PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Open-label, Randomized, Clinical Trial to Evaluate the Immunogenicity and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol
AUTHORS	Larivière, Ynke; Zola, Trésor; Stoppie, Elke; Maketa, Vivi; Matangila, Junior; Mitashi, Patrick; De Bie, Jessie; Muhindo-Mavoko, Hypolite; Van geertruyden, Jean-Pierre; Vandamme, Pierre

VERSION 1 – REVIEW

REVIEWER	Morris, Julie South Manchester NHS Trust, Medical Statistics Department
REVIEW RETURNED	10-Dec-2020

GENERAL COMMENTS	This study protocol describing a Phase 2 trial looking at the immunogenicity and safety of a vaccination against Ebola is reasonably detailed and usefully includes a completed SPIRIT checklist. However, there are a number of issues which need to be addressed: 1. The main objective of the study needs to be clarified. Is it to assess binding antibody response post dose 2 vaccination (as reported in Table 1, and as reflected in the title of the paper) or is it to compare the two booster arms (as reported in the Strengths and Limitations section, the last sentence of the Introduction and the first sentence of the Discussion)? If the former, then what does this study add to previous studies which have already assessed the safety and efficacy of the vaccine? 2. Justification of the intended sample size is not currently provided. The protocol simply states that the number of 700 is, "...defined upon the feasibility of recruitment of HCP in the region" (Page 7). It is conventional to report some details of either the precision of estimates (eg based on the width of confidence intervals) for descriptive summary statistics related to the sample size, or detectable differences related to a comparison of treatment arms (depending on the main objective). Also, how many HCP are registered in the area? What proportion of these is

	estimated to agree to take part? What proportion is estimated to be lost to follow-up? 3. The randomization procedure is not described in any detail (only sealed envelopes are mentioned). What process will be used to generate the randomization and who will be involved in creating the sealed envelopes? 4. The intended statistical analysis includes descriptive information only. Is any comparative analysis not planned (to directly compare the two booster arms)? 5. It is stated that a primary interim analysis is to be carried out at 21-day post dose 2 (Page 17). But this timepoint is referred to as the primary objective endpoint in Table 1. This anomaly should be explained. 6. No copies of consent forms are presented.

REVIEWER	Zhu, Fengcai Jiangsu Provincial Center for Disease Control and Prevention
REVIEW RETURNED	18-Dec-2020

GENERAL COMMENTS	This clinical study design is to evaluate the immunogenicity and safety of two booster arms with an Ad26.ZEBOV vaccine administered either 1 or 2 years post Ad26.ZEBOV as first dose and MVA-BN-Filo as second dose vaccination at a 56-day interval in health care providers. However, the objective of this study is to compare the difference of the immune memory response induced by two booster arms, so the primary immunogenicity outcome about boost vaccination is the most significant. The authors should take more considerations for the primary and secondary outcomes selected, and the following points are suggested to be addressed by the authors: 1.In the part of introduction, the author mentioned three previous studies within EBOVAC projects have administered a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494) or two years. If these three studies have published results? If there have, please briefly describe the important results. 2.According to the SPIRIT checklist, Objectives and Trial design are suggested to be included in the introduction, so the author should add some relevant main points in the last paragraph in the part of introduction. 3.The primary outcome is to detect binding antibody levels against the EBOV GP using FANG ELISA, whereas the pre-existing anti-EBOV GP IgG antibodies is using LUMINEX assay. Why they are different, please take some explanations for it. 4. In the study design, a total number of 700 Registered HCP are planned to be recruited,but how it was determined? Please give clinical and statistical assumptions supporting any sample size calculations. In addition, how about the requirement of the ratio of gender or age and how to achieve it? 5. The study mainly compares two booster arms with an Ad26.ZEBOV vaccine administered either 1 or 2 years post

	prophylactic heterologous 2-dose regimen, so why the primary outcome is binding antibody levels against the EBOV GP post-dose 2 vaccination rather than the binding antibody levels after boost vaccination？Also, according to the previous study (NCT02325050) within EBOVAC projects and other clinical trials of EBOLA vaccine, binding antibody levels against the EBOV GP was to be detected at 21 or 28 days post booster vaccination, so why binding antibody levels to be detected at 7 days post booster in this study design? 6.According to the inclusion criteria, HIV-positive subjects can be enrolled as long as their general condition is good. However, before the boost vaccination given 1 or 2 years post first dose, if the general condition of HIV-positive enrolled subjects should be rejudged？So the safety assessment should not be only about vital signs (blood pressure, pulse/heart rate [both at rest] and body temperature), pregnancy test and inquiry after SAE described as Figure 1. Similarly, all the participants should take HIV test before the boost vaccination is given. 7. Boost vaccination procedure written started from line 168 of page 12. The first sentence is directly written by 'At 1 year or 2 years post first dose, depending on the study arm, a booster vaccination with Ad26.ZEBOV is given' but lacking in vital signs collecting and other physical examination. Please add it. 8.Line 170-171 of page 12,'Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant diary starting on the day of the vaccination and continuing for the subsequent 7 days.', so whether the participants are asked to collect solicited and unsolicited AEs after dose 1 and dose 2. If not, why? Also, unsolicited AEs is generally to be collected for the 28 days, so why it is only collected for only 7 days? 9.This is the first randomized vaccine trial that looks into the safety and immunogenicity of prophylactic heterologous 2-dose regimen and boosted with different booster arms of Ad26.ZEBOV and the target population of the study is the health care providers. Why to select this particular population and whether the results from them can be extended to the whole population？In addition, author has mentioned HCP are not only more at risk of disease acquisition but also facilitate the spread of the virus in introduction, and HCP had high rate seroreactive to EBOV protein in discussion, but how about incidence rate？If HCP have high rate of incidence, it will be very significant for phase 3 clinical study.

REVIEWER	Ndwandwe, Duduzile South African Medical Research Council, Cochrane South Africa
REVIEW RETURNED	22-Dec-2020

GENERAL COMMENTS	Thank you for this well written paper. I have enjoyed reading the manuscript. Can the authors just clarity this statement on page 14; line 163-167, especially the last sentence in brackets: "..... the clinical 166 trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity 167 assessment of all participants (where applicable pre-administration of the booster dose)". Why would a booster be given and how long after the pre-booster is given before the assessment of immunogenicity can be

	made given that the secondary objective seeks to assess the effects of a booster dose after 1-2 years. The other question that comes to mind is what would made the study team to consider pre-booster dose at this visit. Similar clarity would be needed also for the 2 year visits in relation to pre-administration of the booster dose

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Ms. Julie Morris, South Manchester NHS Trust

1. The main objective of the study needs to be clarified. Is it to assess binding antibody response post dose 2 vaccination (as reported in Table 1, and as reflected in the title of the paper) or is it to compare the two booster arms (as reported in the Strengths and Limitations section, the last sentence of the Introduction and the first sentence of the Discussion)? If the former, then what does this study add to previous studies which have already assessed the safety and efficacy of the vaccine?
R/ Thank you very much for this very pertinent question. At the start of the project, the protocol initially only included a vaccination strategy with the two-dose regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) and was later adapted to include also the booster vaccination. The purpose of the initial observational trial was, next to obtaining additional immunogenicity data, a way to see if performing a remote vaccine trial in the Democratic Republic of the Congo (DRC) was feasible and accepted by the population. While writing the protocol however, administering a booster dose in this large cohort was added as a novel aspect and thus this was entered as a secondary objective/endpoint. At the moment, it is still unknown whether this booster dose will be required at the moment of an outbreak and what it's effect would be. To explore it's safety and immunogenicity, this study protocol was transformed and became a randomized controlled trial. The main focus therefore remains on collecting descriptive information for the 2-dose vaccine regimen in order to collect enough information and obtain a worldwide licensure of the regimen. In order to make this more clear, we have adapted the wording throughout the manuscript slightly.

2. Justification of the intended sample size is not currently provided. The protocol simply states that the number of 700 is, "...defined upon the feasibility of recruitment of HCP in the region" (Page 7). It is conventional to report some details of either the precision of estimates (eg based on the width of confidence intervals) for descriptive summary statistics related to the sample size, or detectable differences related to a comparison of treatment arms (depending on the main objective). Also, how many HCP are registered in the area? What proportion of these is estimated to agree to take part? What proportion is estimated to be lost to follow-up?
R/ Unfortunately no sample size calculations were performed based on the secondary objectives. When writing the protocol, a monkeypox vaccination study was being conducted in the same geographic area by the Centers for Disease Control and Prevention (1). This study was also enrolling health care providers (HCP) as participants. Based on this trial, we were informed of the high willingness to participate and the high enrolment and retention rate (>90% after 2 years) that they achieved for HCP (CDC, personal communication). With this trial as a reference, it was estimated that recruiting 700 participants (HCP and front-liners) was deemed feasible. This corresponds with approximately 50% of the HCP and front-liners working in the Boende health district. Furthermore, based on the current knowledge of our ongoing trial we expect a very high retention rate (>90%) at the end of the trial. Currently, one year after the first dose, we still have a retention rate of >95%. We have further elaborated on the process in the section "Participant population" in the methods (cfr. Line 134-139).

In addition, while no sample size calculations were performed prior to the writing of the protocol, we have performed a power analysis, based on the available information to us. We have added a paragraph to the "Discussion" section of the manuscript, which notes that the trial is sufficiently powered to compare the two booster arms using comparative analyses (cfr. Line 339-349).

3. The randomization procedure is not described in any detail (only sealed envelopes are mentioned). What process will be used to generate the randomization and who will be involved in creating the sealed envelopes?
R/ Thank you for this comment, we acknowledge that detailed information on the randomization procedures was still lacking and would like to inform you that the this has now been further elaborated on in the methods section of the manuscript under the title "Randomization procedure" (cfr. line 143-165).

4. The intended statistical analysis includes descriptive information only. Is any comparative analysis not planned (to directly compare the two booster arms)?
R/ Thank you for your comment. While no sample size calculations were initially performed, we did perform a power analyses after commencing the trial to determine if the sample size was large enough to compare the two booster arms. Fortunately, this was the case and we will thus be able to perform comparative analysis. This has now been further elaborated in the "Discussion" of the article (cfr. Line 339-349).

5. It is stated that a primary interim analysis is to be carried out at 21-day post dose 2 (Page 17). But this timepoint is referred to as the primary objective endpoint in Table 1. This anomaly should be explained.
R/ As the booster dose administration and it's safety and immunogenicity evaluations were added as a secondary objective of the trial, the primary objective is indeed evaluated after interim data base lock. To make a better distinction, the phrase "primary interim analysis" has been adapted to "primary endpoint analysis" (cfr. Line 316).

6. No copies of consent forms are presented.
R/ The latest available version of the Informed Consent Form has been uploaded.

Reviewer: 2
Dr. Fengcai Zhu, Jiangsu Provincial Center for Disease Control and Prevention

1. In the part of introduction, the author mentioned three previous studies within EBOVAC projects have administered a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494) or two years. If these three studies have published results? If there have, please briefly describe the important results.
R/ Thank you for this comment. We have added the published information for the NCT02325050 trial (cfr. Line 96-99). The NCT02564523 results have been submitted for the publication and the NCT02509494 trial results have been accepted for publication but are not yet published. We can therefore not expand further on these at this moment.

2. According to the SPIRIT checklist, Objectives and Trial design are suggested to be included in the introduction, so the author should add some relevant main points in the last paragraph in the part of introduction.
R/ Thank you for your observation, the last paragraph of the introduction has been adapted to contain this information (cfr. Line 110-113).

3. The primary outcome is to detect binding antibody levels against the EBOV GP using FANG ELISA, whereas the pre-existing anti-EBOV GP IgG antibodies is using LUMINEX assay. Why they are different, please take some explanations for it.
R/ Thank you for this observation, we would like to elaborate why both of these tests are performed for our trial. The first reason being that a broader spectrum of antibodies will be obtained using the LUMINEX assay than the FANG ELISA. Both IgG and IgM results will be obtained using the LUMINEX Assay. These analyses will be performed at the Institut National pour la Recherche Biomedicale (INRB) in the Democratic Republic of the Congo. The reason why FANG ELISA is additionally performed to obtain EBOV GP antibodies at baseline, is because these analyses are performed at a different lab, more precisely at Q^2 Solutions in the United States of America. The latter was a requirement by the vaccine manufacturer as Q^2 Solutions had also performed the FANG ELISA's for all previous EBOVAC studies. This allows the vaccine manufacturer to group results across all trials without possibly introducing a bias by having results of analyses performed by different laboratories. We have added two sentences under "Study procedures" to make this more clear (cfr. Line 176-179).

4. In the study design, a total number of 700 Registered HCP are planned to be recruited, but how it was determined? Please give clinical and statistical assumptions supporting any sample size calculations. In addition, how about the requirement of the ratio of gender or age and how to achieve it?
R/ Unfortunately no sample size calculations were performed based on the secondary objectives. However, while no sample size calculations were performed prior to the writing of the protocol, we have performed a power analysis, based on the available information to us. We have added a paragraph to the "Discussion" section of the manuscript, which notes that the trial is sufficiently powered to compare the two booster arms using comparative analyses (cfr. Line 339-349).
Furthermore, no gender or age ratio was considered for this trial. The focus was on the occupation of the participants who needed to be either registered health care providers or front-liners older than 18 years of age.
5. The study mainly compares two booster arms with an Ad26.ZEBOV vaccine administered either 1 or 2 years post prophylactic heterologous 2-dose regimen, so why the primary outcome is binding antibody levels against the EBOV GP post-dose 2 vaccination rather than the binding antibody levels after boost vaccination? Also, according to the previous study (NCT02325050) within EBOVAC projects and other clinical trials of EBOLA vaccine, binding antibody levels against the EBOV GP was to be detected at 21 or 28 days post booster vaccination, so why binding antibody levels to be detected at 7 days post booster in this study design?
R/ Thank you very much for this very pertinent question. At the start of the project, the protocol initially only included a vaccination strategy with the two-dose regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) and was later adapted to include also the booster vaccination at the request of the vaccine producer. The purpose of the initial observational trial was, next to obtaining additional immunogenicity data, a way to see if performing a remote vaccine trial in the Democratic Republic of the Congo (DRC) was feasible and accepted by the population. While writing the protocol however, administering a booster dose in this large cohort was added as a novel aspect and thus this was entered as a secondary objective/endpoint. At the moment, it is unknown whether this booster dose will be required or not at the moment of an outbreak and what it's effect would be. To explore it's safety and immunogenicity, this study protocol was transformed and became a randomized controlled trial. The main focus therefore remains on collecting descriptive information for the 2-dose vaccine regimen in order to collect enough information and obtain worldwide licensure of the regimen. We have adapted the wording throughout the manuscript slightly to make this distinction clearer.
Concerning your question on the evaluation of the binding antibody response, we believe the recently changed terminology may have led to some misunderstanding. The vaccine manufacturer has recently changed the wording of their heterologous two-dose vaccine regimen (Ad26.ZEBOV followed by MVA-

BN-Filo 56 days later) from “prime-boost” regimen to “two-dose” regimen. To make sure no mix-ups were made between the second dose of the regimen (MVA-BN-Filo: initially referred to as “boost”) and the actual booster dose (Ad26.ZEBOV). In the EBL2007 trial, we also evaluate the response of the second dose (initial “boost”-dose) 21 days after administration of the vaccine, as was the case for previous EBOVAC studies. However, as results from the NCT02325050 trial have shown a rapid immune response after booster vaccination (2), we expect a fast antibody reaction and therefore evaluate the binding antibody response 7 days after vaccination to not miss its peak effect.

6. According to the inclusion criteria, HIV-positive subjects can be enrolled as long as their general condition is good. However, before the boost vaccination given 1 or 2 years post first dose, if the general condition of HIV-positive enrolled subjects should be rejudged? So the safety assessment should not be only about vital signs (blood pressure, pulse/heart rate [both at rest] and body temperature), pregnancy test and inquiry after SAE described as Figure 1. Similarly, all the participants should take HIV test before the boost vaccination is given.
R/ Ongoing and unpublished trials that included HIV-positive participants (on a stable regimen of HAART and in good medical condition) have shown that HIV was not a contra-indication for vaccination (3, 4), as these are non-live vaccines, and not live-attenuated vaccines. The routine control of HIV testing was therefore not chosen for this trial. Furthermore, the prevalence of HIV-positive individuals in the Tshuapa province of DRC is extremely low (0.9% (5)).
The authors would further like to highlight that the HIV-positive participants are followed up very closely throughout the course of the trial. Once enrolled in the trial the wellbeing of a participant is closely monitored. In case the participant experiences symptoms or is unwell during the course of the trial, he/she can return to the study site for a physical examination by the trial medical doctor. If the medical doctor suspects that a participant might be experiencing symptoms as a consequence of an HIV-positive status, the participant will be referred to the general hospital to get tested. The testing itself is however not done in the context of the trial. This also means that their wellbeing and general condition will be rechecked prior to their booster vaccination. If the medical doctor deems the participant unhealthy, he/she will not receive the booster vaccination. In addition, when participants are diagnosed while already enrolled in the trial, the new HIV case is reported as serious adverse event “Other medical important event”. This new case is then followed-up by the principal investigator and provided with anti-retroviral treatment. The newly reported event is also checked by the sponsor medical doctors. If the participant is considered to be in a good condition while on medication, the booster vaccine will be administered.

7. Boost vaccination procedure written started from line 168 of page 12. The first sentence is directly written by ‘At 1 year or 2 years post first dose, depending on the study arm, a booster vaccination with Ad26.ZEBOV is given’ but lacking in vital signs collecting and other physical examination. Please add it.
R/ The following sentence has been added (cfr. Line 210-212): “Prior to vaccination, the general well-being of the participant will be evaluated and urine pregnancy testing (for women of childbearing potential), as well as a vital signs measurement will be performed.”

8. Line 170-171 of page 12,‘Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant diary starting on the day of the vaccination and continuing for the subsequent 7 days.’, so whether the participants are asked to collect solicited and unsolicited AEs after dose 1 and dose 2. If not, why? Also, unsolicited AEs is generally to be collected for the 28 days, so why it is only collected for only 7 days?
R/ For this trial solicited and unsolicited AEs are not collected after dose 1 and dose 2. As the trial location is in a very remote area where travelling from one village to the next can sometimes take days, some trial participants have to travel a long time before reaching the trial site. Previous trials performed with the 2-dose heterologous vaccine regimen had already collected a large amount of safety information

for the first two doses (3, 4). It is for this reason that additional site visits after dose 1 and 2 for the collection of adverse events (AEs) were not taken into account for this trial. However, for the booster dose the collection of AEs was crucial and are thus collected, as only two trials (with small samples sizes n=<40) have previously looked into the safety and immunogenicity effect of a booster dose with Ad26.ZEBOV after a heterologous vaccination with the 2 dose prophylactic regimen, administered 56 days apart.
Furthermore, AEs lasting longer the 7 days post-vaccination are not expected very often and therefore a follow-up of 7 days after booster vaccination is deemed sufficient. However, participants with solicited AEs that still persist at 7 days post booster will be followed up until the solicited AEs are resolved.

9. This is the first randomized vaccine trial that looks into the safety and immunogenicity of prophylactic heterologous 2-dose regimen and boosted with different booster arms of Ad26.ZEBOV and the target population of the study is the health care providers. Why to select this particular population and whether the results from them can be extended to the whole population? In addition, author has mentioned HCP are not only more at risk of disease acquisition but also facilitate the spread of the virus in introduction, and HCP had high rate seroreactive to EBOV protein in discussion, but how about incidence rate? If HCP have high rate of incidence, it will be very significant for phase 3 clinical study.
R/ HCP and front-liners were chosen as trial participants because they are not only more at risk of contracting Ebola but can also facilitate the spread of the disease. While the study population composition cannot be generalized to the general population, we believe that safety and immunogenicity responses can be generalized as the trial itself allows for a broad inclusion of participants, including for example HIV-positive participants.
Concerning your latter query about the incidence rate, though a very pertinent and interesting questions, we will not be able to provide an answer through the set-up of our clinical trial. As we vaccinate our participants, they will start to develop an immune response and we expect an increase in antibodies against Ebola. As we do not have a placebo group, we cannot monitor the incidence rate when no vaccines are administered. Furthermore, as there is currently no ongoing outbreak in the Boende health district, we don't expect an increasing incidence unless asymptomatic Ebola infections are more common than is currently thought. The focus of this trial was thus more to prepare a susceptible population for a future outbreak.

Reviewer: 3
Dr. Duduzile Ndwandwe, South African Medical Research Council

1. Can the authors just clarity this statement on page 14; line 163-167, especially the last sentence in brackets: "..... the clinical 166 trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity 167 assessment of all participants (where applicable pre-administration of the booster dose)". Why would a booster be given and how long after the pre-booster is given before the assessment of immunogenicity can be made given that the secondary objective seeks to assess the effects of a booster dose after 1-2 years. The other question that comes to mind is what would made the study team to consider pre-booster dose at this visit. Similar clarity would be needed also for the 2 year visits in relation to pre-administration of the booster dose
R/ Thank you for your comments. We have provided comments on your queries below:
- Why would a booster be given: For this question, we would like to refer to lines 91-102 of the "Introduction". Within this section, we explain that a booster dose could potentially boost an individual's immune response at times of imminent risk (e.g. during an outbreak).
- how long after the pre-booster is given before the assessment of immunogenicity can be made given that the secondary objective seeks to assess the effects of a booster dose after 1-2 years. The other question that comes to mind is what would made the study team to consider pre-booster dose at this visit.

Similar clarity would be needed also for the 2 year visits in relation to pre-administration of the booster dose: We believe our wording of pre-administration was not completely clear here. We have chosen to adapt the wording from “pre-administration of the booster dose” to “before administration of the booster dose”. We hope that this clarifies that there is no pre-booster dose but that this section refers to a blood sample collection prior to administering the booster dose.

VERSION 2 – REVIEW

REVIEWER	Morris, Julie South Manchester NHS Trust, Medical Statistics Department
REVIEW RETURNED	27-Mar-2021

GENERAL COMMENTS	This study protocol has been revised to take account of some of the points raised in the original statistical report, but there are some issues of concern that remain. The objective of the study has been clarified (see Introduction section in Abstract and last paragraph of the Introduction section in the main text) to be the safety and immunogenicity of the vaccine regimen (which includes a booster vaccination). In this respect the proposed statistical analysis of descriptive statistics alone is appropriate. However, the (late) addition of an RCT relating to the timing of the booster vaccination introduces an element of uncertainty in the main focus of the study. I believe the title of the paper inappropriately emphasizes the randomized part of the clinical study as a formal statistical comparison of the two booster timings does not appear to be intended, and some of the information presented in the protocol also appears inconsistent. Justification of the intended sample size is provided (Participant population section), but the inclusion of a formal power calculation relating to a comparison of the two booster arm groups in the Discussion section is not appropriate given that no formal comparison of the two randomized groups is mentioned in any other part of the paper (in particular a formal comparison of the two groups is not mentioned in Table 1 or the Statistical analysis section). In addition, the power calculation presented is not justified sufficiently as it is unclear whether the effect size mentioned is acceptable for a comparison of two booster arms.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Ms. Julie Morris, South Manchester NHS Trust

R/ Thank you for your very relevant observations. We would like to address this comment together with the below query as they are related to one another. Thus, please find a response to your comment below.

R/ Thank you for your very relevant observations. As we do intend to compare the two booster arms, We have adapted the text accordingly throughout the manuscript to make sure that this intention is clear. We agree that this was not sufficiently elaborated on in the previous resubmission of the manuscript and several adaptations (described below) have now been made throughout the manuscript.

Under Strengths and limitations of this study we have added: “With this randomized vaccine trial, being the first to evaluate the safety and immunogenicity in two different booster vaccine arms 1 or 2 years after the prime dose, new contributions will be added to already existing safety and immunogenicity data. Additionally, it is the first trial to assess the antibody response and (serious) adverse event occurrence of two different booster arms in a large adult cohort.” (crf. Line 47-51)

In the Introduction the following sentence was added: “Additionally, this trial aims to assess the safety and immunogenicity of a booster Ad26.ZEBOV vaccine administered either 1 or 2 years post first dose and to compare the induced immune memory response between both booster arms.” (cfr. Line 113-116)

To determine whether a formal comparison of the induced immune memory of the two booster arms would be possible, a power analysis was performed. The explanation for this has been removed from the discussion and added to the Methods subtitle Participant population and sample size: “However, to determine whether it would be possible to compare the induced immune responses of the two booster arms, a power analysis was performed. A power of 0.99 was calculated based on the following parameters: two-sided t-test, equal samples of 350 participants, significance level of 0.05, an effect size of 0.49 in antibody response. The effect size was calculated based on trial data (NCT02564523 and NCT02509494) available in the first edition of the combined Investigator’s Brochure of the vaccines with samples from 64 participants vaccinated either 1 year or 2 years after the first dose[25]. To obtain the effect size, the difference in geometric mean concentrations (log scale) of the EBOV GP-specific antibody responses between the two groups was divided by the pooled standard deviations[26]. With a power of 0.99 it will thus be possible to perform a formal comparative analysis of the induced immune memory response of the two booster arms.” (cfr. 144-153)

The effect size for the immunogenicity response was calculated based on available information in the investigator’s brochure of the vaccines (confidential at this stage of the vaccine development and approval). While it is our intention to also compare the safety response via formal statistical analysis, it was not possible to perform a power analysis with the available information in the investigator’s brochure as only pooled safety data of all booster doses (irrespective of the timing) are reported. It is our goal to apprehend the unpooled data through the EBOVAC consortium. If obtainable, the power analysis of the safety data will be explained in the manuscript publishing the results of our trial. If unobtainable, safety data will be published in a descriptive manner. To explain this, the following information has been added to the Methods subtitle Participant population and sample size: “Unfortunately no power analysis could be performed to determine whether the sample size is sufficiently large to perform a formal statistical comparison of safety response (AEs and SAEs) from both arms. In the current combined Investigator’s Brochure of the vaccines[25], safety information is pooled for all booster doses independent of the timing of its administration (1 year or 2 years post-dose 1) and thus no effect size can be calculated until the unpooled data from the different trials is obtained.” (cfr. 154-159)

Under the Methods subtitle Statistical analysis we have also added:
1) the intention to perform comparative analyses for the induced immune memory response: “Finally, a formal comparative analysis of the induced immune memory response between the two booster arms will be performed.” (cfr. Line 329-330)
2) the intention to perform a power analysis if safety data can be obtained from within the EBOVAC consortium for the two clinical trials that have assessed the safety of the booster dose at either 1 or 2 years after the first dose: “If the unpooled safety data from the NCT02564523 and NCT02509494 studies can be obtained, a power analysis will be performed to assess whether the safety data of the two booster arms can be compared through formal statistical analysis.” (cfr. 337-340)

Further details of the analyses will of course be published when publishing the results of the trial.

Finally, in the Discussion we acknowledge that it is a limitation of our trial that no formal sample size calculations were performed prior to the study start and that this followed as a consequence of the initial set-up of the trial: “Finally, at the start of the project the protocol initially only included a vaccination strategy with the 2-dose heterologous vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) and was later adapted to include a booster vaccination at the request of the vaccine producer. The purpose of the initial observational trial was, next to obtaining additional immunogenicity data, a way to see if performing a vaccination trial in a remote area of DRC was feasible and accepted by the population. While writing the protocol however, administering a booster dose in this large cohort was added as a novel aspect and thus this was entered as a secondary objective/endpoint. Currently it is unknown whether this booster dose will be required or not at the moment of an outbreak and what its protective effect would be. However, to explore its safety and immunogenicity, this study protocol was transformed and became a randomized clinical trial. Unfortunately, as the comparison of the two booster arm induced immune responses is not required for approval of the licensure of the 2-dose heterologous vaccine regimen and the booster dose was added as a second stage to the study design, no sample size calculations were initially performed for this trial and sample size was selected based on available information from a previous monkeypox vaccine trial in the same area. While this trial thus mainly has a descriptive set-up, scientifically it is interesting to learn if there is a significant difference in the induced immune memory response of the two booster arms. For this reason, a power analysis was retrospectively performed to determine whether it would be possible to compare the induced immune memory response of the two arms. Fortunately this will be possible as a power of 0.99 was calculated and a formal statistical comparison induced immune memory response of the two booster arms has now been foreseen in the Statistical Analysis Plan. It is however important to take into account that a varying antibody response after booster vaccination is not necessarily directly correlated with protective vaccine efficacy[33] and that a high power (99% for this study) can lead to significant differences, even if the difference between both groups is small. Prudent and careful interpretation of the results will thus be crucial[34].”

As we do intend to perform comparative analysis, we would also like to come back to your first query and believe the title of the project is correct. We have however removed the Controlled-element of the title and throughout the manuscript, as we do not have control group and this can be misleading. However, the randomized trial-element of the study could help us to learn more about a potential role of the timing of a booster vaccination on the immune response. While this is not the primary endpoint for the vaccine manufacturer, scientifically this remains a crucial explorative endpoint.

VERSION 3 – REVIEW

REVIEWER **REVIEW RETURNED**	Morris, Julie South Manchester NHS Trust, Medical Statistics Department 29-Aug-2021

GENERAL COMMENTS	This revised paper has now been amended appropriately, and puts into context the addition of the RCT to the study which compares the two booster vaccines. The power calculation is suitably justified, and reference to a formal comparison of the booster vaccines is now included. I have no remaining statistical concerns.