad26 and MVA-BN vectors was studied in rabbits. The studies were in accordance with the Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines (EMA/CHMP/VWP/141697/2009) and in accordance with scientific advice. The studies showed that distribution of both vectors was very limited. Distribution of the Ad26 vector in rabbits was limited to the injection site, the spleen and local lymph nodes. MVA-BN was more widely distributed: mainly to the injection site, but also small amounts in blood, spleen, lung, liver, and pooled lymph nodes, but it was mostly cleared within 7 days. Considering the removal of regions in the genome necessary for replication, its limited distribution and the low integration frequency of adenoviruses, it is considered unlikely that Ad26 will replicate in human tissues. It is considered unlikely that MVA-BN will replicate or persist in human tissues, because of its limited distribution, its rapid clearance and because it is known not to replicate in human cells. Dissemination of Ad26.ZEBOV and MVA-BN-Filo to breast milk or to/across the placenta has not been specifically assessed in these non-clinical biodistribution studies. Even if a small quantity would be excreted via the milk or disseminated across the placenta, it would not be considered a risk, as Ad26.ZEBOV and MVA-BN-Filo are non-replicating vaccines and do not encode a complete Ebola virus.

The toxicology studies were adequate and in accordance with the WHO Guidelines on Nonclinical Evaluation of Vaccines and with scientific advice. Findings observed were mostly as can be expected following vaccination, i.e. inflammatory changes at the injection site and increased cellularity in iliac lymph node and spleen. Specific neurovirulence testing was not performed, as both Ad26.ZEBOV and MVA-BN-Filo do not replicate in human cells and distribution into the brain was not seen. This is agreed, because the results from the repeated dose studies do not indicate towards damage of the nervous system.

2.3.7 Conclusion on the non-clinical aspects

There are no objections against a marketing authorisation from a non-clinical point of view.

2.4 Clinical aspects

2.4.1 Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Union were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

In the Table 3 an overview of clinical studies submitted to support the current application are listed. In Table 4the NHP studies contributing to the immunobridging are presented.

Table 3: Overview of clinical studies included in AD26.ZEBOV, MVA-BN-Filo dossier

Study ID	Location	Design	Population	Study Objective	Vaccine Regimen, Dose Level[b], and Interval	Subjects
Phase 1 studies						
EBL1001	United Kingdom	Randomized, placebo-controlled, observer-blind, with one uncontrolled, open-label group	Healthy adults (18-50 y)	• Safety & reactogenicity of 2-dose vaccine regimens • Immune response: ELISA, psVNA, ICS, IFN-γ ELISpot • Ad26 (VNA) and MVA (ELISA, PRNT) vector backbone-specific neutralizing antibody responses • Functional antibody characterization	• Ad26, MVA: 28 & 56 days • MVA, Ad26: 28 & 56 days Ad26, MVA: 14 days (uncontrolled)	FAS: 60/12 controlled; 15/0 open-label group
EBL1002	United States	Randomized, placebo controlled, observer-blind	Healthy adults (18-50 y)	•Safety & reactogenicity of 2 dose vaccine regimens and booster • ELISA, psVNA, ICS, IFN γ ELISpot • ELISA responses to MARV & SUDV GP •Ad26 (VNA) and MVA (ELISA, PRNT) vector backbone-specific neutralizing antibody responses	• Ad26, MVA: 28 days • Ad26, MVA(h): 14 days • Ad26(h), MVA(h): 28 days • MVA, Ad26: 7, 14, 28, 56 days • MVA, MVA: 14 days • Ad26, Ad26: 14 days	FAS: 138/26
EBL1003	Kenya	Randomized, placebo controlled, observer-blind	Healthy adults (18-50 y)	•Safety and reactogenicity of 2 dose vaccine regimens •Humoral (ELISA, psVNA) and cellular (ICS, IFN γ ELISpot) immune responses to EBOV GP •Ad26 (VNA) and MVA (ELISA, PRNT) vector backbone-specific neutralizing antibody responses	•Ad26, MVA: 28 and 56 days •MVA, Ad26: 28 and 56 days	FAS: 60/12
EBL1004	Tanzania, Uganda	Randomized, placebo-controlled, observer-blind	Healthy adults (18-50 y)	•Safety and reactogenicity of 2 dose vaccine regimens •Humoral (ELISA, psVNA) and cellular (ICS, IFN γ ELISpot) immune responses to EBOV GP •Ad26 (VNA) and MVA (ELISA, PRNT) vector backbone-specific neutralizing antibody responses	•Ad26, MVA: 28 and 56 days •MVA, Ad26: 28 and 56 days	FAS: 60/12
FLV1001	United States	Randomized, placebo-	Healthy adults (18-50 y)	•Safety and reactogenicity of 2 dose vaccine regimen	Randomized, placebo-	FAS: 15/3

		controlled, double-blind		•Humoral (ELISA, psVNA) responses to EBOV GP •Humoral (ELISA) responses to MARV and SUDV GP •Pre-existing immunity against Ad26 (VNA) vector backbone	controlled, double-blind	
Phase 2 studies						
EBL2001	France, United Kingdom	Randomized, placebo-controlled, observer-blind	Healthy adults (18-65 y)	•Safety and reactogenicity of 2 dose vaccine regimens and Ad26.ZEBOV single dose •Humoral (ELISA, psVNA) and cellular (ICS) immune responses to EBOV GP	Controlled groups •Ad26, MVA: 28, 56, 84 days Vector shedding group (France) •1 dose of Ad26	FAS: 375/46
EBL2002	Burkina Faso, Côte d'Ivoire, Kenya, Uganda	Randomized, placebo-controlled, observer-blind, 2 part	Healthy adults (18-70 y), HIV infected adults (18-50 y), healthy adolescents (12-17 y) and children (4-11 y)	•Safety and reactogenicity of 2 dose vaccine regimens and booster •Humoral (ELISA, psVNA) and cellular (ICS, IFN γ ELISpot) immune responses to EBOV GP •Humoral (ELISA) responses to MARV and SUDV GP •Ad26 (VNA) vector backbone-specific neutralizing antibody responses	Ad26, MVA: 28, 56, 84 days (84 days only for healthy adults)	FAS: Adults: 677/133 Adolescents/ children: 218/45
EBL2003	Kenya, Mozambique, Nigeria, Tanzania, Uganda, US	Randomized, placebo-controlled, observer-blind, 2 part	Healthy adults and HIV-infected adults (18 70 y)	•Safety and reactogenicity of 2 dose vaccine regimens •Humoral (ELISA) immune responses to EBOV GP	•MVA, Ad26: 14 days (Part 1 and Part 2) •Ad26, MVA: 28 days (Part 2)	FAS: (Part 1: 60/15)h (Part 2: 401/98, immunogenicity data not available yet)
Phase 3 studies						
EBL3001	Sierra Leone	Staged study with an open-label, uncontrolled Stage 1 followed by a randomized, controlled, double-blind Stage 2	Adults (≥18 y), Adolescents (12-17 y), children (4-11 and 1 3 y)	•Safety of 2 dose vaccine regimen and booster (Stage 1 adults only) •Humoral (ELISA, psVNA) immune responses to EBOV GP •Humoral (ELISA) responses to MARV and SUDV GP •Ad26 (VNA) and MVA (PRNT) vector backbone-specific neutralizing antibody	•Ad26, MVA: 56 days	FAS: 43/0 open-label; 732/246 controlled Adults: completed, adolescents/ children: ongoing (Adults: 340j/102) (Adolescents /children: 432/144)
EBL3002	United States	Randomized, placebo-controlled, double-blind	Healthy adults (18 50 y)	•Noninferiority immunogenicity assessment of intermediate and low dose versus the selected dose level of 2-dose vaccine regimen •Safety and reactogenicity of 2 dose vaccine regimen at different dose levels •Humoral (ELISA, psVNA) immune responses to EBOV GP	•Ad26, MVA: 56 days •Ad26(i), MVA(l): 56 days •Ad26(l), MVA(l): 56 days	FAS: 450/75
EBL3003	United States	Randomized, placebo-controlled, double-blind	Healthy adults (18 50 y)	•Equivalence immunogenicity assessment of 3 different batches of Ad26.ZEBOV •Safety and reactogenicity of 2 dose vaccine regimen	•Ad26, MVA: 56 days	FAS: 282/47

				•Humoral (ELISA, psVNA) immune responses to EBOV GP		

- Higher dose level (h): 1x1011 vp for Ad26.ZEBOV and 4.4x108 TCID50 for MVA-BN-Filo
- Intermediate dose level (i): 2x1010 vp for Ad26.ZEBOV and 5x107 Inf.U for MVA-BN-Filo
- Low dose level (l): 0.8x1010 vp for Ad26.ZEBOV and 5x107 Inf.U for MVA-BN-Filo

Table 4: Overview of NHP studies forming the basis for the immunobridging

Study Identifier **Type of Study** **(GLP Status)**	**Vaccine Regimens Tested[1]** **Dose 1, Dose 2, Dose 3 (Dose Interval in Days)**	**N (C) control: no active vaccine** **N: all active vaccine regimens[1]** **N*: 56-day dose interval (all regimens and doses)** **N**: intended clinical regimen with 56-day dose interval**
Proof-of-Concept Lethal Challenge Studies, Studies Used for Immunobridging		
study 12 proof-of-concept immunogenicity and efficacy study (non-GLP)	Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} $TCID_{50}$ (42) Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} $TCID_{50}$ (28)	N(C) = 2, N = 10, N* = 0, N** = 0
study C29#1 proof-of-concept immunogenicity and efficacy study (non-GLP)	Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 5x10^{8} $TCID_{50}$ (56) **Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} $TCID_{50}$ (56)** Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} $TCID_{50}$ (28) MVA-BN-Filo 5x10^{8} $TCID_{50}$, Ad26.ZEBOV 5x10^{10} vp (28) MVA-BN-Filo 1x10^{8} $TCID_{50}$, Ad26.ZEBOV 5x10^{10} vp (28)	N(C) = 2, N = 18, N* = 8, **N** = 4**
study C25#1[2] proof-of-concept immunogenicity and efficacy study (non-GLP)	*Ad26.ZEBOV 4x10^{10} vp*, MVA-BN-Filo 5x10^{8} $TCID_{50}$ (56) MVA-BN-Filo 5x10^{8} $TCID_{50}$, *Ad26.ZEBOV 4x10^{10} vp* (56) *Ad26.ZEBOV 4x10^{10} vp*, *Ad26.ZEBOV 4x10^{10} vp*, MVA-BN-Filo 5x10^{8} $TCID_{50}$ (28,28)	N(C) = 2, N = 6, N* = 4, N** = 0
study C29#2[2] proof-of-concept immunogenicity and efficacy study (non-GLP)	*Ad26.ZEBOV 4x10^{10} vp*, MVA-BN-Filo 5x10^{8} $TCID_{50}$ (56) *Ad26.ZEBOV 4x10^{10} vp*, MVA-BN-Filo 5x10^{8} $TCID_{50}$ (28) MVA-BN-Filo 5x10^{8} $TCID_{50}$, *Ad26.ZEBOV 4x10^{10} vp* (56) MVA-BN-Filo 5x10^{8} $TCID_{50}$, *Ad26.ZEBOV 4x10^{10} vp* (28)	N(C) = 2, N = 16, N* = 8, N** = 0
Vaccine Dose-Down Lethal Challenge Studies, Studies Used for Immunobridging		
study C29#8 vaccine dose-down immunogenicity and efficacy study (non-GLP)	Ad26.ZEBOV 1x10^{11} vp, MVA-BN-Filo 5x10^{8} Inf.U (56) **Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} Inf.U (56)** Ad26.ZEBOV 2x10^{10} vp, MVA-BN-Filo 1x10^{8} Inf.U (28) Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{8} Inf.U (56) Ad26.ZEBOV 2x10^{9} vp, MVA-BN-Filo 1x10^{8} Inf.U (28)	N(C) = 1 N = 18 (17 at time of challenge) N* = 18 (17 at time of challenge) **N** = 6**
study TO14#1 vaccine dose-down immunogenicity and efficacy study (non-GLP)	Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{8} Inf.U (56) Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{6} Inf.U (56) Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{4} Inf.U (56) Ad26.ZEBOV 5x10^{8} vp, MVA-BN-Filo 1x10^{4} Inf.U (56) Ad26.ZEBOV 5x10^{7} vp, MVA-BN-Filo 1x10^{4} Inf.U (56)	N(C) = 2, N = 22, N* = 22, N** = 0
study TO14#2 vaccine dose-down immunogenicity and efficacy study (GLP)	Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{8} Inf.U (56) Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{6} Inf.U (56) Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{5} Inf.U (56) Ad26.ZEBOV 5x10^{9} vp, MVA-BN-Filo 1x10^{4} Inf.U (56)	N(C) = 2, N = 21, N* = 21, N** = 0

[1] In some studies, other vaccine products were tested that are out of scope for this application.
[2] In studies C25#1, C29#2, 15 and 16, Ad26.ZEBOV was provided in a trivalent mixture with Ad26.SUDV, and Ad26.MARVA.
[3] N=6 NHP receiving Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} Inf.U were transferred to study 15 for Week 70 challenge; N=2 negative control group was transferred to study 15.
All studies were performed in NHP (cynomolgus macaques; *Macaca fascicularis*).
The route of vaccine administration was intramuscular, except for study C25#1 where MVA-BN-Filo was administered subcutaneously.
An MVA-BN-Filo dose level in infectious units (Inf.U) corresponds to the same dose level expressed in 50% tissue culture infective dose ($TCID_{50}$)
The N represents only the animals receiving the test articles Ad26.ZEBOV and MVA-BN-Filo.

In studies used for immunobridging, the treatment groups that received the vaccine regimen intended for regulatory approval, ie $5x10^{10}$ vp Ad26.ZEBOV, $1x10^{8}$ Inf.U MVA-BN-Filo in a 56-day dose interval, N**, are highlighted in **bold**.
Note that in some studies, Ad26.ZEBOV was administered as part of the trivalent Ad26.Filo vaccine, indicated in *italics*.

2.4.2 Pharmacokinetics

No pharmacokinetic studies were conducted with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. This is acceptable. Pharmacokinetic studies are generally not considered informative for the evaluation of vaccines.

2.4.3 Pharmacodynamics

The pharmacodynamic profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen is defined by the immunogenicity profile, as it is detailed in the CHMP guideline "Guideline on Clinical Evaluation of New Vaccines" (EMEA/CHMP/VWP/164653/2005). Immunogenicity results are described in the Clinical Efficacy sections.

Mechanism of action

Zabdeno is a monovalent vaccine composed of a single recombinant, replication incompetent human adenovirus type 26 vectored vaccine that encodes the Zaire ebolavirus Mayinga variant GP. The EBOV GP encoded by Zabdeno has 100% homology to the one encoded by Mvabea. Following administration, the EBOV GP is expressed locally and stimulates an immune response.

Assays employed

An overview of the immunological assays used in clinical studies is presented in Table 5 For more information on these assays, please refer to the Clinical Assessment report.

Binding total IgG antibody levels were measured by ELISA. The functionality of vaccine-induced antibody responses was investigated by the determination of neutralizing antibody activity in a virus neutralization assay (VNA). T-cell response was investigated using the enzyme-linked immunospot assay (ELISpot) and intracellular cytokine staining (ICS).

Table 5: Summary of Immunological Assays (Humoral and Cellular)

Specific Response Against	Type of Immune Responses	Assay	Type of Response
EBOV GP	Humoral	FANG ELISA	Binding antibody response
		psVNA	Neutralizing antibody response
	Cellular	IFN-γ ELISpot	IFN-γ+ T cell response
		ICS	T cell responses (including IFN-γ, IL-2 and/or TNF-α producing CD4+/CD8+ T cells)
MVA (immune responses against vector backbone)	Humoral	ELISA	Binding antibody response
		PRNT	Neutralizing antibody response
Ad26 (immune responses against vector backbone)	Humoral	VNA	Neutralizing antibody response
MARV and SUDV GPs	Humoral	ELISA	Binding antibody response

For additional immunologic assays performed by the Applicant, refer to Mod2.7.3/Sec2.
In Phase 1, EBOV GP-specific binding antibody concentration assessments were performed using the qualified FANG ELISA at either BBRC (studies EBL1001, EBL1002, and EBL1004) or Q^2 Solutions (study EBL1003). Validated FANG ELISA at BBRC was used for study FLV1001 (refer to Mod2.7.3/Appendix A). However, the high-throughput capacity of Q^2 Solutions was needed to process the large Phase 2 and 3 sample numbers. Although the FANG ELISA assay at Q^2 Solutions is based on the same protocol, using the same critical reagents as the BBRC assay, an interlaboratory comparability assessment demonstrated that reportable values generated in the validated Q^2 Solutions FANG ELISA were 20%-25% lower compared to the validated BBRC FANG ELISA. Refer to Mod2.7.3/Appendix A.
ICS analyses were performed by HIV Vaccine Trials Network, Fred Hutchinson Cancer Research Center (HVTN, FHCRC) for EBL1001, EBL1002, EBL1003, EBL1004, and EBL2002. EBL2001 ICS analysis was performed by Inserm. Data generated in the Inserm ICS cannot be compared to the HVTN ICS since both assays report on different cell populations, for details refer to Mod2.7.3/Appendix A.

FANG EBOV GP-binding antibody ELISA fit for purpose of immunobridging Binding antibodies against EBOV GP, measured by EBOV GP FANG ELISA, were the main immunogenicity endpoint in all clinical studies. As the evaluation of the protective effect of the vaccine regimen for this MAA includes bridging of human immunogenicity results to efficacy and immunogenicity data obtained in non-human primates (NHP), a strategy which was accepted by CHMP in scientific advice procedure EMEA/H/SA/3018/1/FU/3/2017/III, a study was conducted to confirm that the conditions of the anti-EBOV GP IgG ELISA that was validated for human serum are also sufficient for NHP serum and that human and NHP serum demonstrate parallelism.

Four combinations of reference serum standard (RS) and conjugate have been analysed:

- human reference standard (RS) and human conjugate
- human reference standard (RS) and NHP conjugate
- NHP reference standard (RS) and human conjugate
- NHP reference standard (RS) and NHP conjugate

Results measured under the above-mentioned conditions have been provided. The reported ELISA units/mL are converted to µg/mL for both NHP and human samples, which place the ELISA reportable values on the same scale (µg/mL) for the human RS-human conjugate and NHP RS-NHP conjugate test conditions, thereby allowing direct comparison between reportable values for these test conditions.

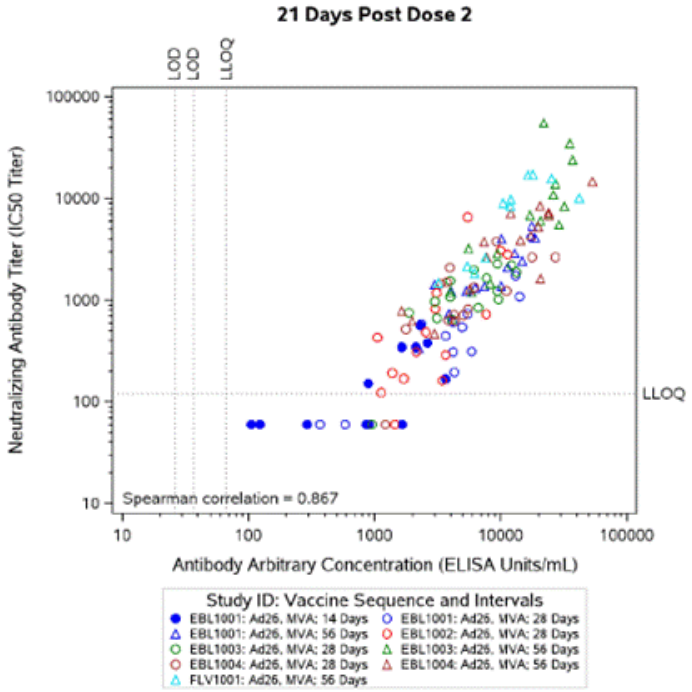
As the anti-EBOV GP IgG levels in NHP serum samples were intertwined when measured using either the human-human test condition or the NHP-NHP test condition, the results support the use of the anti-EBOV GP IgG ELISA that was validated for human serum for the quantitation of anti-GP IgG in NHP serum samples. Parallelism was determined using three methods: the Plikaytis method, the modified 4PL model, and a random coefficients model. The results showed that 83 to 93% of NHP and 88 to 95% of the human TS and QC samples met the Plikaytis method %CV acceptance criteria,

indicating parallelism over at least three sample dilutions.Upon request, the Applicant clarified that the outcome of the two other models are in line with the results of the Plikaytis method. The final anti-EBOV GP IgG ELISA uses human reference serum standard (RS), quality controls (QC), negative control (NC), and secondary antibody conjugate and was fully validated at Battelle (VP2015-291) and Q2 Solutions (formerly Focus Diagnostics (AVAL-119-00116-C)) for testing human serum samples.

Correlation between EBOV GP-specific binding and neutralizing antibody responses

Further investigation of humoral immune responses to EBOV GP included measurement of neutralizing antibodies by the pseudovirion neutralization assay (psVNA).

A positive correlation between EBOV GP-specific binding and neutralizing antibody responses was observed 21 days post Dose 2 for the Ad26.ZEBOV, MVA-BN-Filo regimen in 14-, 28-, and 56-day intervals (Spearman coefficient for pooled data: 0.867) (Figure 2).



The analysis is based on the Immunogenicity Analysis Set.
Placebo participants are excluded from this display.
For EBOV GP FANG ELISA, LOD for EBL1001/1002/1004 is 36.6 EU/mL (qualified assay at BBRC); LOD for EBL1003 is 26.22 EU/mL (qualified assay at Q^2 Solutions); LLOQ for FLV1001 is 66.96 EU/mL (validated assay at BBRC). Values below the LOD (LLOQ) are imputed with half of the LOD (LLOQ).
For EBOV GP psVNA, LLOQ for all studies is 120 IC_{50} titer. Values below the LLOQ are imputed with half of the LLOQ.
Source: [GIMHUMCORR01-P10.RTF][/SAS/Z_VAC52150/VAC52150ZSCE/FILES/RE/EFFICACYPOOL_2019_EMA_FDA/PROGRAMS/OBJECT SERVER] 08JUL2019, 05:54

Figure 2: Correlation Analysis Between EBOV GP Binding Antibody Concentrations and Neutralizing Antibody Titers at 21 Days Post Dose 2 on the Pooled Healthy Adult Data Set From Phase 1 Studies

Human vs. NHP vaccine-induced immunogenicity

The kinetics of the vaccine-induced GP-specific antibody response appear similar in NHP and humans. Regarding the magnitude, there seems to be a lower response in humans as compared to NHP. In both NHP and humans, EBOV GP-specific binding antibody concentrations were detected as early as 14 days after Ad26.ZEBOV vaccination and peaked 14 to 21 days after MVA-BN-Filo vaccination. After the 21 days post Dose 2 time point, the binding antibody responses declined over time in both NHP and

humans, reaching a stable level (10-20 fold lower than 21 days post Dose 2) that persisted at least up to 540 days in NHP and 2 years in humans (last time points assessed). An Ad26.ZEBOV booster dose elicited an approximate 12- to 55-fold increase in EBOV GP binding antibody concentrations by 7 days post booster, which was similar in NHP.

2.4.4 Discussion on clinical pharmacology

The evaluation of the protective effect of the vaccine regimen for this MAA is based on animal data, through the bridging of clinical immunogenicity results to efficacy and immunogenicity data obtained in non-human primates (NHP).

There are no dedicated PK studies. This can be accepted. The PK is not considered informative towards the determination of an optimal dose. Further, the metabolic pathways of vaccines are generally understood. Therefore, PK studies are generally not required for vaccines.

The Applicant has performed several assays to characterise the vaccine-induced immune response. These include the measurement of binding total IgG antibody levels by ELISA, neutralizing antibody activity by virus neutralization assay (VNA), and T-cell responses using the enzyme-linked immunospot assay (ELISpot) and intracellular cytokine staining (ICS). The main immunogenicity studies were studies EBL2001, EBL2002, EBL3001 (Stage 1 and 2), EBL3002, and EBL3003, as results from these studies were included in the immunobridging. Please refer to the Clinical Efficacy section for more information on these studies and the immunobridging. All main assays were appropriately validated.

The EBOV GP FANG ELISA was chosen as the main immune parameter to bridge towards human immune responses and to predict clinical benefit. An extensive analysis was performed to demonstrate parallelism between the human and NHP samples in the EBOV GP FANG ELISA. Three methods were used to determine parallelism. All three methods indicate a sufficient degree of parallelism. Based on the currently provided results, binding antibodies in NHP samples were found to be detected equally well by both the NHP conjugate and human conjugate; it was concluded that the human conjugate cross-reacts fully with NHP samples. For the immunobridging analysis, both the human test samples and the NHP test samples have been analysed using human reference samples and conjugate.

In general, it is preferred that functional immune responses are the focus of the assessment of vaccine immunogenicity. The Applicant however chose to use the EBOV GP-specific *binding* antibody response rather than the functional *neutralizing* antibody response. A positive correlation between binding and neutralizing antibody responses was observed 21 days post Dose 2 for the Ad26.ZEBOV, MVA-BN-Filo regimen in 14-, 28-, and 56-day intervals (Spearman coefficient for pooled data: 0.87). When both the EBOV GP-binding and neutralizing antibody levels were incorporated into a logistic regression model, both covariates became nonsignificant due to variance inflation, indicating multicollinearity. This finding indicates that binding and neutralizing antibody responses reflect each other and do not provide independent information. In addition, neutralizing antibody titers are determined in a cell- and pseudovirion-based assay which is inherently more difficult to control than a GP-binding antibody ELISA due to potential variation of pseudovirions and cell culture. Given this strong correlation and the more robust binding antibody assay, the choice of the Applicant, to use the EBOV GP FANG ELISA rather than the functional psVNA assay as the primary readout of vaccine induced immunogenicity, is acceptable.

Of interest, the psVNA assay could not be used to analyse samples of HIV-1 infected participants, potentially due to the presence of anti-retroviral drugs interfering with the assay. The Applicant clarified that they are in the process of setting up an alternative neutralization assay, in collaboration with the USAMRIID, to measure neutralizing antibody activity against EBOV GP in serum from HIV-infected participants. This effort is appreciated.

There are indications that cell-mediated immunity (CMI) also plays a role in protection against Ebola virus disease (e.g. McElroy AK et al., Curr Opin Virol. 2018; Younan P et al., PLOS Pathogens, 2019). To characterise vaccine-induced CMI, two assays have been deployed, EBOV GP Intracellular Cytokine Staining (ICS) and EBOV GP IFN-γ ELISPOT. These assays have been used to analyse samples of Phase 1 studies, study EBL2002 (both ICS and ELISPOT), and EBL2001 (only ICS). Unfortunately, due to differences in cell populations that could be analysed, the results of study EBL2001 cannot be directly compared with the results of the other studies.

Pre-existing and/or vaccine-induced neutralizing antibody responses against the Ad26 vector backbone were evaluated with an Ad26 virus neutralization assay (Ad26 VNA). The automated Ad26 VNA, used for EBL2002 and EBL3001 sample analysis, has not been validated but a comparability analysis against the validated manual assay was performed. Based on the results presented, the comparability between the manual and automated Ad26 VNA assay can be accepted. MVA vector backbone responses were evaluated with an MVA ELISA in the 4 VAC52150 Phase 1 studies, and with an MVA plaque reduction neutralization test (PRNT)) in the 4 VAC52150 Phase 1 studies and EBL3001.

Given the lack of efficacy studies, to translate human immunogenicity data into likelihood of protection, a logistic regression model was built based on immunogenicity and efficacy data obtained in the NHP EBOV Kikwit challenge model. Importantly, the kinetics of the vaccine-induced GP-specific antibody response appears similar in NHP and humans. The strategy to translate human immunogenicity data into likelihood of protection based on NHP challenge studies has been accepted by CHMP in scientific advice procedure **EMEA/H/SA/3018/1/FU/3/2017/III** and is further described in the Efficacy section of this report.

2.4.5 Conclusions on clinical pharmacology

Overall, the immunogenicity profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen has been sufficiently characterised.

2.5 Clinical efficacy

2.5.1 Dose response studies

The proposed vaccine regimen of Ad26.ZEBOV at $5x10^{10}$ vp per 0.5 mL (not less than 8.75 log_{10} infectious units (Inf.U) in 0.5 mL) dose followed by MVABNFilo at $1x10^{8}$ infectious Units (Inf.U) per 0.5 mL dose with a 56 day interval was selected based upon NHP efficacy data, which is discussed in the Non Clinical section (2.3.2), and early clinical data from phase 1 studies.

In NHP, full protection was obtained with the Ad26.ZEBOV vaccine down to a dose level of $2x10^{9}$ vp (i.e., more than 10 times lower than the selected dose level) when associated with MVA-BN-Filo at the selected dose level. Below these vaccine dose levels, the 2-dose regimen with a 56-day interval led to less than 100% survival. The Ad26.ZEBOV, MVA-BN-Filo sequence with intervals of less than 56 days between doses did not provide full protection in NHP, and neither did the reverse sequence (MVA-BN-Filo first) with a 28-day or 56-day interval.

The dose regimen was evaluated in several phase 1 studies, in which different sequences and dose intervals were evaluated as well as higher doses of Ad26.ZEBOV and MVA-BN-Filo. Phase 2 studies additionally evaluated varying vaccine intervals of 28, 56 and 84 days (EBL2001, EBL2002), and a 14-day interval between a reverse order regimen (MVA, Ad26; EBL2003). Lower dose levels, to support

potency and shelf life, were evaluated in phase 3 study EBL3002. An overview of the regimens as evaluated in these studies can be found in Table 6.

Table 6: Assessments supporting vaccine regimen

Study	Regimen		Interval	Vaccine Dose Level[a]
EBL1001	Heterologous	MVA, Ad26	28 days	Selected
		MVA, Ad26	56 days	Selected
		Ad26, MVA	14 days	Selected
		Ad26, MVA	28 days	Selected
		Ad26, MVA	56 days	Selected
EBL1002	Heterologous	MVA, Ad26	7 days	Selected
		MVA, Ad26	14 days	Selected
		MVA, Ad26	28 days	Selected
		MVA, Ad26	56 days	Selected
		Ad26, MVA	28 days	Selected
	Homologous	MVA, MVA	14 days	Selected
		Ad26, Ad26	14 days	Selected
	Heterologous	Ad26, MVA	14 days	Higher
		Ad26, MVA	28 days	Higher
EBL1003	Heterologous	MVA, Ad26	28 days	Selected
		MVA, Ad26	56 days	Selected
		Ad26, MVA	28 days	Selected
		Ad26, MVA	56 days	Selected
EBL1004	Heterologous	MVA, Ad26	28 days	Selected
		MVA, Ad26	56 days	Selected
		Ad26, MVA	28 days	Selected
		Ad26, MVA	56 days	Selected
EBL2003	Heterologous	MVA, Ad26	14 days	Selected
EBL3002	Heterologous	Ad26, MVA	56 days	Selected
		Ad26, MVA	56 days	Lower
		Ad26, MVA	56 days	Lower

Ad26: Ad26.ZEBOV; MVA: MVA-BN-Filo.
Light grey shading: Ad26.ZEBOV, MVA-BN-Filo vaccine regimens; No shading: MVA-BN-Filo, Ad26.ZEBOV vaccine regimens; Dark grey shading: homologous vaccine regimens.
[a] Selected dose level: $5x10^{10}$ vp for Ad26.ZEBOV and $1x10^{8}$ $TCID_{50}$ or Inf.U for MVA-BN-Filo.
Higher dose level: $1x10^{11}$ vp for Ad26.ZEBOV and $4.4x10^{8}$ $TCID_{50}$ for MVA-BN-Filo.
Lower dose level: $2x10^{10}$ vp or $0.8x10^{10}$ vp for Ad26.ZEBOV and $5x10^{7}$ Inf.U for MVA-BN-Filo.

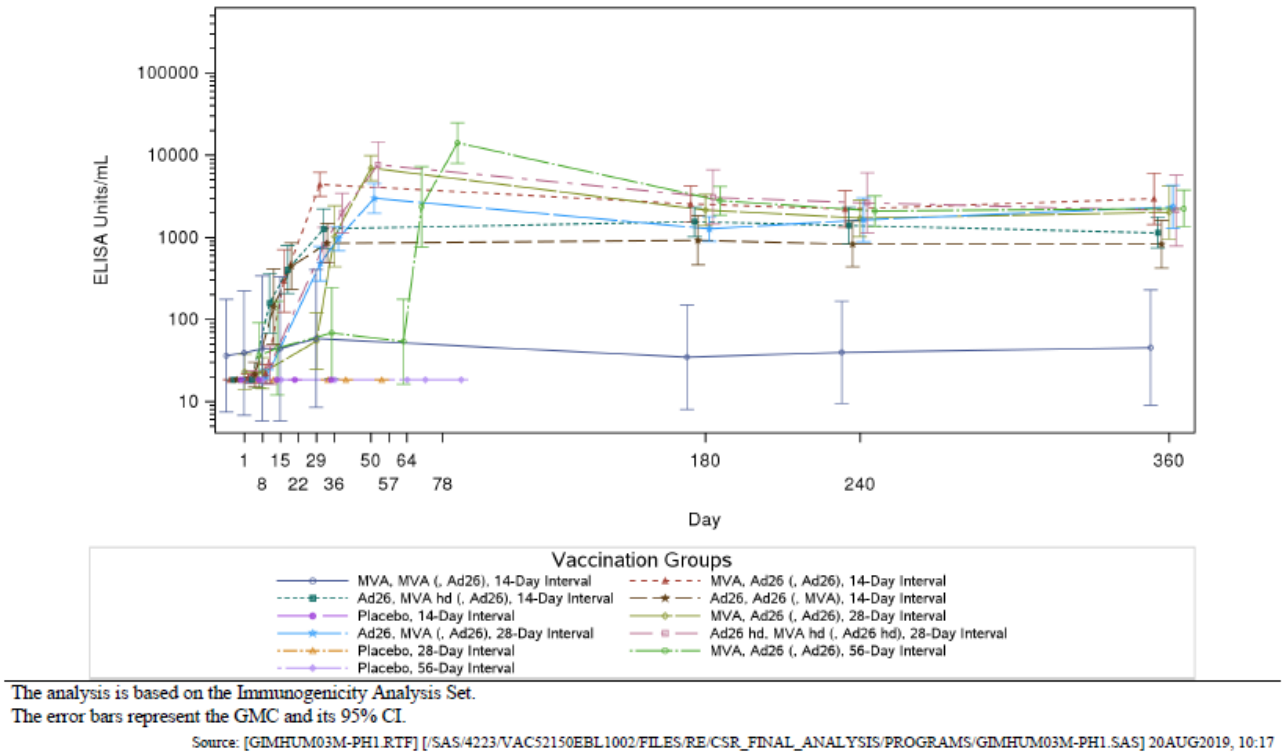
In all studies, the vaccine regimen was found to be immunogenic.

A higher **dose level** was evaluated in **EBL1002,** a randomized, placebo-controlled, observer-blind, Phase 1 study to evaluate the safety, reactogenicity, and immunogenicity of heterologous and homologous 2-dose vaccine regimens using Ad26.ZEBOV and MVA-BN-Filo administered in different doses, sequences, and intervals in healthy adults (18-50 years). Data from this study suggested that higher dose levels of MVA-BN-Filo ($4.4x10^{8}$ TCID50) and/or Ad26.ZEBOV ($1x10^{11}$ vp) resulted in an approximate 2-fold increase in GMC compared to the responses induced by the selected dose level. The relevance of a two-fold increase in titres is not known.

Although based on very limited numbers, reactogenicity was not clearly increased with the higher doses and no significant safety issues were identified (see safety section). Therefore logically, in a usual clinical development the higher dose would have been pursued. Development was expedited due to the ongoing epidemic in West Africa at the time. Further, selection of dose was primarily based on NHP studies in which a 10-fold lower dose was found to result in 100% protection against lethal challenge. Therefore, the decision not to further investigate higher doses is understandable.

Studies EBL1001, EBL1003, and EBL1004 evaluated the impact of changing the **sequence of vaccines** (i.e. Ad26.ZEBOV/MVA-BN-Filo vs MVA-BN-Filo/Ad26.ZEBOV) and heterologous vs homologous regimens.

The choice of the heterologous regimen is supported by human immunogenicity data as the responses are better to the heterologous regimen compared to the homologous regimens when given 14 days apart (EBL1002, see Figure 3).



The analysis is based on the Immunogenicity Analysis Set.
The error bars represent the GMC and its 95% CI.
Source: [GIMHUM03M-PH1.RTF] [/SAS/4223/VAC52150EBL1002/FILES/RE/CSR_FINAL_ANALYSIS/PROGRAMS/GIMHUM03M-PH1.SAS] 20AUG2019, 10:17

Figure 3: EBOV GP Binding Antibody Concentrations (ELISA, EU/mL) From Study EBL1002, Restricted to Pre-booster Time Points (Study EBL1002)

Responses to the Ebola GP were elicited more rapidly with the Ad26/MVA sequence compared to the MVA/Ad26 sequence. In study EBL1002, at 28 days post Dose 1, higher responses were observed after Ad26.ZEBOV (100% responder rate, GMC: 477 EU/mL) as first vaccination compared to MVA-BN-Filo as first vaccination (27%-47% responder rate, GMC range: 55-68 EU/mL). In study EBL1003, at 28 days post Dose 1, higher responses were observed after Ad26.ZEBOV (93%-100% responder rate, GMC range: 302-365 EU/mL) as first vaccination compared to MVA-BN-Filo as first vaccination (47%-60% responder rate, GMC range: 42-86 EU/mL). Similar results were seen in study EBL1004 where at 28 days post Dose 1, higher responses were observed after Ad26.ZEBOV (80%-93% responder rate, GMC range: 255-412 EU/mL) as first vaccination compared to MVA-BN-Filo as first vaccination (13%-21% responder rate, GMC: <LOD).

In conclusion, the selection of the Ad26/MVA sequence is understood, mainly as a faster immune response is seen with the Ad26/MVA sequence. Importantly, vaccination with the reverse sequence did not provide full protection in NHP challenge studies (see non-clinical section).

The use of the Ad26.ZEBOV, MVA-BN-Filo sequence at the selected vaccine dose levels in the 56-day **dose interval** led to 100% survival in the otherwise lethal EBOV challenge in NHP. Shortening of the intervals led to gradually lower survival rates: the 6-week interval led to 80% protection and the 4-week interval to more variable survival rates. Phase 1 clinical data confirmed that the 56-day interval selected for Phase 2 and 3 development was appropriate as providing the highest immune response in a schedule (8 weeks) that was practicable for prophylactic vaccination.

Table 7: GMCs 21 days after 2nd dose (Ad26/MVA except EBL1002) as reported for the individual studies.

		Vaccination interval between Ad26.ZEBOV and MVA-BN-Filo					
Study (analysis population)		**7 days***	**14 days**	**28 days**	**56 days**	**84 days**	**>98 days**
EBL1001 (Immunogenicity Analysis Set)	GMC		915	4274	7553		
	95% CI		432; 1936	2350; 7775	5114; 11156		
EBL1002 (Immunogenicity Analysis Set)	GMC	5655*	4418*	6987*†	14048*		
	95% CI	3426; 7759	3135; 6225	4916; 9931	7982; 24725		
EBL1003 (Immunogenicity Analysis Set)	GMC			5156	16341		
	95% CI			3426; 7759	10812; 24697		
EBL1004 (Immunogenicity Analysis Set)	GMC			5256	10613		
	95% CI			3376; 8183	6092; 18492		
EBL2001 (Per Protocol Analysis Set)	GMC			4627	10131	11312	19432
	95% CI			3649; 5867	8554; 11999	9072; 14106	8786;42977
EBL2002 (Per Protocol Analysis Set)	GMC			3085	7518	7300	
	95% CI			2648; 3594	6468; 8740	5116; 10417	

*MVA/Ad26 sequence only
† The Ad26/MVA sequence with a 28 day interval gave a GMC response of 2976 (1951; 4541)

As can be seen in Table 7 above, extending the interval between doses for Ad26.ZEBOV, MVA-BN-Filo, or MVA-BN-Filo, Ad26.ZEBOV in study EBL1002, generally resulted in higher antibody concentrations.

In study EBL1003, the 56-day interval induced an antibody response at 21 days post Dose 2 three times that of the 28-day interval. However, similar responses were observed for both intervals in the long-term immunogenicity follow-up (GMC year post Dose 1: 403 EU/ml compared to 449 EU/mL). Similarly, in study EBL1004 the 56-day interval induced an antibody response at 21 days post Dose 2 twice as high as compared to the 28-day interval. Here too similar responses were observed for both intervals in the long-term immunogenicity follow-up (GMC 1-year post Dose 1: 550 EU/mL for the 56 day interval compared to 551 EU/ml for the 28 day interval).

A longer interval of 84 days between doses was evaluated in Phase 2 studies EBL2001 and EBL2002, which resulted EBOV GP-specific binding antibody GMCs that were not markedly higher compared to the 56-day interval (see Table 7).

As study EBL2001 was paused (see safety section) some subjects received dose 2 later than planned. Binding antibody concentrations in participants who received Dose 2 later than planned per protocol (i.e. between 98 and 355 days post Dose 1) were at least as high as the 56-day interval (see Table 7).

The data from the four phase 1 and two phase 2 studies discussed above demonstrate that lengthening of the interval to 56 days increased the binding antibody response against EBOV GP, with GMCs which were two to three-fold higher dependent on the study. Further prolonging the interval to 84 days had no clear impact on the GMCs. In conclusion, the human immunogenicity data comparing different intervals supports the selection of the 56-day interval.

Of note, the height of the anti EBOV GP binding antibody response after the second dose does not predict the decay curve. When questioned, the Applicant hypothesized that the differences at 21 days post Dose 2 are due to different levels of short-lived plasma cells, while the persisting level of circulating antibodies 1 year after vaccination would typically be produced by long-lived plasma cells which may have been comparable across groups, regardless of the level of the acute immune response. Additional analyses suggested that, at the individual level, there may be some relationship between the 21 days post Dose 2 and 21 days post booster antibody levels, although this correlation appears relatively weak and its implication is unclear. The differences between groups post booster dose are unlikely to have a clinical relevance.

2.5.2 Main studies

Immunogenicity data obtained with the selected vaccine regimen from the following studies has been used in the immunobridging: studies EBL2001, EBL2002, EBL3001 (Stage 1 and 2), EBL3002, and EBL3003, which is pivotal to this application. As such, the methods of these studies are described below rather than under supportive studies. Where possible the presentation of methods has been integrated.

Methods

EBL2001 was a randomized, observer-blind, placebo-controlled, parallel-group, multicenter, Phase 2 clinical study to evaluate the safety, tolerability and immunogenicity of three 2-dose heterologous vaccination regimens using Ad26.ZEBOV at $5x10^{10}$ vp as dose 1 followed by MVA-BN-Filo at $1x10^{8}$ Inf.U (nominal titer) as dose 2 at a 28-, 56- or 84-day interval in healthy adult subjects in Europe (United Kingdom [UK] and France). Group 2 is the only group that represents the to be marketed dose for both vaccines, given 8 weeks apart as recommended in the SmPC. Viral shedding, safety, tolerability and immunogenicity of the Ad26.ZEBOV vaccine (at $5x10^{10}$ vp) as a single-dose vaccination were also evaluated.

A schematic overview of the design of EBL2001 is provided in Table 8.

The study was conducted in healthy men and women aged between 18 and 65 years (inclusive), who had no prior exposure to Ebola virus (including travel to West Africa within 1 month prior to screening) or a diagnosis of Ebola virus disease.

Table 8: Schematic Overview of Study Design, Cohorts and Groups (Study EBL2001)

Groups 1, 2 and 3 (2-dose heterologous vaccination regimens)

Study Cohorts	Randomization Ratio (Ad26,MVA:Placebo)	Group 1 (28-day interval) N=204	Group 2 (56-day interval) N=204	Group 3 [a] (84-day interval) N=204	Cohort Total N=612	UK [b] N=321	France [b,c] N=291
Cohort I [d]	-	10/0	10/0	10/0	**30**	30	-
Cohort II [e]	14:1	84/6	84/6	84/6	**270**	135	135
Cohort III [f]	10:3	80/24	80/24	80/24	**312**	156	156

Group 4 (single-dose vaccination regimen) [g]

Randomization Ratio (Ad26:Placebo)	Group 4 N=18	Group Total N=18	France [b] N=18
5:1	15/3	**18**	18

N: number of subjects to receive study vaccine (Ad26.ZEBOV, MVA-BN-Filo or placebo); UK: United Kingdom
Groups 1, 2 and 3: first dose on Day 1, followed by second dose on Day 29, Day 57 or Day 85, respectively.
Group 4: vaccination on Day 1.

[a] Randomization to Group 3 was stopped per Amendment 4 (see Section 3.1.2) to focus on the schedules for which an indication will be sought.

[b] Enrollment in the entire study (UK and France) was stopped per Amendment 5 (see Section 3.1.2).

[c] To ensure to have a sufficient number of subjects in France for blood sample collection for additional immunogenicity assessments, subjects in France were allowed to switch from Cohort III to Cohort II if needed.

Assessments planned per protocol:

[d] Cohort I (UK only): safety and tolerability, and immunogenicity assessments for the evaluation of additional exploratory endpoints planned by the consortium partners.

[e] Cohort II (UK and France): safety and tolerability, core immunogenicity assessments (humoral and cellular assays) for the evaluation of secondary and exploratory endpoints planned by the sponsor, and additional immunogenicity assessments for the evaluation of additional exploratory endpoints planned by the consortium partners.

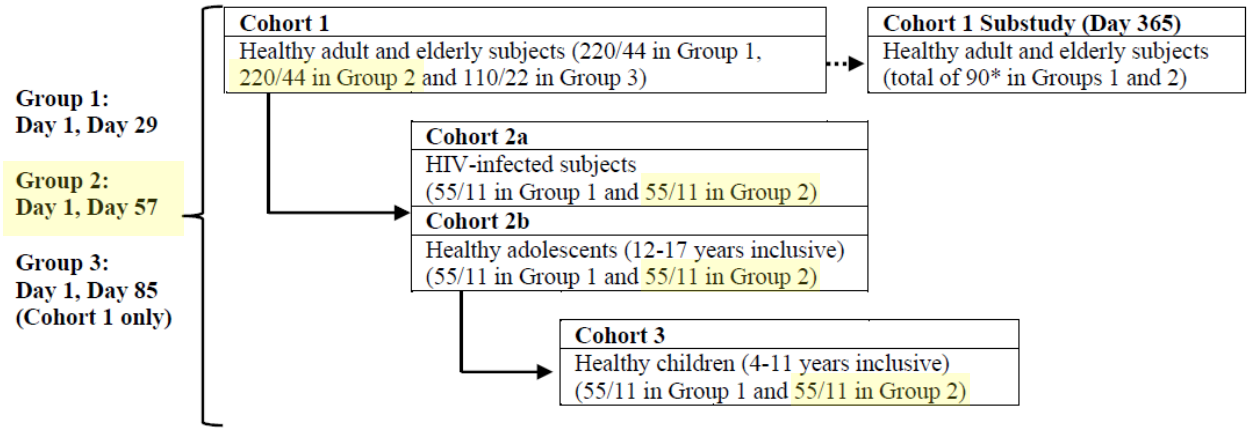
[f] Cohort III (UK and France): safety and tolerability, and core immunogenicity assessments (humoral and cellular assays) for the evaluation of secondary and exploratory endpoints planned by the sponsor.

[g] Group 4 (France only): core immunogenicity assessments (humoral assays) and Ad26 vector shedding assessments.

EBL2002 was a randomized, observer-blind, placebo-controlled, parallel-group, multicenter, Phase 2 clinical study in Africa to evaluate the safety, tolerability and immunogenicity of different 2-dose heterologous vaccination regimens using Ad26.ZEBOV $5x10^{10}$ vp as dose 1 and MVA-BN-Filo $1x10^{8}$ Inf.U as dose 2 at a 28-, 56- or 84-day interval in HIV-uninfected adult and elderly participants (Cohort 1). The same schedules, except for the 84-day interval schedule, were evaluated in HIV-infected adult participants (Cohort 2a) and in HIV-uninfected adolescents and children (Cohort 2b and Cohort 3, respectively).

At selected sites in Cohort 1 (Groups 1 and 2), a booster dose of Ad26.ZEBOV (or placebo) was administered at 1-year post dose 1 (window: +3 months) in those participants who consented to this (Cohort 1 substudy).

A schematic overview of the study design is presented in Figure 4. In each cohort, Group 2 is the only group that represents the to be marketed dose for both vaccines, given 8 weeks apart as recommended in the SmPC.



N/n: planned number of subjects per group to be randomized to Ad26.ZEBOV, MVA-BN-Filo regimen/placebo regimen.

- Cohorts 2a and 2b: started when 25% of subjects from Cohort 1 had reached the 7-day post-dose 1 visit.
- Cohort 3: started when 50% of subjects from Cohort 2b had reached the 7-day post-dose 1 visit.

* Subjects who received Ad26.ZEBOV and MVA-BN-Filo received Ad26.ZEBOV as a booster dose at 1 year post dose 1 (window: +3 months). Subjects who received placebo received placebo as a booster dose at 1 year post dose 1 (window: +3 months).

Figure 4: Schematic Overview of the Study (Study EBL2002)

EBL3001 was a 2-staged Phase 3 clinical study with an open-label uncontrolled stage (Stage 1) and a double-blinded controlled stage (Stage 2) to evaluate the immunogenicity and safety of a 2-dose heterologous vaccination regimen where Ad26.ZEBOV at $5x10^{10}$ vp was administered as the first dose and MVA-BN-Filo at $1x10^{8}$ Inf.U as the second dose 56 days later. The study was conducted in Sierra Leone. In Stage 1, a booster dose (Ad26.ZEBOV $5x10^{10}$ vp) was given at 2 years (window: +3 months) post dose 1 to participants who consented to this.

The study was conducted as follows:

- Stage 1: Approximately 40 adult participants aged ≥18 years were planned to be vaccinated.
- Stage 2: A total of 976 participants aged ≥1 year were planned to be individually randomized in a 3:1 ratio to the 2-dose experimental vaccination regimen or an active control vaccine followed by placebo: 400 participants aged ≥18 years and 576 participants aged ≥1 year, in 3 age groups (12-17 years, 4-11 years, and 1-3 years). Enrollment of participants was staggered, starting with the eldest group. The decision to proceed to the next age group was based on evaluations by the Independent Data Monitoring Committee (IDMC) installed for this study. Randomization was stratified by age group.

EBL3002 was a randomized, double-blind, placebo-controlled, parallel group, multicenter, Phase 3 study in adult subjects in the USA, performed to support the lower specification for potency over the expected shelf life for both Ad26.ZEBOV and MVA-BN-Filo. The vaccination regimens in this study differed in dose levels of Ad26.ZEBOV ($5x10^{10}$ vp, $2x10^{10}$ vp or $0.8x10^{10}$ vp, respectively, referred to as Groups 1, 2 and 3) and of MVA-BN-Filo ($1x10^{8}$ Inf.U [Group 1] or $5x10^{7}$ Inf.U [Groups 2 and 3]), while the timing of dose 2 (56 days post dose 1) and order of the vaccinations were identical. Group 1 is the only group that represents the to be marketed dose for both vaccines, given 8 weeks apart as recommended in the SmPC.

The study population consisted of healthy men and women, aged ≥18 to ≤50 years, with no known prior exposure to EBOV (including travel to West Africa less than 1 month prior to screening) or diagnosis of Ebola virus disease.

A schematic overview of the study design and groups is shown in Table 9.

Table 9: Schematic Overview of Study Design and Groups (Study EBL3002)

Group	N	Dose 1 Vaccination	Dose 2 Vaccination
		Day 1	Day 57
1	150	Ad26.ZEBOV $5x10^{10}$ vp	MVA-BN-Filo $1x10^{8}$ Inf.U
2	150	Ad26.ZEBOV $2x10^{10}$ vp	MVA-BN-Filo $5x10^{7}$ Inf.U
3	150	Ad26.ZEBOV $0.8x10^{10}$ vp	MVA-BN-Filo $5x10^{7}$ Inf.U
4	75	Placebo (0.9% saline)	Placebo (0.9% saline)

N: number of subjects to receive study vaccine (active or placebo)
Inf.U: infectious units; vp: viral particles

EBL3003 was a randomized, double-blind, placebo-controlled, parallel-group, multicenter Phase 3 clinical study, designed to evaluate immunogenic equivalence of a 2-dose heterologous vaccination regimen using 3 different batches of Ad26.ZEBOV ($5x10^{10}$ vp) followed by MVA-BN-Filo from a single batch ($1x10^{8}$ infectious units [Inf.U]) 56 days later in healthy adult subjects in the USA. The drug substance batches used in Ad26.ZEBOV drug product were manufactured according to the 2x10L scale process from WVS in Leiden, the Netherlands manufacturing facility (Group 1), from WVS in Bern, Switzerland manufacturing facility (Group 2), and from MVS in Leiden, the Netherlands manufacturing facility (Group 3, identical to Batch used in Phase 2 studies). All three active groups in this study received the to be marketed dose for both vaccines, given 8 weeks apart as recommended in the SmPC.

The study population consisted of healthy men and women, aged ≥18 to ≤50 years, with no known prior exposure to EBOV (including travel to West Africa less than 1 month prior to screening) or diagnosis of Ebola virus disease.

A schematic overview of the study design and groups is shown in Table 10.

Table 10: Schematic Overview of Study Design and Groups (Study EBL3003)

Group	N	Dose 1 Vaccination	Dose 2 Vaccination
		Day 1	Day 57
1	94	Ad26.ZEBOV – (V)	MVA-BN-Filo – (A)
2	94	Ad26.ZEBOV – (B)	MVA-BN-Filo – (A)
3	94	Ad26.ZEBOV – (C)	MVA-BN-Filo – (A)
4	47	Placebo (0.9% saline)	Placebo (0.9% saline)

N: number of subjects to receive study vaccine (active or placebo)
A: batch Kvistgård; B: WVS batch Bern; C: MVS batch Leiden (identical to a Batch used in Phase 2 studies);
V: WVS batch Leiden
Ad26.ZEBOV dose level: $5x10^{10}$ viral particles (vp); MVA-BN-Filo dose level: $1x10^{8}$ infectious units (Inf.U).

Study Participants

All studies enrolled **healthy adult men and women** (in the investigator's clinical judgment on the basis of medical history, physical examination, and/or vital signs, and/or ECG assessments) from whom written informed consent was obtained and who could comply with the protocol requirements.

In addition, **healthy adolescents and children** were enrolled in EBL2002 and EBL3001.

Studies EBL2002 and EBL2003 additionally enrolled separate cohorts of **HIV-infected adults**. All inclusion and exclusion criteria for healthy adults had to be met by HIV-infected adults, with the requirement that they had to have documented HIV infection for at least 6 months prior to screening (EBL2002) or have had a positive HIV serology test within 6 months of screening (EBL2003), a screening CD4+ cell count >200 (EBL2003) or >350 (EBL2002) cells/µL, be on a stable regimen of highly active antiretroviral therapy (HAART) for 4 weeks prior to inclusion, and were in good medical condition.

For all studies, the main exclusion criteria were:

- participants with any medical condition that could potentially interfere with the evaluation of the immune response (such as participants with prior exposure/diagnosis of EVD, participants who had HIV type 1 or type 2 infection [applicable for healthy participants; HIV-infected adults were enrolled in studies EBL2002 and EBL2003),
- participants with any medical condition that could potentially interfere with the evaluation of safety (such as known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products; participants with an acute illness or body temperature ≥38.0ºC on Day 1; participants positive for HBsAg or HCV at screening [not applicable for EBL2002 and EBL3001]),
- participants taking concomitant medication or receiving other vaccinations that could potentially interfere with the evaluation of the immune response to study vaccine, as well as with regard to attribution of AEs (such as any candidate Ebola vaccine or candidate Ad26- or MVA-based vaccine in the past; investigational products, routine immunizations with inactivated vaccines or with live attenuated vaccines within a specified time window before and after administration of study vaccine).

Treatments

For the immunobridging analysis, only those groups included in the studies which received the final vaccine regimen ($5x10^{10}$ virus particles (vp) (not less than 8.75 $\log_{10}$ infectious units (IU) in 0.5 mL) for Ad26.ZEBOV and $1x10^{8}$ infectious units (Inf.U,) for MVA-BN-Filo given 56 days apart) were included.

The Ad26.ZEBOV and MVA-BN-Filo vaccines are suspensions for injection provided in single-dose vials with an extractable volume of 0.5 mL for IM injection. Placebo was formulated as a sterile 0.9% saline for injection (as commercially available).

Objectives

In all study protocols, the main immunogenicity objective was to assess binding antibody responses to EBOV GP (as measured by EBOV GP FANG ELISA) at 21 days post Dose 2.

To support specification settings for potency over the expected shelf life of the 2 vaccines, the primary objective in study EBL3002 (Section 2.3.2) was to demonstrate noninferiority of the 2 dose heterologous vaccine regimen in the 56-day interval administered at an intermediate dose level (Ad26.ZEBOV $2x10^{10}$ vp followed by MVA-BN-Filo $5x10^{7}$ Inf.U) versus the same regimen at the selected dose level (release titers: Ad26.ZEBOV $5x10^{10}$ vp, MVA-BN-Filo $1x10^{8}$ Inf.U). A low dose level (Ad26.ZEBOV $0.8x10^{10}$ vp, MVA-BN-Filo $5x10^{7}$ Inf.U) was also evaluated.

Study EBL3003 (Section 2.3.3) was designed to compare immune responses between 3 batches of Ad26.ZEBOV from different virus seeds (Working Virus Seed [WVS] or Master Virus Seed [MVS])

produced at different manufacturing sites (Leiden or Bern). The primary objective was to demonstrate the immunological equivalence of the batch derived from WVS produced in Bern versus the batch derived from MVS produced in Leiden. Other comparisons (WVS Leiden versus WVS Bern; WVS Leiden versus MVS Leiden) were performed as secondary objectives. All these objectives were assessed in terms of the geometric mean concentration (GMC) of binding antibodies 56 days after Ad26.ZEBOV vaccination (given as Dose 1).

Outcomes/endpoints

The primary immunogenicity endpoint was:

- Binding antibody levels against EBOV GP, as measured by EBOV GP FANG ELISA (unit: ELISA units/mL) at 56 days after dose 1. **All Studies**

Secondary/additional immunogenicity endpoints were:

- Binding antibody levels against EBOV GP, as measured by EBOV GP FANG ELISA (unit: ELISA units/mL) at all other timepoints. **All Studies**
- Neutralizing antibody levels against EBOV GP, as measured by psVNA in titers inhibiting viral infection by 50% (IC50). **All Studies**
- Number of IFN-γ producing T-cells, as measured in an IFN-γ ELISpot assay, at selected timepoints. **Study EBL2002**
- Percentage of $CD4^+$ T-cells and/or $CD8^+$ T-cells producing IFN-γ and/or IL-2 and/or TNF-α, as measured by ICS, at selected timepoints. **Study EBL2001 and EBL2002**
- Binding antibody levels against MARV GP, as measured by MARV GP ELISA (unit: ELISA units/mL). **Study EBL2002 and EBL3001**
- Binding antibody levels against SUDV GP, as measured by SUDV GP ELISA (unit: ELISA units/mL). **Study EBL2002 and EBL3001**
- Neutralizing antibody levels against the Ad26 vector backbone, as measured by Ad26 VNA (unit: IC90). **Study EBL2002 and EBL3001**
- Neutralizing antibody levels against the MVA vector backbone, as measured by MVA PRNT (unit: IC50). **Study EBL3001**

Randomisation and blinding (masking)

Central randomization was implemented in all studies for all blinded arms. Participants were randomly assigned to study groups (i.e. vaccine regimen and interval)/cohorts (i.e. study population), and within groups/cohorts randomly assigned to receive active vaccine or control (placebo or active control), if applicable. Randomization within each group was balanced by using randomly permuted blocks. The interactive web response system assigned a unique code that dictated the assignment and matching study vaccine for the participant.

Randomization within groups/cohorts was stratified:

- By age (≤50 years or >50 years) in EBL2001, EBL2002, EBL2003.
- By country (UK/France) in Cohorts II and III in EBL2001; By site (within USA) in EBL3002 and EBL3003.

- By peripheral blood mononuclear cell (PBMC) sampling capability of the selected sites in EBL2002.
- Randomization was also stratified for healthy and HIV-infected adults in studies EBL2002 and EBL2003.

Statistical methods

In all study protocols, the main immunogenicity objective was to assess binding antibody responses to EBOV GP (as measured by EBOV GP FANG ELISA) at 21 days post Dose 2. All these objectives were assessed in terms of the geometric mean concentration (GMC) of binding antibodies 56 days after Ad26.ZEBOV vaccination (given as Dose 1).

Summary statistics of actual values (on the log10 scale) were be presented for ELISA (EU/mL) at Day 21 post boost for the Ad26.ZEBOV/MVA-BN-Filo 56-day interval regimen. Geometric mean concentration together with the 95% CI will also be presented.

Study EBL3002

Non-inferiority of a dose level versus the release titer was demonstrated if the 95% CI of the estimated GMC ratio: (GMC intermediate (or low) dose level / GMC release titer) was entirely above 2/3. If non-inferiority of the intermediate dose level was demonstrated, non-inferiority of a low dose level (Ad26.ZEBOV 0.8x1010 vp and MVA-BN-Filo 5x107 Inf.U) versus the release titer was investigated in the same way. Hierarchical testing was applied. The analysis of immune responses was performed on the Per Protocol Analysis Set.
Prior to study unblinding, a non-inferiority assessment using a margin of 0.5 was also planned. This was done because, after the present study was designed, regulatory agreement was reached with the Food and Drug Administration (FDA) to assess consistency of manufacturing in the lot-to-lot studyVAC52150EBL3004 with equivalence limits of 0.5 and 2.0. Therefore, non-inferiority was assessed with the same limits for consistency.

Study EBL 3003

For evaluation of the primary endpoint (levels of binding antibodies against EBOV GP using EBOV GP FANG ELISA at 56 days post dose 1), only subjects in Group 2 (WVS batch Bern [batch B]) and Group 3 (MVS batch Leiden [batch C]) were considered. Estimated differences in ELISA concentrations (ELISA units/mL) 56 days after dose 1 were expressed as the ratio of GMCs with corresponding 95% confidence intervals (CIs). This was determined by comparing the log10-transformed ELISA concentrations between groups and back-transformation of the estimated difference and 95% CIs. If the 95% CI of the estimated GMC ratio was entirely within the range of 2/3 through 3/2, the immunological equivalence of the batch derived from WVS produced in Bern versus the batch derived from MVS produced in Leiden was demonstrated and bridging accomplished. The analysis of immune responses was performed on the Per Protocol Analysis Set.
Other comparisons (WVS Leiden versus WVS Bern; WVS Leiden versus MVS Leiden) were performed as secondary objectives.

Immunobridging model

To translate human immunogenicity data into likelihood of protection, a logistic regression model was built based on immunogenicity and efficacy data obtained in the NHP EBOV Kikwit challenge model. This NHP challenge model bears close resemblance to human EVD after parenteral exposure such as needle stick infection, but, in contrast to human EVD, has a faster disease course to death and is fully lethal in unvaccinated NHP. Logistic regression analysis was used to assess the relationship between

immunogenicity and survival outcome based on the pooled data from 7 NHP studies. EBOV GP-specific binding antibody responses, as measured by EBOV GP FANG ELISA, were identified to be the most suitable and adequately correlating immune response with survival in NHP after Ebola virus challenge, as agreed with EMA and FDA.

First a model was built using penalized logistic regression with Firth's method, with survival outcome as the dependent variable and the FANG ELISA concentrations (EU/mL, log10) at Day 21 post dose 2 of the 2-dose vaccine regimen as the independent variable using the NHP data from the challenge studies. Logistic regression model: Log(p/1-p) = intercept + slope*log10(ELISA). The logistic model fitted on the NHP data was then used to estimate the survival probability for a given human ELISA value detected at 3 weeks post dose 2 of the 2-dose vaccine regimen.
Subsequently, the individual predicted human survival probabilities were averaged for the 0,56-day schedule to calculate the mean predicted survival probability. The mean predicted survival probability together with its 95% CI was calculated for the 2 analysis sets. For the mean predicted survival probability, a 95% CI was then calculated using a nonparametric double-bootstrap method. The NHP and human datasets were resampled 10,000 times each with replacement and the logistic regression model was refitted for each resampled NHP dataset. Subsequently, predictions were made for the resampled clinical dataset based on this updated logistic regression curve. As a result, 10,000 mean predicted survival probabilities were obtained. The 95% CI were then derived as the 250th and 9,750th values when sorting the resulting mean predicted survival probabilities. To evaluate success, the lower bound of this CI on the mean predicted survival probability for the 0,56-schedule was compared to the pre-specified success criterion of 20%.

The dataset consisting of all NHP immunized with the Ad26.ZEBOV, MVA-BN-Filo regimen in the 56-day interval (also referred to as the main regimen, N=66, i.e. excluding unvaccinated controls) was used to build the logistic model for the primary immunobridging analysis. The dataset consisting of all immunized NHP (i.e. all regimens combined, N=108), was used to build a second logistic model that was used in an immunobridging sensitivity analysis.

Analysis sets

For the immunobridging analysis, the primary analysis population will be the Per Protocol Immunogenicity Analysis Set (PPI analysis set).

The following definitions for the analysis sets were used:
The *Per Protocol Immunogenicity (PPI)* analysis set includes all randomized and vaccinated subjects, who received the 2 dose vaccination regimen (administered within the protocol-defined window), have at least 1 post-vaccination evaluable immunogenicity sample, and have no major protocol violations influencing the immune response. For the immunogenicity analyses, only subjects with a Day 21 post boost ELISA result were included.
The *Full Analysis set* includes all subjects who were randomized and received at least 1 dose of study vaccine, regardless of the occurrence of protocol deviations. For the immunogenicity and the immunobridging analyses, only subjects with a Day 21 post boost ELISA result were included.

Primary analysis and sensitivity analysis

The primary analysis was done on the pooled Phase 2/3 data of healthy adults (18-50 years of age) vaccinated with the main regimen (Ad26.ZEBOV/MVA-BN-Filo prime-boost with a 56-day interval) using the logistic model based on NHP data of the main regimen.
Four sensitivity analyses were done. All 4 sensitivity analyses were done on the pooled phase 2/3 data of healthy adults 18-50 years of age vaccinated with the main regimen (Ad26.ZEBOV/MVA-BN-Filo prime-boost with a 56-day interval):

1. Using the logistic model based on all available NHP data.

2. Stratified per baseline EBOV GP ELISA level (<LLOQ, LLOQ-100, >100- 1,000, above 1,000 EU/mL) using the logistic model based on NHP data of the main regimen. For each of these 4 subgroups, the mean predicted survival probability and its 95% CI was calculated.
3. Excluding the subjects of the Sierra Leone study (EBL3001) using the logistic model based on NHP data of the main regimen.
4. Stratified by age (18-30 and 31-50 years of age), sex, race (Asian, Black or African American, White and Other) and geographic region (East Africa [Kenya, Uganda, Mozambique, Tanzania], West Africa [Burkina Faso, Cote d'Ivoire, Sierra Leone, Nigeria], Europe and US) using the logistic model based on NHP data of the main regimen. For each of these subgroups, the mean predicted survival probability and its 95% CI was calculated.

Success criterion of 20%

As the level of vaccine-induced immune responses needed to achieve protection in NHP and people is unknown, a predefined success criterion for the immunobridging analysis was agreed according to which the lower limit of the CI has to be above 20% to be able to conclude on the inferred likelihood of protection.

This lower limit of the CI was chosen taking into account the following elements:

- In unvaccinated NHP having undetectable binding antibody concentrations (<LLOQ), the predicted survival probability is expected to be at most 0.00956% (i.e. the upper limit of the 95% CI around the predicted survival probability at the LLOQ). Any lower limit of above 10% would already exclude the uncertainty of the logistic regression model since it can rule out a false positive outcome. A lower limit of 20% provides an additional margin to indicate a true protective effect.

- In addition, a 20% limit is similar to thresholds employed in vaccine field efficacy studies to conclude on efficacy and support regulatory approval (e.g. dengue vaccine).

Interim analysis

The SAP mentions: A futility analysis was done to evaluate whether the lot-to-lot consistency study (EBL3004) can start and the development program can be continued. This futility analysis only evaluated the main regimen in healthy adults (18-50 years of age) using the logistic model based on the main regimen. Evaluation was done by calculating the mean predicted survival probability and its 95% CI based on pooled data of the following clinical studies: EBL2001, EBL2002, EBL3001, EBL3002 and EBL3003. As this is a futility analysis, this interim analysis is not intended to stop earlier for efficacy in case the primary objective would be met. Therefore, no adjustment of the CI alpha level was done, as only stopping the clinical development for futility is allowed.

The following non-binding futility criterion was used: If the lower bound of the 95% CI for the mean predicted survival probability is below 15%, the outcome of the analysis was declared futile. If the outcome is considered futile, it may be decided not to start the lot-to-lot consistency study. Otherwise, the lot-to-lot consistency study may start as planned and the final mean predicted survival probability is evaluated including the abovementioned studies as well as the lot-to-lot consistency study. The lower bound of the 95% CI of the mean predicted survival probability is then evaluated against the 20% success criterion.

The analysis was stopped for efficacy based on the interim results, as this might give a too optimistic estimate of the efficacy, adapted confidence intervals should be used for interpretation of the success criterion (see results).

Subgroups

Forest plots for the mean predicted survival probability together with its 95% CI for the primary analysis, as well as the subgroup analyses stratified by baseline EBOV GP ELISA level, age, sex, race and geographic region are shown.

Analyses of different vaccine interval regimens (28- and 84-day interval), paediatric and elderly data and other subgroups (e.g. HIV+ subjects) will not be evaluated using the immunobridging approach and these analyses will be described in the individual study SAPs.

Results

Participant flow and recruitment

Study EBL2001, Group 2 (Ad26,MVA; 56-day interval)

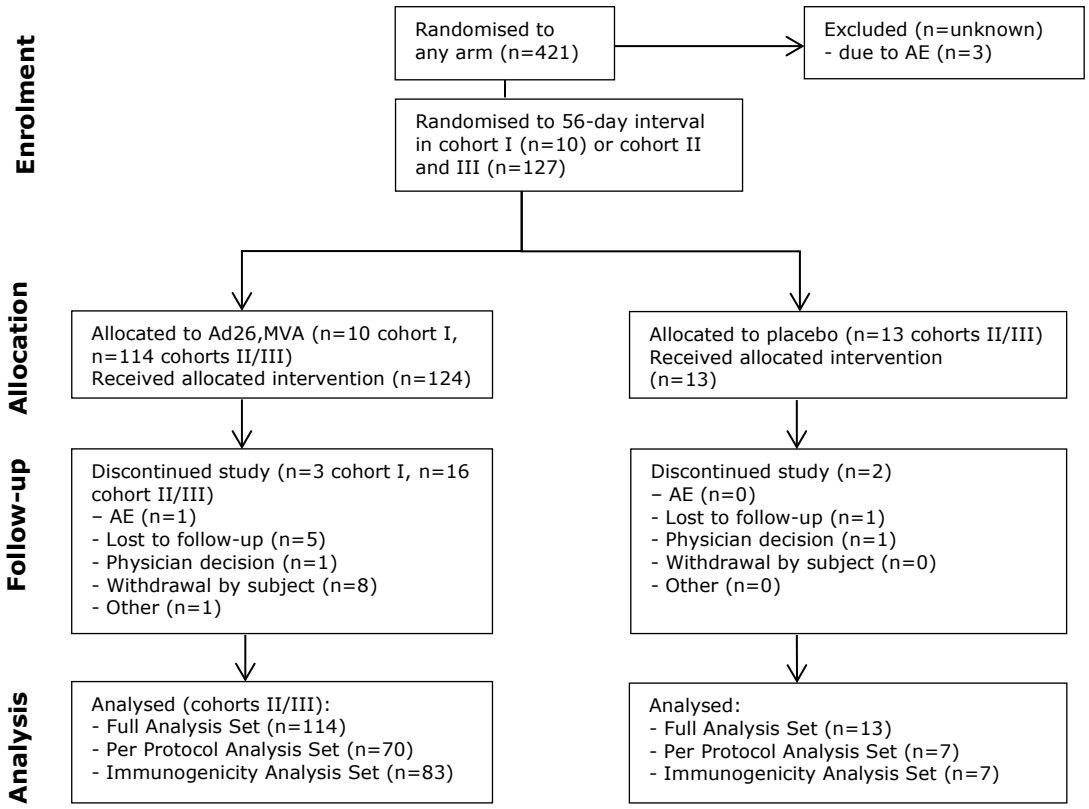


Figure 5: Participant flow in study EBL2001, group 2

The study was conducted at 2 sites in the **UK** and 7 sites in **France**.

First subject first visit: 18 June 2015, Last subject last visit: 19 January 2018.

Study EBL2002 (Ad26,MVA; 56-day interval)

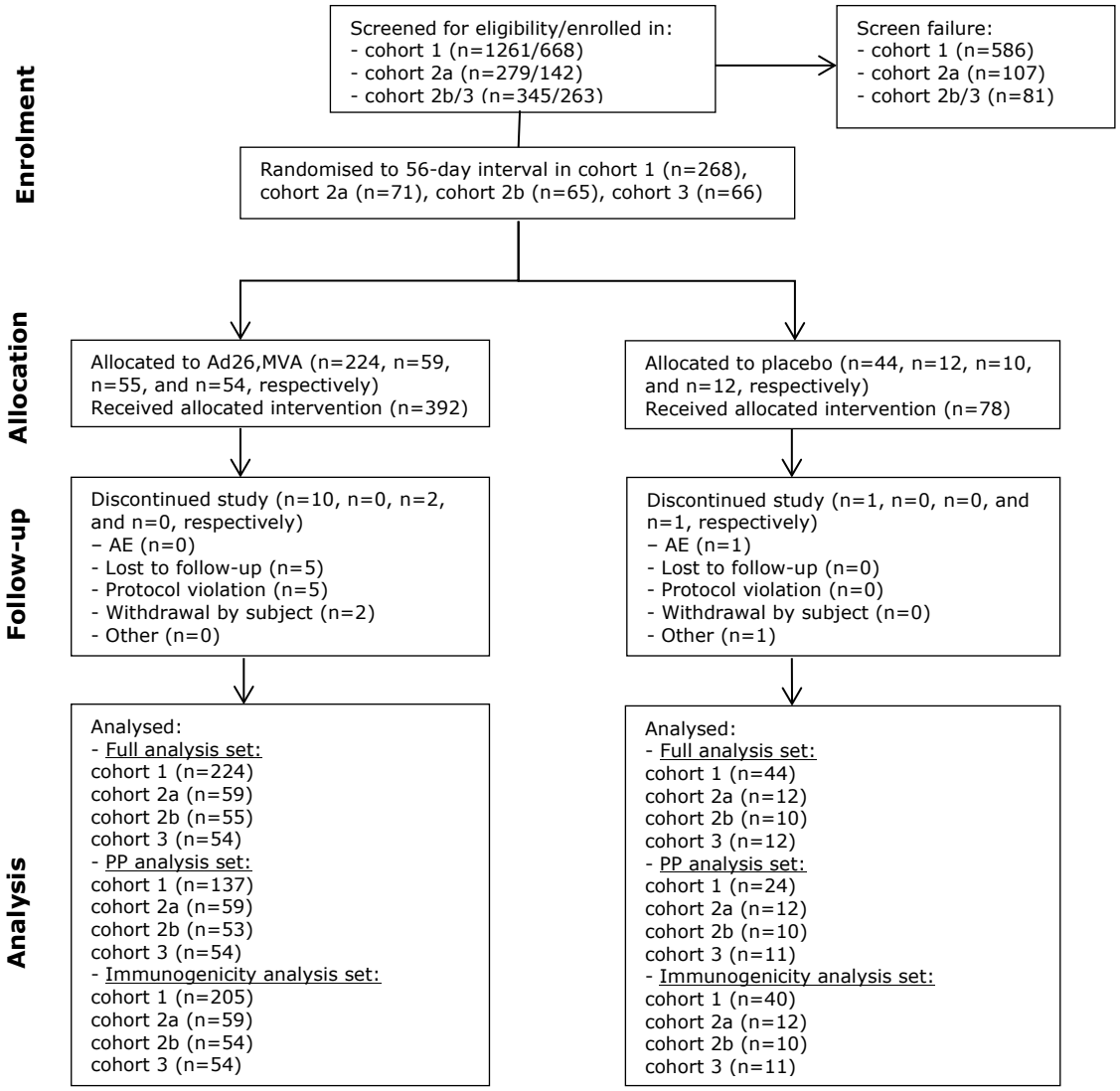


Figure 6: Participant flow in study EBL2002

The study was conducted in 4 countries in Africa: **Burkina Faso** (2 sites), **Cote d'Ivoire** (2 sites), **Kenya** (1 site), and **Uganda** (2 sites)

First subject first visit: 9 November 2015, Last subject last visit: 12 February 2019.

Study EBL3001

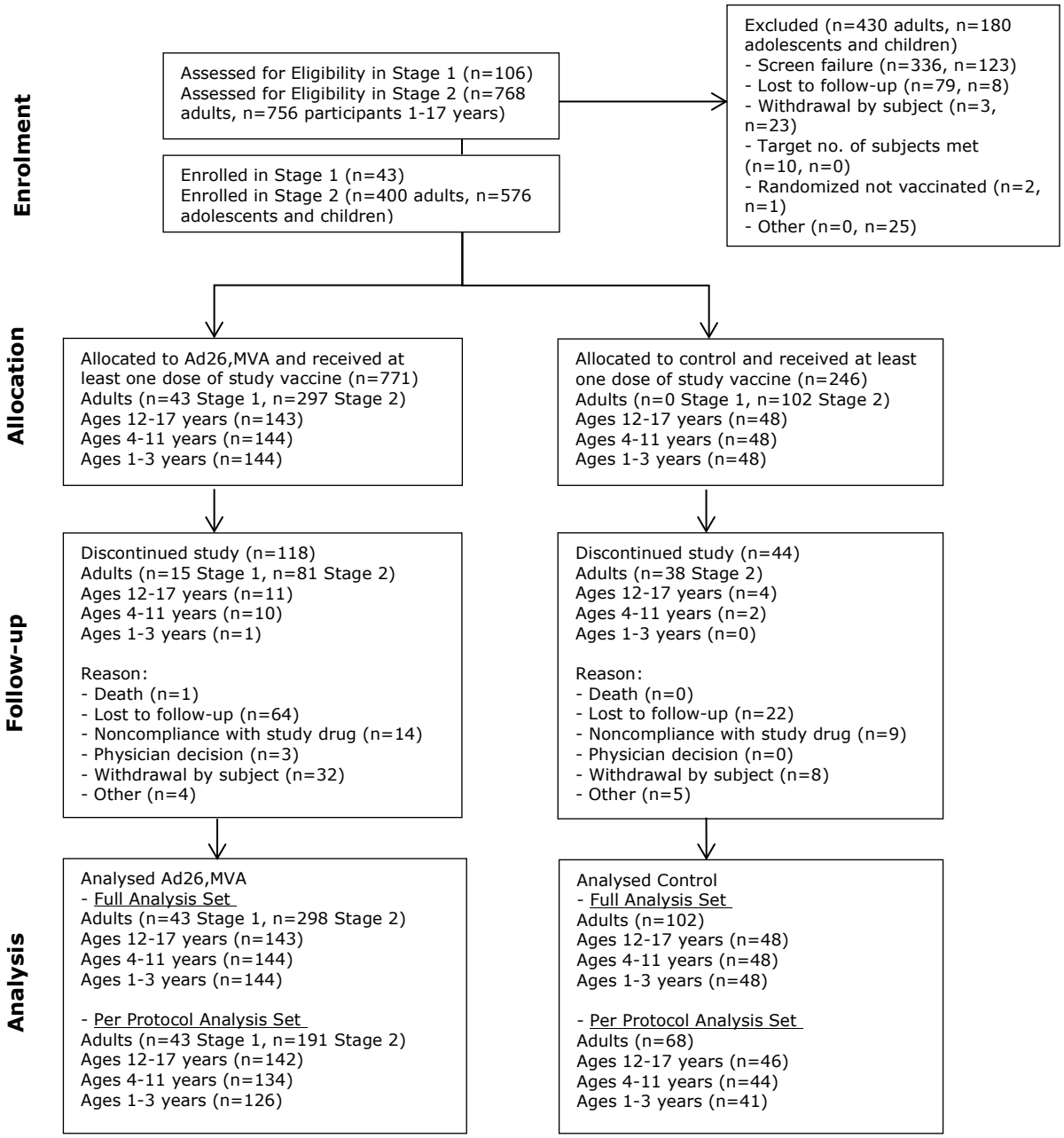


Figure 7: Participant flow in study EBL3001

The study was conducted at 1 site with 3 locations in **Sierra Leone**. Recruitment was as follows:

- Adults aged ≥18 years: First subject first visit: 30 September 2015, Last subject last visit for interim analysis: 28 November 2018.

- Adolescents aged 12-17 years: First subject first visit: 31 March 2016, Last subject last visit for interim analysis: 24 August 2018.

- Children aged 4-11 years: First subject first visit: 28 July 2017, Last subject last visit for interim analysis: 09 October 2018.

- Children aged 1-3 years: First subject first visit: 23 October 2017, Last subject last visit for interim analysis: 04 October 2018.

Study EBL3002 (Ad26,MVA; 56-day interval (group 1))

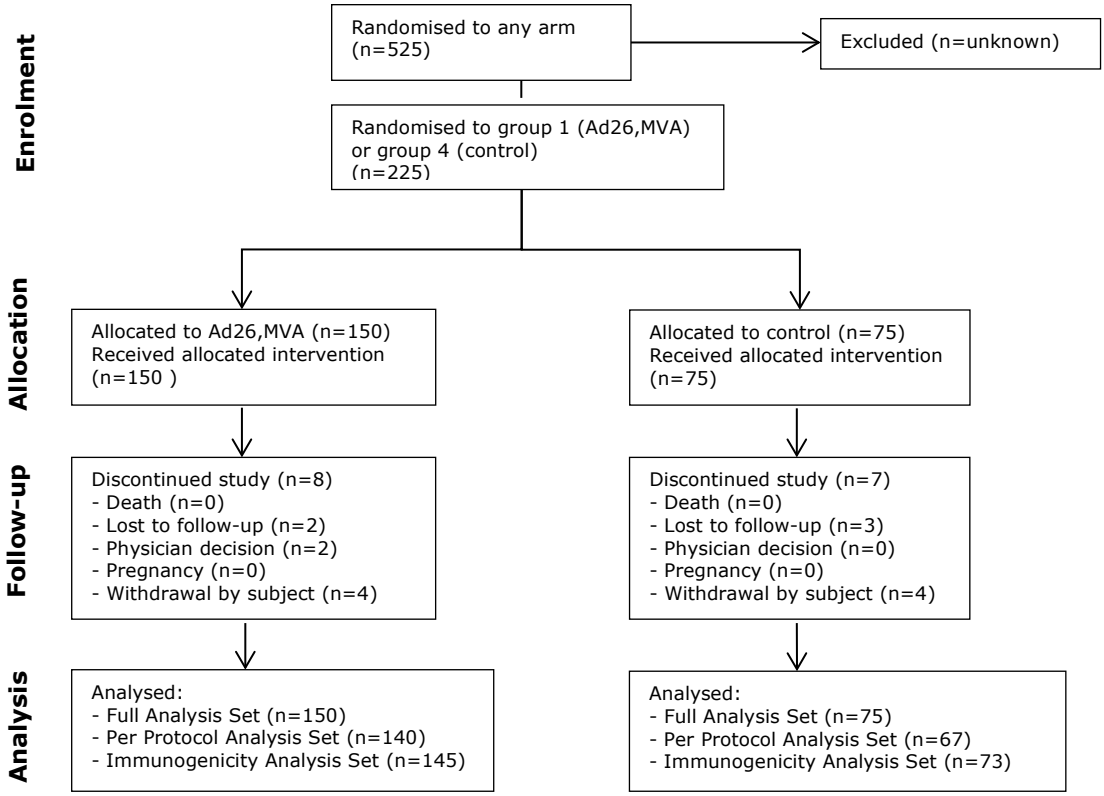


Figure 8: Participant flow in study EBL3002

The study was conducted at 4 sites in the **USA**.

First subject first visit: 30 July 2015, Last subject last visit: 29 November 2016

Study EBL3003

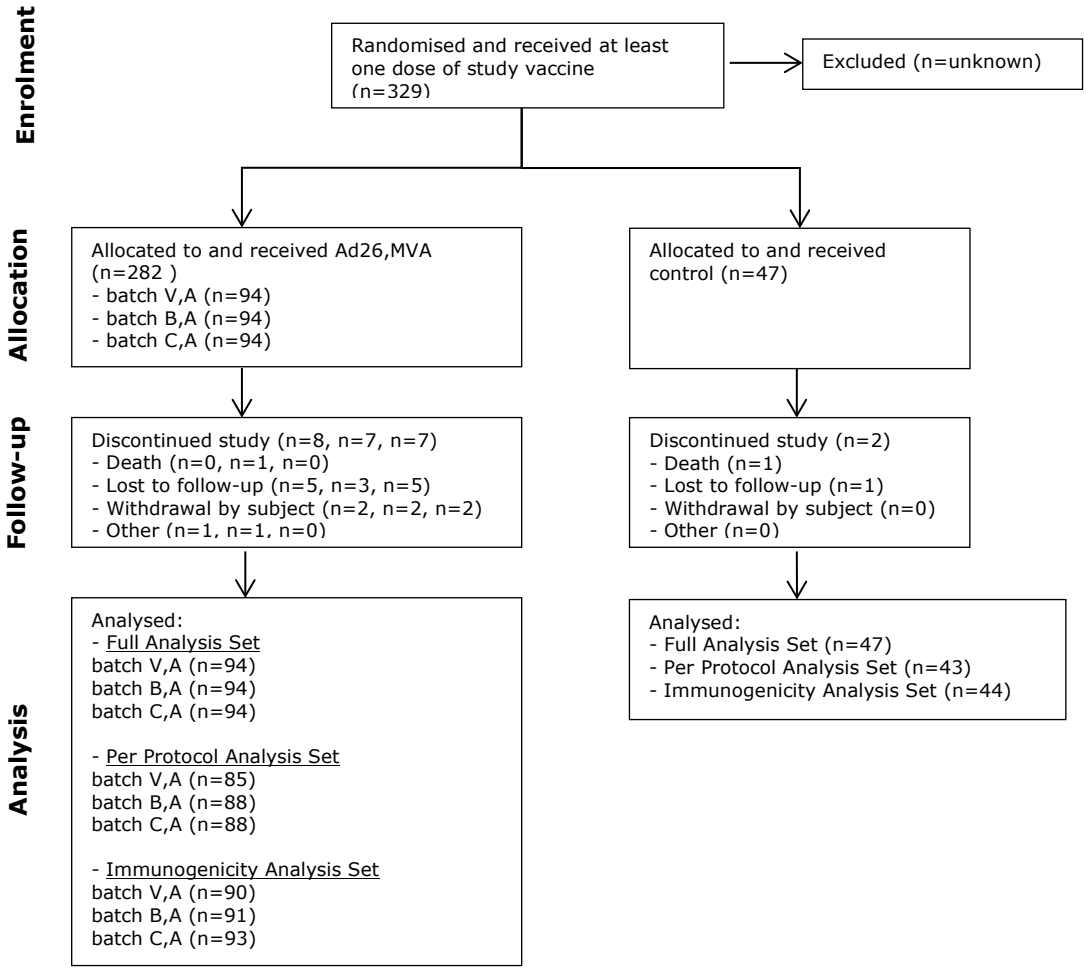


Figure 9: Participant flow in study EBL3003

The study was conducted at 3 sites in the **USA**.

First subject first visit: 21 September 2015, Last subject last visit: 20 July 2016.

Conduct of the study

The protocol of each of the main studies was amended several times; see Clinical AR for more information. Below, the main issues are discussed.

On 27 April 2016, study vaccinations in EBL2001 were halted due to the occurrence of a serious adverse event (Miller Fisher syndrome). Following IDMC recommendation, further evaluations and analyses were performed, and all study vaccinations were halted until the safety language of the ICF was updated. The study resumed on 09 May 2016 in France. On 11 May 2016, a second serious adverse event was reported ('possible cervical myelitis', later determined to be small fiber neuropathy). As a result of this second report, all screening and all study vaccinations across the program were halted per sponsor decision on 20 May 2016. The Medicines and Healthcare Products Regulatory Agency (MHRA) approved the resumption of the study in the UK on 27 September 2016. Per Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) decision, no further screening and study vaccinations took place in France. The initial report of 'possible cervical myelitis'

triggered a clinical hold issued by the Food and Drug Administration (FDA) on 26 May 2016 for all of the clinical studies of Ad26.ZEBOV and MVA-BN-Filo ongoing at that time. After receipt of follow-up information, on 16 June 2016, the FDA lifted the hold. The pause interrupted vaccination of subjects, some awaiting the first vaccination and some awaiting the second vaccination. This impacted all ongoing studies and resulted in subjects receiving the second vaccination later than planned (outside the window allowed by the protocol), or sometimes not at all.

The design of Study EBL3001 was changed, from a 3-stage study as originally planned to a 2-stage study only investigating safety and immunogenicity. This was due to the Ebola outbreak that subsided before any clinical efficacy data could be generated. Also, for Study EBL3003 the design was changed when the study was already ongoing. In this case, the aim of the study was changed from demonstration of immunologic equivalence of 3 different batches of Ad26.ZEBOV from 3 different virus seeds, to show equivalence between 2 batches only (WVS batch Bern and MVS batch Leiden). Also the timing of the primary endpoint analysis was changed from 21 days post dose 2 to 56 days post dose 1. As these changes were implemented <2 months after first subject first visit date, these changes did not result in differential treatment of subjects, and overall results are not impacted.

Baseline data

Adults

The mean age across participants included in the immunobridging analysis was 30.5 years. Most participants were male (65%) and had a baseline EBOV GP binding antibody concentration <LLOQ (74%). The majority of participants were from the United States (51%) and from African countries (43% in total, 29% from Sierra Leone). Other countries included France, United Kingdom, Burkina Faso, Côte d'Ivoire, Kenya, and Uganda. See Table 11

Table 11: Summary of Baseline and Demographic Characteristics for All Adults From Phase 1/2/3 Studies

Study (Country)	Age (years) N Mean (SD) Min; Max	BMI (kg/m^2) N Mean (SD) Min; Max	Sex N Male (%) Female (%)	Race N White (%) Black or African American (%) Other (%)
Phase 2				
EBL2001 (FRA, GBR)	223 41.9 (14.49) (19; 65)	223 25.03 (4.055) (17.7; 42.2)	223 101 (45.3%) 122 (54.7%)	223 194 (87%) 20 (9%) 9 (4%)
EBL2002 (BFA, CIV, KEN, UGA) – Healthy adults	400 33.5 (12.21) (18; 69)	400 23.36 (4.148) (16.8; 44.2)	400 256 (64%) 144 (36%)	400 1 (0.3%) 396 (99%) 3 (0.8%)
EBL2002 (BFA, CIV, KEN, UGA) – HIV-infected adults	140 38.8 (6.79) (18; 50)	140 24.16 (4.41) (15.8; 36.5)	140 43 (30.7%) 97 (69.3%)	140 0 140 (100%) 0
Phase 3				
EBL3001 (SLE)	302 27.5 (10.25) (18; 69)	302 21.92 (3.226) (15.4; 40.1)	302 258 (85.4%) 44 (14.6%)	302 0 302 (100%) 0
EBL3002 (USA)	207 33.8 (8.93) (18; 50)	207 28.11 (4.861) (19; 40.6)	207 110 (53.1%) 97 (46.9%)	207 168 (81.2%) 35 (16.9%) 4 (1.9%)
EBL3003 (USA)	304 32.4 (9.52) (18; 50)	304 28.18 (5.087) (17.4; 43.6)	304 161 (53%) 143 (47%)	304 175 (57.6%) 104 (34.2%) 25 (8.2%)
All studies				
Healthy adults	1891	1891	1891	1891

Study (Country)	Age (years) N Mean (SD) Min; Max	BMI (kg/m²) N Mean (SD) Min; Max	Sex N Male (%) Female (%)	Race N White (%) Black or African American (%) Other (%)
	33 (11.71) (18; 70)	25.04 (4.849) (15.4; 46.4)	1144 (60.5%) 747 (39.5%)	740 (39.1%) 1093 (57.8%) 58 (3.1%)
HIV-infected adults	164 40 (8.24) (18; 67)	164 24.53 (4.528) (15.8; 36.5)	164 64 (39%) 100 (61%)	164 6 (3.7%) 156 (95.1%) 2 (1.2%)

The analysis is based on the Immunogenicity Analysis Set for the Phase 1 studies, and on the Per Protocol Analysis Set for the Phase 2/3 studies.

[a] Only immunogenicity data of Part 1 (USA) of study EBL2003 are included in the Summary of Clinical Efficacy.
Source: [TSIDEM01-P123.RTF] [/SAS/Z_VAC52150/VAC52150ZSCE/FILES/RE/EFFICACYPOOL_2019_EMA_FDA/PROGRAMS/OBJECT SERVER] 28JUN2019, 03:18

Adolescents and children

The baseline and demographic characteristics for adolescents and children in the Per Protocol Analysis Set from studies EBL2002 and EBL3001 are provided in Table 12. The mean age of adolescents (12-17 years) and children (4 11 years) was 14.2 and 7.8 years, respectively. The mean age of the youngest children (1 3 years) in EBL3001 was 1.9 years. The majority of youngest children were male (58%), no relevant imbalance in sex distribution was observed for adolescents (53% male) or children (48% male). Studies EBL2002 and EBL3001 were conducted in Burkina Faso, Côte d'Ivoire, Kenya, Uganda, and Sierra Leone. The majority of adolescents and children (4 11 and 1-3 years) were of Black or African American heritage (99%-100%).

Table 12: Summary of Baseline and Demographic Characteristics for the Adolescents and Children From Studies EBL2002 and EBL3001

Study (Country) Age Group	Age (years) N Mean (SD) Min; Max	BMI (kg/m²) N Mean (SD) Min; Max	Weight-for-age Percentile N Median Q1; Q3	Weight-for-length Percentile N Median Q1; Q3	Sex N Male (%) Female (%)	Race N White (%) Black or African American (%) Other (%)
EBL2002 (BFA, CIV, KEN, UGA)						
12-17 years	127 14.3 (1.67) (11; 17)	127 19.03 (3.045) (13.3; 33.3)	- - -	- - -	127 69 (54.3%) 58 (45.7%)	127 0 127 (100%) 0
4-11 years	130 7.6 (2.11) (4; 11)	130 15.71 (1.8) (12.8; 26.6)	130 24.44 (13.93; 43.23)	- - -	130 65 (50%) 65 (50%)	130 0 130 (100%) 0
EBL3001 (SLE)						
12-17 years	188 14.2 (1.58) (12; 17)	188 18.71 (2.929) (13.5; 28)	- - -	- - -	188 99 (52.7%) 89 (47.3%)	188 2 (1.1%) 186 (98.9%) 0
4-11 years	178 8 (1.76) (4; 11)	178 15.45 (1.333) (8.9; 20.7)	178 23.02 (11.48; 44.82)	- - -	178 83 (46.6%) 95 (53.4%)	178 1 (0.6%) 177 (99.4%) 0
1-3 years	167 1.9 (0.77) (1; 3)	167 16.13 (1.359) (13.4; 25.3)	112 28.86 (11.97; 50.94)	55 44.99 (25.08; 60.82)	167 96 (57.5%) 71 (42.5%)	167 0 167 (100%) 0
All Studies						
12-17 years	315 14.2 (1.61) (11; 17)	315 18.84 (2.975) (13.3; 33.3)	- - -	- - -	315 168 (53.3%) 147 (46.7%)	315 2 (0.6%) 313 (99.4%) 0

Study (Country) Age Group	Age (years) N Mean (SD) Min; Max	BMI (kg/m²) N Mean (SD) Min; Max	Weight-for-age Percentile N Median Q1; Q3	Weight-for-length Percentile N Median Q1; Q3	Sex N Male (%) Female (%)	Race N White (%) Black or African American (%) Other (%)
4-11 years	308 7.8 (1.92) (4; 11)	308 15.56 (1.55) (8.9; 26.6)	308 23.48 (11.74; 43.89)	- - -	308 148 (48.1%) 160 (51.9%)	308 1 (0.3%) 307 (99.7%) 0
1-3 years	167 1.9 (0.77) (1; 3)	167 16.13 (1.359) (13.4; 25.3)	112 28.86 (11.97; 50.94)	55 44.99 (25.08; 60.82)	167 96 (57.5%) 71 (42.5%)	167 0 167 (100%) 0

The analysis is based on the Per Protocol Analysis Set.

Source: [TSIDEM01-PD.RTF][/SAS/Z_VAC52150/VAC52150ZSCE/FILES/RE/EFFICACYPOOL_2019_EMA_FDA/ PROGRAMS/OBJECT SERVER] 28JUN2019, 03:18

Numbers analysed

Table 13: Total number of subjects analyzed per study

	EBL 2001			EBL 2002				EBL 3001				EBL 3002				EBL 3003			
	Gr1 (C)	Gr2 (C)	Gr3 (C)	Adults (C)	HIV+ (C)	Adol. 12-17 (C)	Child. 4-11 (C)	Adults Stage 1+2 (C)	Adol. 12-17 (C)	Child. 4-11 (C)	Child. 1-3 (C)	Gr1	Gr2	Gr3	C	Gr1	Gr2	Gr3	C
FAS	112 (13)	114 (13)	106 (18)	559 (109)	118 (24)	110 (21)	108 (24)	43+298 (102)	143 (48)	144 (48)	144 (48)	150	150	150	75	94	94	94	47
PP	80 (8)	70 (7)	52 (6)	337 (63)	117 (23)	107 (20)	107 (23)	43+191 (68)	142 (46)	134 (44)	126 (41)	140	130	136	67	85	88	88	47
IG	92 (10)	83 (7)	62 (11)	527 (101)	117 (24)	109 (20)	108 (23)	-	-	-	-	145	146	144	73	90	91	93	44
Immunobridging Set*	45			115				215				135				254			

Gr.: Group; (C): Control; Adol.: Adolescents; Child.: Children. FAS: Full Analysis Set; PP: Per protocol population; IG: Immunogenicity population.

*Per protocol set with healthy adults (18-50 years of age) vaccinated with Ad26.ZEBOV, MVA-BN-Filo in a 56 day interval from 5 Phase 2/3 studies who had immunogenicity data at 21 days post Dose 2.

The main analysis, i.e. the immunobridging, was based on the "Per Protocol Immunogenicity Analysis Set". This included all randomized [and nonrandomized open label Stage 1 of study EBL3001] and vaccinated participants, who received both Dose 1 and Dose 2 vaccinations within the protocol defined window, had no major protocol deviations influencing the immune response, and had a 21-day post Dose 2 ELISA result). Not all studies contributed equally to the immunobridging analysis. The proportion of participants included in the PP analysis set (Ad26/MVA 56-day interval only), who have been included in the immunobridging set, are: study EBL2001: 45/70 (64%), EBL2002:115/137 (84%) , EBL3001: 215/234 (92%), EBL3002: 135/140 (96%) and EBL3003: 254/261 (97%). This is most likely due to the impact of the temporary study pause, which had a more pronounced impact on the phase 2 studies as compared to the phase 3 studies. More important is that all studies are represented, given the wide range of GMC values across studies. This seems to be the fact, as the study with the lowest GMC (EBL3001) and the study with the highest GMC (EBL3003) are both well represented.

Outcomes and estimation

- ***EBOV GP-specific Binding Antibody Responses of 2-dose Ad26.ZEBOV, MVA-BN-Filo Vaccine Regimen***

All intervals induced binding antibody responses after the first vaccination (i.e. 28, 56, or 84 days post Dose 1, depending on the interval) with GMC ranging between 236 and 1,156 EU/mL, which further increased at 21 days post Dose 2. In none of the control groups, a significant increase in GMCs was observed after either dose.

Lengthening the interval between the 2 doses from 28 to 56 days (in studies EBL2001 and EBL2002) increased the magnitude of the responses at 21 days post Dose 2 by approximately 2-fold, from 4,627 to 10,131 EU/mL for EBL2001 and from 3,085 to 7,518 EU/mL for EBL2002. GMC observed at 21 days post Dose 2 in the 56-day interval ranged between 3,810 and 11,790 EU/mL across the studies (Table 14). There was no additional increase in GMC for the 84-day interval in studies EBL2001 (11,312 EU/mL) and EBL2002 (7,300 EU/mL). The responder rates were similar for all intervals, ranging from 98% to 100%. Table 14 provides an overview of the GMC values for the main studies.

Table 14: EBOV GP Binding Antibody Concentrations (ELISA, EU/mL) at Selected Time Points From Phase 2/3 Studies

Study (Country) Regimen; Interval	Baseline N GMC (95% CI)	Pre-Dose 2 N GMC (95% CI) (% Responder)	21 Days Post Dose 2 N GMC (95% CI) (% Responder)
EBL2001 (FRA, GBR)			
Healthy adults	70 **<LLOQ** (<LLOQ; <LLOQ)	69 **880** (709; 1093) (96%)	69 **10131** (8554; 11999) (100%)
EBL2002 (BFA, CIV, KEN, UGA)			
Healthy adults	134 **39** (<LLOQ; 48)	136 **361** (307; 423) (80%)	136 **7518** (6468; 8740) (99%)
HIV+ adults	58 **<LLOQ** (<LLOQ; <LLOQ)	59 **291** (233; 364) (88%)	N59 **5283** (4094; 6817) (100%)
Adolescents 12-17 years	53 **<LLOQ** (<LLOQ; <LLOQ)	53 **619** (490; 782) (93%)	53 **13,532** (10,732; 17,061) (100%)
Children 4-11 years	52 **<LLOQ** (<LLOQ; <LLOQ)	54 **658** (556; 780) (98%)	53 **17,388** (12,973; 23,306) (100%)
EBL3001 (SLE)			
Healthy adults (Stage 1 Open Label)	43 **60** (40; 90)	43 **269** (208; 347) (65%)	42 **4784** (3736; 6125) (98%)
Healthy adults (Stage 2 Randomized)	188 **69** (56; 85)	190 **236** (206; 270) (54%)	182 **3810** (3312; 4383) (98%)
Adolescents 12-17 years	142 **65** (52; 81)	142 **314** (269; 366) (64%)	134 **9,929** (8,172; 12,064) (98%)
Children 4-11 years	130 **62** (49; 78)	133 **390** (334; 456) (71%)	124 **10,212** (8,419; 12,388) (99%)

Study (Country) Regimen; Interval	Baseline N GMC (95% CI)	Pre-Dose 2 N GMC (95% CI) (% Responder)	21 Days Post Dose 2 N GMC (95% CI) (% Responder)
Children 1-3 years	123 **<LLOQ** (<LLOQ; <LLOQ)	125 **693** (591; 812) (94%)	123 **22,452** (18,305; 27,538) (98%)
EBL3002 (USA)			
Healthy adults[a]	140 **<LLOQ** (<LLOQ; <LLOQ)	140 **793** (698; 902) (96%)	135 **11054** (9673; 12633) (100%)
EBL3003 (USA)			
Healthy adults (Ad26 Batch V[b])	85 **<LLOQ** (<LLOQ; <LLOQ)	85 **813** (632; 1046) (96%)	81 **11089** (9323; 13189) (100%)
Healthy adults (Ad26 BatchB[b])	86 **<LLOQ** (<LLOQ; <LLOQ)	88 **745** (603; 921) (96%)	87 **10337** (8660; 12339) (100%)
Healthy adults (Ad26 BatchC[b])	87 **<LLOQ** (<LLOQ; <LLOQ)	88 **851** (720; 1006) (100%)	86 **11790** (9701; 14328) (100%)

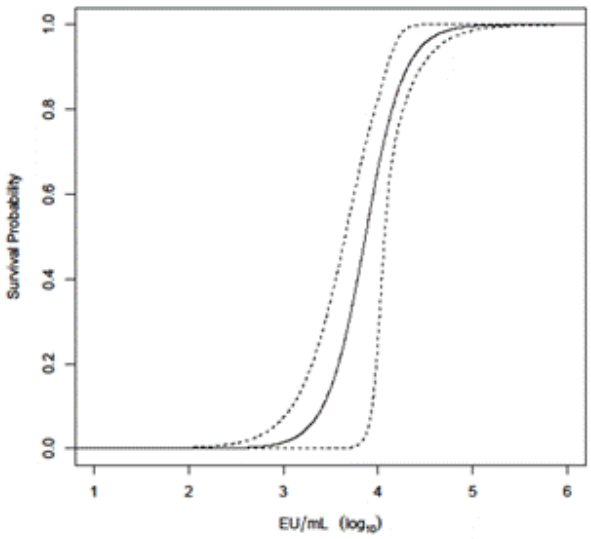
The analysis is based on the Per Protocol Analysis Set.

a Includes only participants in the Ad26.ZEBOV 5x10^{10} vp, MVA-BN-Filo 1x10^{8} Inf.U regimen.
Source: [TIMHUM03-P23.RTF][/SAS/Z_VAC52150/VAC52150ZSCE/FILES/RE/EFFICACYPOOL_2019_EMA_FDA/PROGRAMS/OBJECT SERVER] 28JUN2019, 03:18

b B: WVS batch Bern; C: MVS batch Leiden, V: WVS batch Leiden

- ***Immunobridging***

Based on the 66 NHPs vaccinated with the selected dose regimen and 56-day interval with ELISA data available, a logistic regression model with 95% confidence band (bootstrap-derived using 10,000 bootstraps of the NHP data of the main regimen) was constructed (Figure 10). This model was used for the immunobridging of human immunogenicity results. As a sensitivity analysis, a similar model was constructed based on the 108 NHPs vaccinated with Ad26.ZEBOV/Ad26.Filo and MVA-BN-Filo (independent of the order and interval between the 2 vaccine doses) with ELISA data available.



Black line: fitted logistic regression model. Dashed lines indicate the 95% confidence band around the fitted logistic regression model.

Figure 10: Logistic Regression Model for Main Regimen

An interim immunobridging analysis was performed 21 days post Dose 2 on the pooled dataset of healthy adults (18-50 years of age) vaccinated with Ad26.ZEBOV, MVA-BN-Filo in a 56-day interval from 5 Phase 2/3 studies. As this pre-specified interim immunobridging analysis was originally intended as a futility analysis, no adjustment of the 95% CI alpha level was foreseen. Since this interim analysis now serves the purpose of an efficacy interim analysis with the ability to conclude on the likely efficacy of the vaccine regimen, a post hoc O'Brien-Fleming approach was adopted, as this approach is conservative and regularly used in interim analyses. Assuming that 65% of the data was collected, a 98.68% CI (one-sided alpha=0.0066, number of bootstraps increased from 10,000 to 100,000) was used to correct for the fact that multiple analyses would be performed. As a sensitivity analysis, an even more stringent correction (one-sided alpha=0.0001, 99.98% CI) was also applied.

Based on the pooled data from 764 healthy adults, the mean predicted survival probability is 53.4% and the lower limit of the 95% CI is 33.8% using post-hoc O'Brien Fleming correction, well above the pre-specified success criterion of 20% (Table 15). This analysis demonstrates the likelihood of protection of the Ad26.ZEBOV, MVA-BN-Filo regimen in healthy adults.

The SAP specified that the immunobridging analyses will be provided for the PPI population and the FAS. The results of the immunobridging based on the FAS were provided upon request. There are no major differences between the FAS and PP GMC values, except for study EBL2002 in which a 1.3-fold increased GMC value is observed in the FAS (10042) as compared to the PP (7518) population. As the FAS GMCs are not lower than the PP GMCs, for none of the studies nor subgroups, the differences in the number of participants included in the FAS and PP population have not negatively affected the outcome of the studies.

Table 15: Immunobridging Analysis Using the Logistic Regression Model Based on Data From NHP Vaccinated With the Ad26.ZEBOV, MVA-BN-Filo Vaccine Regimen in a 56-day Interval, Including O'Brien-Fleming Adjustment

	Ad26.ZEBOV, MVA-BN-Filo 56-day Interval
N	764
Interim Analysis	
Mean Predicted Survival Probability (95% CI)	53.4% (36.7%; 67.4%)
Post hoc analyses	
Mean Predicted Survival Probability (98.68% CI) O'Brien-Fleming Adjustment (one-sided alpha of 0.0066)[a]	**53.4% (33.8%; 70.9%)**
Mean Predicted Survival Probability (99.98% CI) (one-sided alpha of 0.0001)[b]	53.4% (28.4%; 80.8%)

CI: bootstrapped confidence interval.
The interim analysis is based on the pooled Phase 2/3 data of healthy adults (18-50 years of age) (EBL2001, EBL2002, EBL3001, EBL3002, and EBL3003), using the logistic regression model based on NHP data from the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval; Per Protocol Immunogenicity Analysis Set.

[a] The first post hoc analysis applies the O'Brien-Fleming alpha spending rule: with approximately 65% of the data being available at the time of the interim analysis, the O'Brien-Fleming adjusted one-sided alpha is 0.0066, leading to a 98.68% CI. The CI is calculated based on 100,000 bootstraps.

[b] The second post hoc analysis does not apply a formal alpha spending rule but utilizes a very low one-sided alpha level of 0.0001 (and hence a CI of 99.98%). The CI is calculated based on 100,000 bootstraps.

Source: [TIBHUM01-PP.RTF][/SAS/4223/Z_IM_BRIDGING/FILES/RE/POOLEDIMBRIDGING/PROGRAMS /TIBHUM01.SAS]01OCT2018, 11:54; [TIBHUM01-B-PP.RTF][/SAS/4223/Z_IM_BRIDGING/FILES/RE/POOLEDIMBRIDGING/PROGRAMS /TIBHUM01B.SAS]26APR2019, 05:14; [TIBHUM01-C-PP.RTF][/SAS/4223/Z_IM_BRIDGING/FILES/RE/POOLEDIMBRIDGING/PROGRAMS /TIBHUM01C.SAS]26APR2019, 03:31

Using the logistic model based on the NHP dataset from all vaccine regimens combined, the mean predicted survival probability is 45.6%, with a lower limit of the 95% CI of 34.8%. The outcome of this sensitivity analysis is consistent with the results obtained in the primary interim immunobridging analysis.

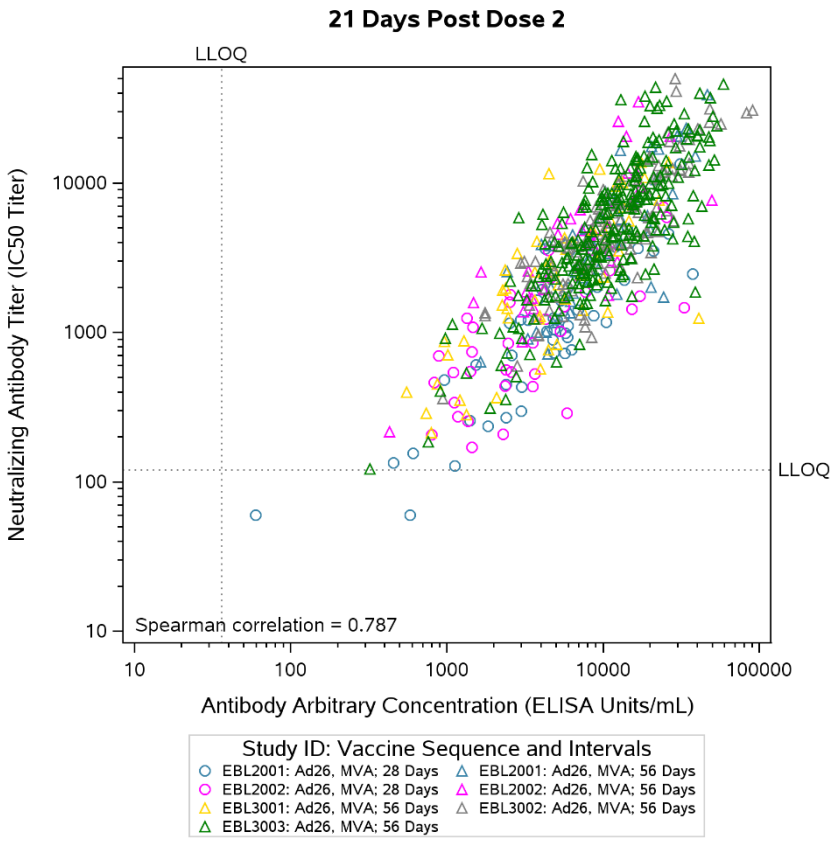
Forest plots for the mean predicted survival probability together with its 95% CI for the primary analysis, as well as the subgroup analyses stratified by baseline EBOV GP ELISA level, age, sex, race, and geographic region were generated The pre-specified sensitivity analyses were repeated on the 'model from all vaccine regimens combined', based on the NHP dataset containing all available NHP data (including data from NHP vaccinated with other regimens and intervals).

- ***Other Immunological Assessments***

EBOV GP-Specific Neutralizing Antibody Responses (psVNA)

EBOV GP-specific neutralizing antibody responses were analysed in all 5 clinical studies. Across studies, 97% to 100% of participants showed a neutralizing antibody response at 21 days post Dose 2 with GMTs ranging between 1,700 and 6,555 IC50 titer.

A positive correlation was observed between EBOV GP-specific binding antibody concentrations (ELISA) and neutralizing antibody titers (psVNA) measured 21 days post Dose 2, as shown in Figure 11 for the pooled dataset of the 5 Phase 2 and 3 studies (Spearman coefficient: 0.787).



The analysis is based on the Per Protocol Analysis Set.
Placebo/control participants are excluded from this display. For ELISA, LLOQ for all studies is 36.11 EU/mL. Values below the LLOQ are imputed with half of the LLOQ. For psVNA, LLOQ for all studies is 120 IC_{50} titer. Values below the LLOQ are imputed with half of the LLOQ Source: [GIMHUMCORR01-P23.RTF]
[/SAS/Z_VAC52150/VAC52150ZSCE/FILES/RE/EFFICACYPOOL_2019_EMA_FDA/ PROGRAMS/OBJECT SERVER] 01AUG2019, 10:51

Figure 11: Correlation Analysis Between EBOV GP Binding Antibody Concentrations and Neutralizing Antibody Titers at 21 Days Post Dose 2 on the Pooled Healthy Adult Data Set From Phase 2 and 3 Studies

<u>BOV GP-Specific Cellular Immune Responses (CMI)</u>

EBOV GP-specific cellular immune responses were evaluated in a subset of participants from 4 VAC52150 Phase 1 studies, study EBL2002 (IFN γ ELISpot and ICS), and study EBL2001 (ICS).

In both phase 2 studies, low T cells responses were observed at 21 days post MVA-BN-Filo vaccination.

EBL2001, ICS:

- CD4+ T cell responses: responder rate 37%, median 0.15% (IQR: 0.11%; 0.20%)
- CD8+ T cell responses: responder rate 55%, median 0.12% (IQR: 0.07%; 0.95%)

EBL2002, IFN-γ ELISpot:

- Healthy adults and elderly: responder rate 27%, median 61 SFU/10^6 PBMC (IQR: <50; 105)
- HIV-1 infected adults: responder rate 17%, median <50 SFU/10^6 PBMC (IQR: <50; 105),
- Adolescents: responder rate: 29%, median: 99 SFU/10^6 PBMC (IQR: <50; 122)

- Children aged 4-11 years: responder rate: 25%, median: 70 SFU/10^6 PBMC (IQR: <50; 117)

In EBL2002, IFN-γ and/or IL-2 and/or TNF-α producing CD4+ and CD8+ T-cells were also determined using ICS. In healthy adults and elderly, CD4+ T-cell responses were observed 21 days post dose 2 in 50% and 32% of subjects following the 28-day and 56-day schedule respectively. The median percentage of CD4+ T-cells producing at least 1 of the 3 investigated cytokines (IFN-γ, IL-2, TNF-α) tended to be higher for the 28-day (0.11%) compared to the 56-day (0.06%) interval schedule. Considering the small numbers of participants, the CD4+ and CD8+ T-cell responses in HIV-1 infected adults, or in healthy adolescents and children, were not different from those observed in the HIV-uninfected adult and elderly population.

At 1-year post dose 1 (Day 365), CD4+ T-cell responses were observed in 7% of participants in the 28-day interval schedule (median observed value: <LLOQ) and 9% of participants in the 56-day interval schedule (median observed value: <LLOQ).

Limited EBOV GP-specific CD8+ T-cell responses were observed at 21 days post dose 2 in 29% of the participants in the 28-day interval schedule (median: 0.05%) and 30% of the participants in the 56-day interval (median: <LLOQ). At 1-year post dose 1 (Day 365), CD8+ T-cell responses were observed in 16% of the participants in the 28-day interval schedule (median: <LLOQ) and 3% of the participants in the 56-day interval schedule (median: <LLOQ).

- ***Antibody persistence and Booster dose***

To assess whether vaccination with the 2-dose Ad26.ZEBOV, MVA-BN-Filo vaccine regimen had induced immune memory, a booster vaccination with Ad26.ZEBOV was provided to participants who had received the 2-dose vaccine regimen in a 28-day interval in studies EBL1002 and EBL2002, or in a 56 day interval in studies EBL2002 and EBL3001. The booster dose was administered at 1 year (EBL1002 and EBL2002) or at 2 years (EBL3001) post Dose 1.

One year post Dose 1, binding antibody responses persisted in 100% of participants in study EBL1002 (GMC: 2,343 EU/mL), and in 77%-80% of participants in the African studies EBL2002 and EBL3001 Stage 1 (GMC range: 313-342 EU/mL), for the 28- or 56-day intervals. Two years post Dose 1, binding antibody responses persisted in 68% of participants in study EBL3001 Stage 1 (GMC: 279 EU/mL).

In studies EBL1002 and EBL2002, the booster vaccination administered 1 year after the initial vaccination resulted in a rapid activation of an anamnestic response at 7 days post booster in 100% of participants, with an approximate 12-fold increase in study EBL1002 (from 2,243 to 27,920 EU/mL; 28 day interval) and 55 fold increase in study EBL2002 (from 301 to 16,639 EU/mL for the 28-day interval and from 366 to 20,416 EU/mL for the 56-day interval) in binding antibody GMC as compared to the pre booster time point. The binding antibody responses were further increased at the 21-day post booster time point, irrespective of the initial vaccination interval (GMC range: 29,315 to 42,237 EU/mL).

Similarly, in study EBL3001, the booster vaccination administered 2 years post Dose 1 resulted in a rapid activation of an anamnestic response at 7 days post booster in 96% of participants, with an approximate 40-fold increase (from 274 to 11,166 EU/mL) in binding antibody GMC. The magnitude of the observed binding antibody responses further increased 2-fold towards the 21-day post booster time point (GMC: 30,411 EU/mL; 100% responder rate) (Table 16). In all studies, the GMC at 7 days post booster were 2- to 9-fold higher than the GMC observed at 21 days post Dose 2 (Table 16).

In study EBL1002, a booster vaccination with Ad26.ZEBOV also induced an anamnestic response in 100% of participants vaccinated with the MVA-BN-Filo, Ad26.ZEBOV vaccine regimen (irrespective of the interval).

Table 16: EBOV GP Binding Antibody Concentrations (ELISA, EU/mL) Pre- and Post Ad26.ZEBOV Booster for the Healthy Adult Data Set From Phase 1/2/3 Studies

Study (Country) Regimen; Interval	Pre-Booster N GMC (95% CI)	2/4 Days Post Booster[a] N GMC (95% CI) (% Responder)	7 Days Post Booster N GMC (95% CI) (% Responder)	21 Days Post Booster N GMC (95% CI) (% Responder)
EBL1002 (USA)				
Ad26, MVA: 28 days; Ad26 booster 1 year post Dose 1	13 **2243** (1131; 4447)	13 **2422** (1155; 5080) (100%)	13 **27920** (15517; 50237) (100%)	13 **42237** (25545; 69836) (100%)
EBL2002 (BFA, CIV, KEN, UGA)				
Ad26, MVA: 28 days; Ad26 booster 1 year post Dose 1	32 **301** (215; 422)	33 **386** (268; 558) (77.4%)	33 **16639** (12567; 22030) (100%)	33 **29315** (20614; 41689) (100%)
Ad26, MVA: 56 days; Ad26 booster 1 year post Dose 1	39 **366** (273; 491)	39 **551** (401; 756) (73%)	39 **20416** (15432; 27009) (100%)	39 **41643** (32045; 54116) (100%)
EBL3001 (SLE)				
Ad26, MVA: 56 days; Ad26 booster 2 years post Dose 1 (Stage 1 Open Label)	29 **274** (193; 387)	27 **304** (211; 440) (70.4%)	25 **11166** (5881; 21201) (96%)	29 **30411** (21972; 42091) (100%)

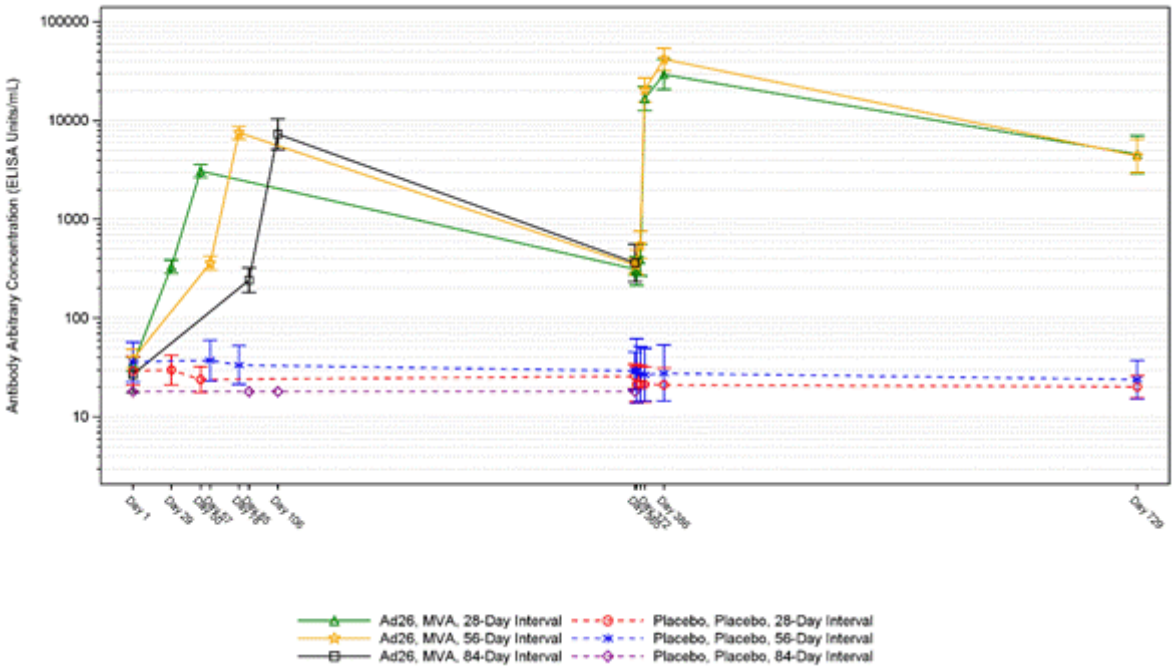
The analysis is based on the Immunogenicity Analysis Set for the Phase 1 studies, and on the Per Protocol Analysis Set for the Phase 2/3 studies. The regimens where high doses of Ad26.ZEBOV and MVA-BN-Filo were administered are not shown. Responder rates are calculated versus baseline (Day 1).

[a] Two days post booster in study EBL1002, 4 days post booster in study EBL2002 and EBL3001.

[b] Placebo/control groups are pooled.

Source: [TIMHUM03-B123.RTF][/SAS/Z_VAC52150/VAC52150ZSCE/FILES/RE/EFFICACYPOOL_2019_EMA_FDA/PROGRAMS/OBJECT SERVER] 19JUL2019, 09:26

After the 21-day post booster time point, the binding antibody concentrations gradually decreased, as shown for EBL2002 (Figure 12) and confirmed in EBL1002 and EBL3001. One-year post booster responses persisted in 97%-100% of participants with similar GMC observed across the 3 studies (GMC range: 3,237 4,534 EU/mL), that were higher than the level observed prior to administration of the booster dose. In studies EBL2002 and EBL3001, the GMC at 1-year post booster was approximately 10 fold higher compared to 1 and 2 years post Dose 1 (pre booster), respectively (Table 16).



The analysis is based on the Per Protocol Analysis Set.
The error bars represent the GMC and its 95% CI.
Day 1: Baseline; Day 29: 28 days post Dose 1; Day 50, Day 78, Day 106: 21 days post Dose 2;
Day 57: 56 days post Dose 1; Day 85: 84 days post Dose 1; Day 365: 364 days post
Dose 1; Day 372: 7 days post booster; Day 386: 21 days post booster; Day 729: 364 days post booster.
Labels for following time point tickmarks are omitted: Pre-booster; Day 369 (4 days post booster).

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Figure 12: EBOV GP Binding Antibody Responses (ELISA, EU/mL) for the Healthy Adult Data Set From Study EBL2002

Ancillary analyses

Since variation in EBOV GP-specific binding antibody GMC post vaccination was observed across countries, a linear regression analysis was performed to identify potential confounding factors. Evaluated factors included age, sex, BMI, baseline EBOV GP binding antibody concentrations, and geographical location that may have an effect on the pooled EBOV GP-specific binding antibody concentrations measured in the 5 Phase 2/3 studies including all healthy adult participants. EBOV GP-specific binding antibody concentrations at 21 days post Dose 2 were regressed on sex, age, BMI, baseline EBOV GP binding antibody concentrations, and country, with Burkina Faso arbitrarily chosen as reference country.

The results indicated that at 21 days post Dose 2, vaccinated male participants are expected to have EBOV GP-specific binding antibody concentrations that are approximately 21% (0.1022 log10 units) lower compared to vaccinated female participants.

The factor age was negatively associated with vaccine-induced EBOV GP-specific binding antibody concentrations measured at 21 days post Dose 2. In the analysis, one age unit constitutes 10 years, meaning that with every 10 years increase in age, the 21-days post Dose 2 EBOV GP-specific binding antibody concentrations are expected to decrease with approximately 11% (0.0506 log10 units).

Baseline positivity in the EBOV GP FANG ELISA was positively associated with vaccine induced EBOV GP-specific binding antibody concentrations measured at 21 days post Dose 2. For each log10 unit increase in baseline binding antibody concentrations, the 21 days post Dose 2 EBOV GP-specific binding antibody concentrations are expected to increase with approximately 25% (0.0973 log10 units).

While the United Kingdom, Sierra Leone, and the United States had statistically significant different EBOV GP-specific binding antibody concentrations at 21 days post Dose 2 than the reference country Burkina Faso, the F-test is more informative since it tests all countries simultaneously without choosing one particular reference country. The F-test reached statistical significance ($p<0.0001$), demonstrating that inclusion of the control variables sex, age, BMI, and baseline EBOV GP binding antibody concentrations did not sufficiently explain the differences observed in EBOV GP-specific binding antibody concentrations between the different countries. If the observed country differences were solely attributable to differences in terms of age, sex, BMI, and/or baseline ELISA values across the countries, then the country variable would no longer have reached statistical significance in this analysis.

Although the regression analysis indicated that several variables were statistically associated with the 21 days post Dose 2 EBOV GP-specific binding antibody concentrations, the goodness-of-fit measure for linear regression models, adjusted R^2, had a value of 0.2325, indicating that only about 23% of the variability in 21 days post Dose 2 EBOV GP-specific binding antibody concentrations can be explained by the included variables.

To directly assess a **potential impact of baseline EBOV GP FANG ELISA positivity**, which was observed in 0%-59% of participants across studies, on mean predicted survival probability, a post hoc immunobridging subanalysis stratified by baseline EBOV GP ELISA level restricted to the EBL3001 Sierra Leone participants was performed. Results were suggestive that baseline ELISA values did not significantly influence the post vaccination GMCs.

To further explore a potential link between baseline ELISA values and values 21 days post Dose 2 on an individual level, a correlation analysis was also performed. If baseline positivity was an indication of priming of the immune system, one would expect to see a positive correlation between baseline and post Dose 2 ELISA values. A negligible correlation (Spearman coefficient: 0.104) was observed.

In summary, these additional analyses do not indicate an obvious positive (anamnestic response) or negative (immune interference) effect of baseline ELISA positivity on the immunogenicity and mean predicted survival probability for participants from Sierra Leone (EBL3001). The observed baseline positivity could be due to either previous exposure to natural Ebola virus infection, or due to assay cross-reactivity and/or nonspecific binding. Unexpected baseline positivity was already observed during EBOV GP FANG ELISA assay qualification, prior to assay validation. Several investigations aiming to improve the specificity and reduce the baseline background signal were unsuccessful and baseline positivity was found to be in part explained by cross-reactivity caused by CMV-specific antibodies. In the same investigation, a 2-fold or greater increase in EBOV GP-specific antibody concentration post vaccination was shown to predominantly represent a response specific to the vaccination.

Pre-existing immune responses against Ad26 and MVA vector were assessed in several clinical trials. In the Phase 1 studies **EBL1001, EBL1002**, and FLV1001 conducted in the United Kingdom and the United States, Ad26 neutralizing antibodies were present in few participants (3% - 13%), at low titers prior to vaccination. In the African studies EBL1003, EBL1004, EBL2002, and EBL3001, Ad26 neutralizing antibodies were present at baseline in the majority of participants (82%-93%), but GMTs were relatively low (106-152 IC90 titer).

The impact of Ad26 pre-existing immunity on the vaccine-induced EBOV GP-specific immune responses at 21 days post Dose 2 was assessed by summarizing 21-day post Dose 2 data by baseline Ad26 antibody level in all individual studies, as well as by correlation analyses. In the individual studies, there was no apparent influence of the presence of pre-existing Ad26 antibodies on the geometric mean binding antibody response levels at 21 days post Dose 2. Furthermore, negligible or low negative correlations were observed between pre-existing Ad26 neutralizing antibodies and 21 days post Dose 2 EBOV GP-specific binding antibody responses (Spearman coefficient Phase 1: 0.047, Phase 2/3: 0.063). In line with the binding antibody responses, Ad26 pre-existing immunity also had no impact on the EBOV GP-specific neutralizing antibody responses and IFN-γ, CD4+, and CD8+ T cell responses.

In the Phase 1 studies EBL1001 and EBL1002, conducted in the United Kingdom and the United States, MVA neutralizing antibodies were present in few participants, at low titers prior to vaccination (11% and 15%). Pre-existing immunity to MVA was very low to absent in the African studies EBL1003 and 1004 (0% and 1%). In the Phase 3 African study EBL3001, MVA neutralizing antibodies were present at baseline in 5%-8% of adult participants.

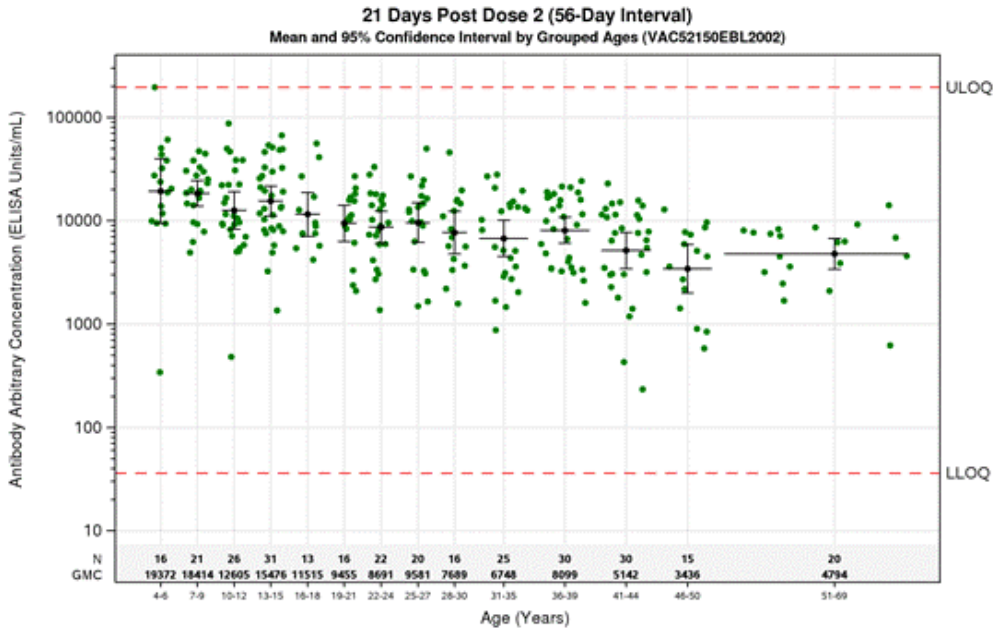
A negligible correlation was observed between pre-existing MVA neutralizing antibodies and EBOV GP-specific binding antibody responses 21 days post Dose 2 for the pooled Phase 1 data (Spearman coefficient: 0.041), indicating that pre-existing immunity for the MVA vector did not have an impact on the vaccine-induced binding antibody responses.

Analysis performed across trials (pooled analyses and meta-analysis)

Comparative Immunogenicity Across Age Groups

In adults, a weak trend towards decreasing EBOV GP-specific binding antibody concentrations with increasing age was observed in study EBL2002, but no apparent differences were observed in any of the other studies across the different age categories. In addition, as indicated in the immunobridging analysis stratified by age showed a similar mean predicted survival probability in the 18-30 and 31-50 years age categories. Also, data from studies EBL2002 and EBL3001 indicate no decrease in the binding antibody response in adults above the age of 50 years (51-69 years) compared to adults below the age of 50 (Figure 13 and Figure 14). The regimen can therefore be expected to provide similar protection in older and younger adults.

In studies EBL2002 and EBL3001 that included adolescents (12-17 years) and children (4-11 years and 1-3 years), an age-dependent trend in the magnitude of the response to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was apparent, with higher binding antibody responses observed at any time point in adolescents (12-17 years) and children (4-11 and 1-3 years) as compared to adults vaccinated in the same interval within the same study (Figure 13 and Figure 14).



The analysis is based on the Per Protocol Analysis Set.
Green dots represent individual data points (jittering applied). Solid black dots represent geometric mean of the age category, vertical bars represent 95% CI, horizontal bars represent the age covered in the age category.

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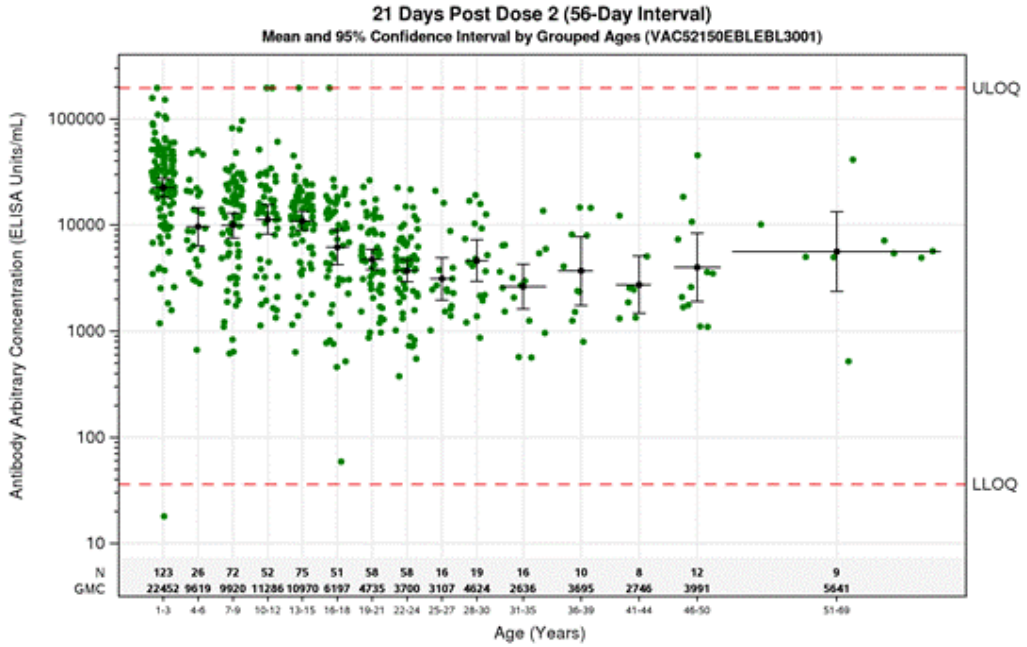
Figure 13: EBOV GP Binding Antibody Concentrations (ELISA, EU/mL) at 21 Days Post Dose 2 by Age Groups for All Participants Vaccinated in the 56-day Interval (Study EBL2002)



The analysis is based on the Per Protocol Analysis Set.
Green dots represent individual data points (jittering applied). Solid black dots represent geometric mean of the age category, vertical bars represent 95% CI, horizontal bars represent the age covered in the age category.

Source: [GIMHUM03-AGE-P23.RTF]
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Figure 14: EBOV GP Binding Antibody Concentrations (ELISA, EU/mL) at 21 Days Post Dose 2 by Age Groups for All Participants Vaccinated in the 56-day Interval (Study EBL3001)

Clinical studies in special populations

No dedicated studies in special populations have been performed. However, children and adolescents have been included in Study EBL2002 and EBL3001. Please see section -2.5 Clinical Efficacy for more information on these studies and their outcomes. From the total number of adult participants (2,341) who received the active vaccine, placebo, or active control regimen, only 9 were >65 years old (see safety section 2.6.

HIV-1 infected subjects

Studies EBL2002 (conducted in Burkina Faso, Côte d'Ivoire, Kenya, and Uganda) and EBL2003 (conducted in the United States, Kenya, Mozambique, Nigeria, Uganda, and Tanzania) included HIV-infected individuals on a stable regimen of HAART and in good medical condition.

In EBL2002, at 21 days post MVA-BN-Filo dosing (Day 50 for the 28-day interval schedule and Day 78 for the 56-day interval schedule), binding antibody responses against EBOV GP were observed in all (100%) participants, with similar GMC values between schedules and no remarkable differences in comparison to the HIV-uninfected adult and elderly participants in Cohort 1:

- Day 50: GMC: 4207 ELISA units/mL (95% CI: 3233; 5474)

  (versus 3085 ELISA units/mL [95% CI: 2648; 3594] in healthy adults and elderly)
- Day 78: GMC: 5283 ELISA units/mL (95% CI: 4094; 6817)

  (versus 7518 ELISA units/mL [95% CI: 6468; 8740] in healthy adults and elderly)

In EBL2003, HIV-infected and HIV-uninfected adults were vaccinated with the reverse sequence (MVA-BN-Filo, Ad26.ZEBOV) of the vaccine regimen in a compressed 14-day interval, which is not the recommended vaccine regimen. In Part 1 of this study performed in the United States, approximately 3-fold lower binding antibody concentrations were observed at any time point in HIV-infected participants. Although there was no overlap between the 95% CIs of both groups, the number of HIV-infected participants in the Per Protocol Analysis Set (N=19) was half the size of the number of HIV-uninfected participants (N=38) and substantial variation was observed within the HIV-infected group. Immunogenicity data from Part 2 of the study including twice as many HIV-infected participants as Part 1 will help clarify whether MVA BN-Filo, Ad26.ZEBOV vaccination with a very short interval of only 14 days induces lower immune responses in HIV-infected compared to HIV-uninfected adults.

2.5.3 Supportive studies

Immunological Data to Support the Manufacturing Process **Release specifications and shelf life limits**

The release specification for Ad26.ZEBOV is 0.5×10^{11} vp/ml – 2.0×10^{11} vp/ml, which translates to 0.25×10^{11} vp/dose – 1.0×10^{11} vp/dose. The upper specification limit of 2.0×10^{11} vp/ml is supported by clinical Phase 1 study EBL1002.

For MVA-BN-Filo the proposed commercial acceptance criterion for Infectious virus titre in stability is $\geq 1.00 \times 10^{8}$ Inf.U./mL which is justified by EBL3002. The upper specification limit is 8.80×10^{8} Inf.U./mL which was justified in clinical Phase 1 study EBL1002.

In **study EBL1002** Ad26.ZEBOV hd (injection of 1 mL dose of vaccine at concentration of 1.0×10^{11} vp /mL) was administered to 15 subjects as dose 1 in a 2-dose heterologous vaccination regimen, followed by MVA-BN-Filo 4.4×10^{8} TCID50 (hd) as dose 2, four weeks later in study EBL1002.

No apparent influence of the high dose level on the overall occurrence of adverse events was observed in study EBL1002.

Study EBL3002, a randomized, double-blind, placebo-controlled study in healthy adults conducted in the USA, was performed to support potency specification settings over the expected shelf life for both Ad26.ZEBOV and MVA-BN-Filo vaccines. The intention was to identify the minimum acceptable potencies for Ad26.ZEBOV and MVA-BN-Filo through the evaluation of vaccination with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later at lower dose levels than the selected dose levels of both vaccines. To do so, the 2 vaccines were diluted to mimic the end-of-shelf life potency. The dose levels evaluated in this study were selected based on the provisional limits for stability set during the development program.

The primary objective was to demonstrate non-inferiority of the vaccine regimen using Ad26.ZEBOV 2×10^{10} vp (Dose 1) and MVA-BN-Filo 0.5×10^{8} Inf.U (Dose 2) (Group 2) versus the same regimen using the release titers (Ad26.ZEBOV 5×10^{10} vp and MVA-BN-Filo 1×10^{8} Inf.U; Group 1) at 21 days post Dose 2. A lower dose level of Ad26.ZEBOV (0.8×10^{10} vp) was also evaluated: the objective was to demonstrate non-inferiority of the vaccine regimen using Ad26.ZEBOV 0.8×10^{10} vp (Dose 1) and MVA-BN-Filo 0.5×10^{8} Inf.U (Dose 2) (Group 3) versus the same regimen using the release titers. The assessments of non-inferiority versus the release titer group were based on a non-inferiority margin of 2/3 (0.67) for the 95% CI of the GMC ratio.

Table 17: Schematic Description of study EBL3002

Group	N	Dose 1 Vaccination	Dose 2 Vaccination
		Day 1	Day 57
1	150	Ad26.ZEBOV $5x10^{10}$ vp	MVA-BN-Filo $1x10^{8}$ Inf.U
2	150	Ad26.ZEBOV $2x10^{10}$ vp	MVA-BN-Filo $5x10^{7}$ Inf.U
3	150	Ad26.ZEBOV $0.8x10^{10}$ vp	MVA-BN-Filo $5x10^{7}$ Inf.U
4	75	Placebo (0.9% saline)	Placebo (0.9% saline)

N: number of subjects to receive study vaccine (active or placebo)
Inf.U: infectious units; vp: viral particles

Testing was performed in a hierarchical fashion starting with the intermediate dose level (Group 2) and moving to the lower dose level (Group 3) if the objective had been met for Group 2. Due to unavailability of aged material at the time of study start, diluted material was used to mimic aged material.

The results at different time points in each group are displayed in Table 18.

Table 18: EBOV GP-Specific Binding Antibody Responses (ELISA, ELISA units/mL): Summary Statistics; Per Protocol Analysis Set (study EBL3002)

	Ad26 $5x10^{10}$ vp, MVA $1x10^{8}$ Inf.U	**Ad26 $2x10^{10}$ vp, MVA $5x10^{7}$ Inf.U**	**Ad26 $0.8x10^{10}$ vp, MVA $5x10^{7}$ Inf.U**	**Placebo, Placebo**
Day 1 (Baseline)				
N	140	131	136	66
GMC (95% CI)	<LLOQ (<LLOQ; <LLOQ)	<LLOQ (<LLOQ; <LLOQ)	<LLOQ (<LLOQ; <LLOQ)	<LLOQ (<LLOQ; <LLOQ)
Day 57 (56 days post dose 1)				
N	140	131	136	67
GMC (95% CI)	793 (698; 902)	669 (571; 784)	496 (422; 582)	<LLOQ (<LLOQ; <LLOQ)
GMI (95% CI) from predose 1	20.7 (18.1; 23.6)	17.6 (15.1; 20.7)	12.3 (10.5; 14.5)	1.0 (1.0; 1.0)
Responder (n/N* (%)) (95% CI)	135/140 (96.4%) (91.9%; 98.8%)	127/131 (96.9%) (92.4%; 99.2%)	131/136 (96.3%) (91.6%; 98.8%)	0/66 (0.0%) (0.0%; 5.4%)
Day 78 (21 days post dose 2)				
N	135	123	130	65
GMC (95% CI)	11054 (9673; 12633)	7524 (6472; 8746)	8538 (7338; 9934)	<LLOQ (<LLOQ; <LLOQ)
GMI (95% CI) from predose 1	290.2 (251.0; 335.6)	197.7 (169.1; 231.2)	210.5 (178.0; 248.8)	1.0 (1.0; 1.1)
GMI (95% CI) from predose 2	14.2 (12.4; 16.3)	10.9 (9.2; 13.0)	16.5 (14.3; 19.0)	1.0 (1.0; 1.0)
Responder (n/N* (%)) (95% CI)	135/135 (100.0%) (97.3%; 100.0%)	123/123 (100.0%) (97.0%; 100.0%)	130/130 (100.0%) (97.2%; 100.0%)	0/65 (0.0%) (0.0%; 5.5%)
Day 237 (180 days post dose 2)				
N	131	121	129	60
GMC (95% CI)	1263 (1100; 1450)	962 (822; 1125)	831 (716; 965)	<LLOQ (<LLOQ; <LLOQ)
GMI (95% CI) from predose 1	32.7 (28.2; 38.0)	25.4 (21.6; 29.9)	20.6 (17.7; 24.1)	1.0 (1.0; 1.0)
GMI (95% CI) from predose 2	1.6 (1.4; 1.8)	1.5 (1.3; 1.7)	1.6 (1.4; 1.9)	1.0 (0.9; 1.0)
Responder (n/N* (%)) (95% CI)	129/131 (98.5%) (94.6%; 99.8%)	119/121 (98.3%) (94.2%; 99.8%)	127/129 (98.4%) (94.5%; 99.8%)	0/59 (0.0%) (0.0%; 6.1%)

	Ad26 $5x10^{10}$ vp, MVA $1x10^8$ Inf.U	Ad26 $2x10^{10}$ vp, MVA $5x10^7$ Inf.U	Ad26 $0.8x10^{10}$ vp, MVA $5x10^7$ Inf.U	Placebo, Placebo
N: Number of subjects with data at that timepoint; N*: number of subjects with data at baseline and at that timepoint CI: confidence interval; GMC: geometric mean concentration; GMI: geometric mean increase; LLOQ: lower limit of quantification A subject was a responder at a considered timepoint if the sample interpretation was negative at baseline and positive post baseline and the post-baseline value was greater than 2.5x LLOQ, or sample interpretation was positive both at baseline and post baseline and there was a greater than 2.5-fold increase from baseline. The GMC and corresponding CI are shown on the reported scale (ELISA units/mL). Exact Clopper-Pearson confidence interval is shown for the corresponding responder rate. Ad26: Ad26.ZEBOV; MVA: MVA-BN-Filo				

At 21 days post dose 2 (Day 78), the GMC ratio of the intermediate dose level (Group 2) versus the release titer (Group 1) was 0.7, with the 95% CI ranging from **0.56** to 0.83. The non-inferiority criterion of 2/3 (0.67) for the lower limit of the 95% CI was therefore not met and non-inferiority could not be concluded for the intermediate dose level (Group 2) and the low dose level (Group 3). The GMC ratio of the lower dose level versus release titer was 0.8, (95% CI: **0.63**; 0.94).

In a post-hoc exploratory analysis at 56 days post dose 1 (Day 57, prior to dose 2) using the non-inferiority margin of 2/3 (0.67), the non-inferiority criteria were met for the intermediate dose level (Group 2) versus the release titer (Group 1), but not for the low dose level (Group 3) versus the release titre.

Consistency of manufacturing processes and sites

Study EBL3003 was conducted to support the optimization of the manufacturing process by assessing the immunological equivalence of the Ad26.ZEBOV vaccine from different virus seeds produced at different manufacturing sites. Healthy US adults were assigned in a 2:2:2:1 ratio to receive 1 of 3 batches of Ad26.ZEBOV as first vaccination followed by a single batch of MVA-BN-Filo, or placebo as first and second vaccination, all at a 56-day interval. The 3 Ad26.ZEBOV batches included MVS Batch Leiden, WVS batch Leiden, and WVS batch Bern. Results are presented in Table 19.

Table 19: EBOV GP Binding Antibody Concentrations (ELISA, EU/mL) From Study EBL3003

Group	N	Dose 1 Vaccination	Dose 2 Vaccination	Baseline N GMC (95% CI)	Pre-Dose 2 N GMC (95% CI)	21 Days Post Dose 2 N GMC (95% CI)
		Day 1	Day 57			
1	94	Ad26.ZEBOV – Batch (V)	MVA-BN-Filo – Batch (A)	85 **<LLOQ** (<LLOQ; <LLOQ)	85 **813** (632; 1046)	81 **11089** (9323; 13189)
2	94	Ad26.ZEBOV – Batch (B)	MVA-BN-Filo – Batch (A)	86 **<LLOQ** (<LLOQ; <LLOQ)	88 **745** (603; 921)	87 **10337** (8660; 12339)
3	94	Ad26.ZEBOV – Batch (C)	MVA-BN-Filo – Batch (A)	87 **<LLOQ** (<LLOQ; <LLOQ)	88 **851** (720; 1006)	86 **11790** (9701; 14328)
4	47	Placebo (0.9% saline)	Placebo (0.9% saline)	43 <LLOQ (<LLOQ; <LLOQ)	43 <LLOQ (<LLOQ; <LLOQ)	41 <LLOQ (<LLOQ; <LLOQ)

The analysis is based on the Per Protocol Analysis Set.
Evaluated batches: Ad26.ZEBOV: Ad26 WVS Bern batch, Ad26 MVS Leiden batch, Ad26 WVS Leiden Batch, MVA-BN-Filo: Kvistgård batch

The primary objective to demonstrate immunologic equivalence between WVS batch Bernand MVS batch Leiden at 56 days post Dose 1 was not met; the GMC ratio was 0.9 with a 95% CI of 0.65-1.17, the lower limit of the 95% CI being just outside the lower limit of the equivalence criterion of 0.67. Although a statistical conclusion could not be drawn about the other comparisons evaluated as secondary objective, the equivalence criteria were met for WVS batch Leiden and WVS batch Bern (GMC ratio of 1.1, 95% CI of 0.81-1.47), and for WVS batch Leiden and MVS batch Leiden (GMC ratio of 1.0, 95% CI of 0.71-1.29).

Study EBL3008

In the ongoing EVD outbreak, in addition to ring vaccination with the rVSV ZEBOV GP vaccine, the Strategic Advisory Board of Experts (SAGE) recommended vaccination of lower risk populations with the Ad26.ZEBOV, MVA-BN-Filo regimen under informed consent. A clinical study (study EBL3008) has been designed for that purpose and, at the time of file writing (September 2019), the study protocol has been approved. In this study, the Institut National de Recherche Biomédicale (DRC National Institute of Biomedical Research (INRB) and the Ministry of Health (MOH, EPI) and the London School of Hygiene and Tropical Medicine (LSHTM; Sponsor) are evaluating the effectiveness and safety of VAC52150 (Ad26.ZEBOV, MVA-BN-Filo) against Ebola Virus Disease (EVD), in collaboration with Janssen Vaccines and Prevention B. V. (Janssen), Epicentre, Médicines Sans Frontières (MSF) France, and a consortium led by the Coalition for Epidemic Preparedness Innovations (CEPI), United Kingdom Public Health Rapid Support Team (UK-PHRST), the Wellcome Trust and the World Health Organization (WHO).

The study is an open-label, non-randomised, population-based study. Ad26.ZEBOV ($5x10^{10}$ viral particles [vp]) will be given as the first dose and MVA-BN-Filo ($1x10^{8}$ infectious units [Inf.U]) will be given as the second dose 56 (-14 day +28 day) days later, to adults, and children aged 1 year or over. Evaluation of this intervention will include the estimation of the effectiveness of the two-dose vaccine regimen to prevent Ebola Virus Disease (EVD) via a test negative case control design.

The primary evaluation outcome for estimating vaccine effectiveness is laboratory confirmed EVD. Outcomes will be collected until the end of the study, which is until the end of the current EVD outbreak for the evaluation of vaccine efficacy. If a vaccinated person is suspected of having EVD s/he will be referred to the nearest Ebola Treatment Centre (ETC) or Transit Centre (TC). Laboratory testing for EVD will be done following the Ebola response guidelines, using a qualified polymerase chain reaction (PCR) test performed in the approved EVD diagnostic laboratories.

If an Ebola outbreak occurs in a community that is receiving the Ad26.ZEBOV, MVA -BN-Filo vaccination, vaccinations will be discontinued until the management of the included participants is clarified with the relevant authorities. For example, if there is an outbreak between first and second vaccinations in a specific community, the participants may be eligible for ring vaccination with the rVSV-ZEBOV-GP vaccine. A decision on continuation with dose 2 for those not eligible for the rVSV-ZEBOV-GP vaccine will be taken after discussion with the relevant authorities.

Participants will be observed for 15 minutes after vaccination for immediate adverse events. SAEs will be assessed at the next vaccination visit and through passive reporting up to 1-month post-dose 2. Vaccinated subjects who will be provided with a contact phone number to report any AEs or other medical concerns up to 1-month post-dose 2. These will be recorded as SAEs (passive reporting).

Standardised forms will be used to record symptom type, symptom onset and end dates, diagnosis and final outcomes of any SAEs reported to the team. Investigators will assess and determine any causal relationship to the study vaccine. Participants will be seen if required.

In the safety subset, the first 500 adults and the first 500 children in the study will be actively followed by telephone one month after the administration of the second dose. Participants that are pregnant at the time of vaccination or who become pregnant up to one month after the second vaccination will be followed after birth to document the outcome of pregnancy and neonatal events.

The primary analysis will exclude individuals known to have received the rVSV -ZEBOV-GP vaccine, those who received the first but not the second dose of the Ad26.ZEBOV, MVA-BN-Filo vaccine prior to testing for Ebola, and those who received the second dose less than 21 days prior to testing for Ebola. Secondary analyses will examine the effectiveness based on consideration of receiving at least one dose of the vaccine, or development of disease within 21 days after the second dose.

In the primary analysis, the odds of having been fully vaccinated (having received the 2 doses, in the right order, at least one month apart, at least 21 days before the onset of symptoms) will be compared with the odds of not being vaccinated between the cases and the controls through the odds ratio (OR). ORs for being an EVD case will be calculated and used to estimate vaccine effectiveness: VE(%)=(1-OR)x100. Regression analyses will allow for appropriate account of any matching and adjustment for potential confounders, where available information allows.

Vaccination of approximately 500,000 people with Ad26.ZEBOV and MVA-BN-Filo in EVD transmission areas should achieve the sample size (110 confirmed EVD cases) required for primary evaluation of vaccine effectiveness.

2.5.4 Discussion on clinical efficacy

Design and conduct of clinical studies

Design

The proposed vaccine regimen of Ad26.ZEBOV at 5x1010 vp per 0.5 mL dose followed by MVA-BN-Filo at 1x108 infectious Inf.U per 0.5 mL dose with a 56-day interval was selected based on NHP efficacy data and on clinical data from phase 1 studies. Dose selection, sequence, and interval are sufficiently supported by human immunogenicity data and NHP challenge studies.

The general design of the main studies and overall development program is endorsed. All 5 clinical studies used for immunobridging were randomized, observer-blind, placebo/active-controlled studies.

In the main clinical studies, 4 different Ad26.ZEBOV batches have been used). Also for MVA-BN-Filo, 4 batches have been used. MVA-BN-Filo drug product batches produced by the latest process variants (DP4 and DP5) are however observed to be significantly less stable (weakened) than the batches that have been used in the clinical studies. This may affect the immunogenicity or safety of the vaccine and more information should be provided. Please refer to the quality section in the MVA-BN-Filo AR for more information.

In all studies, the main immunogenicity objective was to assess binding antibody responses to EBOV GP (as measured by EBOV GP FANG ELISA) at 21 days post Dose 2. This is considered an acceptable objective for this clinical development program. The following additional endpoints were assessed: neutralizing antibodies against EBOV GP (all studies), cell-mediated immunity (study EBL2001 and EBL2002), binding antibody levels against MARV GP and SUDV GP (study EBL2002 and EBL3001), and neutralizing antibody levels against vector backbones (study EBL2002 and EBL3001 for Ad26, Study

EBL3001 for MVA). Two studies had additional immunogenicity objectives, to demonstrate non-inferiority of 2 dose levels to support specification settings for potency over the expected shelf life (study EBL3002), and to compare immune responses between 3 different vaccine batches (study EBL3003).

Patient population

The population enrolled in the clinical studies consisted of healthy adults, adolescents and children (from the age of 1 year onwards), as well as HIV-1 infected adults who were on a stable regimen of highly active antiretroviral therapy (HAART). All studies have been conducted in Europe, the United States, or Africa. Of note, the enrolled population is broader than the population used for the immunobridging analysis, which only included healthy adults between 18 and 50 years of age. This is further discussed below.

Conduct of the studies

During the study, study vaccinations in EBL2001 were temporarily halted due to the occurrence of a serious adverse event (Miller Fisher syndrome). This decision had a clear impact on all clinical studies. For most of the studies, this resulted in subjects receiving the second vaccination later than planned (outside the window allowed by the protocol), or sometimes not at all. Following the protocol, this delay in vaccination resulted in exclusion from the PP analysis set for these subjects.

The design of Study EBL3001 was changed during the study, from a 3-stage study also including an efficacy assessment as originally planned, to a 2-stage study only investigating safety and immunogenicity. This was due to the Ebola outbreak subsiding before any clinical efficacy data could be collected. Also for Study EBL3003 the design was changed when the study was already ongoing. In this case, the aim of the study was changed from demonstration of immunologic equivalence of 3 different batches of Ad26.ZEBOV from 3 different virus seeds, to show equivalence between 2 batches only (WVS batch Bern and MVS batch Leiden). Also, the timing of the primary endpoint analysis was changed from 21 days post dose 2 to 56 days post dose 1 (i.e. pre-dose 2). As these changes were implemented <2 months after first subject first visit date, these changes did not result in some subjects being treated differently than others, and overall results are not expected to have been impacted.

Efficacy data and additional analyses

No efficacy data could be generated for this vaccine regimen. The evaluation of the protective effect of the vaccine regimen for this MAA is based on the bridging of clinical immunogenicity results to efficacy and immunogenicity data obtained in non-human primates (NHP). To translate human immunogenicity data into likelihood of protection, a logistic regression model was built based on immunogenicity and efficacy data obtained in the NHP EBOV Kikwit challenge model. Data from the following studies has been used in the immunobridging and are considered pivotal to this application: studies EBL2001, EBL2002, EBL3001 (Stage 1 and 2), EBL3002, and EBL3003.

There is no correlate of protection known for Ebola. The primary immunogenicity endpoint in the studies was the level of EBOV GP-specific binding antibodies, as measured by the EBOV GP FANG ELISA. Immunogenicity results based on the Full Analysis set showed no major differences between the FAS and PP GMC values, except for study EBL2002 in which a 1.3-fold increased GMC value was observed in the FAS (10,042) as compared to the PP (7,518) population. Due to the lack of an established threshold value associated with clinical benefit, interpretation of the outcome is difficult. No specific hypothesis was tested in EBL2001, EBL2002 and EBL3001. All 5 main studies showed high responder rates for all schedules tested. Specifically looking at the to be marketed dose, given in the

right order (Ad26 first, followed by MVA) and with a 56 day interval, the GMCs 21 days after the second dose ranged between 3,810 EU/mL in Study EBL3001 (conducted in Sierra Leone) and 11,790 EU/mL in Study EBL3003 (conducted in the USA). The observed variability could only to some extent be explained (see below under "Subgroup analyses"), and the Applicant was requested to discuss whether differences in potency, storage or transportation conditions between batches may also have contributed to the observed variation. The Applicant argued that the potency of the batches at release does not provide an explanation for the differences in GMCs. Storage temperatures may however have had an impact, although it does not explain the full variation. Additional information on storage conditions and expected potency for Study EBL2002 was provided but did not result in a better understanding on the impact that storage temperature may have had on the induced immune response.

A positive correlation was observed between neutralizing antibody activity and binding antibody responses in those studies in which both assays were investigated. Given this strong correlation, the choice of the Applicant to use the EBOV GP FANG ELISA rather than the functional psVNA assay as the primary readout of vaccine induced immunogenicity, is acceptable.

Cell-mediated immunity was investigated in 2 studies (EBL2001 and 2002). Both CD4+ and CD8+ T cell responses were low after vaccination, with only few subjects showing relevant responses. No conclusions can be drawn from these results.

Immunobridging

The strategy to translate human immunogenicity data into likelihood of protection based on NHP challenge studies was discussed with the Applicant during development. Having acknowledged that a pre-licensure assessment of vaccine efficacy was not feasible given the circumstances, it is acceptable that an assumption of benefit can be derived only from nonclinical data together with clinical immunogenicity data via an immunobridging exercise. NHPs are considered the most relevant animal model for EVD since the symptoms of disease in NHPs closely resemble human EVD. Regarding the disease course, the NHP model is more stringent than natural Ebola infection in humans. After infection, NHPs have a shorter time to onset of symptoms and a much faster disease progression. Case fatality rate in NHPs is 100%, which is higher than in humans. Overall, the NHP model is considered a representative model of a human worst-case scenario.

The kinetics of the vaccine-induced EBOV GP-specific antibody response appears similar in NHP and humans. In both NHPs and humans, EBOV GP-specific binding antibody concentrations were detected from 14 days after Ad26.ZEBOV vaccination onwards and peaked 14 to 21 days after MVA-BN-Filo vaccination. After the 21 days post Dose 2 time point, the binding antibody responses declined over time in both NHPs and humans, reaching a stable level (10-20 fold lower than 21 days post Dose 2) that persisted at least up to 540 days (1.5 years) in NHPs and 2 years in humans (last time points assessed). Regarding the magnitude, there seems to be a lower response in humans as compared to NHPs.

To be able to compare antibody responses between NHP and humans, the Applicant applied a one-assay/one-laboratory strategy and used the validated EBOV GP FANG ELISA for the analysis of Phase 2/3 clinical samples and the NHP sera that served as a basis to construct the logistic curve. An extensive analysis was performed to demonstrate parallelism between the human and NHP samples in the EBOV GP FANG ELISA. Binding antibodies in NHP samples were found to be detected equally well by both the NHP conjugate and human conjugate; it was concluded that the human conjugate cross-reacts fully with NHP samples. For the immunobridging analysis, both the human test samples and the NHP test samples have been analysed using human reference samples and conjugate. Overall, the strategy to translate human immunogenicity data into likelihood of protection based on NHP challenge studies is considered acceptable.

In the prediction model it is assumed that the subjects belong to one population, while they were treated in different studies/countries. However, as the prediction model was built using data from the NHPs, this cannot be accounted for in the human prediction model. The estimated mean EBOV GP-specific antibody concentration in subjects enrolled in study EBL3001 (Sierra Leone, GMC 3,810 EU/mL) are lower than the estimated mean EBOV GP-specific antibody concentrations observed in the other studies (all subjects GMC 7,781 EU/mL). In the SAP the Applicant pre-planned a sensitivity analyses to assess the effect of possible pre-exposure on the immunobridging analysis, and repeated the analyses excluding the subjects of the Sierra Leone study EBL3001.

It was also confirmed post-hoc that baseline exposure was not linked to lower titres. No other explanation could be found.

Only an interim analysis for futility was planned. However, the study was stopped based on the unplanned interim analysis, while it is known that the estimation of an effect determined during an interim analysis is likely overestimated. In principle the alpha correction for this should be pre-planned, and there is no generally accepted method to correct for this in hindsight. However, a correction with a conservative and frequently used O'Brien Fleming alpha spending rule was performed, which combined with the one-sided alpha level of 0.00001, can be accepted. The lower bound of the corrected confidence interval should be used for interpretation.

Outcome/interpretation

Based on the pooled data from 764 healthy adults, the mean predicted survival probability was determined to be 53.4% and the lower limit of the 95% CI 36.7%. The outcome of the analysis is suggestive of a clinically relevant protective effect, and it can be concluded that a certain level of protection of the Ad26.ZEBOV, MVA-BN-Filo regimen in healthy adults is highly likely. There are however many unknowns and assumptions, and the actual beneficial effect in humans can only be derived from properly designed field efficacy trials, which are currently not considered feasible to conduct. A test-negative case-control study in currently being organised in DRC, but whether or not this study will be able to answer the outstanding questions on the beneficial effect in humans remains to be seen.

The immunobridging model is fully dependent on peak antibody titers measured 21 days after the second vaccination. Although this can be understood from a development perspective, it is evident that titers wane after this time point and decline to levels that are >10 fold lower than the peak titers within 1-2 years after the initial dose. Whether or not such a level of circulating antibodies will be sufficient to protect a subject from EVD upon natural challenge is unknown. In the NHP challenge model, challenge during steady state did not result in survival of the animals, but it may be that results from the NHP challenge model may not be fully applicable to humans given the difference in disease course between these species. However, all or most animals survived when challenged after a booster dose given 1.5 years post-primary vaccination. See below under 'duration of immune responses'.

Therefore section 4.4 of the SmPC reflects that the exact level of protection afforded by the vaccine regimen as well as the duration of protection is unknown. Furthermore, in section 4.2 of the SmPC, the administration of an Ad26.ZEBOV booster in previously vaccinated individuals, when considered at imminent risk of exposure to Ebola virus, is recommended from 4 months after the 2nd dose (MVA-BN-Filo) or anytime thereafter, for an optimal response.

A lower immune response upon vaccination was observed in certain populations, most notably subjects from Sierra Leone, and to a lesser extent also in HIV-infected subjects. The clinical relevance of the lower level of vaccine-induced EBOV-specific antibodies remains unknown.

Paediatric subjects showed higher immune responses upon vaccination as compared to healthy adult subjects. Upon request, immunobridging analyses were provided for paediatric, elderly and HIV

positive subjects. The mean predicted survival probability yielded by the model (based on the PP analysis set) ranged from 42.0% (HIV-infected participants) to 82.6% (children 1-3 years) with a lower limit of the 95% CI ranging from 22.4% to 74.9%. While this seems consistent with the mean predicted survival probability of 53.4% in adults, these predictions are based on the assumption that the relation between antibody titter and survival is the same in children, elderly and HIV-infected subjects. As this association has been studied only in adult NHP, some uncertainty remains.

Subgroup analyses

Female participants had somewhat higher EBOV GP-specific binding antibody concentrations than males, and a linear regression analysis suggested that with every 10 years increase in age, the 21-days post Dose 2 EBOV GP-specific binding antibody concentrations are expected to decrease with approximately 11% (0.0506 log10 units). However, the statistical significance of the model parameters does not indicate a marked effect of age on the 21 days post Dose 2 EBOV GP antibody concentrations, and the clinical relevance of this observation is unknown. Moreover baseline antibody concentrations may impact the 21-days post Dose 2 EBOV GP-specific binding antibody concentrations, although baseline positivity could be also due to assay cross-reactivity and/or nonspecific binding.

Well suppressed HIV-infected adult subjects with relatively high CD4 counts and without clinical symptoms of immune deficiency were included in Study EBL2002 (Cohort 2a). In this study, a lower GMC 21 days post dose-2 (GMC: 5,283 (95%CI 4,094-6,817) was observed for HIV-1 infected adults as compared to healthy adults (GMC: 7,518 (95% CI 6,468-8,740)). The clinical relevance of this difference is unknown.

Immunological data to support the manufacturing process

The Applicant claims that the proposed batch release and shelf life limits are supported by clinical data from study EBL3002. This study however clearly shows a detrimental effect of the intermediate and lower potencies on the immune response to the vaccines. Importantly, in the NHP challenge model it was found that whilst survival of 100% was obtained for regimens with Ad26.ZEBOV dosed down to $2x10^9$ vp combined with $1x10^8$ Inf.U MVA-BN-Filo, combinations with lower doses of MVA-BN-Filo were not protective. This was also applicable for the Ad26.ZEBOV shelf life limit for infectious units. The specification limits for potency at end of shelf life were thus increased to 9.05 $\log_{10}$ IU/mL for Ad26.ZEBOV DP and to 1.40 x 10^8 Inf.U/mL for MVA-BN-Filo DP. The lowest expected potency of MVA-BN-Filo at administration was 1.5-2.0 x 10^8 Inf.U/mL in stage 2 of Study EBL3001, which is in the range of the newly proposed limit of 1.40 x 10^8 Inf.U/mL. The response as observed in study EBL3001 can still be considered of clinical benefit, therefore the concerns with the lower potency due to the set shelf life limits are considered addressed.

The expected potency was markedly lower in study EBL3001 than what is estimated for the other studies, which seems to be due, at least in part, to the higher temperatures at which the vaccine was stored in this study EBL3001 as compared to the other studies. Shelf life of 4 years including long term storage at -85 to -55 °C, short term storage and shipment at -25 to -15 °C, and short term storage at 2 to 8 °C is considered crucial for the efficient roll-out of a large scale vaccination campaign in regions such as Sub-Saharan Africa.

An in-depth discussion on the feasibility of the proposed storage conditions, especially for use outside of Europe provided reassurance that the storage can be implemented in the field using routinely available vaccine storage infrastructure. The updated storage instructions for Zabdeno and Mvabea in the SmPC and Leaflet provide adequate guidance for central facilities and local vaccinating centres. It is however recommended to monitor the compliance with storage conditions closely after authorisation, and the Applicant is requested to notify the EMA as soon as a signal would arise that the storage conditions are routinely not met.

Study EBL3003 was conducted to support the optimization of the manufacturing process by assessing the immunological equivalence of the Ad26.ZEBOV vaccine from different virus seeds produced at different manufacturing sites. The primary objective of this study was not met. Equivalence between the WVS batch Bern and MVS batch Leiden could not be demonstrated as the GMC ratio was 0.9 with a 95% CI of 0.65-1.17, crossing the lower equivalence margin of 0.67. Although here too the relevance of the equivalence margins is not known, the point estimates are all close to 1 and GMCs are very similar, so it is concluded that the three batches of Ad26.ZEBOV are consistent.

Effectiveness data (EBL3008)

The Applicant, together with L'Institut National de Recherche Biomédicale, the Ministère de la Santé de la République Démocratique du Congo, the London School of Hygiene and Tropical Medicine, CEPI, Epicentre, MSF, UK-PHRST, Wellcome Trust, and WHO is implementing an observational study in DRC in order to estimate the effectiveness of the AD26.ZEBOV, MVA-BN-Filo vaccine regimen in preventing EVD. The plan is to vaccinate approximately 500,000 people with Ad26.ZEBOV and MVA-BN-Filo in areas at risk of EVD transmission; this would achieve the sample size (110 confirmed EVD cases) required for primary evaluation of vaccine effectiveness. Evaluation of this intervention on vaccine effectiveness to protect against EVD will be done through a retrospective test-negative case control study of laboratory confirmed EVD cases and matching controls who test negative for EVD. Note however that if a case of EVD were to occur, subjects around a case may become eligible for vaccination with rVSV. It is unclear at this stage whether the proposed study can be successful in acquiring sufficient cases to estimate vaccine effectiveness.

Duration of the immune response

There is no clinical efficacy data to inform on the duration of protection. Immune responses in vaccinated subjects have been followed-up for 2 years after the first dose, but as it is unknown what antibody level is required for protection after Ebola virus exposure, duration of protection cannot currently be established. In non-clinical study, no anamnestic response was observed in NHP who were challenged 70 weeks post-dose 2. Administration of a booster dose induced a rapid and strong immune response. This was consistent across studies. The ability to boost the vaccine-induced immune response was also evident when looking at neutralising antibody titers (i.e. a 24-fold increase in titers was seen 7 days post booster in EBL1002 compared to pre-booster titers). The administration of an Ad26.ZEBOV booster in previously vaccinated individuals, when considered at imminent risk of exposure to Ebola virus, is recommended from 4 months after the 2nd dose (MVA-BN-Filo) or anytime thereafter, for an optimal response.

Pre-existing immunity to MVA vector

Data on the effect of pre-existing immunity to MVA on vaccine immunogenicity is limited. The prevalence of neutralizing anti-MVA antibodies was low or absent in the populations tested. The ongoing study EBL2007, which will be submitted post-authorisation, may provide more information, as part of the enrolled population has been previously immunized with a smallpox vaccine and baseline MVA neutralizing antibodies will be evaluated.

2.5.5 Conclusions on the clinical efficacy

The evaluation of the protective effect of the vaccine regimen was based on immunobridging of clinical immunogenicity results to efficacy and immunogenicity data obtained in non-human primates (NHP). To translate human immunogenicity data into likelihood of protection, a logistic regression model was built based on immunogenicity and efficacy data obtained in the NHP EBOV Kikwit challenge model. Data from the following studies has been used in the immunobridging and are considered pivotal to

this application: studies EBL2001, EBL2002, EBL3001 (Stage 1 and 2), EBL3002, and EBL3003.

Although the results of the NHP challenge studies demonstrate protection in an otherwise lethal challenge model, it remains uncertain what the effect size in humans will be, as there is no efficacy or effectiveness data available.

The level of antibodies induced upon vaccination with the selected dose, sequence and interval across the different studies is wide (ranging between 7,553 and 16,341 EU/mL in phase 1 studies and between 3,810 and 11,790 EU/mL in phase 2/3 studies), for unknown reasons and with unknown consequences for the level of protection against naturally acquired Ebola virus disease.

Duration of protection is unknown, but antibodies were detected up to 1/2 years post-primary vaccination and administration of a booster dose induced a rapid and strong immune response.

Immunogenicity of the vaccine regimen has not been assessed in immunocompromised individuals, including those receiving immunosuppressive therapy. Immunocompromised individuals may not respond as well as immunocompetent individuals to the vaccine regimen.

The CHMP agrees with the claim by the applicant that conducting a randomised (placebo) controlled efficacy study is not feasible for ethical reasons considering the high mortality of EVD, due to the security situation in the current DRC outbreak and due to operational difficulties of conducting such a study during an ongoing Ebola outbreak and that therefore it will not be possible to submit a comprehensive clinical data package in the future. Consequently, the applicant's request for consideration of a marketing authorisation under exceptional circumstances is considered approvable by the Committee. In this context, and to address the lack of effectiveness data overall, the CHMP agreed with the Applicant's proposal to provide annual status reports and data from the post authorisation non interventional study VAC52150EBLXXXX: Evaluation of a heterologous, two dose preventive Ebola vaccine for field effectiveness, which is consequently imposed as a specific obligation.

2.6 Clinical safety

Patient exposure

The safety profile of Ad26.ZEBOV is based on available safety data from 11 clinical studies in addition to SAEs including fatal outcomes from ongoing studies up to the cut-off date of 12 August 2019. For EBL3001 & EBL4001 this concerns unblinded safety data; for other ongoing studies safety data is blinded.

The Applicant defined different pooling sets in order to describe the safety of the selected vaccination regimen. See Figure 15 for an overview of the adult safety pooling.

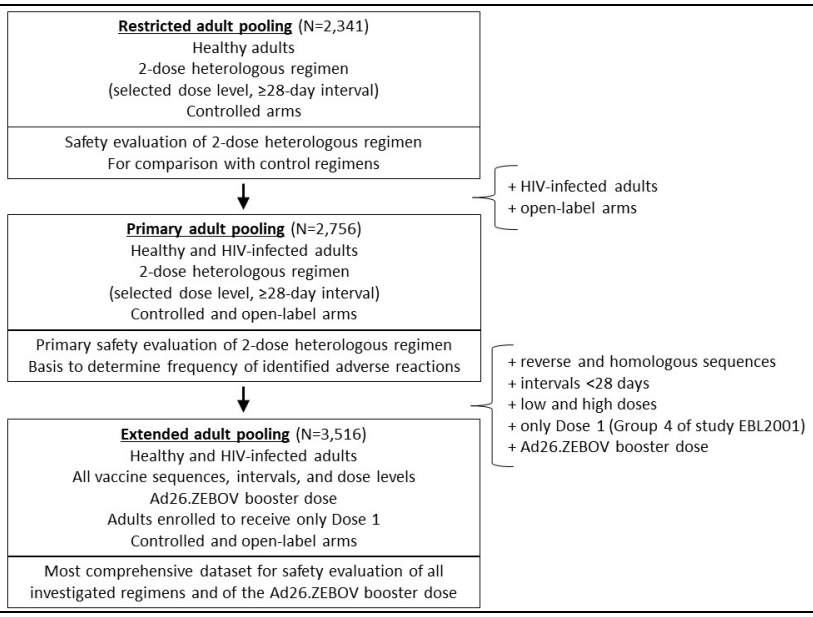
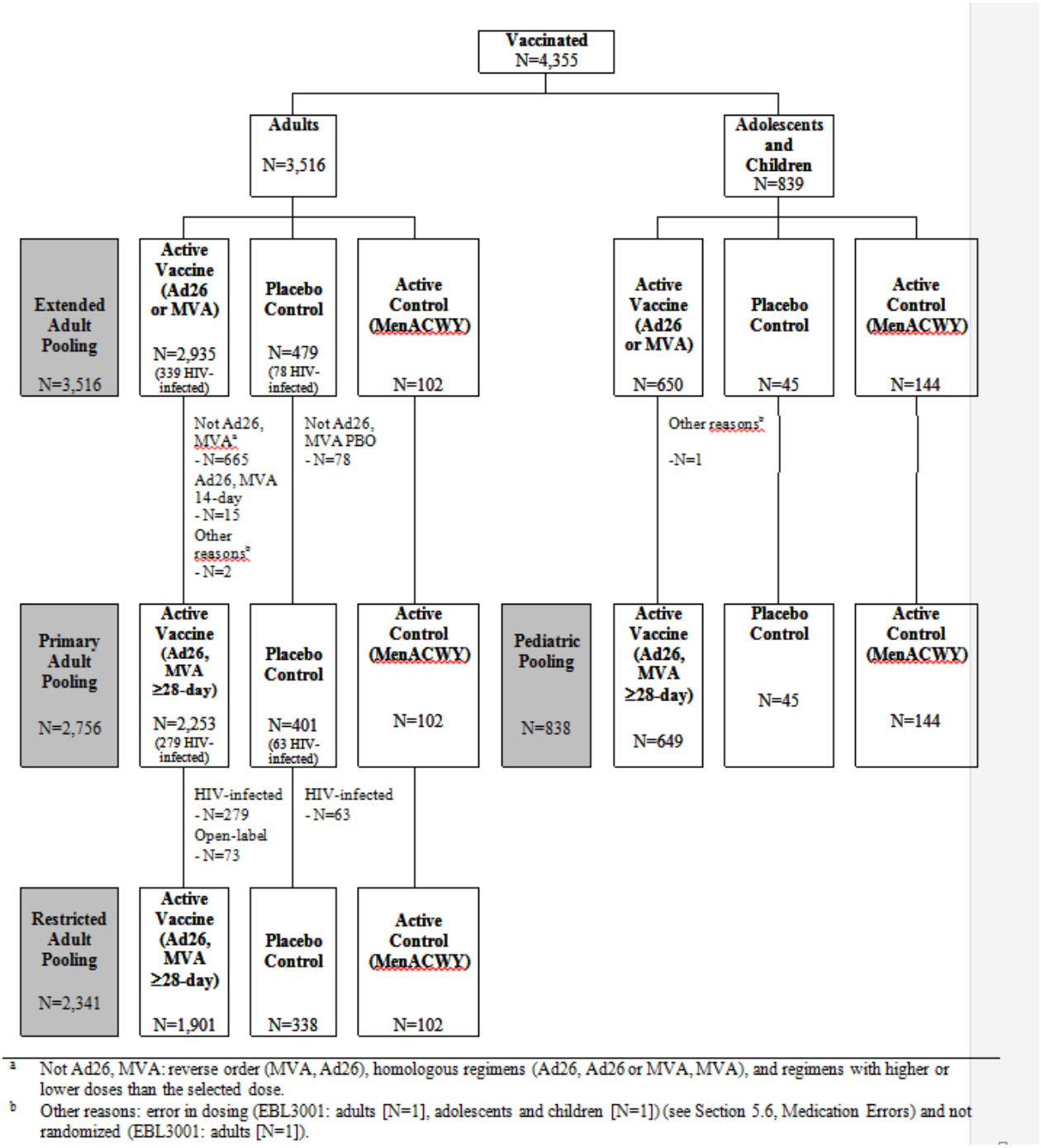


Figure 15: Schematic Overview of Safety Pooling Analysis Strategy in Adults

In addition, there was a fourth safety pooling, a paediatric pooling, including all available safety data from paediatric participants aged 1-17 years enrolled to receive Ad26.ZEBOV, MVA-BN-Filo at the selected dose level with an interval ≥28 days between doses.

For adults, the description of safety data is based on the restricted adult pooling, which allows comparison with the control regimens in healthy participants. To further support the safety profile, relevant safety data from the primary and extended adult pooling are described, as appropriate. For adolescents and children, the description of safety data is based on the paediatric pooling.



[a] Not Ad26, MVA: reverse order (MVA, Ad26), homologous regimens (Ad26, Ad26 or MVA, MVA), and regimens with higher or lower doses than the selected dose.

[b] Other reasons: error in dosing (EBL3001: adults [N=1], adolescents and children [N=1]) (see Section 5.6, Medication Errors) and not randomized (EBL3001: adults [N=1]).

Figure 16: Participant Disposition, All Vaccinated (Safety Pooling)

Adverse events

No notable differences were observed between the extended and primary adult pooling with regard to solicited and unsolicited AEs reported after vaccination with Ad26.ZEBOV and MVA-BN-Filo at the selected dose levels compared to placebo and/or MenACWY. Therefore, the presentation of AEs below focusses on the restricted pooling.

Local solicited adverse events in adults

Local solicited adverse events in adults are presented in Table 20. For the restricted adult pooling, by regimen, solicited local AEs were more frequently reported for participants who received the active vaccine regimen (64.1%) versus placebo or active control regimens (32.5% and 20.6%, respectively). By dose, no notable differences in frequencies of solicited local AEs were observed after Ad26.ZEBOV and MVA-BN-Filo vaccination (e.g. injection site pain was reported for 47.6% and 46.6% of participants, respectively, injection site warmth for 24.2% and 20.0% of participants, respectively, and injection site swelling for 10.5% and 10.4% of participants, respectively). The 3 most frequently reported solicited local AEs by preferred term (PT) for the vaccine regimen were injection site pain, warmth, and swelling reported for 60.5%, 33.3%, and 16.3% of active vaccine recipients, respectively.

Table 20: Solicited Adverse Events: Solicited Local Adverse Events by Worst Severity Grade by Dose - Adults (Restricted Pooling)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Number of Doses	1901	1688	728	102
Any local event	972 (51.1%)	844 (50%)	156 (21.4%)	17 (16.7%)
Grade 1	814 (42.8%)	686 (40.6%)	143 (19.6%)	14 (13.7%)
Grade 2	142 (7.5%)	148 (8.8%)	13 (1.8%)	3 (2.9%)
Grade 3	16 (0.8%)	10 (0.6%)	0	0
N doses in studies where Injection Site Erythema was collected	1901	1688	728	102
Any grade	10 (0.5%)	10 (0.6%)	6 (0.8%)	0
Grade 1	7 (0.4%)	9 (0.5%)	5 (0.7%)	0
Grade 2	2 (0.1%)	0	1 (0.1%)	0
Grade 3	1 (0.1%)	1 (0.1%)	0	0
N doses in studies where Injection Site Induration was collected	105	104	42	-
Any grade	1 (1%)	1 (1%)	0	-
Grade 1	0	1 (1%)	0	-
Grade 2	1 (1%)	0	0	-
N doses in studies where Injection Site Pain was collected	1901	1688	728	102
Any grade	904 (47.6%)	787 (46.6%)	129 (17.7%)	16 (15.7%)
Grade 1	764 (40.2%)	642 (38%)	122 (16.8%)	13 (12.7%)
Grade 2	131 (6.9%)	136 (8.1%)	7 (1%)	3 (2.9%)
Grade 3	9 (0.5%)	9 (0.5%)	0	0
N doses in studies where Injection Site Pruritus was collected	1901	1688	728	102
Any grade	168 (8.8%)	154 (9.1%)	48 (6.6%)	3 (2.9%)
Grade 1	151 (7.9%)	131 (7.8%)	44 (6%)	2 (2%)
Grade 2	16 (0.8%)	23 (1.4%)	4 (0.5%)	1 (1%)
Grade 3	1 (0.1%)	0	0	0
N doses in studies where Injection Site Swelling was collected	1901	1688	728	102
Any grade	199 (10.5%)	175 (10.4%)	48 (6.6%)	1 (1%)
Grade 1	178 (9.4%)	153 (9.1%)	44 (6%)	0
Grade 2	14 (0.7%)	21 (1.2%)	4 (0.5%)	1 (1%)
Grade 3	7 (0.4%)	1 (0.1%)	0	0
N doses in studies where Injection Site Warmth was collected	120	115	47	-
Any grade	29 (24.2%)	23 (20%)	7 (14.9%)	-
Grade 1	23 (19.2%)	20 (17.4%)	7 (14.9%)	-
Grade 2	4 (3.3%)	3 (2.6%)	0	-
Grade 3	2 (1.7%)	0	0	-

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*

n (%): number (percentage) of doses with 1 or more events;
Solicited adverse events with unknown severity are not taken into account in this table.
The denominator is the number of doses with available reactogenicity data.
* MenACWY: active control present in EBL3001 only.

Local solicited adverse events in adolescents and children (>1 years – <18 years)

Solicited local adverse events reported in children and adolescents are presented in Table 21. In children aged 1-3 years, the most frequently reported solicited local AEs by PT were injection site pain and injection site pruritus reported for 17.4% and 2.1% of active vaccine recipients, respectively. All local AEs were grade 1 or grade 2 in severity.

In children aged 4-11 years, the most frequently reported solicited local AE by PT was injection site pain reported for 39.3% of active vaccine recipients. Apart from grade 3 injection site pain (0.8%) and injection site swelling (0.4%), no other grade 3 solicited local AEs were reported for active vaccine recipients.

In adolescents aged 12-17 years, the most frequently reported solicited local AE by PT was injection site pain reported for 38.3% of active vaccine recipients. Other solicited local AEs were reported for <10% of active vaccine recipients. Apart from grade 3 injection site swelling (0.4%), no other grade 3 solicited local AEs were reported for active vaccine recipients.

By dose, no notable differences in frequencies of solicited local AEs were observed after Ad26.ZEBOV and MVA-BN-Filo vaccination for children aged 4-11 years (e.g., injection site pain reported for 29.8% and 22.3% participants, respectively) and adolescents aged 12-17 years (e.g. injection site pain reported for 24.9% and 27.5% participants, respectively). For children aged 1-3 years, the frequency of injection site pain reported after Ad26.ZEBOV vaccination (13.9%) and MenACWY vaccination (10.4%) was higher than after vaccination with MVA-BN-Filo (4.9%).

Table 21: Solicited Adverse Events: Solicited Local Adverse Events by Worst Severity Grade by Dose - Pediatric Pooling

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Age group: 1-3 years				
Number of Doses	144	143	48	48
Any local event	21 (14.6%)	7 (4.9%)	0	5 (10.4%)
Grade 1	20 (13.9%)	6 (4.2%)	0	5 (10.4%)
Grade 2	1 (0.7%)	1 (0.7%)	0	0
N doses in studies where Injection Site Pain was collected	144	143	48	48
Any grade	20 (13.9%)	7 (4.9%)	0	5 (10.4%)
Grade 1	19 (13.2%)	6 (4.2%)	0	5 (10.4%)
Grade 2	1 (0.7%)	1 (0.7%)	0	0
N doses in studies where Injection Site Pruritus was collected	144	143	48	48
Any grade	3 (2.1%)	0	0	0
Grade 1	3 (2.1%)	0	0	0
Age group: 4-11 years				
Number of Doses	252	251	95	48
Any local event	85 (33.7%)	66 (26.3%)	20 (21.1%)	2 (4.2%)
Grade 1	77 (30.6%)	59 (23.5%)	19 (20%)	2 (4.2%)
Grade 2	5 (2%)	7 (2.8%)	1 (1.1%)	0
Grade 3	3 (1.2%)	0	0	0
N doses in studies where Injection Site Erythema was collected	252	251	95	48
Any grade	8 (3.2%)	8 (3.2%)	2 (2.1%)	0
Grade 1	7 (2.8%)	8 (3.2%)	2 (2.1%)	0
Grade 2	1 (0.4%)	0	0	0
N doses in studies where Injection Site Pain was collected	252	251	95	48
Any grade	75 (29.8%)	56 (22.3%)	15 (15.8%)	2 (4.2%)
Grade 1	69 (27.4%)	51 (20.3%)	14 (14.7%)	2 (4.2%)
Grade 2	4 (1.6%)	5 (2%)	1 (1.1%)	0
Grade 3	2 (0.8%)	0	0	0
N doses in studies where Injection Site Pruritus was collected	252	251	95	48
Any grade	14 (5.6%)	12 (4.8%)	7 (7.4%)	0
Grade 1	12 (4.8%)	11 (4.4%)	6 (6.3%)	0
Grade 2	2 (0.8%)	1 (0.4%)	1 (1.1%)	0
N doses in studies where Injection Site Swelling was collected	252	251	95	48
Any grade	19 (7.5%)	21 (8.4%)	4 (4.2%)	0
Grade 1	17 (6.7%)	20 (8%)	4 (4.2%)	0
Grade 2	1 (0.4%)	1 (0.4%)	0	0
Grade 3	1 (0.4%)	0	0	0
Age group: 12-17 years				
Number of Doses	253	251	87	48
Any local event	70 (27.7%)	70 (27.9%)	15 (17.2%)	3 (6.3%)
Grade 1	59 (23.3%)	57 (22.7%)	11 (12.6%)	3 (6.3%)
Grade 2	11 (4.3%)	12 (4.8%)	3 (3.4%)	0
Grade 3	0	1 (0.4%)	1 (1.1%)	0
N doses in studies where Injection Site Erythema was collected	253	251	87	48
Any grade	1 (0.4%)	0	0	0

Grade 1	1 (0.4%)	0	0	0
N doses in studies where Injection Site Pain was collected	253	251	87	48
Any grade	63 (24.9%)	69 (27.5%)	7 (8%)	3 (6.3%)
Grade 1	53 (20.9%)	57 (22.7%)	4 (4.6%)	3 (6.3%)
Grade 2	10 (4%)	12 (4.8%)	2 (2.3%)	0
Grade 3	0	0	1 (1.1%)	0
N doses in studies where Injection Site Pruritus was collected	253	251	87	48
Any grade	16 (6.3%)	15 (6%)	6 (6.9%)	0
Grade 1	15 (5.9%)	13 (5.2%)	4 (4.6%)	0
Grade 2	1 (0.4%)	2 (0.8%)	1 (1.1%)	0
Grade 3	0	0	1 (1.1%)	0
N doses in studies where Injection Site Swelling was collected	253	251	87	48
Any grade	20 (7.9%)	14 (5.6%)	10 (11.5%)	0
Grade 1	18 (7.1%)	12 (4.8%)	8 (9.2%)	0
Grade 2	2 (0.8%)	1 (0.4%)	1 (1.1%)	0
Grade 3	0	1 (0.4%)	1 (1.1%)	0

n (%): number (percentage) of doses with 1 or more events;
Solicited adverse events with unknown severity are not taken into account in this table.
The denominator is the number of doses with available reactogenicity data.
* MenACWY: active control present in EBL3001 only.

Systemic solicited adverse events in adults

Solicited systemic adverse events reported in adults are presented in Table 22. In adults, *higher frequencies of solicited systemic AEs were observed after Ad26.ZEBOV vaccination than after MVA-BN-Filo vaccination (*e.g., fatigue was reported for 46.2% and 29.8% of participants, respectively, headache for 45.1% and 26.7% of participants, respectively, and myalgia for 36.8% and 25.8% of participants, respectively). The frequency of grade 3 solicited local AEs was low and higher after vaccination with Ad26.ZEBOV (4.1%) compared to MVA-BN-Filo (1.5%), placebo (2.1%), and MenACWY (0%). The most frequently reported grade 3 solicited systemic AEs (ie, fatigue, headache, and chills) were all reported more frequently after Ad26.ZEBOV vaccination (for 2.0%, 1.8%, and 1.4% of active vaccine recipients, respectively) than after MVA BN-Filo vaccination (for 0.7%, 0.4% and 0.4% of active vaccine recipients, respectively).

The majority of solicited systemic AEs had a median duration of 1 or 2 days and rash had a median duration of 5 or 6 days, after both Ad26.ZEBOV and MVA-BN-Filo vaccination.

Table 22: Solicited Adverse Events: Systemic Adverse Events by Worst Severity Grade by Dose - Adults (Restricted Pooling)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Number of Doses	1901	1688	728	102
Any systemic event	1276 (67.1%)	834 (49.4%)	328 (45.1%)	51 (50%)
Grade 1	832 (43.8%)	613 (36.3%)	257 (35.3%)	45 (44.1%)
Grade 2	367 (19.3%)	195 (11.6%)	56 (7.7%)	6 (5.9%)
Grade 3	77 (4.1%)	26 (1.5%)	15 (2.1%)	0
N doses in studies where Arthralgia was collected	1901	1688	728	102
Any grade	468 (24.6%)	265 (15.7%)	86 (11.8%)	23 (22.5%)
Grade 1	343 (18%)	216 (12.8%)	73 (10%)	20 (19.6%)
Grade 2	114 (6%)	45 (2.7%)	13 (1.8%)	3 (2.9%)
Grade 3	11 (0.6%)	4 (0.2%)	0	0
N doses in studies where Chills was collected	1901	1688	728	102
Any grade	451 (23.7%)	178 (10.5%)	67 (9.2%)	7 (6.9%)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Grade 1	290 (15.3%)	139 (8.2%)	56 (7.7%)	7 (6.9%)
Grade 2	135 (7.1%)	33 (2%)	9 (1.2%)	0
Grade 3	26 (1.4%)	6 (0.4%)	2 (0.3%)	0
N doses in studies where Fatigue was collected	1901	1688	728	102
Any grade	879 (46.2%)	503 (29.8%)	200 (27.5%)	16 (15.7%)
Grade 1	596 (31.4%)	391 (23.2%)	168 (23.1%)	15 (14.7%)
Grade 2	245 (12.9%)	100 (5.9%)	27 (3.7%)	1 (1%)
Grade 3	38 (2%)	12 (0.7%)	5 (0.7%)	0
N doses in studies where Headache was collected	1901	1688	728	102
Any grade	858 (45.1%)	451 (26.7%)	208 (28.6%)	39 (38.2%)
Grade 1	609 (32%)	363 (21.5%)	169 (23.2%)	38 (37.3%)
Grade 2	214 (11.3%)	82 (4.9%)	35 (4.8%)	1 (1%)
Grade 3	35 (1.8%)	6 (0.4%)	4 (0.5%)	0
N doses in studies where Myalgia was collected	1901	1688	728	102
Any grade	699 (36.8%)	435 (25.8%)	117 (16.1%)	20 (19.6%)
Grade 1	514 (27%)	348 (20.6%)	97 (13.3%)	19 (18.6%)
Grade 2	165 (8.7%)	80 (4.7%)	18 (2.5%)	1 (1%)
Grade 3	20 (1.1%)	7 (0.4%)	2 (0.3%)	0
N doses in studies where Nausea was collected	1901	1688	728	102
Any grade	220 (11.6%)	97 (5.7%)	61 (8.4%)	0
Grade 1	166 (8.7%)	79 (4.7%)	53 (7.3%)	0
Grade 2	46 (2.4%)	16 (0.9%)	6 (0.8%)	0
Grade 3	8 (0.4%)	2 (0.1%)	2 (0.3%)	0
N doses in studies where Pruritus Generalised was collected	120	115	47	-
Any grade	8 (6.7%)	7 (6.1%)	3 (6.4%)	-
Grade 1	4 (3.3%)	3 (2.6%)	1 (2.1%)	-
Grade 2	4 (3.3%)	4 (3.5%)	2 (4.3%)	-
N doses in studies where Pyrexia was collected**	1901	1688	728	102
Any grade	134 (7%)	70 (4.1%)	31 (4.3%)	1 (1%)
Grade 1	82 (4.3%)	42 (2.5%)	13 (1.8%)	1 (1%)
Grade 2	38 (2%)	16 (0.9%)	11 (1.5%)	0
Grade 3	14 (0.7%)	12 (0.7%)	7 (1%)	0
N doses in studies where Rash was collected	120	115	47	-
Any grade	2 (1.7%)	7 (6.1%)	2 (4.3%)	-
Grade 1	2 (1.7%)	6 (5.2%)	1 (2.1%)	-
Grade 2	0	1 (0.9%)	1 (2.1%)	-
N doses in studies where Vomiting was collected	120	115	47	-
Any grade	5 (4.2%)	3 (2.6%)	0	-
Grade 1	5 (4.2%)	3 (2.6%)	0	-

n (%): number (percentage) of doses with 1 or more events;
Solicited adverse events with unknown severity are not taken into account in this table.
The denominator is the number of doses with available reactogenicity data.
* MenACWY: active control present in EBL3001 only.
** Pyrexia grading according to DMID toxicity tables for adolescents and adults (Grade 1: 38.0°C - 38.4□C, Grade 2: 38.5°C - 38.9°C, Grade 3: >38.9°C).

Systemic solicited adverse events in adolescents and children (>1 years – <18 years)

Solicited systemic AEs reported in children and adolescents per dose are presented in Table 23. Solicited systemic AEs (any grade) were more frequently reported after vaccination with Ad26.ZEBOV than after vaccination with MVA-BN-Filo (e.g., headache was reported for 13.5% and 8.4% [children aged 4-11 years] and 34.8% and 21.5% [adolescents aged 12-17 years] of participants, respectively). Grade 3 solicited systemic AEs were only reported after Ad26.ZEBOV vaccination and not after MVA-BN-Filo vaccination, except for pyrexia in children aged 1-3 years which was reported for 1 child after Ad26.ZEBOV vaccination and for 1 child after MVA-BN-Filo vaccination.

In children aged 1-3 years the 3 most frequently reported AEs by PT were decreased appetite, decreased activity, and pyrexia reported for 21.5%, 19.4%, and 18.1% of active vaccine recipients (i.e. by regimen), which is similar to the frequency reported in the control vaccine recipients (16.7%, 20.8% and 22.9% decreased appetite, decreased activity and pyrexia.In children aged 4-11 years the 4 most frequently reported AEs by PT were headache, fatigue and decreased activity reported for 18.7%, 9.1%, and 9.1% of active vaccine recipients, similar to or less than the frequency reported in the control vaccine recipients (37.5%,16.7% and 12.5% for headache, fatigue and decreased activity respectively. Pyrexia was reported in 14.7% in active vaccine accine recipients and in 4.2% of control vaccine recipients. In adolescents aged 12-17 years, the 3 most frequently reported AEs by PT were headache, fatigue, and myalgia reported for 42.3%, 29.2%, and 19.8% of active vaccine recipients, similar to the frequency reported in the placebo participants (38.1%, 33.3% and 14.3%) for headache, fatigue and myalgia respectively.

A trend for higher frequencies of pyrexia in the active vaccine and control groups was observed in children aged 1-3 years and children aged 4-11 years compared to adolescents aged 12-17 years. The majority of solicited systemic AEs of pyrexia were grade 1 or grade 2 in severity. Grade 3 pyrexia (>40°C for children and >38.9°C for adolescents) was reported for 1.4% of children aged 1 - 3 years who received the active vaccine regimen and none who received the active control regimen; and 1.2% and 2.1% of adolescents aged 12-17 years who received the active vaccine and active control regimens, respectively.

Table 23: Solicited Adverse Events: Systemic Adverse Events by Worst Severity Grade by Dose - Pediatric Pooling

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Age group: 1-3 years				
Number of Doses	144	143	48	48
Any systemic event	36 (25%)	23 (16.1%)	14 (29.2%)	12 (25%)
Grade 1	25 (17.4%)	13 (9.1%)	9 (18.8%)	10 (20.8%)
Grade 2	10 (6.9%)	9 (6.3%)	3 (6.3%)	2 (4.2%)
Grade 3	1 (0.7%)	1 (0.7%)	2 (4.2%)	0
N doses in studies where Decreased Activity was collected	144	143	48	48
Any grade	19 (13.2%)	12 (8.4%)	5 (10.4%)	6 (12.5%)
Grade 1	17 (11.8%)	12 (8.4%)	3 (6.3%)	5 (10.4%)
Grade 2	2 (1.4%)	0	1 (2.1%)	1 (2.1%)
Grade 3	0	0	1 (2.1%)	0
N doses in studies where Decreased Appetite was collected	144	143	48	48
Any grade	20 (13.9%)	14 (9.8%)	6 (12.5%)	3 (6.3%)
Grade 1	18 (12.5%)	14 (9.8%)	4 (8.3%)	3 (6.3%)
Grade 2	2 (1.4%)	0	1 (2.1%)	0
Grade 3	0	0	1 (2.1%)	0
N doses in studies where Irritability was collected	144	143	48	48
Any grade	15 (10.4%)	6 (4.2%)	3 (6.3%)	4 (8.3%)
Grade 1	13 (9%)	6 (4.2%)	2 (4.2%)	4 (8.3%)
Grade 2	2 (1.4%)	0	1 (2.1%)	0
N doses in studies where Pyrexia was collected**	144	143	48	48
Any grade	16 (11.1%)	12 (8.4%)	7 (14.6%)	4 (8.3%)
Grade 1	7 (4.9%)	4 (2.8%)	2 (4.2%)	4 (8.3%)
Grade 2	8 (5.6%)	7 (4.9%)	5 (10.4%)	0
Grade 3	1 (0.7%)	1 (0.7%)	0	0

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
N doses in studies where Vomiting was collected	144	143	48	48
Any grade	9 (6.3%)	8 (5.6%)	4 (8.3%)	1 (2.1%)
Grade 1	7 (4.9%)	6 (4.2%)	3 (6.3%)	0
Grade 2	2 (1.4%)	2 (1.4%)	0	1 (2.1%)
Grade 3	0	0	1 (2.1%)	0
Age group: 4-11 years				
Number of Doses	252	251	95	48
Any systemic event	92 (36.5%)	47 (18.7%)	19 (20%)	15 (31.3%)
Grade 1	67 (26.6%)	38 (15.1%)	16 (16.8%)	12 (25%)
Grade 2	24 (9.5%)	9 (3.6%)	3 (3.2%)	3 (6.3%)
Grade 3	1 (0.4%)	0	0	0
N doses in studies where Arthralgia was collected	252	251	95	48
Any grade	7 (2.8%)	4 (1.6%)	0	1 (2.1%)
Grade 1	7 (2.8%)	4 (1.6%)	0	1 (2.1%)
N doses in studies where Chills was collected	252	251	95	48
Any grade	17 (6.7%)	4 (1.6%)	0	5 (10.4%)
Grade 1	12 (4.8%)	4 (1.6%)	0	4 (8.3%)
Grade 2	5 (2%)	0	0	1 (2.1%)
N doses in studies where Decreased Activity was collected	252	251	95	48
Any grade	20 (7.9%)	10 (4%)	4 (4.2%)	0
Grade 1	16 (6.3%)	9 (3.6%)	3 (3.2%)	0
Grade 2	3 (1.2%)	1 (0.4%)	1 (1.1%)	0
Grade 3	1 (0.4%)	0	0	0
N doses in studies where Decreased Appetite was collected	252	251	95	48
Any grade	16 (6.3%)	9 (3.6%)	4 (4.2%)	0
Grade 1	13 (5.2%)	9 (3.6%)	3 (3.2%)	0
Grade 2	2 (0.8%)	0	1 (1.1%)	0
Grade 3	1 (0.4%)	0	0	0
N doses in studies where Fatigue was collected	252	251	95	48
Any grade	15 (6%)	9 (3.6%)	0	8 (16.7%)
Grade 1	11 (4.4%)	7 (2.8%)	0	8 (16.7%)
Grade 2	4 (1.6%)	2 (0.8%)	0	0
N doses in studies where Headache was collected	252	251	95	48
Any grade	34 (13.5%)	21 (8.4%)	8 (8.4%)	14 (29.2%)
Grade 1	29 (11.5%)	20 (8%)	8 (8.4%)	12 (25%)
Grade 2	5 (2%)	1 (0.4%)	0	2 (4.2%)
N doses in studies where Irritability was collected	252	251	95	48
Any grade	19 (7.5%)	12 (4.8%)	5 (5.3%)	0
Grade 1	15 (6%)	10 (4%)	3 (3.2%)	0
Grade 2	3 (1.2%)	2 (0.8%)	2 (2.1%)	0
Grade 3	1 (0.4%)	0	0	0
N doses in studies where Myalgia was collected	252	251	95	48
Any grade	6 (2.4%)	6 (2.4%)	1 (1.1%)	1 (2.1%)
Grade 1	6 (2.4%)	6 (2.4%)	1 (1.1%)	1 (2.1%)
N doses in studies where Nausea was collected	252	251	95	48
Any grade	8 (3.2%)	5 (2%)	1 (1.1%)	2 (4.2%)
Grade 1	5 (2%)	5 (2%)	1 (1.1%)	2 (4.2%)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Grade 2	3 (1.2%)	0	0	0
N doses in studies where Pyrexia was collected**	252	251	95	48
Any grade	30 (11.9%)	9 (3.6%)	2 (2.1%)	2 (4.2%)
Grade 1	14 (5.6%)	4 (1.6%)	1 (1.1%)	2 (4.2%)
Grade 2	16 (6.3%)	5 (2%)	1 (1.1%)	0
N doses in studies where Vomiting was collected	252	251	95	48
Any grade	9 (3.6%)	7 (2.8%)	2 (2.1%)	0
Grade 1	7 (2.8%)	7 (2.8%)	2 (2.1%)	0
Grade 2	2 (0.8%)	0	0	0
Age group: 12-17 years				
Number of Doses	253	251	87	48
Any systemic event	111 (43.9%)	78 (31.1%)	24 (27.6%)	14 (29.2%)
Grade 1	87 (34.4%)	68 (27.1%)	18 (20.7%)	12 (25%)
Grade 2	20 (7.9%)	10 (4%)	6 (6.9%)	1 (2.1%)
Grade 3	4 (1.6%)	0	0	1 (2.1%)
N doses in studies where Arthralgia was collected	253	251	87	48
Any grade	25 (9.9%)	23 (9.2%)	10 (11.5%)	0
Grade 1	18 (7.1%)	20 (8%)	8 (9.2%)	0
Grade 2	7 (2.8%)	3 (1.2%)	2 (2.3%)	0
N doses in studies where Chills was collected	253	251	87	48
Any grade	34 (13.4%)	28 (11.2%)	5 (5.7%)	1 (2.1%)
Grade 1	29 (11.5%)	25 (10%)	4 (4.6%)	1 (2.1%)
Grade 2	4 (1.6%)	3 (1.2%)	1 (1.1%)	0
Grade 3	1 (0.4%)	0	0	0
N doses in studies where Fatigue was collected	253	251	87	48
Any grade	61 (24.1%)	36 (14.3%)	11 (12.6%)	1 (2.1%)
Grade 1	50 (19.8%)	30 (12%)	9 (10.3%)	1 (2.1%)
Grade 2	11 (4.3%)	6 (2.4%)	2 (2.3%)	0
N doses in studies where Headache was collected	253	251	87	48
Any grade	88 (34.8%)	54 (21.5%)	18 (20.7%)	11 (22.9%)
Grade 1	70 (27.7%)	48 (19.1%)	13 (14.9%)	10 (20.8%)
Grade 2	18 (7.1%)	6 (2.4%)	5 (5.7%)	1 (2.1%)
N doses in studies where Myalgia was collected	253	251	87	48
Any grade	33 (13%)	28 (11.2%)	7 (8%)	1 (2.1%)
Grade 1	30 (11.9%)	23 (9.2%)	5 (5.7%)	1 (2.1%)
Grade 2	3 (1.2%)	5 (2%)	2 (2.3%)	0
N doses in studies where Nausea was collected	253	251	87	48
Any grade	8 (3.2%)	6 (2.4%)	2 (2.3%)	1 (2.1%)
Grade 1	7 (2.8%)	4 (1.6%)	1 (1.1%)	1 (2.1%)
Grade 2	1 (0.4%)	2 (0.8%)	1 (1.1%)	0
N doses in studies where Pyrexia was collected**	253	251	87	48
Any grade	10 (4%)	5 (2%)	2 (2.3%)	1 (2.1%)
Grade 1	5 (2%)	5 (2%)	0	0
Grade 2	2 (0.8%)	0	2 (2.3%)	0
Grade 3	3 (1.2%)	0	0	1 (2.1%)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*

n (%): number (percentage) of doses with 1 or more events;
Solicited adverse events with unknown severity are not taken into account in this table.
The denominator is the number of doses with available reactogenicity data.
* MenACWY: active control present in EBL3001 only.
** ** Pyrexia grading according to DMID toxicity tables for adolescents and adults (Grade 1: 38.0°C - 38.4°C, Grade 2: 38.5°C - 38.9°C, Grade 3: >38.9°C) and for children more than 3 months of age (Grade 1: 38.0°C - 38.4°C, Grade 2: 38.5°C - 40°C, Grade 3: >40°C).

Unsolicited Adverse events in adults

Unsolicited adverse events in adults are presented in Table 24. No notable differences in frequencies of unsolicited AEs were observed after Ad26.ZEBOV and MVA-BN-Filo vaccination (e.g. malaria was reported for 5.8% and 4.6% of participants, respectively, upper respiratory tract infection for 3.1% and 3.9% of participants, respectively, and headache for 3.3% and 2.5% of participants, respectively). The frequency of grade 3 unsolicited AEs was low and similar after vaccination with Ad26.ZEBOV (2.6%), MVA-BN-Filo (2.3%), placebo (3.4%), and MenACWY (2.0%).

Table 24: Unsolicited Adverse Events: Most Frequent Unsolicited Adverse Events by System Organ Class and Dictionary-derived Term (at Least 1% in any of the Active Vaccines) by Dose- Adults (Restricted Pooling)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Number of Doses	1901	1688	728	102
Any event, n (%)	695 (36.6%)	554 (32.8%)	231 (31.7%)	65 (63.7%)
Infections and infestations	311 (16.4%)	260 (15.4%)	108 (14.8%)	40 (39.2%)
Malaria	111 (5.8%)	78 (4.6%)	34 (4.7%)	26 (25.5%)
Upper respiratory tract infection	59 (3.1%)	65 (3.9%)	29 (4%)	5 (4.9%)
Nasopharyngitis	37 (1.9%)	22 (1.3%)	11 (1.5%)	3 (2.9%)
Rhinitis	20 (1.1%)	16 (0.9%)	5 (0.7%)	0
Gastroenteritis	14 (0.7%)	14 (0.8%)	3 (0.4%)	2 (2%)
Urinary tract infection	12 (0.6%)	18 (1.1%)	7 (1%)	2 (2%)
Conjunctivitis	10 (0.5%)	7 (0.4%)	4 (0.5%)	4 (3.9%)
Furuncle	7 (0.4%)	6 (0.4%)	0	3 (2.9%)
Respiratory tract infection	7 (0.4%)	14 (0.8%)	4 (0.5%)	1 (1%)
Typhoid fever	7 (0.4%)	8 (0.5%)	6 (0.8%)	3 (2.9%)
Gonorrhoea	3 (0.2%)	0	0	1 (1%)
Sexually transmitted disease	2 (0.1%)	2 (0.1%)	0	2 (2%)
Fungal infection	1 (0.1%)	1 (0.1%)	0	1 (1%)
Sinusitis	1 (0.1%)	3 (0.2%)	0	1 (1%)
Nematodiasis	0	0	0	1 (1%)
Pneumonia	0	3 (0.2%)	1 (0.1%)	1 (1%)
Nervous system disorders	98 (5.2%)	59 (3.5%)	30 (4.1%)	10 (9.8%)
Headache	63 (3.3%)	42 (2.5%)	19 (2.6%)	10 (9.8%)
Investigations	92 (4.8%)	47 (2.8%)	18 (2.5%)	2 (2%)
Haemoglobin decreased	12 (0.6%)	9 (0.5%)	2 (0.3%)	1 (1%)
White blood cell count decreased	12 (0.6%)	5 (0.3%)	2 (0.3%)	1 (1%)
Granulocyte count decreased	0	0	0	1 (1%)
Gastrointestinal disorders	88 (4.6%)	75 (4.4%)	31 (4.3%)	7 (6.9%)
Abdominal pain	16 (0.8%)	9 (0.5%)	2 (0.3%)	3 (2.9%)
Peptic ulcer	8 (0.4%)	11 (0.7%)	0	1 (1%)
Toothache	6 (0.3%)	5 (0.3%)	4 (0.5%)	2 (2%)
Abdominal discomfort	3 (0.2%)	3 (0.2%)	1 (0.1%)	2 (2%)
Gastrooesophageal reflux disease	2 (0.1%)	1 (0.1%)	0	1 (1%)
Musculoskeletal and connective tissue disorders	71 (3.7%)	62 (3.7%)	23 (3.2%)	14 (13.7%)
Back pain	29 (1.5%)	17 (1%)	12 (1.6%)	6 (5.9%)
Arthralgia	16 (0.8%)	12 (0.7%)	7 (1%)	2 (2%)
Myalgia	12 (0.6%)	11 (0.7%)	1 (0.1%)	4 (3.9%)
Musculoskeletal pain	5 (0.3%)	10 (0.6%)	2 (0.3%)	1 (1%)
Pain in extremity	3 (0.2%)	4 (0.2%)	0	2 (2%)
Blood and lymphatic system disorders	56 (2.9%)	40 (2.4%)	16 (2.2%)	4 (3.9%)
Neutropenia	25 (1.3%)	13 (0.8%)	12 (1.6%)	1 (1%)
Leukopenia	8 (0.4%)	12 (0.7%)	2 (0.3%)	1 (1%)
Lymphadenopathy	3 (0.2%)	1 (0.1%)	0	1 (1%)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Lymphadenitis	2 (0.1%)	0	0	1 (1%)
General disorders and administration site conditions	50 (2.6%)	30 (1.8%)	15 (2.1%)	7 (6.9%)
Pain	13 (0.7%)	5 (0.3%)	6 (0.8%)	4 (3.9%)
Non-cardiac chest pain	4 (0.2%)	2 (0.1%)	1 (0.1%)	1 (1%)
Asthenia	3 (0.2%)	0	1 (0.1%)	1 (1%)
Fatigue	0	4 (0.2%)	1 (0.1%)	1 (1%)
Skin and subcutaneous tissue disorders	50 (2.6%)	29 (1.7%)	8 (1.1%)	7 (6.9%)
Pruritus generalised	11 (0.6%)	5 (0.3%)	1 (0.1%)	5 (4.9%)
Rash	2 (0.1%)	7 (0.4%)	0	1 (1%)
Penile ulceration	0	0	0	1 (1%)
Respiratory, thoracic and mediastinal disorders	48 (2.5%)	40 (2.4%)	12 (1.6%)	2 (2%)
Cough	15 (0.8%)	11 (0.7%)	6 (0.8%)	2 (2%)
Injury, poisoning and procedural complications	28 (1.5%)	17 (1%)	10 (1.4%)	5 (4.9%)
Ligament sprain	5 (0.3%)	1 (0.1%)	2 (0.3%)	1 (1%)
Contusion	3 (0.2%)	2 (0.1%)	1 (0.1%)	1 (1%)
Skin abrasion	2 (0.1%)	2 (0.1%)	0	1 (1%)
Limb injury	1 (0.1%)	1 (0.1%)	1 (0.1%)	1 (1%)
Abdominal wall wound	0	0	0	1 (1%)
Reproductive system and breast disorders	16 (0.8%)	6 (0.4%)	5 (0.7%)	1 (1%)
Genital rash	0	2 (0.1%)	0	1 (1%)
Metabolism and nutrition disorders	15 (0.8%)	18 (1.1%)	7 (1%)	0
Eye disorders	11 (0.6%)	8 (0.5%)	0	1 (1%)
Visual impairment	0	0	0	1 (1%)
Vascular disorders	10 (0.5%)	5 (0.3%)	2 (0.3%)	1 (1%)
Thrombophlebitis	0	0	0	1 (1%)
Ear and labyrinth disorders	5 (0.3%)	5 (0.3%)	3 (0.4%)	1 (1%)
Ear pain	0	0	0	1 (1%)

Adverse events are coded using MedDRA version 22.0.
n (%): number (percentage) of doses with 1 or more events
This table only includes adverse events that were reported between Dose 1 and 28 days post Dose 1, and between the Dose 2 and up to 28 days post Dose 2.
* MenACWY: active control present in EBL3001 only.

Unsolicited AEs considered related to the study vaccine were reported for 11.6%, 7.7%, and 5.9% of participants in the active vaccine, placebo control, and active control regimens, respectively.

After review of all AEs that appeared specifically in the Ad26.ZEBOV, MVA-BN-Filo group, taking into account the medical plausibility as well as the timing of events, only dizziness (n=20, 0.9%) was identified as an related adverse event not already captured in the solicited adverse events.

In the CSR for study EBL3001, which was the only study to include a MenACWY group (n=102; these subjects received MenACWY as a first dose and placebo as a second dose), pruritus generalised was reported in 11 subjects in the Ad26/MVA group and 5 subjects in the control group. It is further stated that generalized pruritus considered related to study vaccine was reported in 3 (1.0%) participants after Ad26.ZEBOV dosing and 2 (0.8%) participants after MVA-BN-Filo dosing, versus no participants after MenACWY or placebo dosing during stage 2 of the study. Additionally, a SUSAR of generalized pruritus in ongoing study EBL2004 was reported, which was considered related to MVA-BN-Filo by the investigator and sponsor, due to temporal plausibility and lack of alternative causes.

Unsolicited Adverse events in children and adolescents (>1 – <18 years)

Unsolicited adverse events reported in children and adolescents are presented in Table 25.

Generally, differences in frequencies of unsolicited AEs observed after Ad26.ZEBOV and MVA-BN-Filo vaccination were small (e.g., malaria was reported for 36.8% and 35.7% in children aged 1-3 years and 10.7% and 10.0% in adolescents aged 12-17 years] of participants, respectively; for children aged 4 – 11 years 19.0% (n=48) and 8.4% (n=21) reported malaria. The frequency of grade 3 unsolicited

AEs was low and similar after vaccination with Ad26.ZEBOV, MVA-BN-Filo, placebo, and MenACWY across the 3 age categories.

Table 25: Unsolicited Adverse Events: Most Frequent Unsolicited Adverse Events by System Organ Class and Dictionary-derived Term (at Least 1% in any of the Active Vaccines) by Dose - Pediatric Pooling

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Age group: 1-3 years				
Number of Doses	144	143	48	48
Any event, n (%)	88 (61.1%)	77 (53.8%)	29 (60.4%)	28 (58.3%)
Infections and infestations	78 (54.2%)	72 (50.3%)	26 (54.2%)	25 (52.1%)
Malaria	53 (36.8%)	51 (35.7%)	17 (35.4%)	14 (29.2%)
Upper respiratory tract infection	17 (11.8%)	10 (7%)	2 (4.2%)	7 (14.6%)
Respiratory tract infection	6 (4.2%)	12 (8.4%)	4 (8.3%)	1 (2.1%)
Furuncle	4 (2.8%)	2 (1.4%)	2 (4.2%)	1 (2.1%)
Nasopharyngitis	4 (2.8%)	2 (1.4%)	1 (2.1%)	4 (8.3%)
Acarodermatitis	2 (1.4%)	1 (0.7%)	1 (2.1%)	1 (2.1%)
Gastroenteritis	2 (1.4%)	6 (4.2%)	2 (4.2%)	3 (6.3%)
Rhinitis	2 (1.4%)	2 (1.4%)	0	0
Bullous impetigo	1 (0.7%)	2 (1.4%)	0	0
Pneumonia	1 (0.7%)	2 (1.4%)	0	0
Tinea capitis	1 (0.7%)	2 (1.4%)	1 (2.1%)	2 (4.2%)
Fungal skin infection	0	1 (0.7%)	0	1 (2.1%)
Otitis media acute	0	2 (1.4%)	0	0
Septic rash	0	0	0	1 (2.1%)
Tonsillitis	0	1 (0.7%)	0	1 (2.1%)
Gastrointestinal disorders	9 (6.3%)	2 (1.4%)	3 (6.3%)	3 (6.3%)
Diarrhoea	7 (4.9%)	2 (1.4%)	1 (2.1%)	3 (6.3%)
Skin and subcutaneous tissue disorders	5 (3.5%)	4 (2.8%)	2 (4.2%)	2 (4.2%)
Rash pruritic	3 (2.1%)	1 (0.7%)	1 (2.1%)	1 (2.1%)
Rash	1 (0.7%)	1 (0.7%)	0	1 (2.1%)
General disorders and administration site conditions	4 (2.8%)	1 (0.7%)	1 (2.1%)	1 (2.1%)
Pyrexia	4 (2.8%)	1 (0.7%)	1 (2.1%)	1 (2.1%)
Blood and lymphatic system disorders	3 (2.1%)	14 (9.8%)	2 (4.2%)	1 (2.1%)
Anaemia	3 (2.1%)	12 (8.4%)	0	1 (2.1%)
Thrombocytopenia	0	4 (2.8%)	1 (2.1%)	0
Investigations	2 (1.4%)	3 (2.1%)	0	1 (2.1%)
Alanine aminotransferase increased	1 (0.7%)	1 (0.7%)	0	1 (2.1%)
Aspartate aminotransferase increased	1 (0.7%)	2 (1.4%)	0	0
Injury, poisoning and procedural complications	1 (0.7%)	1 (0.7%)	1 (2.1%)	1 (2.1%)
Contusion	0	0	0	1 (2.1%)
Age group: 4-11 years				
Number of Doses	252	251	95	48
Any event, n (%)	102 (40.5%)	88 (35.1%)	36 (37.9%)	18 (37.5%)
Infections and infestations	83 (32.9%)	54 (21.5%)	18 (18.9%)	15 (31.3%)
Malaria	48 (19%)	21 (8.4%)	7 (7.4%)	11 (22.9%)
Upper respiratory tract infection	9 (3.6%)	4 (1.6%)	2 (2.1%)	3 (6.3%)
Nasopharyngitis	5 (2%)	3 (1.2%)	0	0
Bronchitis	3 (1.2%)	3 (1.2%)	3 (3.2%)	0
Gastroenteritis	3 (1.2%)	3 (1.2%)	1 (1.1%)	1 (2.1%)
Influenza	3 (1.2%)	2 (0.8%)	1 (1.1%)	0
Respiratory tract infection	3 (1.2%)	1 (0.4%)	1 (1.1%)	4 (8.3%)
Rhinitis	3 (1.2%)	7 (2.8%)	1 (1.1%)	0
Tinea capitis	3 (1.2%)	1 (0.4%)	0	1 (2.1%)
Body tinea	1 (0.4%)	1 (0.4%)	0	1 (2.1%)
Furuncle	1 (0.4%)	0	1 (1.1%)	1 (2.1%)
Tinea infection	1 (0.4%)	0	0	2 (4.2%)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
Injury, poisoning and procedural complications	8 (3.2%)	4 (1.6%)	0	1 (2.1%)
Soft tissue injury	0	1 (0.4%)	0	1 (2.1%)
Blood and lymphatic system disorders	6 (2.4%)	11 (4.4%)	7 (7.4%)	1 (2.1%)
Anaemia	2 (0.8%)	8 (3.2%)	5 (5.3%)	1 (2.1%)
Leukopenia	0	3 (1.2%)	1 (1.1%)	0
Gastrointestinal disorders	5 (2%)	5 (2%)	3 (3.2%)	2 (4.2%)
Abdominal pain	3 (1.2%)	4 (1.6%)	0	2 (4.2%)
Metabolism and nutrition disorders	5 (2%)	3 (1.2%)	2 (2.1%)	0
Hypernatraemia	5 (2%)	2 (0.8%)	2 (2.1%)	0
Skin and subcutaneous tissue disorders	5 (2%)	4 (1.6%)	1 (1.1%)	0
Investigations	4 (1.6%)	7 (2.8%)	2 (2.1%)	1 (2.1%)
Aspartate aminotransferase increased	2 (0.8%)	1 (0.4%)	0	1 (2.1%)
Nervous system disorders	3 (1.2%)	2 (0.8%)	1 (1.1%)	0
Headache	3 (1.2%)	2 (0.8%)	1 (1.1%)	0
Respiratory, thoracic and mediastinal disorders	3 (1.2%)	7 (2.8%)	5 (5.3%)	0
Cough	3 (1.2%)	2 (0.8%)	4 (4.2%)	0
Productive cough	0	3 (1.2%)	1 (1.1%)	0
Age group: 12-17 years				
Number of Doses	253	251	87	48
Any event, n (%)	113 (44.7%)	93 (37.1%)	30 (34.5%)	20 (41.7%)
Infections and infestations	61 (24.1%)	51 (20.3%)	13 (14.9%)	11 (22.9%)
Malaria	27 (10.7%)	25 (10%)	6 (6.9%)	7 (14.6%)
Upper respiratory tract infection	8 (3.2%)	5 (2%)	2 (2.3%)	1 (2.1%)
Nasopharyngitis	5 (2%)	4 (1.6%)	3 (3.4%)	0
Conjunctivitis	4 (1.6%)	2 (0.8%)	0	0
Respiratory tract infection	4 (1.6%)	3 (1.2%)	0	2 (4.2%)
Tonsillitis	2 (0.8%)	0	0	1 (2.1%)
Furuncle	1 (0.4%)	1 (0.4%)	0	1 (2.1%)
Helminthic infection	1 (0.4%)	0	0	1 (2.1%)
Paronychia	0	0	0	1 (2.1%)
Investigations	25 (9.9%)	12 (4.8%)	8 (9.2%)	4 (8.3%)
Blood urea decreased	5 (2%)	1 (0.4%)	1 (1.1%)	0
Haemoglobin decreased	5 (2%)	5 (2%)	3 (3.4%)	2 (4.2%)
Blood sodium decreased	4 (1.6%)	0	1 (1.1%)	0
Aspartate aminotransferase increased	3 (1.2%)	0	1 (1.1%)	2 (4.2%)
Alanine aminotransferase increased	1 (0.4%)	1 (0.4%)	0	1 (2.1%)
Blood and lymphatic system disorders	13 (5.1%)	7 (2.8%)	5 (5.7%)	3 (6.3%)
Neutropenia	3 (1.2%)	2 (0.8%)	1 (1.1%)	0
Thrombocytosis	3 (1.2%)	1 (0.4%)	0	1 (2.1%)
Leukocytosis	2 (0.8%)	1 (0.4%)	0	1 (2.1%)
Anaemia	1 (0.4%)	2 (0.8%)	2 (2.3%)	1 (2.1%)
Nervous system disorders	12 (4.7%)	7 (2.8%)	1 (1.1%)	1 (2.1%)
Headache	12 (4.7%)	7 (2.8%)	1 (1.1%)	1 (2.1%)
Injury, poisoning and procedural complications	9 (3.6%)	2 (0.8%)	1 (1.1%)	1 (2.1%)
Limb injury	4 (1.6%)	0	0	1 (2.1%)
Metabolism and nutrition disorders	8 (3.2%)	13 (5.2%)	3 (3.4%)	0
Hypernatraemia	6 (2.4%)	10 (4%)	2 (2.3%)	0
Hypercreatininaemia	3 (1.2%)	4 (1.6%)	1 (1.1%)	0
Gastrointestinal disorders	6 (2.4%)	3 (1.2%)	1 (1.1%)	3 (6.3%)
Gastritis	1 (0.4%)	0	0	1 (2.1%)
Angular cheilitis	0	0	0	1 (2.1%)
Peptic ulcer	0	0	0	1 (2.1%)
Respiratory, thoracic and mediastinal disorders	3 (1.2%)	4 (1.6%)	2 (2.3%)	0
Skin and subcutaneous tissue disorders	3 (1.2%)	2 (0.8%)	0	1 (2.1%)
Skin ulcer	0	0	0	1 (2.1%)

	Ad26.ZEBOV	MVA-BN-Filo	Placebo	MenACWY*
General disorders and administration site conditions	2 (0.8%)	5 (2%)	1 (1.1%)	2 (4.2%)
Pain	1 (0.4%)	2 (0.8%)	0	1 (2.1%)
Pyrexia	0	0	0	1 (2.1%)
Cardiac disorders	1 (0.4%)	1 (0.4%)	1 (1.1%)	1 (2.1%)
Tachycardia	0	1 (0.4%)	0	1 (2.1%)
Musculoskeletal and connective tissue disorders	1 (0.4%)	4 (1.6%)	0	1 (2.1%)
Pain in extremity	0	0	0	1 (2.1%)
Vascular disorders	1 (0.4%)	0	1 (1.1%)	1 (2.1%)
Hypotension	0	0	0	1 (2.1%)

Adverse events are coded using MedDRA version 22.0.
n (%): number (percentage) of doses with 1 or more events
This table only includes adverse events that were reported between Dose 1 and 28 days post Dose 1, and between the Dose 2 and up to 28 days post Dose 2.
* MenACWY: active control present in EBL3001 only.

Unsolicited AEs considered related to the study vaccine were reported for 0.7% and 4.2% of children aged 1-3 years in the active vaccine and active control regimens, respectively. Unsolicited AEs considered related to the study vaccine were reported for 6.0%, 12.5%, and 0% of children aged 4-11 years who received the active vaccine, placebo control, and active control regimens, respectively and 12.3%, 14.3%, and 4.2% of adolescents aged 12-17 years who received the active vaccine, placebo control, and active control regimens, respectively.

Adverse events of special interest

Cardiac safety

Modified vaccinia Ankara (MVA), which is a highly attenuated strain of vaccinia virus derived from a replication-competent Ankara vaccinia strain, has been used to vaccinate >120,000 people in Germany in the 1970s against smallpox, without significant side effects. In contrast, vaccinations with the replicating smallpox vaccine Dryvax® or the second generation replicating vaccinia vaccine ACAM2000® revealed increased rates of myocarditis and myopericarditis, which were all considered at least possibly related to the study vaccine. Therefore, a requirement for prospective cardiac monitoring to clinical studies employing MVA issued by the Food and Drug Administration (FDA) was adopted by Bavarian Nordic (BN), who decided to monitor cardiac AESIs in all clinical studies using the MVA BN® vector.

In the Phase 1 studies with MVA-BN-Filo, i.e. EBL1001, EBL1003, and EBL1004, any cardiac related signs or symptoms (including increases in troponin I greater than twice the normal value) and electrocardiogram (ECG) changes determined to be clinically significant by the investigator were to be reported as AESIs for the MVA-BN-Filo vaccine.

In total, 2 AESIs were reported after vaccination with MVA-BN-Filo

- Asymptomatic grade 1 ECG T wave inversion was reported for 1 participant 3 days after vaccination with MVA-BN-Filo, which was considered possibly related to the study vaccine. When the ECG was repeated 8 days later, 11 days after MVA-BN-Filo vaccination, the ECG intervals were normal and the T wave inversion had resolved. (Participant ID:, study EBL1003).

- Grade 3 bradycardia was reported for 1 participant 1 hour after MVA-BN-Filo vaccination on Day 1, which was considered probably related to the study vaccine and which was a protocol-specific contraindication to the second vaccination. Symptoms resolved within 1 hour without medication (Participant ID:, study EBL1004).

Additionally, the following cardiac events were also reported, although not as AESIs:

- Increased troponin I (twice the normal value) was reported for 2 participants (0.07 and 0.08 µg/L, respectively) after Ad26.ZEBOV vaccination and was initially considered an AESI. No clinical manifestations associated with the troponin increases were noted. As these increases in troponin occurred prior to MVA-BN-Filo vaccination, they were no longer considered AESIs after unblinding of the study (participant IDs:, study EBL1003).

- Grade 1 palpitations (transient 'awareness of heart beat') was reported for 1 participant 7 days after vaccination with MVA-BN-Filo and was not reported as an AESI (Participant ID:, study EBL1003).

- Grade 1 hypertension was reported for 1 participant on Day 1, after Ad26.ZEBOV vaccination and was initially considered an AESI. This participant received antihypertensive treatment. As the hypertension was reported after Ad26.ZEBOV vaccination and prior to receiving MVA-BN-Filo, it was no longer considered an AESI after unblinding of the study (Participant ID:, study EBL1004.

In conclusion, following the outcome of these Phase 1 studies and the outcome of the analysis of the MVA-BN safety database, cardiac events were no longer considered an AESI for the vaccine regimen, which is supported by the data from Phase 2 and Phase 3 studies.

Neuro-inflammatory Events

In 2016, two SAEs of potential neuro-inflammatory nature (Miller Fisher syndrome and small fibre neuropathy) were reported in the Phase 2 study EBL2001, conducted in France and the United Kingdom. The study was halted and the blind to study vaccine assignment was broken by the sponsor for the reporting to regulatory authorities and for the review by the external neurology expert panel.

- One subject experienced a serious and very rare condition called 'Miller Fisher syndrome', which consists of double vision, pain on moving the eye, and difficulty with balance while walking. Miller Fisher syndrome most commonly occurs after a recent infection. The subject experienced the symptoms about a week after a respiratory tract infection and about a month after dose 2 vaccination with MVA-BN-Filo. The subject had to go to hospital for treatment and recovered. This serious adverse event was initially reported as possibly related to dose 2. After extensive evaluation, the event was considered to be doubtfully related to study vaccine and most likely related to the prior upper respiratory tract infection by the investigator and the sponsor.
- One subject experienced intermittent episodes of paraesthesia of the palms and soles, which was initially reported as a serious adverse event of 'possible cervical myelitis' after dose 1 vaccination with Ad26.ZEBOV, and considered to be possibly related to study vaccine by the investigator. Based on initial magnetic resonance imaging (MRI), the neurology team treating the subject was not unanimous in agreement of the diagnosis of cervical myelitis; therefore, a second MRI assessment was performed, which was declared normal by the neurology team. As such, the diagnosis of cervical myelitis was withdrawn and the event downgraded to a nonserious adverse event of intermittent paraesthesia of the palms and soles. However, due to the subsequent clinical evolution of the subject's symptoms, with persistent limitation and disability in daily life activities as well as iterative hospitalizations, and after thorough evaluations, the diagnosis of nonserious paraesthesia of the palms and soles was replaced by the investigator with a diagnosis of a serious case of small fibre neuropathy. The causality assessment per the investigator and sponsor was possibly related to study vaccine.

Following the assessment of the above mentioned SAEs, the Medicines and Healthcare Products Regulatory Agency (MHRA) and Ethics Committee approved the restart of study vaccinations in study EBL2001 in the United Kingdom on 27 September 2016 and 07 November 2016, respectively. The

Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) also approved the restart of study vaccinations in France, however, the study did not restart as approval was not granted by the French Ethics Committee.

Per request of the ANSM, neuroinflammatory events occurring in any of the ongoing or subsequent clinical studies that were considered serious were to be reported in an expedited manner. Consequently, a process for IRE collection and monitoring of neuroinflammatory events, as agreed with the panel of external neurology experts and communicated to FDA, was implemented in the ongoing Phase 1 studies EBL1002 and FLV1001, the ongoing Phase 2 studies EBL2001, EBL2002 and EBL2003, the ongoing Phase 3 studies EBL3001 and EBL3002, and subsequent clinical studies. In addition, the Applicant's databases were used to identify any potential neuroinflammatory events using search criteria. The neurology expert panel, employed to assess the 2 SAEs described above, was retained to assess any future neuroinflammatory events as needed.

Overall, the frequency of AEs of potential neuro-inflammatory nature was similar between active vaccine and placebo control groups. No events reported through the IRE process were confirmed to be neuro-inflammatory diseases or disorders which could be associated with the study vaccines.

The most commonly reported AE of potential neuroinflammatory nature was paraesthesia which occurred in a similar rate following the vaccine regimen as following placebo: considering the adverse events related to study vaccination in the restricted adult pooling, paraesthesia was reported in 7 subjects (0.4%) following the vaccine regimen compared to 1 subject (0.3%) following placebo.

Adverse Events Following Ad26.ZEBOV Booster Dose

A summary of solicited AEs by worst severity grade, reported during the 7-day post Dose 1 (Ad26.ZEBOV) versus post booster (Ad26.ZEBOV) vaccination phase, is provided by dose in Table 26 (local) and Table 27 (systemic).

Similar frequencies of solicited local AEs were reported for participants who received Ad26.ZEBOV as booster dose (42.9%) compared to participants who received Ad26.ZEBOV as Dose 1 (45.2%). The frequency of solicited systemic AEs tended to be lower for participants who received Ad26.ZEBOV as booster dose (43.7%) compared to participants who received Ad26.ZEBOV as Dose 1 (54.8%).

Table 26: Solicited Adverse Events: Solicited Local Adverse Events by Worst Severity Grade of Post Dose 1 (Ad26.ZEBOV) Versus Post Booster Dose (Ad26.ZEBOV) - Adults (Extended Pooling)

	Post dose 1		Post booster dose	
	Ad26.ZEBOV	Placebo	Ad26.ZEBOV	Placebo
Number of Doses	126	17	126	17
Any local event	57 (45.2%)	6 (35.3%)	54 (42.9%)	4 (23.5%)
Grade 1	48 (38.1%)	5 (29.4%)	48 (38.1%)	4 (23.5%)
Grade 2	9 (7.1%)	1 (5.9%)	6 (4.8%)	0
N doses in studies where Injection Site Erythema was collected	126	17	126	17
Any grade	1 (0.8%)	2 (11.8%)	0	1 (5.9%)
Grade 1	1 (0.8%)	2 (11.8%)	0	1 (5.9%)
N doses in studies where Injection Site Induration was collected	24	-	24	-
No data to report	-	-	-	-
N doses in studies where Injection Site Pain was collected	126	17	126	17
Any grade	51 (40.5%)	4 (23.5%)	44 (34.9%)	4 (23.5%)
Grade 1	42 (33.3%)	4 (23.5%)	38 (30.2%)	4 (23.5%)

	Post dose 1		Post booster dose	
	Ad26.ZEBOV	Placebo	Ad26.ZEBOV	Placebo
Grade 2	9 (7.1%)	0	6 (4.8%)	0
N doses in studies where Injection Site Pruritus was collected	126	17	126	17
Any grade	14 (11.1%)	4 (23.5%)	15 (11.9%)	2 (11.8%)
Grade 1	14 (11.1%)	4 (23.5%)	13 (10.3%)	2 (11.8%)
Grade 2	0	0	2 (1.6%)	0
N doses in studies where Injection Site Swelling was collected	126	17	126	17
Any grade	16 (12.7%)	4 (23.5%)	15 (11.9%)	2 (11.8%)
Grade 1	15 (11.9%)	3 (17.6%)	15 (11.9%)	2 (11.8%)
Grade 2	1 (0.8%)	1 (5.9%)	0	0
N doses in studies where Injection Site Warmth was collected	24	-	24	-
Any grade	1 (4.2%)	-	4 (16.7%)	-
Grade 1	0	-	4 (16.7%)	-
Grade 2	1 (4.2%)	-	0	-

n (%): number (percentage) of doses with 1 or more events.
The denominator is the number of doses with available reactogenicity data.
The placebo booster doses are from the EBL2002 study only.

Table 27: Solicited Adverse Events: Solicited Systemic Adverse Events by Worst Severity Grade of Post Dose 1 (Ad26.ZEBOV) Versus Post Booster Dose (Ad26.ZEBOV) - Adults (Extended Pooling)

	Post dose 1		Post booster dose	
	Ad26.ZEBOV	Placebo	Ad26.ZEBOV	Placebo
Number of Doses	126	17	126	17
Any systemic event	69 (54.8%)	11 (64.7%)	55 (43.7%)	6 (35.3%)
Grade 1	50 (39.7%)	10 (58.8%)	43 (34.1%)	5 (29.4%)
Grade 2	16 (12.7%)	1 (5.9%)	11 (8.7%)	1 (5.9%)
Grade 3	3 (2.4%)	0	1 (0.8%)	0
N doses in studies where Arthralgia was collected	126	17	126	17
Any grade	28 (22.2%)	3 (17.6%)	18 (14.3%)	4 (23.5%)
Grade 1	21 (16.7%)	3 (17.6%)	14 (11.1%)	3 (17.6%)
Grade 2	6 (4.8%)	0	4 (3.2%)	1 (5.9%)
Grade 3	1 (0.8%)	0	0	0
N doses in studies where Chills was collected	126	17	126	17
Any grade	20 (15.9%)	3 (17.6%)	15 (11.9%)	1 (5.9%)
Grade 1	14 (11.1%)	3 (17.6%)	14 (11.1%)	1 (5.9%)
Grade 2	4 (3.2%)	0	1 (0.8%)	0
Grade 3	2 (1.6%)	0	0	0
N doses in studies where Fatigue was collected	126	17	126	17
Any grade	47 (37.3%)	9 (52.9%)	36 (28.6%)	3 (17.6%)
Grade 1	34 (27%)	9 (52.9%)	33 (26.2%)	3 (17.6%)
Grade 2	13 (10.3%)	0	3 (2.4%)	0
N doses in studies where Headache was collected	126	17	126	17
Any grade	49 (38.9%)	6 (35.3%)	38 (30.2%)	4 (23.5%)
Grade 1	37 (29.4%)	6 (35.3%)	37 (29.4%)	4 (23.5%)
Grade 2	11 (8.7%)	0	1 (0.8%)	0
Grade 3	1 (0.8%)	0	0	0
N doses in studies where Myalgia was collected	126	17	126	17
Any grade	32 (25.4%)	2 (11.8%)	20 (15.9%)	3 (17.6%)

	Post dose 1		Post booster dose	
	Ad26.ZEBOV	Placebo	Ad26.ZEBOV	Placebo
Grade 1	22 (17.5%)	2 (11.8%)	18 (14.3%)	3 (17.6%)
Grade 2	9 (7.1%)	0	2 (1.6%)	0
Grade 3	1 (0.8%)	0	0	0
N doses in studies where Nausea was collected	126	17	126	17
Any grade	14 (11.1%)	2 (11.8%)	14 (11.1%)	1 (5.9%)
Grade 1	13 (10.3%)	2 (11.8%)	13 (10.3%)	1 (5.9%)
Grade 2	1 (0.8%)	0	1 (0.8%)	0
N doses in studies where Pruritus Generalised was collected	24	-	24	-
Any grade	1 (4.2%)	-	0	-
Grade 1	1 (4.2%)	-	0	-
N doses in studies where Pyrexia was collected	126	17	126	17
Any grade	6 (4.8%)	2 (11.8%)	5 (4%)	0
Grade 1	4 (3.2%)	1 (5.9%)	2 (1.6%)	0
Grade 2	2 (1.6%)	1 (5.9%)	2 (1.6%)	0
Grade 3	0	0	1 (0.8%)	0
N doses in studies where Rash was collected	24	-	24	-
Any grade	1 (4.2%)	-	0	-
Grade 1	1 (4.2%)	-	0	-
N doses in studies where Vomiting was collected	24	-	24	-
Any grade	1 (4.2%)	-	0	-
Grade 1	1 (4.2%)	-	0	-

n (%): number (percentage) of doses with 1 or more events.
The denominator is the number of doses with available reactogenicity data.
The placebo booster doses are from the EBL2002 study only.

Adverse events following Other Regimens

The **reverse order** (MVA-BN-Filo followed by Ad26.ZEBOV) was explored in the Phase 1 studies EBL1001, EBL1002, EBL1003, EBL1004 and further evaluated in the Phase 2 study EBL2003. There was no indication of a divergent safety profile when Ad26.ZEBOV was given after MVA-BN-Filo as compared to Ad26.ZEBOV followed by MVA-BN-Filo when given 28 days apart in study EBL1001, albeit based on limited numbers (MVA, Ad26: n=15; Ad26,MVA: N=15; Placebo, Placebo: N=6). Similar results were seen with a 56 day interval. No safety concerns were identified with reverse order regimens.

Different dosing intervals were studied in several studies. In studies EBL2001 and EBL2002, the safety and reactogenicity of the heterologous vaccine regimen consisting of Ad26.ZEBOV ($5x10^{10}$ vp) and MVA-BN-Filo ($1x10^{8}$ Inf.U) with vaccination intervals of 28, 56, and 84 days was evaluated in adults. In study EBL2002, the safety and reactogenicity of the heterologous vaccination regimen (Ad26.ZEBOV [$5x10^{10}$ vp], MVA-BN-Filo [$1x10^{8}$ Inf.U]) with 28-day and 56 day vaccination intervals were evaluated in adolescents (12-17 years) and children (4-11 years). There was no apparent influence of the time interval of 28, 56, or 84 days (or ≥98 days due to study pause) between the Ad26.ZEBOV and MVA-BN-Filo doses on the frequency of reported solicited and unsolicited AEs. No clinically relevant safety concerns were identified in adults after vaccination with the heterologous Ad26.ZEBOV, MVA-BN-Filo regimen in a 14-day interval, as evaluated in the open label part of study EBL1001.

Homologous regimens (MVA-BN-Filo/ MVA-BN-Filo and Ad26.ZEBOV/ Ad26.ZEBOV) were studied in EBL1002. There were more solicited adverse events with the Ad26/Ad26 regimen which may be

reflective of the increased reactogenicity of Ad26 compared to MVA-BN-Filo. No safety concerns were identified using 2-dose homologous vaccine regimens; hence no safety issues are expected in case the same vaccine component would erroneously be administered twice instead of the recommended 2-dose heterologous vaccine regimen.

Vaccination with High dose and low dose formulations

Higher and lower dose levels for Ad26.ZEBOV ($0.8x10^{10}$ vp [low], $2x10^{10}$ vp [intermediate], $5x10^{10}$ vp [selected], $1x10^{11}$ vp [high]) and MVA-BN-Filo ($5x10^{7}$ Inf.U [low], $1x10^{8}$ Inf.U [selected], $4.4x10^{8}$ Inf.U [high]) were evaluated in adults in studies EBL1002 (high and selected) and EBL3002 (intermediate and low).

An overview of solicited local and systemic AEs with higher and lower dose levels of the active vaccines is provided for the extended adult pooled analyses in Table 28.

There was a trend for a lower frequency of solicited local and systemic AEs with the low dose level of both active vaccines, compared to the selected dose level. The majority of solicited local and systemic AEs were grade 1 and grade 2 in severity.

In general, across vaccines, the 3 most frequently reported solicited local AEs after vaccination were injection site pain, injection site warmth, and injection site swelling. All other solicited local AEs were reported in <10% of participants. The 3 most frequently reported solicited systemic AEs were fatigue, headache, and myalgia. All other solicited systemic AEs were reported in ≤40% of participants for all vaccine components.

Lower dose levels of both active vaccines as well as higher dose levels up to $1x10^{11}$ vp Ad26.ZEBOV and/or up to $4.4x10^{8}$ Inf.U MVA-BN-Filo did not identify a safety issue.

Table 28: Solicited Adverse Events by Worst Severity (Any Grade and Grade 3) by Dose - Adults (Extended Pooling)

	Ad26 (l)	Ad26 (i)	Ad26	**Ad26 (h)**	MVA (l)	MVA	**MVA (h)**	Placebo	MenACWY*
Number of Doses	150	150	2610	15	287	2369	29	1006	102
Any local event	37 (24.7%)	61 (40.7%)	1386 (53.1%)	7 (46.7%)	95 (33.1%)	1190 (50.2%)	18 (62.1%)	214 (21.3%)	17 (16.7%)
Grade 3	0	0	30 (1.1%)	0	2 (0.7%)	10 (0.4%)	0	0	0
N doses in studies where Injection Site Erythema was collected	150	150	2610	15	287	2369	29	1006	102
Injection Site Erythema	1 (0.7%)	0	31 (1.2%)	0	0	17 (0.7%)	0	6 (0.6%)	0
Grade 3	0	0	11 (0.4%)	0	0	1 (< 0.1%)	0	0	0
N doses in studies where Injection Site Induration was collected	-	-	312	15	-	299	29	124	-
Injection Site Induration	-	-	1 (0.3%)	0	-	2 (0.7%)	0	0	-
Grade 3	-	-	0	0	-	0	0	0	-
N doses in studies where Injection Site Pain was collected	150	150	2610	15	287	2369	29	1006	102
Injection Site Pain	28 (18.7%)	58 (38.7%)	1272 (48.7%)	7 (46.7%)	89 (31%)	1105 (46.6%)	18 (62.1%)	177 (17.6%)	16 (15.7%)
Grade 3	0	0	13 (0.5%)	0	2 (0.7%)	9 (0.4%)	0	0	0
N doses in studies where Injection Site Pruritus was collected	150	150	2610	15	287	2369	29	1006	102
Injection Site Pruritus	6 (4%)	5 (3.3%)	254 (9.7%)	0	11 (3.8%)	230 (9.7%)	0	66 (6.6%)	3 (2.9%)
Grade 3	0	0	1 (< 0.1%)	0	1 (0.3%)	0	0	0	0

	Ad26 (l)	Ad26 (i)	Ad26	**Ad26 (h)**	MVA (l)	MVA	**MVA (h)**	Placebo	MenACWY*
N doses in studies where Injection Site Swelling was collected	150	150	2610	15	287	2369	29	1006	102
Injection Site Swelling	10 (6.7%)	4 (2.7%)	284 (10.9%)	0	11 (3.8%)	221 (9.3%)	0	57 (5.7%)	1 (1%)
Grade 3	0	0	9 (0.3%)	0	1 (0.3%)	1 (< 0.1%)	0	0	0
N doses in studies where Injection Site Warmth was collected	-	-	327	15	-	310	29	129	-
Injection Site Warmth	-	-	74 (22.6%)	3 (20%)	-	58 (18.7%)	3 (10.3%)	14 (10.9%)	-
Grade 3	-	-	2 (0.6%)	0	-	0	0	0	-
Any systemic event	53 (35.3%)	74 (49.3%)	1728 (66.2%)	10 (66.7%)	102 (35.5%)	1194 (50.4%)	8 (27.6%)	446 (44.3%)	51 (50%)
Grade 3	2 (1.3%)	2 (1.3%)	104 (4%)	0	6 (2.1%)	32 (1.4%)	1 (3.4%)	18 (1.8%)	0
N doses in studies where Arthralgia was collected	150	150	2610	15	287	2369	29	1006	102
Arthralgia	9 (6%)	17 (11.3%)	618 (23.7%)	3 (20%)	20 (7%)	372 (15.7%)	0	119 (11.8%)	23 (22.5%)
Grade 3	0	0	14 (0.5%)	0	2 (0.7%)	4 (0.2%)	0	0	0
N doses in studies where Chills was collected	150	150	2610	15	287	2369	29	1006	102
Chills	6 (4%)	12 (8%)	599 (23%)	6 (40%)	10 (3.5%)	253 (10.7%)	0	95 (9.4%)	7 (6.9%)
Grade 3	0	0	40 (1.5%)	0	1 (0.3%)	6 (0.3%)	0	2 (0.2%)	0
N doses in studies where Fatigue was collected	150	150	2610	15	287	2369	29	1006	102
Fatigue	25 (16.7%)	51 (34%)	1196 (45.8%)	4 (26.7%)	45 (15.7%)	740 (31.2%)	4 (13.8%)	274 (27.2%)	16 (15.7%)
Grade 3	1 (0.7%)	0	49 (1.9%)	0	1 (0.3%)	15 (0.6%)	0	6 (0.6%)	0
N doses in studies where Headache was collected	150	150	2610	15	287	2369	29	1006	102
Headache	26 (17.3%)	39 (26%)	1151 (44.1%)	5 (33.3%)	35 (12.2%)	654 (27.6%)	4 (13.8%)	275 (27.3%)	39 (38.2%)
Grade 3	1 (0.7%)	2 (1.3%)	48 (1.8%)	0	2 (0.7%)	6 (0.3%)	0	4 (0.4%)	0
N doses in studies where Myalgia was collected	150	150	2610	15	287	2369	29	1006	102
Myalgia	25 (16.7%)	31 (20.7%)	924 (35.4%)	6 (40%)	54 (18.8%)	625 (26.4%)	3 (10.3%)	149 (14.8%)	20 (19.6%)

	Ad26 (l)	Ad26 (i)	Ad26	**Ad26 (h)**	MVA (l)	MVA	**MVA (h)**	Placebo	MenACWY*
Grade 3	0	0	24 (0.9%)	0	3 (1%)	8 (0.3%)	0	2 (0.2%)	0
N doses in studies where Nausea was collected	150	150	2610	15	287	2369	29	1006	102
Nausea	5 (3.3%)	13 (8.7%)	326 (12.5%)	2 (13.3%)	10 (3.5%)	178 (7.5%)	2 (6.9%)	88 (8.7%)	0
Grade 3	1 (0.7%)	0	10 (0.4%)	0	1 (0.3%)	5 (0.2%)	0	2 (0.2%)	0
N doses in studies where Pruritus Generalised was collected	-	-	327	15	-	310	29	129	-
Pruritus Generalised	-	-	19 (5.8%)	0	-	18 (5.8%)	0	6 (4.7%)	-
Grade 3	-	-	0	0	-	0	0	0	-
N doses in studies where Pyrexia was collected	150	150	2610	15	287	2369	29	1006	102
Pyrexia	4 (2.7%)	4 (2.7%)	195 (7.5%)	3 (20%)	10 (3.5%)	91 (3.8%)	1 (3.4%)	45 (4.5%)	1 (1%)
Grade 3	0	0	20 (0.8%)	0	2 (0.7%)	14 (0.6%)	1 (3.4%)	9 (0.9%)	0
N doses in studies where Rash was collected	-	-	327	15	-	310	29	129	-
Rash	-	-	8 (2.4%)	0	-	11 (3.5%)	0	4 (3.1%)	-
Grade 3	-	-	0	0	-	0	0	0	-
N doses in studies where Vomiting was collected	-	-	327	15	-	310	29	129	-
Vomiting	-	-	14 (4.3%)	0	-	8 (2.6%)	0	4 (3.1%)	-
Grade 3	-	-	0	0	-	1 (0.3%)	0	0	-

n (%): number (percentage) of doses with 1 or more events;
Solicited adverse events with unknown severity are not taken into account in this table.
The denominator is the number of doses with available reactogenicity data.
Ad26: Ad26.ZEBOV ($5x10^{10}$ vp); MVA: MVA-BN-Filo ($1x10^{8}$ Inf U);
h: high dose ($1x10^{11}$ vp for Ad26.ZEBOV and $4.4x10^{8}$ Inf.U for MVA-BN-Filo);
i: intermediate dose ($2x10^{10}$ vp for Ad26.ZEBOV);
l: low dose ($0.8x10^{10}$ vp for Ad26.ZEBOV and $5x10^{7}$ Inf.U for MVA-BN-Filo);
* MenACWY: active control present in EBL3001 only.

Serious adverse events and deaths

Deaths

Up to the cut-off date of the pooled safety analysis, 6 deaths were reported in adults and one death in children which were all considered unrelated to the study vaccine:

- One participant enrolled in the Ad26.ZEBOV, MVA-BN-Filo group died on Day 197 post Dose 2 (MVA-BN-Filo) due to severe dehydration as a result of severe vomiting (participant ID:, study EBL3001).
- One participant enrolled in the Ad26.ZEBOV, MVA-BN-Filo group died on Day 216 post Dose 2 (MVA-BN-Filo) due to the toxic effects of chronic prescription drug abuse (present for years per death certificate but unknown to the investigator at the time of enrolment) (participant ID:, study EBL3003).
- One participant enrolled in the placebo control group died on Day 54 post Dose 1 (placebo) due to the toxic effects of benzodiazepines, cocaine, and opiates (participant ID:, study EBL3003).
- One participant (HIV-infected adult) died on Day 283 post Dose 2 (MVA-BN-Filo) due to alcohol poisoning (heavy alcohol consumption for 2 consecutive days) (participant ID:, study EBL2002).
- One participant died on Day 12 post Dose 1 (Ad26.ZEBOV $2x10^{10}$ vp) due to toxicity to various agents (accidental fentanyl intoxication). The participant used the fentanyl recreationally without knowledge of the study site personnel (participant ID:, study EBL3002).
- One participant experienced multiple gunshot wounds on Day 16 post Dose 1 (Ad26.ZEBOV $2x10^{10}$ vp) and died the next day in hospital (participant ID:, study EBL3002).
- The pediatric pooling includes 1 adolescent aged 12-17 years of the Ad26.ZEBOV, MVA-BN-Filo vaccine group who died on Day 55 post Dose 2 (MVA-BN-Filo) of typhoid fever and malaria (reported duration: 4 days) which were considered unrelated to the study vaccine (participant ID:, study EBL2002).

No deaths were observed post booster vaccination or during the post booster vaccination follow up phase.

Other Serious Adverse Events

Overall, few SAEs were observed in adults (≥18 years), adolescents (12-17 years) and children (4-11 years and 1-3 years), with no notable differences between the active vaccine and control regimens.

SAEs reported in adults (extended pooling) are presented in Table 29. SAEs in children and adolescents are presented in Table 30.

In **adults**, one SAE of small fibre neuropathy, reported for a participant who received a single vaccination with Ad26.ZEBOV, was considered related to the study vaccine. No other SAEs were considered related to either active vaccine.

In the extended adult pooling, a total of 118 SAEs were reported for 92 participants. Of these, 22 SAEs were reported for 19 participants within 28 days after vaccination.

In the restricted adult pooling, 55 (2.9%) participants in the Ad26.ZEBOV, MVA-BN-Filo group had a total of 76 SAEs, 7 (2.1%) participants in the placebo control group had a total of 9 SAEs, and 4 (3.9%) participants in the active control group had a total of 5 SAEs.

Across all vaccine regimens, the most frequent SAEs reported during the entire study conduct in adults were:

- Malaria: reported for 10 participants in the Ad26.ZEBOV, MVA-BN-Filo group, 1 participant in the MVA-BN-Filo, Ad26.ZEBOV group, and 1 participant in the placebo control group
- Inguinal hernia: reported for 4 participants in the Ad26.ZEBOV, MVA-BN-Filo group
- Spontaneous abortion: reported for 5 participants in the Ad26.ZEBOV, MVA-BN-Filo group and 1 participant in the Ad26.ZEBOV, Ad26.ZEBOV group
- Gastroenteritis: reported for 2 participants in the Ad26.ZEBOV, MVA-BN-Filo group and 3 participants in the MenACWY, placebo group

Other SAEs were reported for at most 2 participants in any of the vaccine groups. Three SAEs led to permanent stop of study vaccination: facial paralysis (Bells palsy) and death (due to toxic effects of benzodiazepines, cocaine, and opiates) reported for 2 participants who were enrolled to receive the placebo regimen and cholecystitis reported for a participant who was enrolled to receive the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.

No SAEs were observed within 28 days post booster vaccination.

Table 29: Adverse Events: Serious Adverse Events Reported Within 28 days after Vaccination by System Organ Class and Dictionary-derived Term - Adults (Extended Pooling)

	Ad26 (l)	Ad26 (i)	Ad26	Ad26 (h)	MVA (l)	MVA	MVA (h)	Placebo	Men-ACWY*
Number of Doses	150	150	2610	15	287	2369	29	1006	102
Any event, n (%)	0	2 (1.3%)	6 (0.2%)	0	0	6 (0.3%)	0	4 (0.4%)	1 (1%)
Ear and labyrinth disorders	0	0	0	0	0	0	0	1 (0.1%)	0
Meniere's disease	0	0	0	0	0	0	0	1 (0.1%)	0
Eye disorders	0	0	0	0	0	1 (< 0.1%)	0	0	0
Cataract	0	0	0	0	0	1 (< 0.1%)	0	0	0
Gastrointestinal disorders	0	0	0	0	0	1 (< 0.1%)	0	1 (0.1%)	0
Gastritis	0	0	0	0	0	0	0	1 (0.1%)	0
Inguinal hernia	0	0	0	0	0	1 (< 0.1%)	0	0	0
Hepatobiliary disorders	0	0	1 (< 0.1%)	0	0	0	0	0	0
Cholecystitis acute	0	0	1 (< 0.1%)	0	0	0	0	0	0
Infections and infestations	0	0	3 (0.1%)	0	0	0	0	0	1 (1%)
Brain abscess	0	0	1 (< 0.1%)	0	0	0	0	0	0
Cellulitis	0	0	1 (< 0.1%)	0	0	0	0	0	0
Gastroenteritis	0	0	0	0	0	0	0	0	1 (1%)
Malaria	0	0	1 (< 0.1%)	0	0	0	0	0	0
Subcutaneous abscess	0	0	1 (< 0.1%)	0	0	0	0	0	0
Typhoid fever	0	0	1 (< 0.1%)	0	0	0	0	0	0

	Ad26 (l)	Ad26 (i)	Ad26	Ad26 (h)	MVA (l)	MVA	MVA (h)	Placebo	Men-ACWY*
Injury, poisoning and procedural complications	0	2 (1.3%)	1 (< 0.1%)	0	0	1 (< 0.1%)	0	0	0
Forearm fracture	0	0	0	0	0	1 (< 0.1%)	0	0	0
Gun shot wound	0	1 (0.7%)	0	0	0	0	0	0	0
Ligament sprain	0	0	1 (< 0.1%)	0	0	0	0	0	0
Skin laceration	0	0	1 (< 0.1%)	0	0	0	0	0	0
Toxicity to various agents	0	1 (0.7%)	0	0	0	0	0	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	0	0	0	0	0	0	1 (0.1%)	0
Osteosarcoma	0	0	0	0	0	0	0	1 (0.1%)	0
Nervous system disorders	0	0	1 (< 0.1%)	0	0	1 (< 0.1%)	0	1 (0.1%)	0
Facial paralysis	0	0	0	0	0	0	0	1 (0.1%)	0
Miller fisher syndrome	0	0	0	0	0	1 (< 0.1%)	0	0	0
Small fibre neuropathy	0	0	1 (< 0.1%)	0	0	0	0	0	0
Respiratory, thoracic and mediastinal disorders	0	0	0	0	0	1 (< 0.1%)	0	0	0
Respiratory disorder	0	0	0	0	0	1 (< 0.1%)	0	0	0
Vascular disorders	0	0	0	0	0	1 (< 0.1%)	0	0	0
Arterial occlusive disease	0	0	0	0	0	1 (< 0.1%)	0	0	0

Adverse events (AE) are coded using MedDRA version 22.0.
n (%): number (percentage) of doses with 1 or more events.
This table only includes adverse events that were reported between Dose 1 and 28 days post Dose 1, and between the Dose 2 and up to 28 days post Dose 2.
Ad26: Ad26.ZEBOV ($5x10^{10}$ vp); MVA: MVA-BN-Filo ($1x10^{8}$ Inf.U);
h: high dose ($1x10^{11}$ vp for Ad26.ZEBOV and $4.4x10^{8}$ Inf.U for MVA-BN-Filo);
i: intermediate dose ($2x10^{10}$ vp for Ad26.ZEBOV);
l: low dose ($0.8x10^{10}$ vp for Ad26.ZEBOV and $5x10^{7}$ Inf.U for MVA-BN-Filo);
* MenACWY: active control present in EBL3001 only.

In **children aged 1-3 years**, 6 (4.2%) participants in the Ad26.ZEBOV, MVA-BN-Filo group had a total of 14 SAEs and 2 (4.2%) participants in the active control group had a total of 3 SAEs. The majority (13 of 17) of the SAEs were reported within 28 days after vaccination. All other SAEs were reported during the post vaccination follow-up phase. The most frequently reported SAEs were malaria (reported for 6 participants in the Ad26.ZEBOV, MVA-BN-Filo group and 1 participant in the active control group) and pneumonia (reported for 3 participants in the Ad26.ZEBOV, MVA-BN-Filo group). Other SAEs were reported for at most 1 participant in any of the vaccine groups. One SAE, a case of severe thrombocytopenia in a participant enrolled in study EBL3001, was considered related to the MenACWY vaccine by the investigator (participant ID:, study EBL3001, see details above). All other SAEs were considered unrelated to the study vaccine.

In **children aged 4-11 years**, 6 (2.4%) participants in the Ad26.ZEBOV, MVA-BN-Filo group had a total of 8 SAEs and 1 (4.2%) participant in the placebo control group had 1 SAE. All SAEs were reported during the post vaccination follow-up phase. The most frequently reported SAE was malaria (reported for 3 participants in the Ad26.ZEBOV, MVA-BN-Filo group). Other SAEs were reported for at most 1 participant in any of the vaccine groups. All SAEs were considered unrelated to the study vaccine.

In **adolescents aged 12-17 years**, 1 (0.4%) participant in the Ad26.ZEBOV, MVA-BN-Filo group had 2 SAEs (malaria and typhoid fever) reported during the post vaccination follow-up phase which were considered unrelated to the study vaccine.

Table 30: Adverse Events: Serious Adverse Events by System Organ Class and Dictionary-derived Term - Paediatric Pooling

	Ad26.ZEBOV, MVA-BN-Filo	Placebo, Placebo	MenACWY*, Placebo
Age group: 1-3 years			
Entire study	144	-	48
Any event, n (%)	6 (4.2%)	-	2 (4.2%)
Infections and infestations	6 (4.2%)	-	1 (2.1%)
Malaria	6 (4.2%)	-	1 (2.1%)
Pneumonia	3 (2.1%)	-	0
Meningitis bacterial	1 (0.7%)	-	1 (2.1%)
Sepsis	1 (0.7%)	-	0
Blood and lymphatic system disorders	2 (1.4%)	-	1 (2.1%)
Anaemia	1 (0.7%)	-	0
Iron deficiency anaemia	1 (0.7%)	-	0
Thrombocytopenia	0	-	1 (2.1%)
Nervous system disorders	1 (0.7%)	-	0
Febrile convulsion	1 (0.7%)	-	0
Age group: 4-11 years			
Entire study	252	24	48
Any event, n (%)	6 (2.4%)	1 (4.2%)	0
Infections and infestations	5 (2%)	0	0
Malaria	3 (1.2%)	0	0
Gastroenteritis	1 (0.4%)	0	0
Osteomyelitis chronic	1 (0.4%)	0	0
Respiratory tract infection	1 (0.4%)	0	0
Blood and lymphatic system disorders	1 (0.4%)	0	0
Anaemia	1 (0.4%)	0	0
Respiratory, thoracic and mediastinal disorders	1 (0.4%)	0	0
Asthma	1 (0.4%)	0	0
Injury, poisoning and procedural complications	0	1 (4.2%)	0
Burns second degree	0	1 (4.2%)	0
Age group: 12-17 years			
Entire study	253	21	48
Any event, n (%)	1 (0.4%)	0	0
Infections and infestations	1 (0.4%)	0	0
Malaria	1 (0.4%)	0	0
Typhoid fever	1 (0.4%)	0	0

Adverse events (AE) are coded using MedDRA version 22.0.
n (%): number (percentage) of participants with 1 or more events.
* MenACWY: active control present in EBL3001 only.

In ongoing studies, one SAE was reported in study EBL1007 (removal of ovarian cysts, not related) and 82 SAEs in study EBL2004. Infections were the most commonly reported SAEs in Study EBL2004, and this particular clinical pattern reflected incident infections in the local populations rather than an issue related to the study vaccines. In addition there was a cluster of 8 SAEs of appendicitis in 2 sites in Guinea; this cluster of appendicitis SAEs was not considered by the Applicant as a confirmed safety signal for the study vaccines based on the review of these cases and due to a lack of temporal association and biological plausibility for vaccines to cause appendicitis. One SUSAR of generalized pruritus reported in study EBL2004, which fully resolved within a few days, was considered related to Dose 2 vaccination (unblinded to MVA-BN-Filo) by the investigator and sponsor, due to temporal plausibility and lack of alternative causes.

Thirteen paediatric SAEs were reported for study EBL3001 and 25 SAEs for study EBL4001. Again, these were mostly infections not considered related to study vaccination. Two SAEs with a fatal outcome, considered unrelated to the study vaccines, were reported: a male participant with severe malaria, severe anaemia, sepsis, and disseminated intravascular coagulation died 3 weeks after Dose 2 vaccination (MVA-BN-Filo) and a male participant with severe typhoid fever died 10 months after administration of the active control vaccine (MenACWY).

Laboratory findings

Overall, after vaccination (28-day and 56-day regimens) with Ad26.ZEBOV, MVA-BN-Filo, placebo, and MenACWY in adolescents and children, the majority of the observed laboratory abnormalities were grade 1 or grade 2 in severity and no clinically relevant differences in the frequency of grade 3 laboratory abnormalities were observed. Laboratory abnormalities were not reported more frequently following either Ad26 or MVA than following placebo.

Safety in special populations

HIV infected subjects

The potential influence of HIV infection on the safety profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was evaluated in studies EBL2002 and EBL2003. For details see section 3.3.5.

Overall, no notable differences with regard to the safety profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen between HIV-infected and healthy participants were observed. Results are consistent with the 56 day interval studied in EBL2002.

Use in Pregnancy and Lactation

To date, active vaccination of pregnant women has not been evaluated, as being pregnant or planning to become pregnant while enrolled in a study was an exclusion criterion in all clinical studies. Birth control methods for female participants of childbearing potential were required for specific periods prior to Dose 1 until after Dose 2 and prior to until after the booster dose, as defined in each clinical study protocol. A pregnancy test was systematically performed in these women prior to each administration of study vaccine, while pregnant women were excluded from receiving study vaccine.

Despite the protocol-specified contraceptive requirements, pregnancies did occur during the vaccination and follow-up phases of the clinical studies. The outcome of the cumulative review of all pregnancies, up to the cut-off date of 12 August 2019, that were reported in the GSDR for female study participants from all completed and ongoing Ebola vaccine clinical studies, is summarized below. Pregnancy data from both Applicant sponsored and collaborative studies were included.

There were 72 pregnancy safety reports in de Global Safety Data Review which includes 6 reports concerning neonates. At the time of this review, 8 pregnancies were reported as ongoing whereas the status of 10 was reported as unknown. Information on pregnancy outcome was available for 48 pregnancies and included live birth (27, including 2 pregnancies in participants who received placebo), spontaneous abortion (9), elective or induced abortion (9), incomplete abortion (1), miscarriage (1), and 1 case of twin pregnancy with reported outcomes as one live birth and one foetal demise. Of the 20 pregnancies with serious pregnancy complications, 7 were initiated outside the per protocol birth control / abstinence period and 7 were initiated within the period. The timing of conception in relation

to vaccination was unknown for remaining six. None of these serious complications or SAEs was considered causally associated with the study vaccines by the investigator or the Applicant.

The review of pregnancy reports does not provide any indication of an unusual risk, safety concern; however exposure to the vaccines in all cases was before the pregnancy. Therefore there is, to date, no clinical data of use of the vaccine in women who are pregnant.

The Applicant is planning a phase 3 randomised controlled trial which should run parallel to the large vaccination programme planned or ongoing in Rwanda (UMURINZI campaign). In this study (EBL3010) 2000 pregnant women randomized (1:1) to receive the 2-dose Ebola vaccine regimen during pregnancy or alternatively, upon completion of their pregnancy. The main outcomes of interest are adverse maternal/fetal outcomes in pregnant women and adverse neonatal/infant outcomes in neonates/infants. This study is expected to provide insight into the safety profile of the vaccine regimen in pregnant women and their infants.

It is not known whether Ad26.ZEBOV or MVA-BN-Filo are excreted in human milk, but it is considered unlikely due to the limited biodistribution observed in nonclinical studies (please refer to non clinical section). Breastfeeding was an exclusion criterion for vaccinating women in all clinical studies conducted to date.

Older adults

From the total number of adult participants (2,341) who received the active vaccine, placebo, or active control regimen, the majority (2,120) were 18-50 years old, 212 were 51-65 years old, and 9 were >65 years old. Across adult age groups (18-50 years and 51-65 years), solicited local and systemic AEs were more frequently reported for participants who received the active vaccine regimen compared to placebo. The rate of solicited AEs was slightly higher in the younger age group, 18-50 years (83.8% vs 78.2% in 51-65 years). Unsolicited adverse events tended to be reported more frequently in the older age group, 48.3% in adults 18-50 years compared to 58.7% in those 51-65 years. A similar effect is seen in the placebo group (42.8% vs 65.4% respectively). Normally, the Applicant would be expected to provide a table of AEs including serious AEs by SOC per age group for the older adults, i.e. Age <65, Age 65-74 Age 75-84, Age 85+. As there were only 9 subjects over the age of 65 years in clinical studies up to date this table is not considered to provide meaningful information.

Sex

From the total number of adult participants (2,341) who received the active vaccine, placebo, or active control regimen, the majority (1,462) were male and 879 were female. From the total of 838 children and adolescents who received the active vaccine, placebo, or active control regimen, 104 were male and 88 were female in the 1-3 years age group, 160 were male and 164 were female in the 4-11 years age group, and 172 were male and 150 were female in the adolescent age group (12-17 years).

Across sexes in adults, solicited local and systemic AEs were more frequently reported for participants who received the active vaccine regimen compared to placebo; 82% of males compared to 85.7% of females reported a solicited AE following the vaccine regimen compared with 66.7% of females and 61.1% of males following placebo. No notable differences in solicited adverse events were observed between boys (41.6%) and girls (47.8%) aged 1-3 years. Similarly, in the 4-11 years age group solicited AEs were reported in 66.1% of boys compared to 62.5% of girls. In the adolescent age group (12-17 years), solicited local and systemic AEs tended to be reported more frequently in girls (73.3%) than in boys (51.9%), after the active vaccine regimens. In adults systemic AEs related to vaccine

were higher in females compared to males (71.6% vs 62.7%). Higher rates of reactogenicity in females have been reported for different vaccines and do not impact the use of the vaccine.

Safety by Region

Across regions, solicited local and systemic AEs were more frequently reported for participants who received the active vaccine regimen compared to placebo, although with smaller differences between the active vaccine regimen and placebo in East and West Africa compared to Europe and the United States. Solicited local and systemic AEs tended to be reported less frequently in West Africa, for both active and placebo regimens, compared to the other 3 regions in adults; solicited AEs were reported by 72.6% of adults from West Africa compared to 84.8%, 89.6% and 91.4% of adults from the US, East Africa and Europe respectively. All 1-3 year old participants (n=192) were from West Africa. For children aged 4-11 years and adolescents, solicited local and systemic AEs tended to be reported less frequently in West Africa compared to East Africa (80.6%, 88.9%), for both active vaccine and placebo regimens. For example, in children aged 4-11 years 61.6% of children from West Africa reported a solicited AE following the vaccine regimen, compared to 80.6% of children from East Africa. For placebo, 40% of children aged 4-11 years from West Africa reported a solicited AE compared to 88.9% of children from East Africa.

Safety by baseline EBOV GP ELISA and baseline Ad26 VNA

The frequency of solicited local and systemic AEs tended to be higher after the active vaccine regimen when baseline **EBOV GP ELISA** was <LLOQ compared to the other baseline ELISA categories (LLOQ 100 EU/mL, 101-1,000 EU/mL, and >1,000 EU/mL), which may indicate that the presence of pre-existing antibodies does not result in an increased reactogenicity to the vaccine regimen. Note that due to the low number of participants in the groups ≥LLOQ and the majority of participants in these groups being from African regions, these results should be interpreted with caution.

Immunological events

The goal of vaccination is to induce antibodies; hence this section is less relevant. Hypersensitivity reactions are discussed elsewhere.

Safety related to drug-drug interactions and other interactions

The safety, immunogenicity, and efficacy of concomitant administration of Ad26.ZEBOV and MVA-BN-Filo with other vaccines have not been evaluated.

Concurrent use of Ad26.ZEBOV and MVA-BN-Filo with immunosuppressive therapies has not been evaluated.

The Applicant currently has not foreseen a specific study to evaluate the concomitant administration with other vaccines or medicinal products (such as antipyretics). Vaccination of the general population in the target age groups (from 1 year of age) is feasible without requiring co-administration with routinely recommended vaccines. Likewise, when the Ad26.ZEBOV, MVA-BN-Filo 56-day regimen is administered as prophylaxis for healthcare workers or others who might be deployed to an area experiencing an EBV outbreak, it should be possible to avoid co-administration with other needed vaccines. A statement to reflect the absence of data on the safe co-administration of Ad26.ZEBOV and MVA-BN-Filo is included in section 4.5 of the SmPC.

Further, the reactogenicity profile of the vaccine regimen is sufficiently mild that systematic (prophylactic) use of antipyretics prior to vaccination is not expected and there is no reason to evaluate the impact of prophylactic use of antipyretics on vaccine response.

Discontinuation due to AES

Discontinuations of subjects from either vaccination or study are discussed per pooling.

In the restricted adult pooling, 6 (0.3%) participants who were enrolled to receive the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen were withdrawn from further vaccination due to nonserious AEs of muscular weakness and paresthesia (both in the same participant) and neutropenia, thrombocytopenia, paresthesia, syncope, and microscopic colitis (the latter unrelated) (each in 1 participant). Of these, 3 participants (with syncope, thrombocytopenia, and microscopic colitis) also discontinued the study..

In the primary adult pooling, 1 additional participant who was enrolled to receive the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was withdrawn from further vaccination due to an SAE of acute cholecystitis (unrelated). In the extended adult pooling, 3 additional participants receiving Ad26.ZEBOV and MVA-BN-Filo in the reverse order (ie, MVA-BN-Filo, Ad26.ZEBOV) were withdrawn from further vaccination due to nonserious AEs of wheezing, bradycardia, and leucocytosis (each in 1 participant). In addition, 2 participants who were enrolled to receive the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen at a 14-day interval were withdrawn from further vaccination due to nonserious AEs of neutropenia.

In the paediatric pooling, 1 (0.4%) adolescent aged 12-17 years in the Ad26.ZEBOV, MVA-BN-Filo vaccine group died after completion of the study vaccinations before planned study end (typhoid fever and malaria). In addition, 1 (4.2%) child aged 4-11 years who was enrolled to receive the placebo regimen was withdrawn from the study prior to Dose 2 due to an SAE (second degree burns).

Post marketing experience

Recently, the vaccine regimen obtained conditional approval in Rwanda. The Ministry of Health of Rwanda will initiate a large-scale deployment of the vaccine regimen, targeting 193,000 Rwandans aged 2 years and above in regions bordering the DRC judged to be at risk.

In Rwanda, to date (25 February 2020), a total of 20,084 participants have started the vaccine regimen, out of these 2,141 have now completed it. Around 76% of the participants vaccinated are adults above 18 years of age and 24% are children above 2 years of age.

Safety monitoring is performed through solicited surveillance. From the day participants receive Dose 1 of the vaccine to approximately one month after they receive Dose 2 they will be reminded to contact the medical team for any adverse event of concern. Women of childbearing potential are also reminded to contact the medical team if they suspect that they have become pregnant.

Currently no safety concerns have been raised. A total of 7 pregnancies and 4 serious adverse events have been reported after Dose 1 and prior to Dose 2. One SAE was considered related to vaccination: vomiting and weakness the day of vaccination with Ad26.ZEBOV in a child. Vomiting is reported after vaccination with Ad26.ZEBOV and therefore there it could be associated with vaccination. However, as no further details are provided therefore there is insufficient information for a causality assessment. As vomiting is included in section 4.8 of the SmPC no further action is needed.

2.6.1 Discussion on clinical safety

The clinical safety of Ad26.ZEBOV and MVA-BN-Filo has been evaluated in 11 clinical trials in which 2,777 adults received Ad26.ZEBOV, and 2,376 received MVA-BN-Filo. The evaluation of safety in children and adolescents aged 1-17 years is based upon 838 subjects who received 649 doses of Ad26.ZEBOV as Dose 1 and 645 doses of MVA-BN-Filo as Dose 2. Follow up of safety in these trials was of an appropriate duration and sufficient to identify AEs potentially related to vaccination. Several clinical trials included either a placebo control or, in one case (EBL3001) an active control, which allows for a proper assessment of safety. In principle this is an adequate safety database for an initial determination of the safety profile of Ad26.ZEBOV (Dose 1 at $5x10^{10}$ viral particles [vp]) and MVA-BN-Filo (Dose 2 at $1x10^{8}$ infectious units [Inf.U]) given approximately 56 days later. Overall, the vaccine regimen's safety profile is characterized quite extensively.

Whilst the Ad26.ZEBOV booster dose was evaluated in 167 adults (n=126 received Ad26.ZEBOV), there is no information on the safety of the Ad26.ZEBOV booster dose in children or HIV-infected subjects. The Applicant appropriately justified the booster dose as now recommended for children and HIV-1 infected subjects in the proposed SmPC in absence of this data. In view of the overall similarity of the safety profile of the primary vaccine regimen between children/adolescents and healthy adults and between HIV-infected adults and healthy adults, and the fact that the Ad26.ZEBOV booster dose in adults is not more reactogenic than the first dose of Ad26.ZEBOV, it was extrapolated that no particular safety concerns with the booster dose in children/adolescents and in HIV-infected subjects on HAART are to be expected.

The dataset for Adverse Reaction identification was derived from the primary adult pooling excluding the open label arms (N=2,683), and determination of relatedness was – in part – based on the comparison of AE frequencies between the active vaccine regimen (Ad26.ZEBOV, MVA-BN-Filo) and the placebo control regimens, which could disregard signals from individual studies.

Solicited Adverse Events

Pain at the injection site was the most frequently reported local reaction, reported by 47.6% of adult subjects following Ad26.ZEBOV and 46.6% of adult subjects following MVA-BN-Filo, and in 24.0% following Ad26.ZEBOV and 20.5% following MVA-BN-Filo in children and adolescents. Local reactogenicity was higher in subjects who received the Ad26/MVA vaccine regimen as compared to placebo and MenACWY. Local reactions were reported in similar frequency following Ad26.ZEBOV as MVA-BN-Filo. Severe local reactions, Grade 3, were rare (<1%).

Local reactions were reported less frequently in children compared to adults. This may be biased as all children were recruited in West African countries, and as was observed in adults, persons in West African countries tended to report less reactions. The Applicant relates this to potential cultural differences in perception of AEs. The implications are that the rates of ADRs as listed in the SmPC for children will likely be lower than what is experienced by children in regions outside Sierra Leone. Therefore, it is key that the frequencies are recalculated once a larger safety database for paediatric patients is available.

In adults, the most frequently reported systemic reactions were fatigue, headache and myalgia reported for 54.1%, 51.7%, and 46.0% of active vaccine recipients, respectively. In children and adolescents, the most frequently reported AEs by PT were decreased appetite, decreased activity, and pyrexia reported for 21.5%, 19.4%, and 18.1% of active vaccine recipients, respectively. Solicited adverse events were mostly grade 1-2.

Unlike local reactogenicity, systemic reactions were reported more commonly following Ad26.ZEBOV compared to MVA-BN-Filo. In adults, the rate of solicited systemic adverse events was 67.2% vs

49.4% following Ad26.ZEBOV and MVA-BN-Filo respectively. This increased rate was observed over all systemic reactions solicited. There were also more grade 3 systemic reactions following Ad26.ZEBOV (4.1% vs 1.5% in adults), which is mostly driven by fatigue, headache and myalgia. This was also seen in children and adolescents. The systemic reactogenicity following MVA-BN-Filo in children and adolescents was similar to placebo.

Unsolicited Adverse Events

In adults, the frequency of unsolicited AEs reported after vaccination with the active vaccine regimen (49.4%) was similar to the placebo regimen (44.4%) and lower compared to the active control regimen (74.5%). The higher frequency of unsolicited adverse events in the active control group is explained by an increase rate of malaria (39.2% compared to 8.6% and 3.3% in the selected regimen group and placebo group respectively). The active control, MenACWY, was given to 102 subjects in study EBL3001 and not in other studies. The rate of malaria was higher in study EBL3001 compared to other studies, i.e. the overall rate of malaria in this study in the Ad26.ZEBOV/MVA-BN-Filo group was 41.3% which is comparable to the MenACWY group. This explains the imbalance as seen in the pooled dataset.

Unsolicited AEs were mostly in the SOC infections and infestations. By preferred term, in adults the most frequently reported unsolicited AEs were malaria (8.6%), upper respiratory tract infection (6.2%), and headache (5.2%). In children and adolescents unsolicited AEs mostly consisted of infections, including malaria and respiratory tract infections.

The occurrence of unsolicited AEs mostly appeared balanced between active groups and placebo groups.

Related AEs

All local reactions solicited were considered related. Systemic reactions solicited were only considered related if they were reported in a higher frequency as compared to the placebo control groups.

The Applicant reports that unsolicited AEs considered **related** to the study vaccine were reported for 11.6%, 7.7%, and 5.9% of adult participants in the active vaccine, placebo control, and active control regimens, respectively. Unsolicited AEs considered related to the study vaccine were reported for 6.0%, 12.5%, and 0% of children aged 4-11 years, and 12.3%, 14.3%, and 4.2% of adolescents aged 12-17 years who received the active vaccine, placebo control, and active control regimens, respectively. In children aged 1-3 years, unsolicited AEs considered related to the study vaccine were reported for 0.7% and 4.2% of children in the active vaccine and active control regimens, respectively.

In adults who received Ad26.ZEBOV, the most common local ARs (≥10%) were pain (46.7%), warmth (24.2%), and swelling (11.0%) at the injection site. The most common systemic ARs (≥10%) were fatigue (45.8%), headache (44.5%), myalgia (36.0%), arthralgia (24.0%), and chills (22.5%). Most ARs occurred within 7 days following vaccination, were mild to moderate in severity, and of short duration (2-3 days). Postural dizziness and pruritus were identified as additional ARs.

In adolescents and children who received Ad26.ZEBOV, the most common local AR (≥10%) was pain (24.3%) at the injection site. The most common systemic ARs (≥10%) were fatigue (19.1%), decreased activity (15.5%), decreased appetite (14.3%), and irritability (13.5%). Most ARs occurred within 7 days following vaccination, were mild to moderate in severity, and of short duration (1-4 days).

Booster regimen

Based upon the limited safety data in adults (n=126) who received Ad26 as a booster dose there is no indication of a worse safety profile following the booster as when Ad26 is given as an initial priming

dose, and some indication that systemic reactions are less frequent and milder following Ad26 given as a booster dose.

Safety of other regimens

The Applicant collected safety data with regimens different to the final recommended vaccine regimen in several phase I and phase II studies. These data suggest that giving the vaccines in the reverse order, so first MVA followed by Ad26, does not impact the safety. Further, limited data obtained with homologous vaccine regimens – meaning Ad26 followed by Ad26 and MVA followed by MVA – does not increase the reactogenicity to the vaccines. Finally, data obtained with varying intervals between the two vaccines do not indicate an impact on the reactogenicity and safety profile of the vaccine regimen as a total. Specifically, data obtained with intervals in varying duration >28 days show a similar occurrence of AEs, providing a justification for pooling of this data for the analysis of safety.

AESIs, deaths and other SAEs

The Applicant considered cardiac events as an AESI due to the MVA vector and neuro-inflammatory events as an AESI due to a report of Miller Fisher syndrome and a small fibre neuropathy early in the development reported in phase II study EBL2001. Based upon the safety data available, which included intensified monitoring in phase I studies, there is no signal for a potential cardiac safety issue with the MVA-BN-Filo vaccine. Further, there is no signal of an increased risk of neuro inflammatory adverse events associated with the selected vaccine regimen A summary analysis of the safety profile of the vaccines will be submitted through the future Ad26.ZEBOV and MVA-BN-Filo PSURs, including specific analyses of neuroinflammatory (for Ad26.ZEBOV) or cardiac (myo/pericarditis for MVA-BN-Filo) AESIs.

There have been no deaths related to Ad26 or MVA in any of the conducted clinical studies or ongoing studies.

In adults, apart from the case of small fibre neuropathy, no SAEs are considered possibly related to either Ad26 or MVA vaccine in the completed studies. In the ongoing studies there was one report of a generalized pruritus considered related to MVA. There were no SAEs considered related to either study vaccine reported in children. SAEs in children mainly involved infections, notably malaria, which is within expectation considering studies including children were conducted in regions where malaria is endemic.

Discontinuations were rare and did not point to any additional safety concerns.

Safety in special populations

The safety of Ad26.ZEBOV/MVA-BN-Filo was evaluated in HIV+ persons in two studies in which 220 HIV-infected subjects received the selected vaccine regimen. Overall, no notable differences with regard to the safety profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen between HIV-infected and healthy participants were observed. Therefore, it is likely that Ad26.ZEBOV will be similarly tolerated given as a booster, and thus a booster can be recommended in HIV+ persons.

Pregnancy

Up to the cut-off date of 12 August 2019, 66 pregnancies have been reported across the clinical trials – of these the status of 10 was unknown, 8 pregnancies were still ongoing. Serious pregnancy complications or SAEs were observed for 20 out of the 66 pregnancies, which were not considered related to vaccine. The review of pregnancy reports does not provide any indication of an unusual risk, safety concern; however, exposure to the vaccines in all cases was before the pregnancy. To date there is no clinical data of use of the vaccine in women who are pregnant. It is important that this is followed up in the field. To evaluate the safety of Ad26.ZEBOV/MVA-BN-Filo in pregnant women, the Applicant is planning a phase 3 randomised controlled trial which should run parallel to the large

vaccination programme planned or ongoing in Rwanda (UMURINZI campaign). In this study (EBL3010) 2000 pregnant women will be randomized (1:1) to receive the 2-dose Ebola vaccine regimen during pregnancy or alternatively once their pregnancy is completed. The main outcomes of interest are adverse maternal/fetal outcomes in pregnant women and adverse neonatal/infant outcomes in neonates/infants.

Safety by other factors

Safety was analysed by region, sex, age, race, baseline EBOV GP ELISA titres >LLOQ and to the baseline positivity to the Ad26 vector.

Solicited local and systemic AEs tended to be reported less frequently in West Africa, for both active and placebo regimens, compared to the other 3 regions. The Applicant argues that there are cultural differences between the centres in West Africa (Sierra Leone) and other regions that may explain the differences in reporting rates of AEs, which is seen consistently across age groups and in vaccine groups. Observations are consistent with studies for Ervebo (Liberia), however not with a small vaccine trial for a different Ebola vaccine (Mali). It is accepted that there may be country differences, or even regional differences, in the perception and hence reporting of AEs.

This imbalance is reflected in the analyses by baseline EBOV GP ELISA, where subjects with baseline ELISA titres >LLOQ tended to report less adverse reactions and to some degree also in the analyses by race with lower rates of adverse reactions reported for *black of African American* compared to *white* and *other race*.

Considering the very limited number of subjects over the age of 65 (n=9), no separate analyses of safety for the elderly are expected. There is limited data in older adults; there is no indication of a worse safety profile with increasing age. Considering the expected target group in Europe consists mostly of persons travelling to Ebola affected areas (presumably to work), the safety profile in older adults (>65 years), particular the more frail persons, is possibly less relevant.

Solicited adverse events tended to be reported more frequently by female compared to male adolescents (solicited AEs: 73.3% vs 51.9%) but not in other age groups. In adults systemic AEs related to vaccine were higher in females compared to males (71.6% vs 62.7%). Higher rates of reactogenicity in females have been reported for different vaccines and do not impact the use of the vaccine.

There is a clear trend for a higher frequency of solicited local and systemic AEs in participants with a baseline Ad26 VNA negative sample compared to participants with a positive Ad26 VNA sample. Although numbers are limited, this effect is observed across the age groups (i.e. in adults as well as children). The trend for higher frequency of solicited local and systemic adverse events observed in adult participants (primary pooling) with a baseline Ad26 VNA negative sample compared to those with a baseline Ad26 VNA positive sample is not replicated in individual studies apart from study EBL2002 which shows a slight trend in this direction. The majority of post Ad26 VNA positive subjects came from study EBL3001, which had a lower reporting frequency of AEs (see issue on regional differences in safety reporting). This could form a plausible explanation for the observation of lower AEs in VNA positive subjects in the pooled safety data. Curiously, for non- Ebola Ad26. vectored vaccines a similar pattern emerges, with lower frequencies of solicited AEs and, in particular, lower frequencies of low severe systemic AEs in those subjects with a baseline Ad26 VNA positive sample. Although interesting, it is agreed with the Applicant there is no clear biological rationale to explain this observation, nor does it have consequences for the use of the vaccine.

It is agreed with the Applicant that there is no indication that the presence of pre-existing antibodies against Ad26 would result in an increased reactogenicity of the Ad26.ZEBOV vaccine.

Finally, it is noted that the proposed vaccine regimen obtained conditional approval in Rwanda. The Ministry of Health of Rwanda will initiate a large-scale deployment of the vaccine regimen, targeting 193,000 Rwandans aged 2 years and above in regions bordering the DRC judged to be at risk. To date (25 February 2020), a total of 20,084 participants have started the vaccine regimen, out of these 2,141 have now completed it. Around 76% of the participants vaccinated are adults above 18 years of age and 24% are children above 2 years of age. Currently no safety concerns have been raised.

There is no information on the occurrence of rare (occurring less frequently than approximately 1 in 1000) but serious adverse events. Whilst in context of study EBL3008 500,000 persons will be vaccinated with the vaccination regimen, active safety surveillance for medically attended AEs and SAEs will only take place in 1,000 persons (500 children, 500 adults). The capture of other (potential) SAEs will be through passive surveillance. Approximately 4,800 subjects have been vaccinated in DRC so far (WHO Ebola Situation report dated 7 January 2020). The Applicant informed that no safety concerns have been raised in context of a large open label non-randomised study which is ongoing in DRC up to 21 January 2020. The Applicant has committed to share relevant new data if and when this becomes available**.**

2.6.2 Conclusions on the clinical safety

Ad26.ZEBOV as part of a vaccine regimen of Ad26.ZEBOV followed by MVA-BN-Filo after 56 days, is well tolerated in children (>1 – 12 years), adolescents and adults.

The safety of the vaccine regimen has been evaluated in 11 clinical trials in which 2,608 adults received Ad26.ZEBOV as a primary dose (2,305), as a second dose (305) or as a booster dose (167), and in which 649 children and adolescents aged 1 to 18 received Ad26.ZEBOV.

Reactogenicity is characterized by pain at the injection site, which was reported by 47.6% of adult subjects and 13.9% of children aged 1-3 years, 29.8% of children aged 4-11 years and 24.9% of adolescents following vaccination with Ad26.ZEBOV, and in adults and adolescents by fatigue (reported by 46.2% and 24.1% respectively), headache (reported by 45.1% and 34.8% respectively), chills (23.7% and 13.4% respectively) and myalgia (36.8% and 13% respectively). In children (aged 1 – 11 years), the most frequently reported systemic solicited AEs by PT were decreased appetite (9.1%), decreased activity (9.8%), and pyrexia (11.6%).

So far there has been only one SAE which was considered possibly related to vaccination with Ad26.ZEBOV, a case of small fibre neuropathy in an adult. In conclusion, the safety profile as determined in 2,777 adults and 649 children and adolescents exposed to Ad26.ZEBOV is acceptable for a vaccine that is likely to prevent EBV.

2.7 Risk Management Plan

2.7.1 Safety concerns

Summary of safety concerns

The Applicant submitted an updated RMP version 1.4. The Applicant proposed the following summary of safety concerns in the RMP:

Table 31: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	Use during pregnancy

2.7.2 Discussion on safety specification

The Applicant has not included any important identified risks, or important potential risks. Based upon the evaluation of safety data as outlined in section 2 of this AR it can be agreed that there are no important identified risks or potential risks. No safety concerns that require follow up through the risk management plan have been identified with the proposed regimen.

There is missing information in pregnant women. Although the Applicants proposes that, as a precautionary measure, the vaccination with Ad26.ZEBOV and MVA-BN-Filo should be avoided during pregnancy unless it is considered that the benefit of preventing EVD outweighs the risk, it is within expectation that pregnant women will be vaccinated whilst pregnant, also within Europe. It is considered relevant that pregnancy outcomes are systematically collected if this is to occur. The Applicant has detailed plans to collect this outcome.

The risks not included in the list of safety concerns as outlined by the Applicant in section SVII.1.1., including hypersensitivity reactions and medication errors, is acceptable.

2.7.3 Conclusions on the safety specification

Having considered the data in the safety specification, the Rapporteurs consider that the current proposal is acceptable. '*Use during pregnancy*' has been included as Missing Information conforming to the Rapporteurs' request.

2.7.4 Pharmacovigilance plan

Routine pharmacovigilance activities

Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection are presented below.

Spontaneous, solicited and clinical trial reports of "exposure during pregnancy" for Janssen products world-wide, even those without association with an adverse event, are collected in the Global Safety Data Repository (GSDR), which serves as the central repository for reports of drug or vaccine exposure during pregnancy. All cases are systematically followed up by the Applicant to collect information about the pregnancy and, around the time of estimated delivery, to gather information on the health of the mother and of the neonate. The data from GSDR will be analysed cumulatively and the results of the analyses will be presented in the PBRERs/PSURs, at least until the results of the clinical study EBL3010 in pregnant women become available.

Summary of additional PhV activities

Clinical study EBL3010 has been included in the Pharmacovigilance Plan as a category 3 study. This is a collaborative randomised study, planned in Rwanda, with 1,000 pregnant women vaccinated and 1,000 non-vaccinated pregnant women enrolled as controls. This study will be conducted with

residents in the catchment area of Gisenyi District Hospital and Gihundwe District Hospital in Western Rwanda bordering the Democratic Republic of Congo (DRC). A preliminary draft design of this study has been provided included. This trial is not sponsored by the Applicant.

Table 32: On-going and planned additional pharmacovigilance activities

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Not applicable				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Not applicable				
Category 3 - Required additional pharmacovigilance activities				
A Phase 3 open-label randomized clinical trial to evaluate the safety, reactogenicity and immunogenicity of a 2-dose Ebola vaccine regimen of Ad26.ZEBOV followed by MVA-BN-Filo in healthy pregnant women. (VAC52150EBL3010) Planned	To evaluate the safety, reactogenicity, and immunogenicity of Ad26.ZEBOV and MVA-BN-Filo in healthy pregnant women	Use during pregnancy	Final study report	31 March 2024

Note: This trial is not sponsored by the Applicant. The Applicant commits to provide the clinical report when it is made available by the study sponsor.

The proposed activities are appropriate and proportionate to document the missing information 'Use in pregnant women'.

Overall conclusions on the PhV Plan

The PRAC Rapporteur, having considered the data submitted, is of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The PRAC Rapporteur also considered that routine PhV remains sufficient to monitor the effectiveness of the risk minimisation measures.

2.7.5 Risk minimisation measures

There are no additional risk minimization measures.

The PRAC Rapporteur having considered the data submitted was of the opinion that:

- the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication(s).

2.7.6 Conclusion

The CHMP and PRAC considered that the risk management plan version 1.4 is acceptable.

2.8 Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list and a new entry will be required. The new EURD list entry uses the IBD to determine the forthcoming Data Lock Points. The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion.

The marketing authorisation holder (MAH) shall submit the first PSUR for this product within 6 months following authorisation.

2.9 New Active Substance

The applicant declared that Ebola vaccine (Ad26.ZEBOV-GP [recombinant]) has not been previously authorised in a medicinal product in the European Union.

The CHMP, based on the available data, considers Ebola vaccine (Ad26.ZEBOV-GP [recombinant]) to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10 Product information

2.10.1 User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2 Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zabdeno (Ebola vaccine (Ad26.ZEBOV-GP [recombinant])) is included in the additional monitoring list as it is approved under exceptional circumstances [REG Art 14(8), DIR Art (22)].

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1 Therapeutic Context

3.1.1 Disease or condition

As part of a 2-dose heterologous vaccine regimen, Ad26.ZEBOV is intended to prevent EVD in adults, adolescents and children 1 years of age and older.

EVD is an acute systemic febrile syndrome caused by Ebola viruses. Zaire Ebola virus is a member of the Filoviridae family, the virus is transmitted through human-to-human contact. Ebola virus disease affects both adults and children with most cases in people aged 20 to 50 years. EVD has a case fatality rate ranging from 30% to 90%, and an incubation period of 2 to 21 days. The pathogenesis of EVD is characterized by an intense inflammatory process, impaired haemostasis and capillary leak, with mortality resulting from septic shock and multi organ system failure.

3.1.2 Available therapies and unmet medical need

Treatment

No specific effective treatment for EVD is currently licensed. Investigational therapies for Ebola virus disease aim at the reduction of viral replication to limit the inflammatory storm triggered by viral expansion.

Without a specific treatment, management of patients with Ebola virus disease consists of the provision of supportive and, as required and when possible, critical care.

Prevention

Ebola vaccine rVSV-ZEBOV-GP was recently licensed against EVD in the EU and in the US for use in individuals of 18 years of age and older at imminent risk of infection.

Two vaccines are approved for human use in China and Russia

Prevention of EVD is accomplished through education on avoidance of risk factors and quarantine of infected individuals.

Unmet medical need

The increased frequency and magnitude of outbreaks in the current decade indicate that Ebola is becoming a prominent part of the epidemiological landscape and possibly a permanent public health threat in sub-Saharan Africa. Therefore, a prophylactic Ebola vaccine providing sufficient long-term protection remains an unmet need, even with the rVSVΔG-ZEBOV-GP vaccine recently licensed and can become an important tool to prevent future outbreaks and to control the current and possible future epidemics.

3.1.3 Main clinical studies

The evaluation of the protective effect of the vaccine regimen for this MAA is based on animal data, through the bridging of clinical immunogenicity results to efficacy and immunogenicity data obtained in non-human primates (NHP). To date, no efficacy or effectiveness data is available.

To translate human immunogenicity data into likelihood of protection, a logistic regression model was built based on immunogenicity and efficacy data obtained in the NHP EBOV Kikwit challenge model. The main clinical studies for this MAA are the studies that have been used for immunobridging: studies EBL2001, EBL2002, EBL3001 (Stage 1 and 2), EBL3002, and EBL3003.

All 5 clinical studies used for immunobridging were randomized, observer-blind, placebo-controlled studies. The population enrolled in the clinical studies consisted of healthy adults, adolescents and children (from the age of 1 year onwards), as well as HIV-1 infected adults who were on a stable regimen of highly active antiretroviral therapy (HAART). In these studies combined, a total of 3,367 subjects were randomized and received at least one dose of study vaccine (including control vaccines). All studies have been conducted in Europe, the United States, or Africa. The population used for the immunobridging analysis only included healthy adults between 18 and 50 years of age (n=764).

3.2 Favourable effects

The main immunogenicity parameter for immunobridging was the binding antibody response to EBOV GP, as measured by EBOV GP FANG ELISA, at 21 days post Dose 2.

In healthy adults, GMC (95% CI) were:

- 10,131 (8,554; 11,999) in EBL2001
- 7,518 (6,468; 8,740) in EBL2002
- 4,784 (3,736; 6,125), and 3,810 (3,312; 4,383) in EBL3001 Stage 1 and 2
- 11,054 (9,673; 12,633) in EBL3002
- 11,089 (9,323; 13,189), 10,337 (8,660; 12,339), and 11,790 (9,701; 14,328) in EBL3003

Immunogenicity and efficacy testing were performed in cynomolgus macaques, challenged 21 days after the 2nd dose with the Zaire Ebola virus of the Kikwit strain. In NHP that were vaccinated with Ad26. MVA as recommended in the draft SmPC (i.e. Ad26 5x10^{10}/dose, followed 8 weeks later by MVA 1x10^{8}/dose), 100% survival was observed after challenge (10/10). The median antibody titer observed in these 10 NHP, 21 days post Dose 2, was 22,927 (range 10,766 – 56,125) EU/mL.

Based on the human immunogenicity data from the 764 healthy adults included in the immunobridging analysis, the mean predicted survival probability in healthy adults aged 18 – 50 years was determined to be 53.4% (95% CI 36.7%; 67.4%).

The population enrolled in the clinical studies included, apart from healthy adults, also adolescents and children (from the age of 1 year onwards), elderly subjects, and HIV-1 infected adults who were on a stable regimen of highly active antiretroviral therapy (HAART). Immunogenicity results for these populations (not included in the immunobridging) were (GMC (95% CI)):

- HIV-infected adults (EBL2002): 5,283 (4,094; 6,817)
- Adolescents: 13,532 (10,732; 17,061) in EBL2002, 9,929 (8,172; 12,064) in EBL3001
- Children 4-11 years of age: 17,388 (12,973; 23,306) in EBL2002, 10,212 (8,419; 12,388) in EBL3001
- Children 1-3 years of age: 22,452 (18,305; 27,538) in EBL3001

The mean predicted survival probability yielded by the model (based on the PP analysis set) ranged from 42.0% (HIV-infected participants) to 82.6% (children 1-3 years) with a lower limit of the 95% CI ranging from 22.4% to 74.9%.

3.3 Uncertainties and limitations about favourable effects

Efficacy/effectiveness data. No efficacy or effectiveness data is available. The Applicant is in the process of conducting a test-negative case-control study in DRC, but it is unknown whether or not this study will be able to answer the outstanding questions on the beneficial effect in humans. Data from this study will be submitted post-authorisation.

Correlate of protection. There is no correlate of protection known for Ebola. This hampers the interpretation of the clinical relevance of the observed vaccine-induced immunogenicity. It also has consequences for the establishment of e.g. release specifications and shelf life limits, as it is unknown what the impact of a certain drop in titers is on the clinical protection afforded by the vaccine regimen.

Immunobridging. Even though the NHP challenge model is likely more stringent than natural EVD in humans, it remains unknown whether the antibody level seen in NHP would induce the same level of protection in both NHP and humans.

The immunobridging model is fully dependent on peak antibody titers measured 21 days after the second vaccination, determined in a narrow population of healthy adults 18 to 50 years of age.

While consistent with the mean predicted survival probability of 53.4% in adults, the predictions of efficacy in children, elderly and HIV-infected individuals are based on the assumption that the relation between antibody titter and survival is the same in children, elderly and HIV-infected subjects. As this association has been studied only in adult NHP, additional uncertainty remains.

Effect size. Based on immunobridging, the Applicant estimated a mean predicted survival probability of 53.4% (95% CI 36.7%; 67.4%). The lower bound of the 95% CI is above the pre-specified 20% criterion that has been accepted by CHMP in scientific advice as a reasonable indicator of a likelihood of clinical benefit. The mean predicted survival probability is difficult to interpret, as it is based on an animal model with 100% lethality and no supportive care available. This is different from the human situation, in which the average case fatality rate is in the range of 50%. The true effect size in humans cannot be determined.

Variation in GMC. The level of EBOV GP-specific binding antibodies induced by vaccination in healthy adults with the selected dose, sequence and interval across the different phase 2 and 3 studies ranged between 3,810 and 11,790 EU/mL. There is a lower response in certain populations, most notably subjects from Sierra Leone, but to a lesser extent also HIV-infected subjects and subjects from other African countries. The reason for, as well as the clinical relevance of, the lower level of vaccine-induced EBOV-specific antibodies is unknown.

Duration of protection. Vaccinated subjects have been followed-up for 2 years after the first dose, but only immunogenicity data is available. As it is unknown what antibody level is required for protection after Ebola virus exposure, duration of protection cannot be established. Need for and timing of a booster dose remain uncertain. However, because of the lethality of the disease, it is recommended that Ad26.ZEBOV booster dose may be administered from 4 months onwards after the 2nd dose (MVA-BN-Filo) of the regimen for individuals at imminent risk of exposure to Ebola virus.

3.4 Unfavourable effects

Across all age groups, the majority of solicited local and systemic AEs were grade 1 or grade 2 in severity. Grade 3 solicited local and systemic AEs were infrequently reported: grade 3 local solicited AEs were reported by 0.4% of adults and by 1/645 children (0.1%) aged 1-18 years in total, grade 3 solicited systemic AEs were reported by 1.5% of adult subjects after vaccination with MVA-BN-Filo and by 1/645 children (0.1%) aged 1-18 years in total. All solicited local and systemic AEs were transient in nature and the majority had a median duration of 1 or 2 days after MVA-BN-Filo vaccination.

Local reactogenicity consisted mainly of pain at the injection site, reported by 46.6% of adult subjects and 4.9% of children aged 1-3 years, 22.3% of children aged 4-11 years and 27.5% of adolescents following vaccination with MVA-BN-Filo.

In adults, most common reported systemic reactions following MVA-BN-Filo were fatigue (29.8%) headache (26.7%) and myalgia (25.8%). In children (1-11 years), the most frequently reported systemic solicited AEs by PT were decreased appetite (5.8%), decreased activity (5.6%;), and irritability (4.6%). In adolescents, the most frequently reported systemic solicited AEs by PT were headache (21.5%), fatigue (14.3%) and myalgia and chills (both 11.2%).

So far there has been only one SAE which was considered possibly related to vaccination with MVA-BN-Filo, a SUSAR of generalized pruritus in an ongoing study.

3.5 Uncertainties and limitations about unfavourable effects

The safety of MVA-BN-Filo has been evaluated in 11 clinical trials in which 2,367 adults received MVA-BN-Filo and in which 645 children aged 1 to 18 received MVA-BN-Filo. This limits the detection of more rare but serious adverse events.

Local reactions were reported less frequently in children compared to adults. It is uncertain whether the safety profile is indeed more favourable in children as in adults as this observation may be biased. All children were recruited in West African countries, and, as was observed in adults, persons in West African countries tended to report less reactions.

Safety has not been assessed in in pregnant women. Safety of exposure of pregnant women to MVA-BN-Filo will need to be systematically collected post licensure.

3.6 Effects Table

Table 33: Effects Table for Zabdeno, Mvabea

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
EBOV GP FANG ELISA	EBOV GP binding antibodies 21 days after 2nd dose, for healthy adults 18-50 years of age (PPI population)	Predicted survival probability)	GMCs correspond to a **mean predicted survival probability** in healthy adults aged 18 – 50 years of 53.4% (95% CI 36.7%; 67.4%).	<LLOQ	The predicted survival probability is based upon EBOV GP binding antibody responses which were correlated to lethal challenge in non human primates. This is considered indicative for clinical benefit in humans however the exact level of protection and duration of protection afforded by the	EBL2001, EBL2002, EBL3001, EBL3002, and EBL3003

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
					vaccine regimen is unknown.	
Unfavourable Effects						
ISR	Pain at injection site	%	Zabdeno: Adults: 47.6% 12-17 years: 24.9% 4-11 years: 29.8% 1-3 years: 13.9% Mvabea: Adults:46.6 % 12-17 years: 27.5% 4-11 years: 22.3% 1-3 years:4.9%	Adults:17.7% 12-17 years:8% 4-11 years:15.8% *1-3 years:10.4%*	Lower reporting of AEs in children may be biased	Pooled data from clinical studies Restricted adult pooling: EBL 1001, 1002, 1002, 1003, 1004, 2001, 2002, 2003, 3001, 3002, 3003 and FLV 1001 Paediatric pooling: EBL 2002 and 3001
	Myalgia	%	Adults: 25.8% 12-17 years: 11.2% 4-11 years: 2.4%	Adults: 16.1% 12-17 years: 8% 4-11 years: 1.1%		

Abbreviations: EBOV: Zaïre ebolavirus; GP: glycoprotein; FANG: Filovirus Animal Nonclinical Group; ELISA: enzyme-linked immunosorbent assay; PPI: Per Protocol Immunogenicity analysis set; EU/mL: ELISA units/mL; LLOQ: lower limit of quantification; CI: confidence interval; ISR: Injection site reactions; AE: adverse event.

Notes: Pain at injection site in children 1-3 years of age was compared against MenACWY vaccination. All other comparisons were against placebo vaccination.

3.7 Benefit-risk assessment and discussion

3.7.1 Importance of favourable and unfavourable effects

Clinical efficacy has not been demonstrated for the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. It is considered that generation of human protective efficacy data against Ebola virus disease is not feasible.

To infer a clinical benefit, the Applicant has bridged the EBOV GP-binding antibody response as observed in humans approximately 21 days after the second dose to the EBOV GP-binding antibody response observed in NHPs approximately 21 days after the second dose using the same assay (EBOV GP FANG ELISA). In a NHP challenge model it was demonstrated that 100% protection to a lethal challenge with EBOV was achieved with the proposed vaccine regimen. The main assumption is that EBOV GP ELISA antibody titres which were protective in the NHP challenge model will also provide protection in humans. This is considered a reasonable assumption.

Through logistic regression modelling it has been estimated that the EBOV GP GMCs as observed in healthy humans aged 18 to 50 years in the phase II and phase III trials conducted by the Applicant would translate into a predicted survival probability of 53.4% with a lower limit of the confidence interval >20% (pre-specified success criterion). Whilst this is suggestive of a clinical benefit of the vaccine regimen, the exact level of protection provided by the vaccine is unknown as it is not clear how the NHP challenge model translates into the situation in humans. The NHP challenge model is likely more stringent than natural EVD in humans. After infection, NHPs have a shorter time to onset of symptoms, a much faster disease progression, and a 100% case fatality rate. A 50% survival probability on this otherwise fully lethal NHP model, may play an important role in controlling an outbreak and preventing death. Therefore, the clinical benefit that this vaccine may provide could be considered of great importance. The application should be seen in the light of the unmet medical need for effective methods to prevent Ebola virus disease, and a higher level of uncertainty may be acceptable in this specific case.

The Applicant has tried to minimize the impact of species-specific aspects by measuring both NHP and human antibody responses in a single ELISA with one cross-reactive detection antibody and one single reference curve based on a human polyclonal sera pool. It remains however unknown if the same antibody concentration detected in a NHP serum and a human serum indeed corresponds to the same actual antibody levels in NHP and humans, and if this antibody level would induce the same level of protection in both species. Despite these caveats, the strategy followed by the Applicant is considered reasonable.

The main immunogenicity parameter used to infer a beneficial effect is the level of EBOV GP-specific binding antibodies. As there is no threshold that can be used to predict clinical benefit, interpretation of the GMC values obtained in clinical trials is challenging. It is unknown what the clinical relevance is of the observed variations between subgroups, vaccine doses, and time after vaccination. This hampers, among others, the interpretation of the impact of lower antibody titers that have been observed in subjects from Sierra Leone and HIV-infected subjects. Based on the analyses provided by the Applicant, there is still a reasonable likelihood of a protective effect based on these lower antibody titers (e.g. for subjects from Sierra Leone a survival probability of 30.9% (95%CI: 13.6;47.0) was predicted) but the actual effect size is not known. These uncertainties cannot be solved with the data that have been generated.

Due to the lack of any threshold value associated with clinical benefit, it is also not possible to establish duration of protection or to advise on when a booster dose should be recommended. A conservative approach was thus recommended in the SmPC.

There are some quality aspects of the MVA vaccine that result in significant uncertainties. Drug product batches produced by the latest process variants (DP4 and DP5) are observed to be significantly less stable (weakened) than the batches that have been used in the clinical studies.

The safety of the vaccine regimen is reasonably characterised and considered acceptable. Both components of the regimen are well tolerated. Reactogenicity was mostly mild and of limited duration. More severe reactions to the vaccine regimen were seen in <1% of subjects across all age groups.

The vaccine regimen is proposed to be licensed under exceptional circumstances as there is no protective efficacy data. Whilst a test negative case control study is currently underway to attempt to estimate vaccine effectiveness, the chances of obtaining sufficient data are considered limited. Further, it is not known whether there will be any future outbreaks in which the protective effect of vaccine regimen can be assessed. Therefore, the likelihood that robust estimation of effectiveness of the vaccine regimen could be provided post approval is considered limited.

3.7.2 Balance of benefits and risks

The occurrence of binding and neutralizing antibodies against the Ebola Zaire GP-protein is considered to be relevant from an immunological point of view, but the clinical relevance in terms of magnitude and duration of protection is unknown, as there is no immunological correlate of protection.

Based upon bridging of the human antibody response level observed in clinical trials to the antibody response level in NHPs and the associated 50% survival as estimated in a fully lethal NHP model, it is considered reasonable to conclude that the Ad26.ZEBOV,MVA-BN-Filo vaccine regimen could provide protection against EVD and that may play an important role in controlling an outbreak and preventing death. Furthermore, the safety profile of both vaccines appears favourable.

3.7.3 Additional considerations on the benefit-risk balance

Despite accepting that the potential benefit of the Ad26.ZEBOV,MVA-BN-Filo vaccine regimen may, even with all the uncertainties, be considered sufficiently demonstrated, careful consideration needs to be given to how this benefit will be framed. It will be important to communicate the exact level of protection afforded by the vaccine regimen cannot be predicted and that therefore it is important to maintain other control measures, in particular when exposed to a high risk of EVD, such as hygiene and personal protection for health care workers. Also, it will be important that if provided with an opportunity, i.e. if there is an outbreak of EVD in a vaccinated population or the vaccine regimen will be used within context of an EVD outbreak, all efforts possible are undertaken to quantify the benefits afforded by the vaccine regimen in terms of prevention of death, prevention of disease and possibly mitigation of disease symptoms.

Marketing authorisation under exceptional circumstances

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was requested by the applicant in the initial submission.

The CHMP considered that the Applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because in the present state of scientific knowledge, comprehensive information cannot be provided, particularly due to the recognized complexity and difficulty around the generation of effectiveness data. Also, the CHMP agrees with the Applicant's claim that it would be contrary to generally accepted principles of medical ethics to collect such information.

The CHMP acknowledges that the Applicant is in the process of conducting a test-negative case-control study to evaluate the effectiveness of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. The CHMP considers that given the difficult-to-predict evolution of the Ebola outbreak, it is possible that the study will not reach enough EVD cases to allow an evaluation of vaccine effectiveness. This could happen in case the outbreak wanes or because of security constraints or community concerns that prevent full vaccine implementation, or because of other external factors which may prevent continuation of the population-based study.

Whether the DRC study will allow the generation of actual effectiveness data will depend on factors beyond the control of the Applicant (epidemiology of the outbreak, vaccination approach taken by the local authorities). Also, whether the ability to determine clinical effectiveness will arise in another setting in a future outbreak cannot be predicted. Therefore, the Applicant is unable to commit to generating effectiveness data within a reasonable time frame, something that would be expected in the

context of the Conditional Approval pathway, and consequently seeks approval for the vaccine regimen under Exceptional Circumstances.

The CHMP is aware of the outbreak and the security constraints in the DRC, as well as the unpredictability of future events. The CHMP recalls the recently licensed Ebola vaccine authorised under a conditional marketing authorisation. There are however relevant differences between Ervebo and the current vaccine regimen, that justify a different licensing strategy. For Ervebo, clinical efficacy data was available at the time of MAA, and considered sufficiently comprehensive for determining the B/R. This is not the case for Ad26/MVA. As comprehensive clinical efficacy data are absent for Ad26/MVA, one could contemplate to approve the vaccine regimen under a conditional marketing authorisation with a condition to supply field effectiveness data post licensure. However, Ad26/MVA is a prophylactic vaccine regimen given 8 weeks apart, hence it will be more than 2 months before a vaccinated person may be protected. This makes it unsuitable for an outbreak response, as in such a situation fast protection is necessary. For these situations Ervebo will be the vaccine of choice. Ad26/MVA will more likely be used in areas where there is no Ebola outbreak, or not yet. Once an Ebola outbreak would occur, it would be within expectation that Ervebo will be used to mitigate the impact and protect potentially exposed persons. These factors will make it very difficult to estimate the effect of the Ad26/MVA regimen in a post licensure setting. Therefore, a conditional approval with an efficacy-related condition is not considered viable, as it is highly unlikely that an efficacy-related condition will ever be met.

Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

3.8 Conclusions

The overall B/R of the Ad26.ZEBOV,MVA-BN-Filo vaccine regimen is considered to be positive, provided that the Applicant agrees to the conditions as identified in section 4 below.

4. Recommendations

Similarity with authorised orphan medicinal products

Not applicable.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Zabdeno is favourable in the following indication:

"*Zabdeno, as part of the Zabdeno, Mvabea vaccine regimen, is indicated for active immunisation for prevention of disease caused by Ebola virus (Zaire ebolavirus species) in individuals ⩾1 year of age.*

The use of the vaccine regimen should be in accordance with official recommendations."
The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Area	Number	Description	Due date
Clinical	001	To ensure adequate monitoring of effectiveness, the applicant will perform the following study to collect data in the context of the intended use of the Ad26.ZEBOV, MVA-BN-Filo prophylactic vaccine regimen. Post-authorisation non-interventional study: - VAC52150EBLXXXX: Evaluation of a heterologous, two-dose preventive Ebola vaccine for field effectiveness	Status to be reported annually within each annual re-assessment application

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that Ebola vaccine (Ad26.ZEBOV-GP [recombinant]) is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Paediatric Data

No significant studies in the agreed paediatric investigation plan P/0116/2019 have been completed, in accordance with Article 45(3) of Regulation (EC) No 1901/2006, after the entry into force of that Regulation.