Warpelin-Decrausaz Loane

Objet: TR: Submission to The Lancet Infectious Diseases

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À : De Santis Olga; olga.desantis80@gmail.com
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Marco

Dr Marco De Ambrogi
Senior Editor TLID
The Lancet Infectious Diseases
Elsevier
Lancet ID
125 London Wall
London EC2Y 5AS
UNITED KINGDOM
Email: m.deambrogi@elsevier.com

Chimpanzee Adenovirus-vectored Ebola Vaccine: Phase IIa randomized, placebo-controlled safety and immunogenicity trial in healthy volunteers

Olga De Santis, M.Res..[1], Régine Audran, Ph.D.[2] , Emilie Pothin, Ph.D.[3], Loane Warpelin-Decrausaz, Ph.D.[4], Laure Vallotton, M.D., Ph.D.[4], Grégoire Wuerzner, M.D.[4], Camille Cochet M.D.[1], Daniel Estoppey, M.D.[1], Viviane Steiner-Monard, M.D.[2], Sophie Lonchampt M.Sc.[1], Anne-Christine Thierry, B. S.[2], Carole Mayor, B.S.[2], Robert T. Bailer, Ph.D.[5], Olivier Tshiani Mbaya, M.D. [5], Yan Zhou, Ph.D.[5], Aurélie Ploquin, Ph.D.[5], Nancy J. Sullivan, Ph.D.[5], Barney S. Graham, M.D.[5], Ph.D.[5], François Roman, M.D., Ph.D.[6], Iris De Ryck, M.D.[6], W. Ripley Ballou, M.D.[6], Marie Paule Kieny, Ph.D.[7], Vasee Moorthy, M.D., Ph.D.[7], François Spertini, M.D.[2], Prof Blaise Genton, M.D., Ph.D.[1,3,8]

1 Policlinique Médicale Universitaire, Lausanne, Switzerland.

2 Division of Immunology and Allergy, Lausanne University Hospital, Switzerland.

3Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland

4 Clinical Trial Unit, Lausanne University Hospital, Switzerland

5 Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA

6 GSK Vaccines, Rixensart, Belgium

7 World Health Organization, Geneva, Switzerland

8 Infectious Disease Service, Department of Medicine, Lausanne University Hospital, Switzerland

Corresponding author:

Olga De Santis
Policlinique Médicale Universitaire (PMU)
Rue du Bugnon 44
1011 Lausanne
Phone : 0041 79 556 29 76
Email: Olga.De-Santis@hospvd.ch

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Abstract

Background

The ongoing Ebola outbreak led to accelerated efforts to test vaccine candidates. Following a request by WHO, a Phase I/IIa clinical trial of the monovalent Ebola (*Zaire*) vaccine ChAd3-EBO-Z was conducted in healthy adults in Lausanne, Switzerland.

Methods

This randomized, double-blind, placebo-controlled, dose-finding trial assessed safety and immunogenicity of ChAd3-EBO-Z vaccine. All volunteers were assigned to three arms, 5 $x10^{10}$ vp dose, $2.5x10^{10}$ vp dose or placebo (ratio 2:2:1). However, 18 volunteers at potential risk of exposure to Ebola virus while deployed in epidemic areas were randomized only into the two vaccine arms ($5x10^{10}$ and $2.5x10^{10}$). The latter, not blinded, were not included in the safety analysis for comparison between the vaccine doses and placebo but were pooled with the non-deployed group to compare immunogenicity between the different arms. Safety and immunogenicity were assessed up to 6 months post vaccination.

Results

120 subjects were recruited. No vaccine-related SAE was observed. Local AEs were observed in 30/40(75%) of $5x10^{10}$, 33/42(78.6%) of $2.5x10^{10}$ and 5/20 (25%) of placebos. Headache was the most frequent systemic AE [26/40 (65%), 29/42 (69%) and 6/20 (30%) respectively] followed by fatigue/malaise [26/40 (65%), 27/42 (64%), 6/20 (30%)]. Fever occurred during the 24h post injection in 30% of vaccinees. Geometric mean concentrations (GMC) of IgG antibodies against Ebola glycoprotein peaked on day 28 (51µg/ml [95% CI 41.1-63.3] in $5x10^{10}$ arm, 44.9µg/ml [25.8-56.3] in $2.5x10^{10}$ arm and 5.2µg/ml [3.5-7.6] in placebos) with respective response rates of 96% [85.7-99.5], 96% [86.5-99.5] and 5% [0.1-24.9]. GMC

decreased to 25.5µg/ml, 22.1µg/ml and 3.2µg/ml on day 180.With regards to cell mediated immunity, 57.1% and 60.8% of vaccinees from the $5x10^{10}$ and the $2.5x10^{10}$ arms developed GP specific CD4+ responses and 67.3% and 68.6% GP specific CD8+ responses respectively.

Conclusion

ChAd3-EBO-Z was safe and well tolerated, although mild to moderate systemic AEs were frequent. A single dose was immunogenic in almost all vaccinees. Antibody responses were still significantly present at 6 months. There was no significant difference between doses for safety and immunogenicity outcomes.

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Introduction

Ebola virus causes a severe, often fatal illness that has occurred in a number of outbreaks since it was first reported in 1976. The largest recorded outbreak of Ebola virus disease (EVD) is ongoing, and there have been more than 28,000 reported cases and more than 11,000 deaths in 3 countries in West Africa by September 2015(1). The World Health Organization (WHO) has declared the current outbreak as an international public health emergency.Thanks to large multilateral public health interventions, the case incidence dropped down to less than 10 cases per week since end of July 2015, but there is as yet no approved treatment or vaccine available against EVD.

Current efforts to develop a vaccine are focused on the viral glycoprotein (GP) encoded by the virus. The most advanced vaccine candidates tested so far are based on the GP from the Zaire ebolavirus species (responsible for the current outbreak of EVD), and/or the Sudan species. Candidates in which viral GP is expressed in either chimpanzee adenovirus (ChAd), Human Adenovirus (Ad5) or vesicular stomatitis (VSV) vector have shown promise in non-human primate models of EVD and in initial clinical trials(2–7). Moreover, preliminary results of a phase III clinical trial using the rVSV-vectored vaccine showed encouraging efficacy results in Guinea(8,9).

The rationale for the development of this vaccine is based on previous human experience with other investigational filovirus vaccines and the development of non-human adenovirus vectors with low seroprevalence in humans(3,10–14).

The present Phase I/IIa study was directed at assessing safety and immunogenicity of the monovalent ChAd3-EBO-Z vaccine construct. It was preceded by Ledgerwood *et al.* and Rampling *et al*. who reported on phase I clinical trials of the bivalent (ChAd3-EBO) and monovalent (ChAd3-EBO-Z) vaccines encoding wild type GP from *Zaire* and *Sudan* species of *Ebolavirus*(4) or *Zaire* only(15). It builds on and extends the clinical development plan for

a ChAd3-vectored vaccine encoding Ebola glycoproteins that has been developed by NIH in collaboration with GSK/Okairos, WHO and University of Oxford. It complements the plan in several key areas: first, the present study, is the only one, among the ChAd3 vectored Ebola vaccine studies, that includes a placebo arm, which allows a precise assessment of the vaccine reactogenicity; second with its large sample size it considerably increases the data already collected in previous studies and allows a better evaluation of safety and two dosage responses, increasing the likelihood of identifying an optimal dose that balances both immunogenicity and reactogenicity; third it is the first report among all Ebola vaccine clinical trials that provides safety and immunogenicity data at 6 months. Altogether, these results have greatly assisted in decision-making for the initiation of further phase IIb and III trials in Africa with a single injection intended for preventing and controlling outbreaks.

Methods

Study design and participants

This is a randomized, double-blind, placebo-controlled, dose finding safety and immunogenicity Phase I/IIa trial conducted at the *Centre Hospitalier Universitaire Vaudois* (CHUV), Lausanne, Switzerland. The study was reviewed and approved by the local ethics review board (CER-VD), by the WHO Research Ethics Review Committee (WHO ERC) and by the Swiss regulatory authorities (Swissmedic). All participants were recruited in the regions of Lausanne using advertisements in the hospital and university halls. Since the study was largely published in the press, many people contacted the team directly to ask for their participation. All subjects provided written informed consent before enrolment.

Inclusion and exclusion criteria summary

Included subjects had to be healthy, aged 18 to 65 years and to practice continuous

contraception during the whole study. The main exclusion criteria were: prior participation to an investigational Ebola or Marburg vaccine or a chimpanzee adenovirus vectored vaccine trial, receipt of any other live or killed vaccine within 28 or 14 days respectively, before the trial, any immunodeficiency state or any acute or chronic disease not well controlled which could increase the risk for the volunteer to have a serious adverse event, or impair interpretation of the data (complete Inclusion and Exclusion criteria are listed in the Supplemental Material).

Vaccine

The recombinant Chimpanzee Adenovirus type-3 vectored Ebola Zaire vaccine (ChAd3-EBO-Z) consists of a recombinant replication-deficient Adenovirus chimpanzee serotype 3 (ChAd3) vector expressing wild-type (WT) Ebola glycoprotein (GP) from the Zaire Mayinga strain. Details about the composition of vaccine and diluent are given in the Supplementary material.

Procedures

For all volunteers, the intra-muscular injection was performed under double-blind conditions. Local and systemic adverse events (AEs) were assessed 1 hour post-injection and at follow-up visits on D1, D7, D14, and D28. In addition, volunteers recorded AEs in a daily notification sheet for the first week. Solicited AEs were adverse events which occurred at any time from injection up until D7, and included both local (pain, erythema and swelling at injection site, plus axillary lymph node enlargement) and systemic AEs (fever, fatigue/malaise, musculo-articular pain, headache, chills, and nausea). Unsolicited AEs were all other AEs not listed above and all AEs which occurred after the 7-day follow-up and up to D28. Grading of AEs for severity and assignment of causal relationship of unsolicited AEs (Supplementary

material) was assessed by clinicians in charge of monitoring the volunteers during the whole study according to pre-defined criteria in the study protocol.

Safety biological monitoring was performed on blood samples taken on D0, D1, D7, D14, and D28 post-injection, and included a full blood count, electrolytes, liver and renal function tests, C-reactive protein (CRP) and activated partial thromboplastin time (aPTT). This assay was performed since an asymptomatic prolongation of aPTT had been observed in the 2 weeks following vaccination in previous adenovirus vaccine trials. This was due to the induction of a non-specific antiphospholipid antibody (APA) and not due to coagulopathy. This effect is actually an artifact of the aPTT test as this test measures the clotting cascade and the assay requires the presence of phospholipid as a reagent(3,15).

At 3 months post injection, a follow-up took place via phone call or email, to record the occurrence of serious adverse events (SAEs) or relevant AEs possibly related to injection. At 6 months post injection, a last follow-up visit was performed to collect SAEs or relevant unsolicited AEs as well as laboratory samples.

Immunogenicity

See methods for antibody measurement and cell mediated immunity evaluation in the Supplemental Material.

Sample size

The sample size of 100 vaccinated was calculated to achieve a total of 250 vaccinated subjects taking into account all three concurrent Phase I trials of the ChAd3-EBO-Z vaccine (Lausanne, Oxford and Mali). This sample size allowed having reliable data on the incidence of frequent adverse events.

Randomisation

Volunteers were randomised in three arms, i) single dose of the Ebola Zaire vaccine ChAd3-EBO-Z $5x10^{10}$ viral particles (vp), ii) single dose of ChAd3-EBO-Z $2.5x10^{10}$ vp or iii) single dose of placebo (diluent only) in a ratio of 2:2:1. The rationale to choose the two doses with only a two-fold difference was based on previous safety experience in clinical trials with ChAd vectors (16).

Since 100% of the non-human primates were protected one month post vaccination, there was a clear signal that this vaccine could be effective in humans. Therefore, deployed volunteers from non-epidemic to epidemic areas could be the first beneficiaries of the vaccine, reason why World Health Organization requested not to include a placebo arm among those volunteers. They were thus randomised in two vaccine arms ($5x10^{10}$ and $2.5x10^{10}$) only, without placebo (Figure 1).

Subjects were randomised following two randomisation runs resulting in two lists, one for the possibly deployed volunteers and one for the non deployed ones. The randomisation lists were computer-generated and kept confidential in the central pharmacy at CHUV.

Data analysis

Only the non-deployed group results were used to compare safety between the $5x10^{10}$, $2.5x10^{10}$ and control arms while all deployed and non-deployed group results were pooled to compare immunogenicity between the different arms as laboratory team performing antibody or cellular responses analyses was blinded to the group assignment. Indeed, blinding is essential for accurate safety and laboratory assessment, in this trial safety evaluation for deployed volunteers was not blinded as mentioned previously, therefore the two groups were not merged for safety analysis. Also, too few of the volunteers had gone to epidemic area

after vaccination to expect potential immunological boost after hypothetical exposure. Anti-Ebola-GP IgG concentrations were described as geometric mean concentration (GMC) with 95% confidence intervals. Allocation arms were compared using the Fisher's exact test for safety and Mann Whitney test for immunogenicity. The lower dose was compared with the higher dose, and the two doses were pooled and named "vaccinated" for comparison with placebo.

For each subject, a positive antibody response was defined as a significant increase in post-vaccination titer from baseline (t-test assuming non-equal variance), using the anti-glycoprotein antibody titers assessed by enzyme-linked immunosorbent assay (ELISA) done in the Vaccine Research Center (VRC) (National Institute of Health, US)(11). Friedman or Kruskal-Wallis with Dunn's post tests were performed for comparison of magnitude of T-cell responses to pre-vaccination or between groups using GraphPrism software v6.07.

Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) was established prior to the trial initiation including two independent clinicians and one epidemiologist. The DSMB reviewed the safety data of days 0 to 7 of the 20 first subjects vaccinated to ensure that holding rules were not met.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and shared the final responsibility with the principal investigator of the trial for the decision to submit for publication.

Results

The WHO request to conduct the trial came on September 1st 2014. Screening of volunteers started on October 24th 2014. Vaccinations were administered from October 31st to December 12th 2014. The 6-month follow-up ended on June 22nd 2015.

Study population

Demographic data of the included participants are detailed in Table 1. All 120 volunteers completed the 6 visits post-injection except two deployed volunteers who missed one visit each (D14 and D28).

Safety

No vaccine-related SAE was observed. Most of the AEs reported were mild and self-limiting, appearing during the first 24h after injection and lasting <48 hours. Seven grade 3 AEs (described below) were observed and all resolved within 3 days with no residual effect. Proportions of volunteers with AEs up to D28 in the vaccine and placebo arms are shown in Figure 2; absolute numbers and differences between arms are detailed in Table 2. Only the placebo-controlled results from the 102 non-deployed volunteers are shown in text below.

The most frequent solicited local AE was pain (91% grade 1) with significant difference between vaccine and placebo arms (77% vs 25% respectively, $p<0.01$), but without difference between vaccine dose arms (75% $5x10^{10}$, 79% $2.5x10^{10}$, $p=0.79$). At least one solicited systemic AE was reported in 87% of subjects in the vaccine arms (93% $5x10^{10}$ and 81% $2.5x10^{10}$) and 50% of placebos ($p<0.01$). The most frequent solicited systemic AEs were headache (65% $5x10^{10}$, 69% $2.5x10^{10}$ and 30% placebo) and fatigue/malaise (65% $5x10^{10}$, 64% $2.5x10^{10}$ and 30% placebo). Musculo-articular pains were also frequently observed (57% $5x10^{10}$, 43% $2.5x10^{10}$ and 25% placebo). Most solicited AEs were mild and resolved within

24 hours after injection. 30% of non-deployed vaccinees developed fever, versus 5% of placebos, with no significant difference between the dose arms (32% $5x10^{10}$ and 29% $2.5x10^{10}$). However, as shown in Figure 1S, the highest vaccine-related temperatures were seen in the $5x10^{10}$ arm.

One relevant unsolicited AE possibly related to the vaccine was an episode of macroscopic haematuria associated with alguria and mild left costovertebral angle tenderness at percussion that occurred within 24 hours after injection ($2.5x10^{10}$). The investigations (urinary sediment and culture, renal US, blood count, coagulation assays) were normal and the episode spontaneously resolved 48 hours after injection. Since no biological cause was found for this episode and since the volunteer never experienced any similar episode before, the AE was considered possibly related to the vaccine. A second relevant unsolicited AE possibly related to the vaccine ($5x10^{10}$) was a herpetiform dermatitis that occurred at day 15 post injection and lasted for 2 weeks. Located in the L2 dermatoma, it was clinically diagnosed as shingles although not confirmed by PCR.

None of the laboratory abnormal values were clinically significant (Tables 1S and 2S and Figure 2S). At D1, 60 grade 1 (<1.5-0.8 G/l) (53% $5x10^{10}$, 55% $2.5x10^{10}$ and 30% placebo) and 4 grade 2 transient lymphopenias (<0.8-0.5 G/l) (2% $5x10^{10}$, 6% $2.5x10^{10}$ and 0% placebo) and 3 transient grade 1 thrombocytopenias (platelets count <150-75G/l) (4% $5x10^{10}$ and 2% $2.5x10^{10}$) were observed. During the one-month follow-up, 8 transient grade 1 anaemias (Hb <117-100g/l) (2% $5x10^{10}$, 14% $2.5x10^{10}$ and 0% placebo) and 14 transient neutropenias were observed (grade 1 (< 1.8-1.5 G/l): 8% $5x10^{10}$, 6% $2.5x10^{10}$ and 5% placebo, grade 2 (<1.5-1 G/l): 2% $5x10^{10}$, 6% $2.5x10^{10}$ and 0% placebo and 2 grade 3 (<1 G/l): 2% $5x10^{10}$, 0% $2.5x10^{10}$ and 5% placebo). Two cases of asymptomatic grade 1 prolonged aPTT were observed at D14 ($5x10^{10}$). One of our two cases of prolonged aPTT had resolved at the following visit (D28) and thus did not go under further investigation.

Investigation of the other one showed no coagulopathy. The antiphospholipid screening was positive for a lupus anticoagulant and doubtful for an anticardiolipin IgM. The aPTT and anticardiolipin had resolved by 3 months. The lupus anticoagulant resolved by 9 months. No associated clinical sign of hypercoagulability was present.

Among the grade 3 AEs, one was an unsolicited local AE, 4 were solicited systemic AEs, and two were laboratory AEs. The one local grade 3 AE was an erythema at injection site of 11 cm of diameter with presence of redness and warmness but no pain, which appeared at day 9 and lasted for less than 24 hours, in the $5x10^{10}$ arm. Among the 4 solicited systemic grade 3 AEs, two were sudden and strong headaches that appeared during the 24 hours following the injection and resolved in less than 2 hours with paracetamol. The other two were fevers with temperatures exceeding 39°C, one during the night post injection ($5x10^{10}$) and lasting less than 24 hours, and the other one appeared at day 4 post injection ($2.5x10^{10}$) but was associated with a streptococcus angina and therefore not related to the vaccine. Two grade 3 neutropenias were observed, the first, at D1 ($5x10^{10}$) and the second, at D14 (placebo). None were associated with symptom or clinical sign and both were resolved at the following visit 3 days later.

At the 3-month follow-up all volunteers except one were reached by phone or email to assess safety. Three mild to moderate AEs were possibly related to the injection. One was a second episode of an axillary lymph node enlargement, at day 63 post injection ($5x10^{10}$), and lasted 2 days (first episode previously described at D1 and lasted 2 days). The two other AEs were a mild fatigue at day 34 and lasted one week ($5x10^{10}$), and a moderate fatigue with several episodes of frontal headache at day 34 and lasted for approximatively 3 weeks ($2.5x10^{10}$).

Upon last visit at 6 month, only one AE was reported as possibly related to the vaccine. The volunteer reported mild arthralgia in the distal interphalangeal joints of the 5th fingers on both hands of one month duration. Neither swelling nor warmth was observed. Mobility was

normal but a light red macula of 2-3 millimetres was observed on the dorsal face of each joint. This volunteer had received the placebo and was sent to a specialized consultation for further investigations.

From D28 to D180, 3 SAE were reported, none related to the injection, all due to trauma**,** namely, an elective hospitalisation for a dislocated shoulder surgery (placebo), an emergency hospitalisation and surgery for a broken radius (placebo), and an elective hospitalisation for a broken anterior cruciate knee ligament surgery (in the $2.5x10^{10}$ arm)

Lastly, at the 3-month follow-up visit, a volunteer ($2.5x10^{10}$) reported the pregnancy of his wife. At this time the pregnancy was in the first trimester. The date of conception was difficult to determine because this was an unexpected pregnancy under oral contraception, but it was estimated at 2 weeks after the vaccination of the volunteer. The pregnancy was terminated 3 weeks later because of a trisomy 21 diagnosed by the gynaecologist. There is no biological plausibility that this diagnosis could be related to the vaccination of the partner.

Immunogenicity

Ebola GP specific antibody response.

Anti-Ebola GP IgG results are summarised in Figure 3, including all data from deployed and non-deployed vaccinees.

Antibody response was detected from D14 onwards and peaked at D28 up to a geometric mean of 51 µg/ml [95%CI: 41.1-63.3] in the $5x10^{10}$ arm and of 44.9 µg/ml [25.8-56.3] in the $2.5x10^{10}$ arm. There was no difference in antibody concentration between the two vaccine dose arms. The percentage of responders was 96% [85.7-99.5] in the $5x10^{10}$, 96% [86.5-99.5] in the $2.5x10^{10}$ and 5% [0.1-24.9] in the placebo arm (table 3S in Supplementary material). Antibody response decreased by approximately half from D28 to D180 with GMC of 25.5

µg/ml [20.6-31.5] in the $5x10^{10}$ arm and of 22.1 µg/ml [19.3-28.6] in the $2.5x10^{10}$ arm 6 months post injection.

At D28, geometric means of the VRC titers were 434.7 [min-max 77.7-5576.3] for the $5x10^{10}$ arm, 467.3 [41.5-4265.3] for the $2.5x10^{10}$, and 33 [6.9-198] for the placebo one (Figure 3B).

Ebola GP specific T cell response.

Mononuclear cell responses to vaccination were evaluated by IFN-γ ELISPOT on D0, D7, D14, D28 and D180. Responses already increased at D7 in arm $5x10^{10}$, to peak similarly at D14 with a significant median response of 177 and 180 SFU / million PBMC in the arms $5x10^{10}$ and $2.5x10^{10}$. Although still significantly higher than at D0 (within group analysis p=0.001, Friedman test), responses at D180 declined in the majority of the subjects and were not significantly different from placebo (Dunn's post tests $p>0.05$) (Figure 3S). Furthermore, T cell specific response was measured by flow cytometry at D0, D14 and D28 and was expressed as frequencies of CD4+ and CD8+ producing IFN-γ, IL-2 or TNF-α after stimulation with GP EBO-Z peptides (Figure 4S). Significant GP specific CD4+ and CD8+ responses were obtained from D14 in vaccinated arms without significant difference between doses. Considering positive responses for at least one of the 3 cytokines, 57.1% and 60.8% of vaccinees from the $5x10^{10}$ and the $2.5x10^{10}$ arms developed GP specific CD4+ responses, and 67.3% and 68.6% GP specific CD8+ responses respectively. The vaccine-specific memory responses showed the same kinetics and were equally distributed between CD4+ and CD8+ T-cells (Figure 4A). Both memory CD4+ and CD8+ T-cells presented poly- and mono-functional phenotypes (Figures 4B and 4C). The CD8+ response consisted mainly of IFN-γ producing cells among which the IFN-γ TNF-α coproducing subsets represented 40% of the response.

ChAd3 neutralizing antibodies

ChAd3 neutralizing antibodies were measured in all volunteers at D0 and D28 (Figure 5S, panel A). Interestingly, the level of neutralizing antibodies at D0 negatively correlated with anti-GP Ab responses as well as with $CD8^+$ IFN-γ responses at D28 (Figure 5S, panels B and C).

Discussion

This is the largest Phase IIa clinical trial reported to date with an experimental Ebola vaccine, and the first to report data with a 6-month follow-up. The placebo-controlled design, the large sample size (120 volunteers) with excellent gender balance, and the extended follow-up provide reliable safety and immunological data, and allow a valid comparison between doses and detection of a possible dose-response effect.

Safety

No vaccine-related SAE was observed during the 6-month follow-up. The ChAd3-EBO-Z vaccine led to more local and systemic AEs than the diluent alone (placebo). The majority of AE were mild and all resolved with no sequelae, for most within the first 24 hours. These results are in line with those observed in other adenovirus-vectored vaccine trials (3,4,15,17,18). More precisely, the reactogenicity was similar to that observed in previous phase I trials using Chimpanzee Adenovirus vector and expression proteins from other pathogens, indicating that adverse events were more likely to be induced by the vector rather than by the Ebola GP(16–18) .

The placebo arm allowed us to demonstrate that local pain and fatigue/malaise, musculo-articular pain, chills, fever and headache, all components of reactogenicity were due to the vaccine. Moreover, no unsolicited AE showed any statistical difference between vaccinated and placebo arms, inferring that larger trials are needed to investigate a potential relationship

with the vaccine. Local reactogenicity was close to that experienced after routine vaccinations (such as influenza, hepatitis B, DTPa or MMR vaccinations(19–23)) with the exception of pain at injection site which was slightly more frequent (77% of recipients) but almost always mild and with little erythema or swelling. On the other hand, the incidence of systemic AEs was markedly higher, especially for headache (65% for $5x10^{10}$ and 69% for $2.5x10^{10}$), musculo-articular pains (57% and 43%) and fever (32% and 29%). Although the safety profile was roughly similar to the data published by Rampling et al. ,with headache, fatigue and malaise being the most frequent AEs (57.5%, 61% and 40% respectively), AEs were more frequent in our study (headache 67%, fatigue/malaise 65%). They reported only 5% (2 cases) of 'objective' fever whereas we did so for 29%. This difference might be explained by measurement technique as feverishness was present in 30% of their subjects. Even if more frequent, AEs were of mild intensity, short-lived and self-limited, which makes them acceptable in a risk-benefit balance in relation to such a severe disease as Ebola. Moreover, 81% of the fevers induced by the vaccine resolved within 24 hours after injection. This rapid resolution makes them manageable, even during an outbreak, by preventing confusion with early onset of a new Ebola case.

Frequencies and intensities of AEs were similar between the two doses, although fevers of higher temperatures and 4 of 7 grade 3 AEs were observed in the $5x10^{10}$ arm. The lack of a significant dose effect observed may be explained by the fact that the two doses differed only by a factor of two. The slight increase of fever in the $5x10^{10}$ arm may become clinically relevant when using the $1x10^{11}$ dose, the one that is currently deployed in Africa. Indeed, in the clinical trial of the bivalent ChAd3-EBO (*Zaire* + *Sudan*) vaccine, the $2x10^{11}$ dose was more reactogenic than the $2x10^{10}$, with 2/10 vaccinees having fever compared with none with the lower dose(4). These data may suggest that the 10^{11} dose will be more reactogenic. The published short-term safety results of the rVSV vaccine trial, the other major promising Ebola

vaccine, showed a similar early reactogenicity profile. Although no vaccine-related SAEs have been reported with either vaccine, it is of note that there were cases of arthritis/arthralgia with maculopapular rash or vesicular dermatitis in some subjects, 2 week post-vaccination after rVSV. These findings were observed at differing rates in different trials with the highest reported rate being 22% (11/52) of recipients in Geneva(5) . While ChAd3 vaccine recipients only complained of transient musculo-articular pain within 3 days post vaccination as part of general 'flu-like symptoms', but without any clinical evidence of arthritis.

Interestingly, in both phase I trials of Ebola vaccine (the rVSV Ebola vaccine (24) and ours), conducted in Switzerland, a higher frequency of AEs was reported than in other trials with the same vaccine. This difference is unlikely to be due to specific genetic traits since our volunteers were of many different origins. This higher frequency of AEs is probably related to the reporting mode.

Immunogenicity

A single vaccination with ChAd3-EBO-Z induced antibody responses in 96% of participants, independently of the dose. The anti-EBO-Z GP titers obtained at D28 (GMT of 434.7 in $5x10^{10}$ and 467 in $2.5x10^{10}$) confirmed the responses obtained with 5 and $2.5x10^{10}$ ChAd3-EBO-Z (GMT of 469 and 402 respectively) in a previous study (15). There was no dose-effect in our trial, probably due to the fact that the two doses were quite close. The 6-month follow-up showed for the first time that antibody titers were maintained at a level significantly different when compared to placebo. Interestingly, the presence of ChAd3 neutralizing antibodies at D0 correlated negatively with the level of anti-GP antibodies at D28 as well as with the $CD8^+$ IFN-γ T cell responses at D28. This was in line with similar observations in a previous preliminary report, although here reaching significance in this much larger study .(4). As far as durability of the T cell response is concerned, the IFN-γ mononuclear cell

responses by ELISPOT decreased but was still present at month 6 despite lack of significant difference with placebo at this later time point. Remarkably, the presence of an Ebola specific CD8+ T-cell response with an IFN-γ TNF-α coproducers component reinforces the potential for protection of the current vaccine formulation, since these markers are associated with vaccine-mediated protection in non-human primates(2) . Proportion of IFN-γ TNF-α coproducers was comparable in this study to a previous study (15) . With comparable dose of 5 and $2.5x10^{10}$, IFN-γ polyfunctional CD8 T cells were also found in proportion similar to our study but appeared to further expend with the highest dose of $2x10^{11}$(4). The promising efficacy provided by the VSV-vectored vaccine in Guinea(8) gives hope that other vaccines based on the Ebola virus GP may be protective. Although correlates of immunity in human vaccination against EBOV is unknown, it is interesting to see that anti-GP titers observed in ChAd3-EBO (bivalent) at a dose of $2x10^{11}$ (4) were equivalent to that obtained with the VSV-vectored vaccine evaluated in the Guinea phase III trial. Available anti-EBO-Z GP ELISA data indicate that the humoral immune responses induced by the $1x10^{11}$ vp dose (for the monovalent form) are higher than those induced by the lower doses, reason why the $1x10^{11}$ vp dose was selected for Phase II and Phase III studies (NCT02485301 on ClinicalTrials.gov). In conclusion, ChAd3-EBO-Z was safe, more reactogenic than routine vaccinations but with only self-limited, usually mild, AEs considering the severity of the disease. This acceptable safety profile linked to Ebola specific antibody response and polyfunctional CD8+ specific T cell response provides a reliable basis for proceeding with efficacy trials in Africa.

Research in context

Evidence before this study

Clinical trial reports were searched for in PubMed up to Aug 17, 2015 using the terms "Ebola" AND "vaccine" with no language or date restriction. Two DNA vaccines and one

recombinant adenovirus serotype 5 (rAd5) using different versions of the Ebola or Marburg GP protein had been tested in the last ten years. Chimpanzee Adenovirus 3 (ChAd3) vectored vaccines using monovalent and bivalent formulations of the Ebola virus glycoprotein (GP) were tested in late 2014 in Phase I clinical trials in the US and UK with limited sample size. A recombinant Vesicular Stomatitis Virus (rVSV) vectored Ebola vaccine was simultaneously tested in a multisite phase I trial. More recently, a report of a Phase I conducted in China using an rAd5 vector-based Ebola vaccine expressing the glycoprotein of the 2014 epidemic strain was published. No safety issues arose from all these trials besides cases of arthritis and rash with rVSV predominantly seen at one site. All these trials conducted simultaneously to ours were published as preliminary reports including safety and immunogenicity data up to day 28 post-injection.

Added value of this study

The present paper provides the most comprehensive results of a phase I/II trial with ChAd3 vector-based vaccine expressing the Ebola GP. This trial was the only one that was placebo-controlled, which allows the most accurate assessment of safety and reactogenicity. Among all Ebola vaccine trials, this is the only one that provides safety and immunogenicity results up to 6 months post-injection, the latter providing some insight on the value of the vaccine over the course of an epidemic. In our trial, no safety signal was observed. All vaccinees showed humoral responses that peaked at day 28, and then decreased by about half at month 6 post-injection. IFN-γ mononuclear cell responses were still present at that time too.

Implications of all the available evidence

Comparing results of the present report with those of the rVSV vectored Ebola vaccine at $2x10^7$ or $5x10^7$ pfu, we can conclude that the safety profile of the ChAd3-EBO-Z at 10^{10} doses

is slightly better, but the humoral responses slightly lower at 1 month post-injection. Considering the good safety profile of ChAd3-EBO-Z at 10^{10} doses in the present trial, it seems appropriate to use the $1x10^{11}$ dose to proceed to Phase II and III trial in Africa as planned, especially so because the few available safety data with ChAd3-EBO-Z at $1x10^{11}$ show acceptable adverse events (AEs) profile and, more importantly, similar antibody responses as those obtained with the $2x10^{7}$ pfu dose of the rVSV vectored vaccine. Assuming that the anti-GP antibody concentration is correlated with protection (even if not protective themselves), we can thus hope that the promising efficacy results observed in the preliminary report of the rVSV vectored vaccine in the Phase III in Guinea could also be obtained using the ChAd3-EBO-Z vaccine at a $1x10^{11}$ dose. The persistence of antibodies at month 6, although at lower concentration, may indicate that some protection remains. This needs to be confirmed though in a thorough Phase III trial. Detailed correlation of immunological data and protection in non-human primates studies may also give some insight on efficacy, if a Phase III trial becomes impossible to conduct because of insufficient number of new Ebola virus disease cases.

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Author contributions

ODS, LWD, LV, GW, FS, MPK, VM, IDR, FR and BG made substantial contributions to the conception and design of the study; ODS, CC, DE, VSM, SL, RA, ACT, CM and BG performed data collection; ODS, EP, LWD, LV, GW, SL, IDR, WRB, FS and BG performed safety data analysis and interpretation; RA, ACT, CM, EP, ODS, BSG, NJS, OTM, YZ, AP, RTB, BG and FS performed immunogenicity data analysis and interpretation; ODS, RA, EP, FS and BG wrote the manuscript; all authors contributed to the revision of the manuscript.

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Conflict of interest.

The authors ODS, RA, BG, FS, LWD, LV, GW, MPK, VM, CC, DE, VSM, SL, ACT, CM, EP, RTB, OTM, YZ, AP, NJS, BSG have reported no conflict of interest. FR, IDR, WRB are employees of GSK.

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Table 1: Characteristics of subjects at baseline

		Non-deployed			Potentially deployed	
		Placebo	**2.5 x 10^{10} vp**	**5 x 10^{10} vp**	**2.5 x 10^{10} vp**	**5 x 10^{10} vp**
	N	20	42	40	9	9
Gender	Male	11 (55%)	22 (52%)	19 (48%)	4 (44%)	3 (33%)
	Female	9 (45%)	20 (48%)	21 (52%)	5 (56%)	6 (67%)
Ethnicity	White	16 (80%)	40 (95%)	35 (88%)	9 (100%)	6 (67%)
	Black	1 (5%)	1 (2%)	1 (2%)	0	2 (22%)
	Hispanic	0	0	1 (2%)	0	0
	Other	3 (15%)	1 (2%)	3 (8%)	0	1 (11%)
Age (years)	Mean(SD)	37.2 (13.4)	30.7 (11.1)	33.2 (13.1)	42 (12.4)	46 (10.8)
	Median[min,max]	37 [19-61]	27.5 [19-63]	27 [19-63]	39 [28-62]	43 [32-64]
BMI (kg/m2)	Mean(SD)	23.5 (3.6)	23.7 (3.3)	24.2 (2.9)	23.4 (4.1)	26.6 (3.9)
	Median[min,max]	22.3 [18.9-33.9]	23.3 [17.6-32]	23.8 [19.4-31.2]	23.4 [18.3-30.3]	27.4 [20.3-33.2]

Table 2: Frequency and maximum intensity of solicited local and systemic AEs (occurring up to D7 +/- 1) and of unsolicited related AEs (up to D28) per arm.

			Non-deployed					Potentially deployed	
						P-value			
			Placebo (N=20)	2.5 x 10^{10} vp (N=42)	5 x 10^{10} vp (N=40)	Placebo vs Vaccinated (N=20 vs N=82)	2.5 vs 5 x 10^{10} vp (N=42 vs N=40)	2.5 x 10^{10} vp (N=9)	5 x 10^{10} vp (N=9)
LOCAL AE	**Pain**	Grade 1	5 (25%)	32 (76%)	25 (62%)			6 (67%)	7 (78%)
		Grade 2	0 (0%)	1 (2%)	5 (12%)			1 (11%)	0 (0%)
		TOTAL	5 (25%)	33 (79%)	30 (75%)	<0.01	0.8	7 (78%)	7 (78%)
	Swelling	Grade 1	0 (0%)	0 (0%)	2 (5%)			0 (0%)	0 (0%)
		Grade 2	0 (0%)	0 (0%)	1 (2%)			0 (0%)	0 (0%)
		TOTAL	0 (0%)	0 (0%)	3 (8%)	1	0.11	0 (0%)	0 (0%)
	Erythema	Grade 1	0 (0%)	2 (5%)	2 (5%)			1 (11%)	0 (0%)
		TOTAL	0 (0%)	2 (5%)	2 (5%)	0.58	1	1 (11%)	0 (0%)
	Axillary lymphatic node enlargement	Grade 1	0 (0%)	0 (0%)	4 (10%)			0 (0%)	0 (0%)
		TOTAL	0 (0%)	0 (0%)	4 (10%)	0.58	0.05	0 (0%)	0 (0%)
SYSTEMIC AE	**Fatigue/Malaise**	Grade 1	6 (30%)	24 (57%)	22 (55%)			4 (44%)	4 (44%)
		Grade 2	0 (0%)	3 (7%)	4 (10%)			1 (11%)	1 (11%)
		TOTAL	6 (30%)	27 (64%)	26 (65%)	0.01	1	5 (56%)	5 (56%)
	Musculo-articula	Grade 1	5 (25%)	17 (40%)	17 (42%)			3 (33%)	2 (22%)
		Grade 2	0 (0%)	1 (2%)	6 (15%)			0 (0%)	1 (11%)
		TOTAL	5 (25%)	18 (43%)	23 (57%)	0.05	0.27	3 (33%)	3 (33%)
	Chills	Grade 1	0 (0%)	6 (14%)	9 (22%)			3 (33%)	3 (33%)
		Grade 2	0 (0%)	3 (7%)	2 (5%)			0 (0%)	0 (0%)
		TOTAL	0 (0%)	9 (21%)	11 (28%)	0.01	0.61	3 (33%)	3 (33%)
	Nausea	Grade 1	4 (20%)	5 (12%)	3 (8%)			2 (22%)	1 (11%)
		Grade 2	0 (0%)	0 (0%)	1 (2%)			0 (0%)	0 (0%)
		TOTAL	4 (20%)	5 (12%)	4 (10%)	0.28	1	2 (22%)	1 (11%)
	Fever	Grade 1	0 (0%)	6 (14%)	7 (18%)			2 (22%)	1 (11%)
		Grade 2	1 (5%)	5 (12%)	5 (12%)			0 (0%)	1 (11%)
		Grade 3	0 (0%)	1 (2%)	1 (2%)			0 (0%)	0 (0%)
		TOTAL	1 (5%)	12 (29%)	13 (32%)	0.02	0.81	2 (22%)	2 (22%)
	Headache	Grade 1	4 (20%)	18 (43%)	15 (38%)			1 (11%)	4 (44%)
		Grade 2	2 (10%)	10 (24%)	10 (25%)			2 (22%)	2 (22%)
		Grade 3	0 (0%)	1 (2%)	1 (2%)			0 (0%)	0 (0%)
		TOTAL	6 (30%)	29 (69%)	26 (65%)	<0.01	0.82	3 (33%)	6 (67%)
UNSOLICITED RELATED AE	**Abdominal pain**	Grade 1	0 (0%)	1 (2%)	1 (2%)			0 (0%)	1 (11%)
		TOTAL	0 (0%)	1 (2%)	1 (2%)	1	1	0 (0%)	1 (11%)
	Conjunctivitis	Grade 1	0 (0%)	0 (0%)	1 (2%)			1 (11%)	0 (0%)
		TOTAL	0 (0%)	0 (0%)	1 (2%)	1	0.49	1 (11%)	0 (0%)
	Rhinitis	Grade 1	0 (0%)	1 (2%)	0 (0%)			0 (0%)	1 (11%)
		TOTAL	0 (0%)	1 (2%)	0 (0%)	1	1	0 (0%)	1 (11%)
	Sweating	Grade 1	0 (0%)	0 (0%)	0 (0%)			0 (0%)	0 (0%)
		Grade 2	0 (0%)	1 (2%)	0 (0%)			0 (0%)	0 (0%)
		TOTAL	0 (0%)	1 (2%)	0 (0%)	1	1	0 (0%)	0 (0%)
	Others	Grade 1	0 (0%)	1 (2%)	1 (2%)			0 (0%)	0 (0%)
		Grade 2	0 (0%)	0 (0%)	0 (0%)			0 (0%)	0 (0%)
		Grade 3	0 (0%)	0 (0%)	1 (2%)*			0 (0%)	0 (0%)
		TOTAL	0 (0%)	1 (2%)	2 (5%)	1	0.61	0 (0%)	0 (0%)

* erythema at injection site of 11 cm of diameter, at D9 post injection. (P-value obtained using Fisher's exact test).

Figure 1

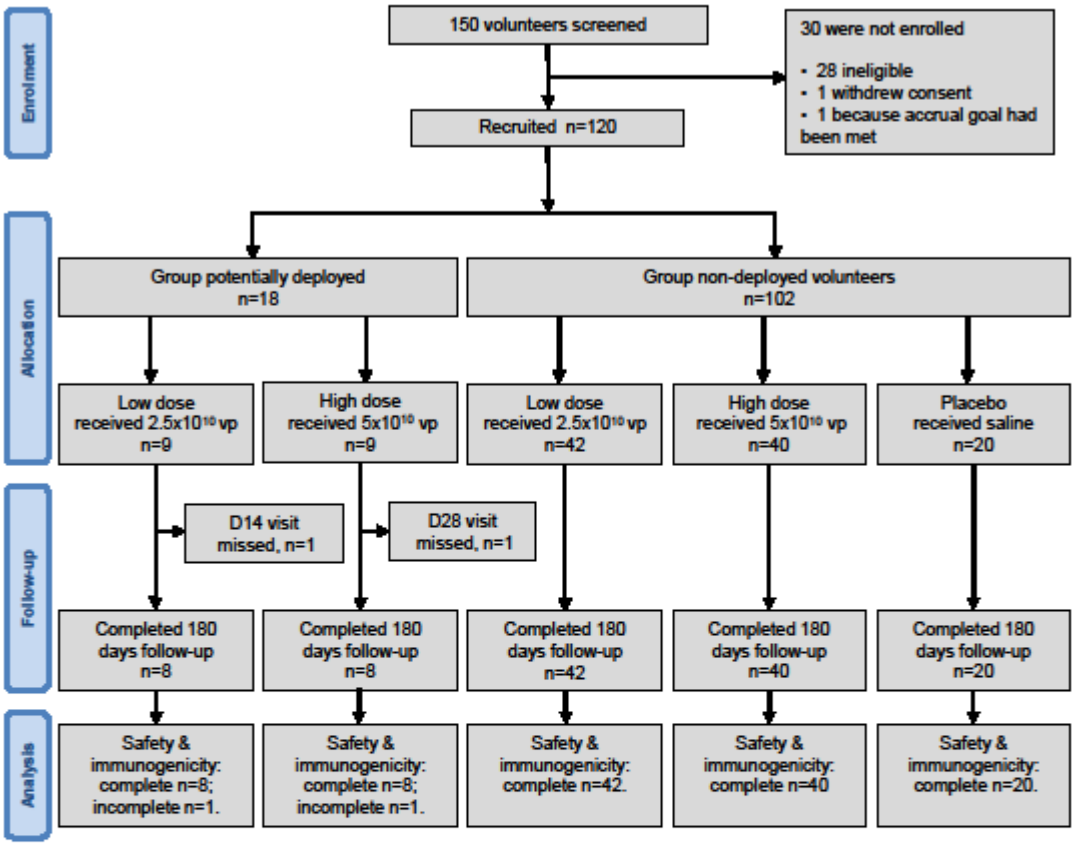

Enrolment
Allocation
Follow-up
Analysis
150 volunteers screened
30 were not enrolled
• 28 ineligible
• 1 withdrew consent
• 1 because accrual goal had been met
Recruited n=120
Group potentially deployed n=18
Group non-deployed volunteers n=102
Low dose received 2.5x10¹⁰ vp n=9
High dose received 5x10¹⁰ vp n=9
Low dose received 2.5x10¹⁰ vp n=42
High dose received 5x10¹⁰ vp n=40
Placebo received saline n=20
D14 visit missed, n=1
D28 visit missed, n=1
Completed 180 days follow-up n=8
Completed 180 days follow-up n=8
Completed 180 days follow-up n=42
Completed 180 days follow-up n=40
Completed 180 days follow-up n=20
Safety & immunogenicity: complete n=8; incomplete n=1.
Safety & immunogenicity: complete n=8; incomplete n=1.
Safety & immunogenicity: complete n=42.
Safety & immunogenicity: complete n=40
Safety & immunogenicity: complete n=20.

Figure 2

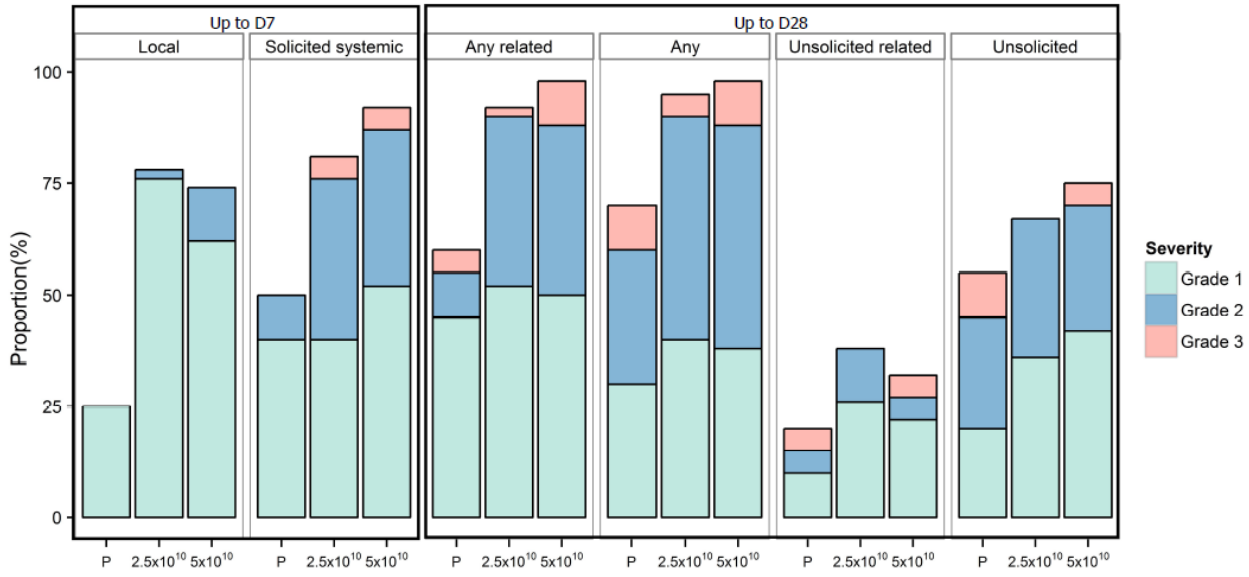

Up to D7
Up to D28
Local
Solicited systemic
Any related
Any
Unsolicited related
Unsolicited
Proportion(%)
100
75
50
25
0
P
2.5x10^10
5x10^10
Severity
Grade 1
Grade 2
Grade 3

Figure 3

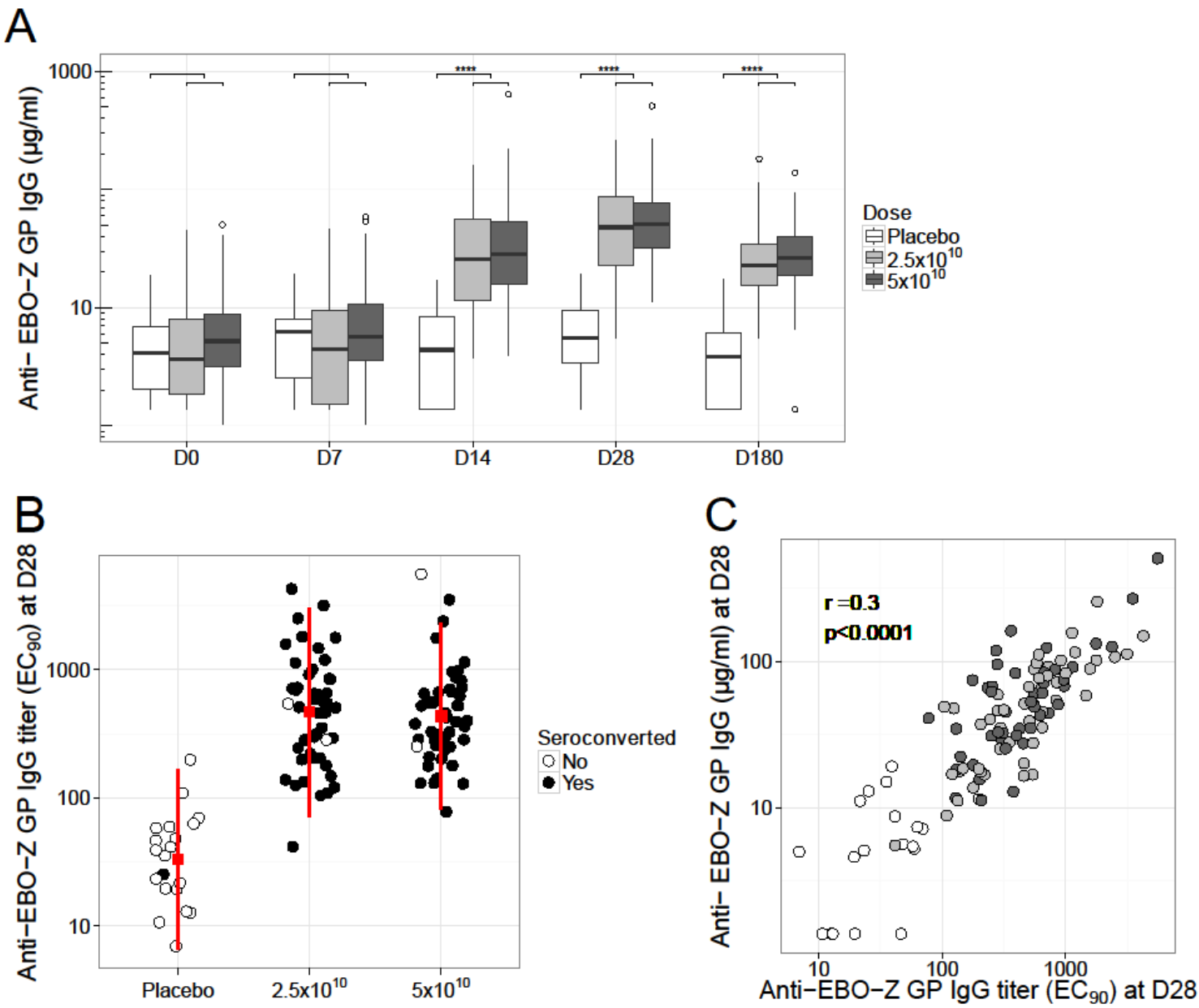
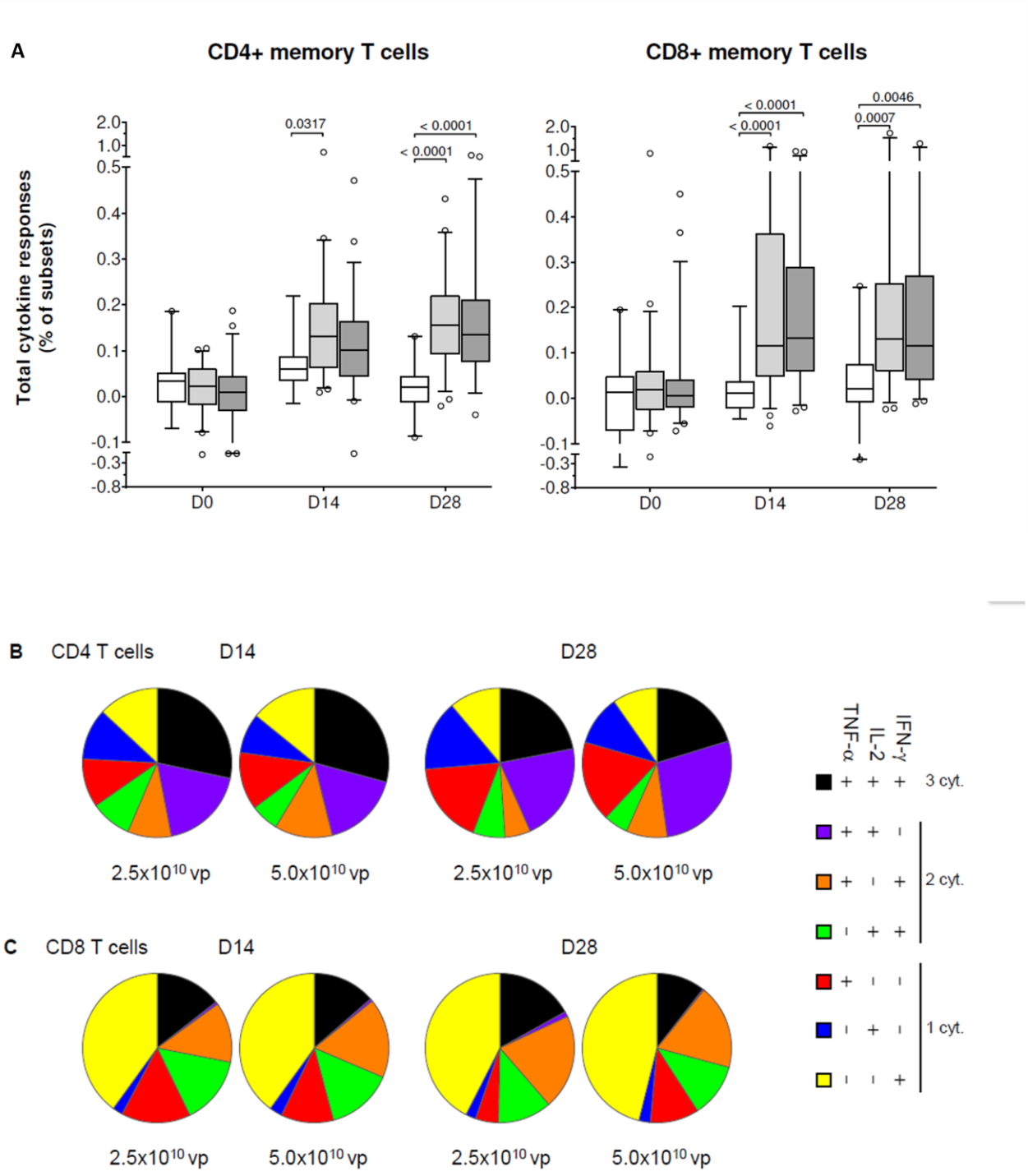

A
Anti− EBO−Z GP IgG (µg/ml)
1000
10
****
****
****
D0
D7
D14
D28
D180
Dose
Placebo
2.5x10^10
5x10^10
B
Anti−EBO−Z GP IgG titer (EC90) at D28
1000
100
10
Placebo
2.5x10^10
5x10^10
Seroconverted
No
Yes
C
Anti− EBO−Z GP IgG (µg/ml) at D28
r =0.3
p<0.0001
100
10
10
100
1000
Anti−EBO−Z GP IgG titer (EC90) at D28

Figure 4


A
CD4+ memory T cells
CD8+ memory T cells
Total cytokine responses (% of subsets)
0.0317
< 0.0001
< 0.0001
< 0.0001
< 0.0001
0.0046
0.0007
D0
D14
D28
B CD4 T cells
D14
D28
2.5x10¹⁰ vp
5.0x10¹⁰ vp
2.5x10¹⁰ vp
5.0x10¹⁰ vp
C CD8 T cells
D14
D28
2.5x10¹⁰ vp
5.0x10¹⁰ vp
2.5x10¹⁰ vp
5.0x10¹⁰ vp
TNF-α
IL-2
IFN-γ
3 cyt.
2 cyt.
1 cyt.

Figures legends

Figure 1: Study flow diagram.

Figure 2: Proportion of volunteers affected and severity of AEs, up to D28, per arm (placebo, dose $2.5x10^{10}$ vp, dose $5.0x10^{10}$ vp) among non-deployed volunteers

Figure 3: Anti-EBOZ-Glycoprotein IgG responses in the different arms. The kinetics of responses as assessed by a commercial ELISA (ADI) are shown in Panel A, where results in boxplots indicate median and quartiles with the 95% Confidence Interval of IgG concentrations (µg/ml) per arm and.where geometric mean concentrations (GMC) (µg/ml) are compared between arms (Mann Whitney; **** $p<0.0001$.). Panel B shows individual VRC endpoint EC90 titers at D28. In red the GMC and the 95%CI. Black dots show volunteers who seroconverted. Panel C shows Spearman's correlation between the two ELISA assays (Lausanne and VRC), placebo arm are in white, $2.5x10^{10}$ vp arm in light grey and $5x10^{10}$ vp arm in dark grey.

Figure 4: EBOZ GP-specific memory T cells responses. Panel A shows the kinetics of individual CD4+ and CD8+ responses expressed as frequencies of subsets expressing at least one cytokine, IFN-γ, IL-2 or TNF-α. Results are shown as boxplots with median, quartiles and 5% centiles, for each arm, placebo in white (n=20), dose $2.5x10^{10}$ vp in light grey (n=51) and dose $5x10^{10}$ in dark grey (n=49). Kruskal-Wallis test was used to assess statistical significance with placebo arm. Panels B and C show the proportions of GP-specific memory CD4 and CD8 T cells that produce any combination of the 3 cytokines, at D14 and D28, in the arms of vaccinees.

Supplemental Materials for

Chimpanzee Adenovirus-vectored Ebola Vaccine: Phase IIa randomized placebo-controlled safety and immunogenicity trial in healthy volunteers

Olga De Santis, M.Res.[1], Régine Audran, Ph.D.[2] , Emilie Pothin, Ph.D.[3], Loane Warpelin-Decrausaz, Ph.D.[4], Laure Vallotton, M.D., PhD[4], Grégoire Wuerzner, M.D.[4], Camille Cochet M.D.[1], Daniel Estoppey, M.D.[1], Viviane Steiner-Monard, M.D.[2], Sophie Lonchampt M.Sc.[1], Anne-Christine Thierry, B.S.[2], Carole Mayor, B.S.[2], Robert T. Bailer, Ph.D.[5], Olivier Tshiani Mbaya, M.D. [5], Yan Zhou, Ph.D.[5], Aurélie Ploquin, Ph.D[5], Nancy J. Sullivan, Ph.D.[5], Barney S. Graham, M.D., Ph.D.[5], François Roman, M.D., Ph.D.[6], Iris De Ryck, M.D.[6], W. Ripley Ballou, M.D.[6], Marie Paule Kieny, Ph.D.[7], Vasee Moorthy, M.D., Ph.D.[7], François Spertini, M.D. [2], Prof. Blaise Genton, M.D., Ph.D.[1,3,8]

1 Policlinique Médicale Universitaire, Lausanne, Switzerland.

2 Division of Immunology and Allergy, Lausanne University Hospital,, Switzerland.

3 Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland

4 Clinical Trial Unit, Lausanne University Hospital, Switzerland

5 Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA

6 GSK Vaccines, Rixensart, Belgium

7 World Health Organization, Geneva, Switzerland

8 Infectious Disease Service, Department of Medicine, Lausanne University Hospital, Switzerland

Corresponding author:

Olga De Santis
Policlinique Médicale Universitaire (PMU)
Rue du Bugnon 44
1011 Lausanne
Phone : 0041 79 556 29 76
Email: Olga.De-Santis@hospvd.ch

Registration trial: ClinicalTrials.gov number NCT02289027

Content

Methods supplemental text

Inclusion and exclusion criteria

The volunteers had to meet all following inclusion and exclusion criteria to be eligible for the study.

Inclusion criteria

1. Healthy adults aged 18 to 65 years
2. Able and willing (in the investigator's opinion) to comply with all study requirements
3. Willing to allow the investigators to discuss the volunteer's medical history with their general practitioner
4. For females of reproductive capacity and male, having practiced continuous effective contraception for 21 days prior to enrolment (see section 6.3.3), and willing to practice continuous effective contraception for 6 months post vaccination
5. For females of reproductive capacity, having a negative pregnancy test on the day(s) of screening and vaccination if >7 days interval
6. Agreement to refrain from blood donation during the course of the study
7. Provide written informed consent

Exclusion criteria

1. Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned use during the study period
2. Prior receipt of an investigational Ebola or Marburg vaccine or a chimpanzee adenovirus vectored vaccine
3. Receipt of any live, attenuated vaccine within 28 days prior to enrolment
4. Receipt of any subunit or killed vaccine within 14 days prior to enrolment (influenza vaccination was encouraged prior to participation)
5. Receipt of any investigational vaccine within 3 months prior to enrollment
6. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate
7. Any confirmed or suspected immunosuppressed or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days)

immunosuppressive medication within the past 6 months (inhaled and topical steroids were allowed)

8. History of allergic reactions likely to be exacerbated by any component of the vaccine,
9. Any history of hereditary angioedema, acquired angioedema, or idiopathic angioedema.
10. Any history of anaphylaxis in reaction to vaccination
11. Pregnancy, lactation or willingness/intention to become pregnant during the study
12. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)
13. History of serious psychiatric condition
14. Poorly controlled asthma or thyroid disease
15. Seizure in the past 3 years or treatment for seizure disorder in the past 3 years
16. Bleeding disorder (eg. Factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture
17. Any other serious chronic illness requiring hospital specialist supervision
18. Current anti-tuberculosis prophylaxis or therapy
19. Suspected or known current alcohol abuse (> 14 units/week for women and >21 units/week for men)
20. Suspected or known injecting drug abuse in the 5 years preceding enrolment
21. Seropositive for hepatitis B surface antigen (HBsAg)
22. Seropositive for hepatitis C virus (antibodies to HCV)
23. Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis
24. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data

Unblinding

Due to the urgency of having results to select the optimal dose for Phase IIb and III to be conducted in Africa from January 2015, the study was unblinded 14 days after the vaccination of the last subject only for the study statistician (investigators and volunteers remained blinded until the end of the study). Tables of results provided by the statistician with no identity or study code (to keep the blinding) allowed investigators and sponsor to best assess safety and immunogenicity results of all ongoing and completed Phase I studies in order to select the most appropriate vaccine dose for further trials in Africa.

Vaccine

The pre-ChAd3 vector is derived from the WT ChAd3 genome isolated from a healthy young chimpanzee housed at New Iberia Research Center facility (New Iberia Research Center; The University of Louisiana at Lafayette). The viral genome was cloned into a plasmid DNA vector and subsequently modified to delete the E1 and E4 region of the viral genome.

The drug substance was manufactured under Good Manufacturing Practice (GMP) conditions by ADVENT S.r.l. (Rome, Italy, under contract to GlaxoSmithKline (GSK) and the NIH) and the vaccine and diluent were manufactured by the VRC Vaccine Pilot Plant (VPP), operated by the Vaccine Clinical Materials Program, Leidos Biomedical Research, Inc., Frederick, MD. ChAd3-EBO-Z was supplied as a sterile, aqueous, buffered solution filled into single dose vials at a final concentration of 9.1×10^{10} vp per ml (after final release). Fill volume was 0.7 ml per vial. The diluent was comprised of formulation buffer and was used to dilute ChAd3-EBOZ to the correct dosage for IM administration. The formulation buffer, pH 7.4, was composed of 10 mM Tris, 10

mM Histidine, 5% Sucrose (w/v), 75 mM Sodium Chloride, 1 mM Magnesium Chloride, 0.02% Polysorbate 80 (PS-80) (w/v), 0.1 mM EDTA, and 0.5% Ethanol (v/v).

Procedures

All AEs, either solicited or unsolicited, were transferred in the source documents and entered in an electronic CRF by the investigator.

Grading

Severity grading criteria for local and systemic AEs :

Grade 0 None

Grade 1 Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required

Grade 2 Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required

Grade 3 Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible

Severity grading for fever :

Grade 1 37.6°C-38.0°C

Grade 2 38.1°C-39.0°C

Grade 3 >39.0°C

Severity grading criteria for local adverse events:

Pain at injection site

Grade 1	Pain that is easily tolerated
Grade 2	Pain that interferes with daily activity
Grade 3	Pain that prevents daily activity

Erythema at injection site diameter

Grade 1	>3 - ≤50 mm
Grade 2	>50 - ≤100 mm
Grade 3	>100 mm

Swelling at injection site diameter

Grade 1	>1 - ≤20 mm
Grade 2	>20 - ≤50 mm
Grade 3	>50 mm

Causality assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine was undertaken. An intervention-related AE referred to an AE for which there was a possible, probable or definite relationship to administration of the vaccine. An interpretation of the causal relationship of the intervention to the AE in question was made, based on the type of event, the relationship of the event to the time of vaccine administration, and the known biology of the vaccine therapy.

No Relationship
No temporal relationship to study product **and**
Alternate aetiology (clinical state, environmental or other interventions); **and**
Does not follow known pattern of response to study product
Unlikely
Unlikely temporal relationship to study product **and**

Alternate aetiology likely (clinical state, environmental or other interventions) **and**
Does not follow known typical or plausible pattern of response to study product.

Possible

Reasonable temporal relationship to study product; **or**
Event not readily produced by clinical state, environmental or other interventions; **or**
Similar pattern of response to that seen with other vaccines

Probable

Reasonable temporal relationship to study product; **and**
Event not readily produced by clinical state, environment, or other interventions **or**
Known pattern of response seen with other vaccines

Definite

Reasonable temporal relationship to study product; **and**
Event not readily produced by clinical state, environment, or other interventions; **and**
Known pattern of response seen with other vaccines

Antibody response

Anti-EBOZ GP IgG responses were assessed by ELISA using a commercial kit (AE 320620-1, Alpha Diagnostics International, Texas, USA) according to the manufacturer's instructions with sera diluted at 1:200 in duplicates. For each volunteer, sera taken at various time-points were evaluated on the same plate. Optical density (OD) was read at 450nm with 630nm substraction on a microplate reader (Opsys MR, Dynex Technologies) and mean OD converted to µg/ml using the standard curve of the kit calibrator. Samples giving a signal above the upper limit of the curve were evaluated at a higher dilution. In parallel, the sera at D0 and D28 were tested for humoral responses by the Vaccine Research Center (VRC) using the methodology previously described[4] for comparison with all phase I trial results.

Cell mediated immunity

Enzyme–linked immunospot (ELISPOT) were performed at all time-points with the use of overlapping peptide pools. Peripheral blood mononuclear cells (PBMC) from blood taken at D0 (pre vaccination), and

D7, D14, D28 and D180 post-injection were separated on a density gradient using Vacutainer CPT (Becton, Dickinson and Company), washed and stored in liquid nitrogen until analysis.

Vaccine-induced T-cell responses were evaluated by means of a qualified intracellular cytokine staining assay performed by the VRC and described elsewhere(1,2). Cryopreserved PBMC obtained at D0, D14, and D28 were stimulated with overlapping peptide pools matching the vaccine insert for glycoprotein Zaire and were quantified to determine the proportion of CD4 and CD8 T cells producing interleukin-2 (IL-2), interferon-γ (IFN-γ), or tumor necrosis factor alpha (TNF-α). Antibodies are from BD Biosciences unless otherwise stated: Anti-CD28-Cy5PE, Anti-CD45RA-Cy7PE, Anti-CCR7-Ax680 (ReaMetrix), Anti-IFN-γ-APC, Anti-IL-2-PE, Anti-TNF-α-FITC, Anti-CD4-ECD (Beckman Coulter), Anti-CD3-Cy7APC, Anti-CD8-Pacific Blue, and Aqua-Blue. Cells are stained with Aqua Blue at room temperature for 20 minutes, followed immediately by staining with the surface markers (CD3, CD28, CD45RA, CCR7) for an additional 20 minutes. Cells are washed twice, permeabilized with 100 µL/well CytoFix-CytoPerm reagent (BD) with twenty minute incubation at 2-8^{o}C minutes, then washed twice with PermWash (BD). Intracellular staining (CD4, CD8, IFN- γ, IL-2, TNF-α) is in a total of 100 µL/well at room temperature for 20 minutes, followed by 3 washes with PermWash. The cells are resuspended in 1% paraformaldehyde and stored at 4°C for no longer than 36 hours prior to flow cytometry analysis. Multi-parameter flow cytometric analysis is performed on a LSR-II flow cytometer (BDIS). Between 50,000 and 250,000 events are acquired. Results are analyzed using FlowJo software (Tree Star Software; Ashland, OR). The same gating strategy is used for all clinical testing (Figure 6S) .A response with a percentage of positive cells stimulated minus unstimulated above 0.05% or 0.08% for CD8 IFN-γ and CD8 TNF-α, was considered positive. A responder had a positive CD4 or CD8 response for at least one cytokine to at least one peptide pool at any time points. In addition, memory T cells were identified on the basis of markers expression and their cytokine production quantified using Boolean gating.

IFN-γ ELISPOT

The frequency of IFN-γ secreting cells per million in response to GP EBOZ was assessed by ELISPOT (Beckton Dickinson). After thawing, 250 000 PBMC per well were stimulated 20h in triplicates with 6 pools of 20-22 peptides covering the sequence of the GP EBOZ protein minus the last C-terminal 16 amino acids or with phytohemagglutinin (PHA) or unstimulated (dimethylsulfoxyde, DMSO alone) as positive and negative controls, respectively. The peptides were 15-mers overlapping by 10 amino acids at a final concentration of 2.5 μg/ml of each peptide. To detect cytokines as discrete spots, a second anti-IFNγ antibody biotinylated, streptavidin-enzyme and an insoluble substrate were used. Results in spot forming units (SFU) per million PBMC were given with the help of computer assisted video image analyzer (EliSpot Robotic Systems with AID EliSpot Software Version 6.x (ELROBO6i, AID, D-Straßberg)), averaged across triplicates, and values in unstimulated wells were substracted. Negative values were set to zero and finally, the response to GP EBOZ calculated as the sum of the responses to the 6 pools of peptides. An ELISPOT was validated if the response to the negative control was less than 50 SFU / million PBMC and the positive control above 500 SFU / million PBMC.

ChAd3 and Ad5 Serologic Assessment

An adenovirus serum neutralization assay was performed to assess neutralizing antibody titers in order to determine baseline and vaccine induced (week 4) neutralization of ChAd3 and human Ad5. Reciprocal antibody titers are reported as the inhibitory concentration 90% (IC90; the titer at which 90% of infectivity is inhibited). The assay was performed according to previous description(3).

Data analysis

To compare the antibody titers IgG obtained in the present study with those obtained in the Ebola challenge studies in macaques(4,5), we used the titers measured at the VRC.

To investigate the effect of demographic characteristics on peak antibody concentration at D28 or maintenance at D180, a regression model was used including age, gender, BMI. The same was done to investigate the relation of safety data with immunological response, by studying the impact of grade 2 and 3 AEs, fever, fatigue, adenopathy and headache on antibody response.

Supplemental tables and figure

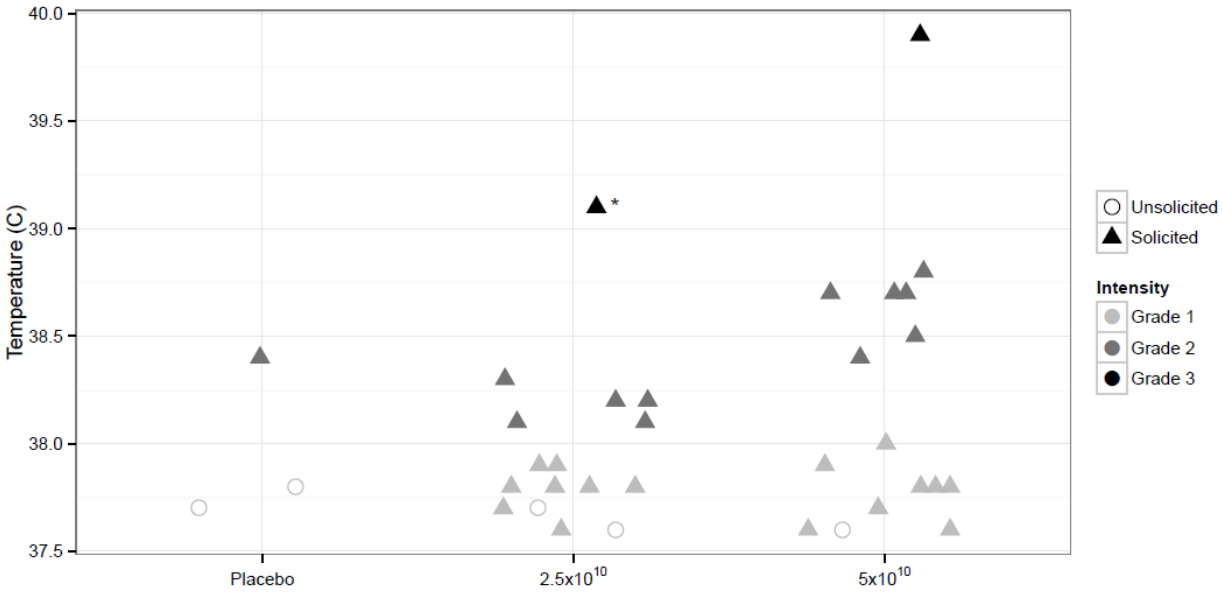


Figure 1S: **Subjects with fever (>37.5°C axillary temperature) per arm.**

*Fever at day 4 associated to a streptococcal angina.

		Placebo			2.5×10^{10} vp			5×10^{10} vp			P-value	
		N	Mean	95%CI	N	Mean	95%CI	N	Mean	95%CI	Placebo vs Vaccinated	Low vs High Dose
Hb - Men (g/l)	*D1*	9	1.9	-4.6-8.4	25	1.3	-8.5-11.1	27	-1.5	-11.3-8.3	0.32	0.03
	D7	9	-1	-10.8-8.8	25	-1.7	-9.7-6.3	27	-4.1	-16.8-8.6	0.31	0.17
	D14	9	-5	-14-4	24	-2.6	-12.4-7.2	27	-7.3	-19.3-4.7	0.97	<0.001
	D28	9	-1.9	-9.5-5.7	25	-2	-13-9	27	-4.4	-16.9-8.1	0.54	0.26
Hb - Women (g/l)	*D1*	11	0.5	-8.9-9.9	26	2.5	-5.7-10.7	21	0.5	-9.5-10.5	0.67	0.24
	D7	11	-1.1	-12.3-10.1	26	-4.1	-14.3-6.1	22	-2.7	-15-9.6	0.27	0.29
	D14	11	-7.1	-19.6-5.4	26	-5.4	-18.3-7.5	22	-4	-16.9-8.9	0.24	0.3
	D28	11	-6.2	-18.2-5.8	26	-3.8	-20.5-12.9	21	-4.2	-15.8-7.4	0.31	0.91
Total white cells (G/l)	*D1*	20	0.1	-1.9-2.1	51	0	-2.4-2.4	48	-0.3	-3-2.4	0.49	0.67
	D7	20	-0.1	-3.2-3	51	0.2	-2.3-2.7	49	-0.2	-2.4-2	0.65	0.09
	D14	20	-0.1	-3.2-3	50	0	-2.5-2.5	49	0.1	-2.4-2.6	0.99	0.67
	D28	20	0.2	-2.2-2.6	51	-0.1	-2.5-2.3	48	-0.3	-2.7-2.1	0.24	0.75
Lymphocytes (G/l)	*D1*	20	0.4	-7.2-8	51	-10.3	-24.2-3.6	48	-8.9	-23.8-6	<0.001	0.32
	D7	20	-0.5	-16.2-15.2	51	-0.5	-14.8-13.8	49	0.8	-13.3-14.9	0.77	0.54
	D14	20	-1.8	-14.9-11.3	50	0.9	-11.1-12.9	49	-0.9	-15.8-14	0.3	0.12
	D28	20	-1.5	-11.9-8.9	51	0.6	-12.1-13.3	48	0.9	-9.9-11.7	0.07	1
Neutrophil (G/l)	*D1*	20	-0.8	-11.8-10.2	51	8.3	-9.7-26.3	48	6.4	-12-24.8	<0.001	0.3
	D7	20	0.2	-18-18.4	51	1.1	-14.4-16.6	49	-0.7	-16.6-15.2	0.89	0.3
	D14	20	1.3	-14.4-17	50	-1.6	-15.1-11.9	49	0.6	-15.1-16.3	0.33	0.21
	D28	20	1.3	-10.1-12.7	51	-0.6	-15.1-13.9	48	-1.3	-13.8-11.2	0.11	0.57
Platelets (G/l)	*D1*	20	4.5	-29-38	51	-20.6	-60.4-19.2	47	-18.8	-53.7-16.1	<0.001	0.72
	D7	20	-5	-48.5-38.5	51	7	-45.1-59.1	48	3.8	-38.7-46.3	0.11	0.33
	D14	20	3.3	-43.9-50.5	50	9.6	-45.1-64.3	48	8.4	-46.9-63.7	0.35	0.93
	D28	20	15.8	-25.9-57.5	51	4.6	-47.1-56.3	47	-0.1	-48.5-48.3	0.02	0.7
aPTT (seconds)	*D1*	20	0.4	-3.1-3.9	51	0	-2.5-2.5	47	0.6	-2.5-3.7	0.79	0.1
	D7	20	-0.2	-4.1-3.7	50	0.1	-2.4-2.6	48	0.4	-2-2.8	0.45	0.29
	D14	20	0.2	-3.3-3.7	50	-0.1	-3-2.8	48	0.4	-4.1-4.9	0.43	0.56
	D28	20	0.2	-3.3-3.7	50	-0.3	-3.8-3.2	47	0.1	-4.4-4.6	0.24	0.76

P-value were calculated with Mann-Whitney test.

Table 1S: Mean changes in haematology values from baseline to D1, D7, D14 and D28 with 95%CI per arm.

		Placebo			2.5 x 10^{10} vp			5 x 10^{10} vp			P-value	
		N	Mean	95%CI	N	Mean	95%CI	N	Mean	95%CI	Placebo vs Vaccinated	Low vs High Dose
Creatinine (umol/l)	***D1***	20	0	-15.3-15.3	51	4.2	-8.5-16.9	49	1.9	-12.2-16	0.1	0.23
	D7	20	-1.7	-17.8-14.4	51	0.1	-10.9-11.1	49	-2.5	-21.5-16.5	0.63	0.26
	D14	20	-1.6	-18.3-15.1	50	1.3	-15.8-18.4	48	-2.6	-20.2-15	0.64	0.03
	D28	20	-1.7	-15.4-12	51	2.6	-11.1-16.3	48	-1.8	-19.6-16	0.18	0.01
CRP (mg/l)	***D1***	2	3	-8.2-14.2	17	2.4	-4.3-9.1	18	4.1	-8.4-16.6	0.81	0.81
	D7	2	-3.5	-7.6-0.6	11	-2.6	-16.1-10.9	14	1.1	-4-6.2	0.07	0.04
	D14	2	-4	-12.2-4.2	12	-2.2	-16.5-12.1	13	1.8	-10.5-14.1	0.1	0.24
	D28	2	2	2-2	10	-2.4	-18.1-13.3	12	0.3	-3.4-4	0.13	0.3
ASAT (U/l)	***D1***	20	-0.4	-8.8-8	50	0.5	-6.8-7.8	49	0.7	-6.6-8	0.16	0.69
	D7	20	-0.9	-13.2-11.4	49	-0.1	-11.1-10.9	49	3	-15.8-21.8	0.65	0.38
	D14	20	0.1	-13.6-13.8	49	0.1	-12.8-13	49	1	-8.4-10.4	0.82	0.59
	D28	20	-1.4	-16.3-13.5	50	-0.3	-14.6-14	48	1.8	-14.5-18.1	0.39	0.68
ALAT (U/l)	***D1***	20	0.8	-11.5-13.1	50	0.4	-7-7.8	49	0	-7.3-7.3	0.59	0.7
	D7	20	0.1	-3.8-4	50	-0.1	-11.1-10.9	49	1.9	-18.9-22.7	0.63	0.07
	D14	20	1.2	-6.1-8.5	49	-0.5	-11.3-10.3	49	1.1	-11.4-13.6	0.38	<0.0001
	D28	20	-0.8	-17.3-15.7	50	-0.7	-13-11.6	48	1.1	-34.8-37	0.44	0.57
γ-GT (U/l)	***D1***	20	0.6	-3.7-4.9	50	0.6	-3.3-4.5	49	0.5	-5-6	0.98	0.25
	D7	20	0.2	-5.5-5.9	50	-0.1	-10.1-9.9	49	1.1	-8.7-10.9	0.49	0.63
	D14	20	-0.9	-8.7-6.9	49	-0.7	-15.8-14.4	49	-0.5	-11.9-10.9	0.93	0.72
	D28	20	0.2	-8.6-9	50	-0.6	-12.6-11.4	48	0.8	-21.2-22.8	0.42	0.86
Bilirubin (umol/l)	***D1***	19	1.5	-5.9-8.9	48	1.1	-3.6-5.8	47	0.9	-6.5-8.3	0.35	0.4
	D7	18	1.1	-4-6.2	49	-0.9	-7.2-5.4	48	-1.7	-14.2-10.8	0.01	0.7
	D14	19	-0.2	-8-7.6	48	-0.7	-7.2-5.8	47	-1.4	-9.8-7	0.43	0.26
	D28	18	0.6	-8.4-9.6	49	-1.1	-7.4-5.2	47	-1.3	-13.8-11.2	0.18	0.75

P-value were calculated with Mann-Whitney test

Table 2S: Mean changes in biochemistry values from baseline to D1, D7, D14 and D28 with 95%CI per arm.

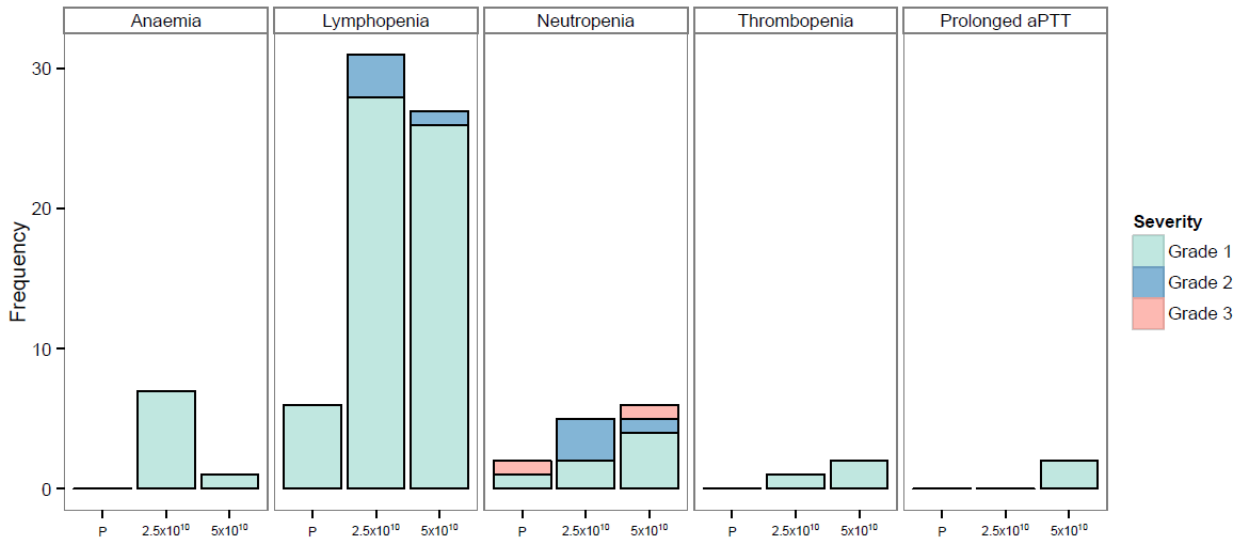


Figure 2S: Frequency of individuals with worsening hematology lab values between D0 and D28 according to vaccine doses.

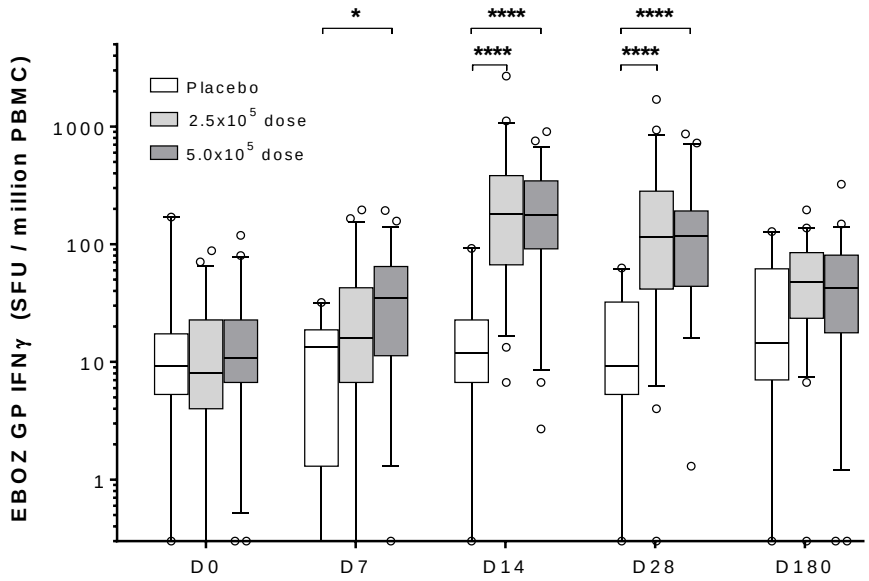
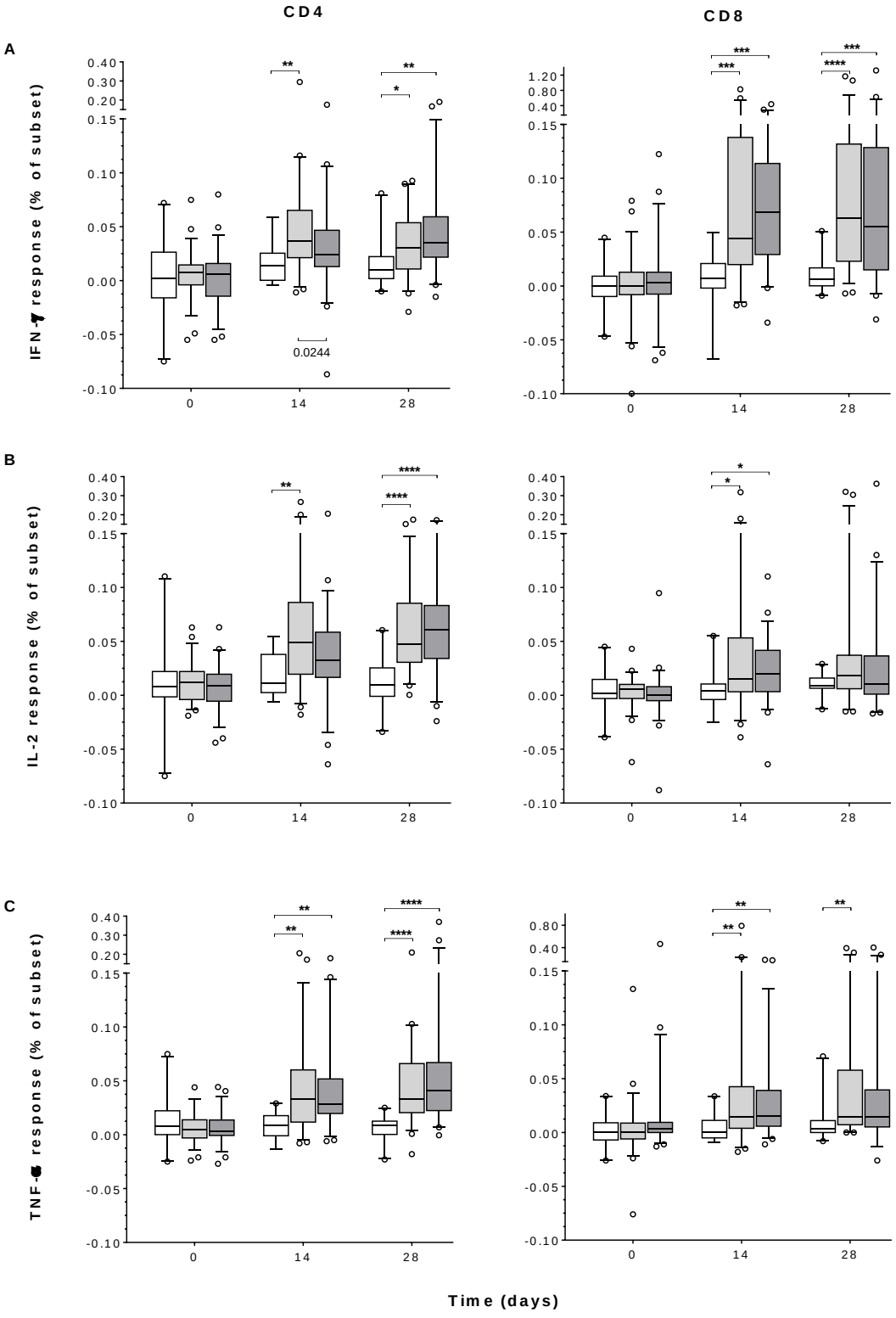


Figure 3S: EBOZ GP specific IFN-γ responses.

The kinetics of individual IFN-γ responses to EBOZ GP peptides was assessed by ELISPOT. Results are shown as boxplots with median, quartiles and 5% centiles, for each group, placebo in white (n=20), dose 2.5x1010 vp in light grey (n=51) and dose 5 x1010 vp in dark grey (n=49). Kruskal-Wallis test was used to assess statistical significance with placebo group. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001. Friedman test was used to assess statistical significance within groups between D0 and D180: for the two vaccines doses, D180 value was higher than D0 value (p=0.001).

19


CD4
CD8
A
B
C
IFN-γ response (% of subset)
IL-2 response (% of subset)
TNF-α response (% of subset)
0.0244
Time (days)

20

Figure 4S: EBOZ GP specific T cells responses.

The kinetics of individual CD4+ (left Panels) and CD8+ (right Panels) responses are expressed as frequencies of subsets expressing IFN-γ (Panels A), IL-2 (Panels B) or TNF-□□(Panels C) separately. Results are shown as boxplots with median, quartiles and 5% centiles, for each group, placebo in white (n=20), dose 2.5x1010 vp in light grey (n=51) and dose 5.0x1010 vp in dark grey (n=49). Kruskal-Wallis test was used to assess statistical significance with placebo group. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Study Group	n/N	%	(95% CI)	Comparisons with Saline [b]	Comparisons with cAd3-EBOZ $2.5x10^{10}$ [b]
Saline					
Week 4 (Day 28)	1/20	5.0	0.1 - 24.9	-	-
By Week 4[c]	1/20	5.0	0.1 - 24.9	-	-
cAd3-EBOZ $2.5x10^{10}$					
Week 4 (Day 28)	49/51	96.1	86.5 - 99.5	**p_f<0.001**	-
By Week 4[c]	49/51	96.1	86.5 - 99.5	**p_f<0.001**	-
cAd3-EBOZ $5.0x10^{10}$					
Week 4 (Day 28)	46/48	95.8	85.7 - 99.5	**p_f<0.001**	p_f=1
By Week 4[c]	46/48	95.8	85.7 - 99.5	**p_f<0.001**	p_f=1

Table 3S: Summary of VRC ELISA positive response rate for the Ebola Mayinga Strain by vaccination dose

n = number of subjects with positive response; N = number of subjects evaluated; CI = Confidence Interval (Clopper-Pearson).

a. Positive ELISA response was defined as a statistically significant increase in titre from baseline.

b. Pairwise comparisons between groups were evaluated using Fisher's exact test (p_f).

c. Subjects were counted as having a positive response by Week 4 if they had a positive response at Week 2 or Week 4.

	GMC at D28			GMC at D180		
	coefficient	std error	p-value	coefficient	std error	p-value
Age	-0.01	0.00	0.12	0.00	0.00	0.76
Gender	-0.03	0.08	0.71	0.02	0.07	0.78
BMI	0.01	0.01	0.45	-0.01	0.01	0.18
Grade 2 and 3 AEs	0.08	0.08	0.27	0.02	0.07	0.77
Fever	-0.20	0.08	**0.02**	-0.15	0.07	**0.05**
Fatigue	-0.02	0.08	0.85	-0.08	0.07	0.27
Adenopathy	-0.09	0.25	0.72	-0.01	0.23	0.98
Headache	-0.15	0.13	0.24	-0.08	0.11	0.47

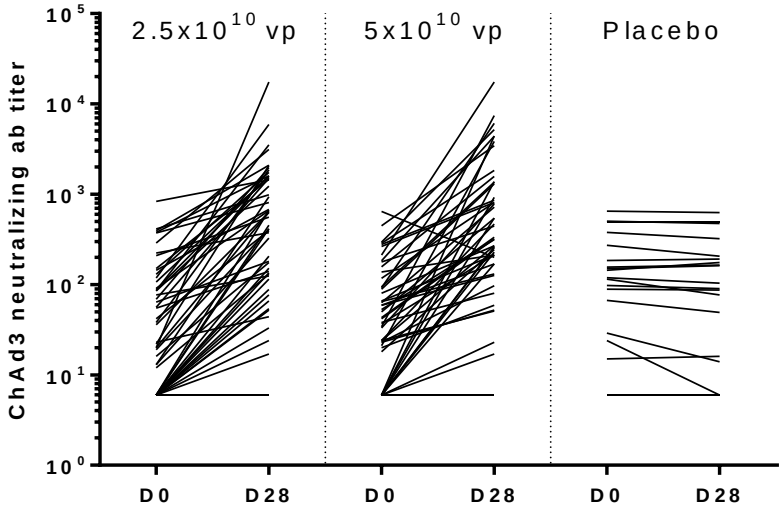
Table 4S: Determinant analysis (ANCOVA) of GMC at D28 and persistence at D180.

Analyzed data is a subset of the full dataset with only individuals who received the vaccine (those who received placebo were omitted in the analysis). An analysis of covariance was performed here (including simultaneously continuous and categorical/binary variables). The outcomes were titres at D28 and D180 (analyzed independently on a log10 scale).

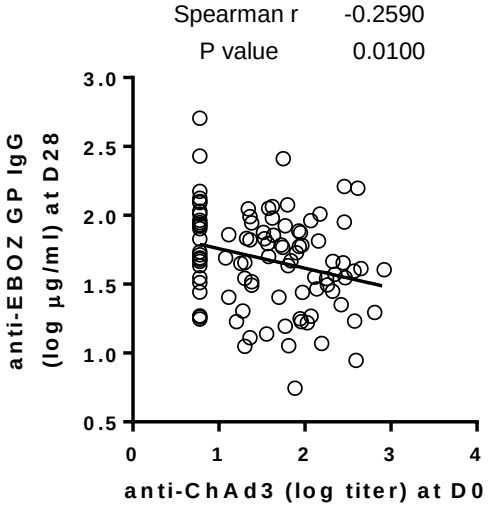
Variables description:

Age	continuous outcome	
Gender	binary outcome	
BMI	continuous outcome	
Grade 2 and 3 AEs	binary outcome	TRUE if person has experienced at least 1 AE with grade ≥2
Fever	binary outcome	TRUE if person has experienced at least 1 fever
Fatigue	binary outcome	TRUE if person has experienced a fatigue at D1
Adenopathy	binary outcome	TRUE if person has experienced an axillary node enlargement at D1
Headache	binary outcome	TRUE if person has experienced a headache at D1 with grade ≥2

A


2.5x10^10 vp
5x10^10 vp
Placebo
ChAd3 neutralizing ab titer
10^5
10^4
10^3
10^2
10^1
10^0
D0
D28
D0
D28
D0
D28

B


Spearman r -0.2590
P value 0.0100
anti-EBOZ GP IgG
(log µg/ml) at D28
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
anti-ChAd3 (log titer) at D0

C

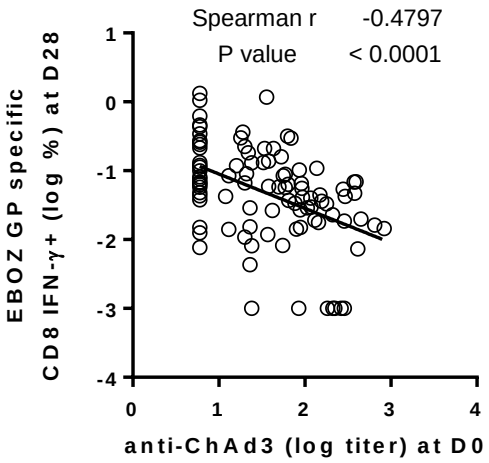

Spearman r -0.4797
P value < 0.0001
EBOZ GP specific
CD8 IFN-γ+ (log %) at D28
1
0
-1
-2
-3
-4
0
1
2
3
4
anti-ChAd3 (log titer) at D0

Figure 5S: Anti-ChAd3 neutralizing antibodies. Panel A show antibody titers pre and 4 weeks post vaccination in volunteers from the 3 arms. Panels B and C show the correlation between anti-ChAd3 antibodies at D0 and EBOZ GP specific responses obtained at D28 in all vaccinees, humoral responses in Panel B and IFN-γ CD8+ responses in Panel C. Spearman r and p values are indicated.

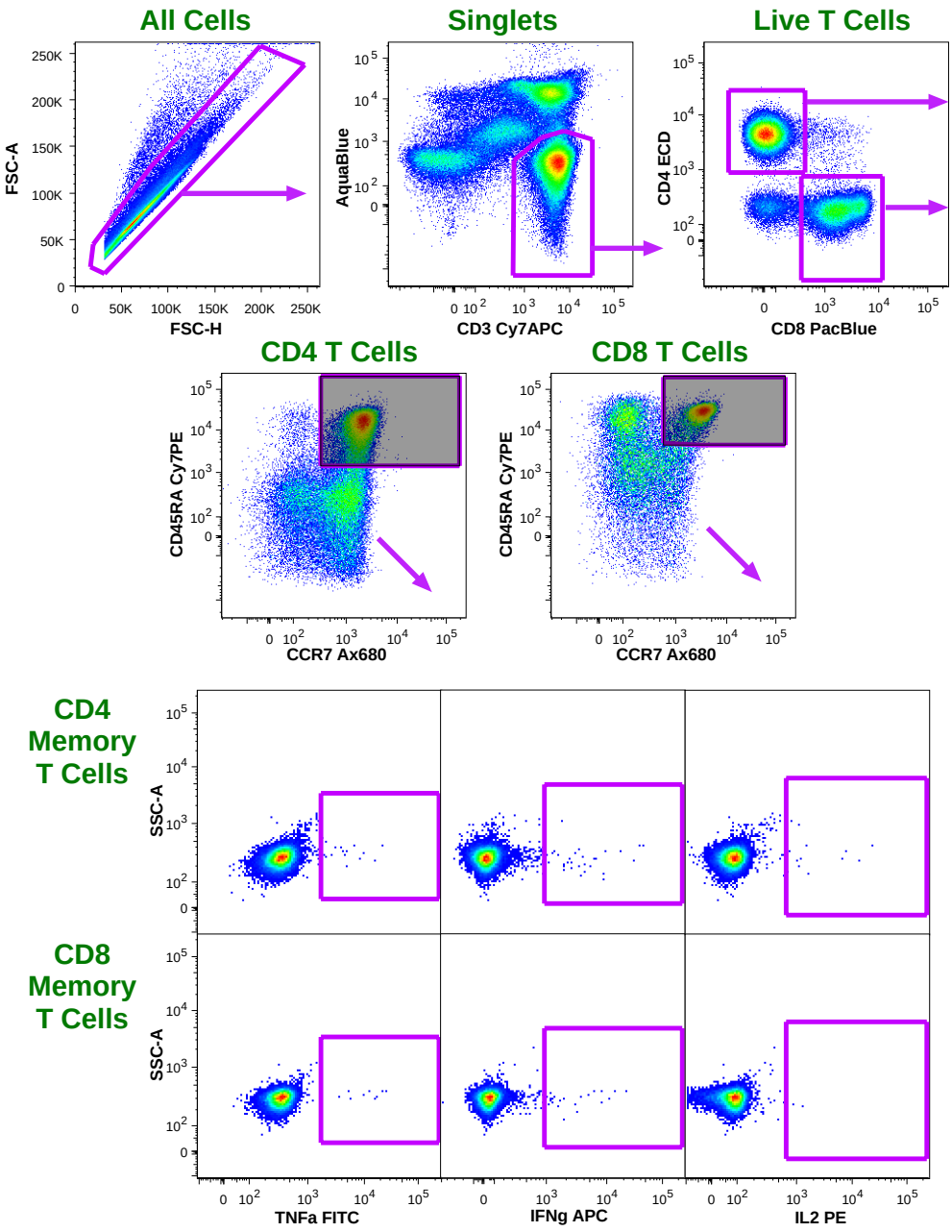


Figure 6S: Gating hierarchy to enumerate antigen-specific T cells.

Stimulated cells were stained as described and analyzed by flow cytometry. For each run, identical gates were applied to all samples; for the study, all fluorescence gates were identical. The sample was progressively gated to identify single cells, live CD3+ T cells, and CD4 or CD8 T cells as shown in the top row. Within these lineages, memory T cells were identified by excluding CD45RA+CCR7+ naïve T cells (second row). Within memory T cells, individual gates for each cytokine were used (bottom).

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