Immunity duration of a recombinant adenovirus type-5 vector-based Ebola vaccine and a homologous prime-boost immunisation in healthy adults in China: final report of a randomised, double-blind, placebo-controlled, phase 1 trial

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Summary

Background The 2013–15 Ebola virus disease epidemic in west Africa greatly accelerated the development of Ebola vaccine. We aimed to analyse the immune persistence induced by one shot of an adenovirus type-5 vector-based Ebola virus vaccine up to 6 months and the effect of boosting with a homologous vector in healthy adults in China.

Methods In a randomised, double-blind, placebo-controlled, phase 1 clinical trial in one site in Jiangsu Province, China, 120 healthy adults aged 18–60 years received an initial dose of intramuscular adenovirus type-5 Ebola virus vaccine of $4·0×10^{10}$ viral particles, $1·6×10^{11}$ viral particles, or placebo, and were followed up to day 168. Participants were subsequently re-recruited to receive a booster dose of the same vaccine or placebo, in the same dose, at month 6. Women who were pregnant, breastfeeding, or planned to become pregnant during the next month were excluded. Randomisation was conducted by computer-generated block randomisation. Randomisation data were unmasked for interim analysis of the data obtained between days 0–28 but not disclosed to participants or site staff. Safety and immunogenicity analysis were done on the intention-to-treat population. We aimed to assess the safety profile of the experimental vaccine and the immunity responses to a single-dose immunisation or a homologous prime-boost regimen. Primary outcomes were Ebola glycoprotein-specific ELISA antibody responses 28 days post-boost and the occurrences of adverse reactions post-boost. The original trial and the extended booster study were registered with ClinicalTrials.gov, numbers NCT02326194 and NCT02533791, respectively.

Findings Between Dec 28, 2014, and Jan 9, 2015, we enrolled 210 volunteers. 90 participants were not randomised due to not meeting inclusion criteria (61), meeting exclusion criteria (4), or withdrawal of consent (25). 120 people were randomly assigned to receive intramuscular Ebola vaccine at $4·0×10^{10}$ viral particles (low dose, n=40), Ebola vaccine at $1·6×10^{11}$ viral particles (high dose, n=40), or placebo (n=40, in two groups of 20). After prime vaccination, the geometric mean titer (GMT) of ELISA EC_{90} peaked at 682·7 (95% CI 424·3–1098·5) in the low-dose vaccine group and 1305·7 (970·1–1757·2) in the high-dose vaccine group at day 28, and then fell gradually through the next a few months to 575·5 (394·8–838·8) in the high-dose vaccine group and 197·9 (107·9–362·7) in the low-dose vaccine group at day 168. No specific response was recorded in the placebo group with a GMT of 5·0. Of the 120 participants involved in the initial trial, ten participants declined to participate, and 110 were included in the boost immunisation: 38 received the low dose, 35 received the high dose, and 37 received the placebo. At day 28 after boost vaccination, the ELISA EC_{90} titres rapidly rose to 6110 (95% CI 4705–7935) in the low-dose group and to 11825 (8904–15705) in the high dose group. 78 of 110 participants reported at least one solicited adverse reaction within the first 7 days after booster administration. Both of the groups who received vaccine showed significantly higher incidence of mild or moderate solicited adverse reactions than did the placebo group.

Interpretation The adenovirus 5-vectored Ebola vaccine of $1·6×10^{11}$ viral particles was highly immunogenic and safe. The lower dose of $4·0×10^{10}$ viral particles was also safe, but immunogenicity seemed to be more vulnerable to the pre-existing immunity of adenovirus 5. A homologous priming-boosting regimen with adenovirus type-5 Ebola vaccine at 6 months interval was able to elicit greater antibody responses with longer duration. These results support an immunisation strategy to implement a booster injection for a more durable protection against Ebola virus disease.

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Research in context

Evidence before this study

We searched PubMed for clinical trial reports with the terms "Ebola" or "Ebolavirus", and "vaccine", and ClinicalTrials.gov for unpublished randomised trials with no date or language restrictions, up to Aug 17, 2016. Since the 2014 Ebola outbreak, a total of 46 clinical trials with various Ebola vaccines candidates were launched according to the registration on Clinicaltrial.gov and Pan African Clinical Trials Registry. Up to now, only three heterologous prime-boost studies have been reported. Results from these trials indicated that some of the heterologous prime-boost combinations could be powerfully immunogenic in elicitation of both anamnestic antibody responses and robust T-cell responses, but some of them were not. An open-label, cluster-randomised ring vaccination trial with a rVSV-ZEBOV in Guinea showed a high efficacy in preventing Ebola virus disease.

A novel adenovirus type-5 Ebola virus vaccine expressing the glycoprotein of the 2014 epidemic strain was assessed in a phase 1 clinical trial in China, of which safety and immunogenicity data up to day 28 after injection was published in a preliminary report. However, the durability of a single dose recombinant adenovirus type-5 vaccination is still unknown, and assessment of whether subsequent boosts will be necessary to maintain or establish sufficient long-term immunity will be important.

Added value of this study

This report includes the follow-up data from the first phase 1 study of the adenovirus type-5 Ebola virus vaccine in Chinese adults up to day 168, and an extra boosting study with a homologous vaccine at a prime-boost interval of 6 months. The humoral responses were followed up to month 12 after boost vaccination. Although strong immune responses were noted after the one-short regimen of adenovirus type-5 Ebola virus vaccine, especially with the high dose, a quick waning of the antibodies were observed during day 56–168.
The homologous prime-boost regimen at month 6 was safe and highly immunogenic. We observed superior antibodies responses induced by the homologous prime-boost regimen to those induced by prime dose alone. However, the boosting effects of specific T-cell responses by the homologous prime-boost regimen seemed small in this study.

Implications of all the available evidence

Adenovirus type-5 Ebola virus vaccine is safe and immunogenic, but the short duration of antibodies raised a need for prime-boost immunisation. A priming-boosting regimen with homologous adenovirus type-5 vector-based Ebola virus vaccine could elicit greater humoral responses, but little cellular immunity response. In future studies, other boosting schedules with a booster vaccination at other prime-boost intervals or with a heterologous Ebola vaccine should be investigated, to provide a longer duration of high protection against Ebola virus.

Introduction

The 2013–15 Ebola virus disease epidemic in west Africa caused by subtype Zaire was the largest in history, spreading across borders and causing a total of 28616 Ebola cases in Guinea, Liberia, and Sierra Leone, with 11310 deaths.[1] Ebola virus disease used to be deemed regional, and with few cases, the development of a vaccine did not get enough attention and progressed slowly. Since this recent outbreak, development of Ebola vaccine accelerated greatly, and clinical trials with various Ebola vaccine candidates were launched as an emergency response to this crisis.[2–7] Most of these studies focused on introducing a quick protective response with a rapidly acting immunisation regimen.[8] However, following the end of the epidemic, more attention must be put in the durability of the vaccine-elicited protection and the potential benefits of a booster injection.

In October, 2014, we launched a first-in-human trial with a novel recombinant adenovirus type-5 vector-based Ebola vaccine expressing the glycoprotein of Ebola.[7] In the preliminary report of this trial, antibody responses elicited by the experimental adenovirus type-5 Ebola virus vaccine have been assessed up to day 28 after vaccination. However, the durability of a single-dose recombinant adenovirus type-5 Ebola virus vaccine immunisation is still unknown, and we must assess whether subsequent boosts will be necessary to maintain or establish sufficient long-term immunity.[9] In this Article, we describe the immune dynamics induced by one dose of the adenovirus type-5 Ebola virus vaccine up to 6 months and the boosting responses to a homologous vector vaccine in healthy adults in China.

Methods

Study design and participants

We did a randomised, double-blind, placebo-controlled, phase 1 clinical trial at one phase 1 vaccine clinical trial site in Taizhou City, Jiangsu Province, China, from Dec 28, 2014.[7] 120 healthy adults of both sexes between the ages of 18 and 60 years were randomly assigned to receive an injection of experimental adenovirus type-5 Ebola virus vaccine or placebo. Women who were pregnant, breastfeeding, or planned to become pregnant during the next month were excluded. After injection, all the participants were followed up for 6 months. We added an extending study for a homologous booster vaccination at month 6 after the prime injection by protocol amendment. Participants who were involved in the initial study were re-recruited and assessed for eligibility. Approval of the modified study protocol was obtained from the ethics committee of the Jiangsu Provincial Center for Disease Control and Prevention before the implementation of the extended boosting

immunisation. Separate written informed consent for the booster study was obtained from each participant. Full details of the study are provided in the protocol online. The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Randomisation and masking

In the initial study, the participants were sequentially enrolled in a two-step manner, and randomly assigned in a 2:1 ratio by computer-generated clock randomisation list to receive the adenovirus type-5 Ebola virus vaccine containing either $4{\cdot}0\times10^{10}$ (low dose, 40 people) viral particles or placebo, or to receive the vaccine containing $1{\cdot}6\times10^{11}$ (high dose, 40 people) viral particles or placebo (40 people), as described in a preliminary report.[7] Both the vaccine and placebo were vialled and had identical packaging with a labelled randomisation code as the only identifier. We unblinded the allocation of the participants for an interim analysis of the data obtained between day 0 and 28. However, the treatment allocation was not disclosed to both the participants and site staff. At the end of the 6-month follow-up period of the initial study, we re-recruited the participants for the following booster study. Ten people declined to participate in the booster trial (two low-dose vaccine, five high-dose vaccine, three placebo) No additional randomisation was implemented, and participants who received the booster received the same dose as their original vaccination. The participants and the staff who assessed adverse events after immunisation or performed antibody detection in the laboratory were masked to the treatment allocation during the whole study.

Procedures

The adenovirus type-5 Ebola virus vaccine consists of a recombinant replication defective adenovirus type-5 vector expressing the glycoprotein of Ebola Zaire Makona variant (GenBank No. KJ660346). Each dose of the vaccine contains $4{\cdot}0\times10^{10}$ adenovirus type-5 viral particles. The placebo contains the vaccine excipients only without any adenovirus type-5 vectors. Participants were tested for any laboratory abnormal changes at protocol-specified study visits, and followed up for 28 days for any adverse events after the prime vaccination, as described previously in a preliminary report.[7] In the extended boosting study, participants were re-recruited and received the same vaccine as the initial treatment allocation. Thus, the participants in the high-dose vaccine group in the initial study were vaccinated with the adenovirus type-5 Ebola virus vaccine of $1{\cdot}6\times10^{11}$ viral particles by receiving two shots of the vaccine (with one shot in each arm); the participants in the low-dose vaccine group in the initial study were vaccinated with a single shot of the adenovirus type-5 Ebola virus vaccine of $4{\cdot}0\times10^{10}$ viral particles; and the participants in the placebo group in the initial study still received placebo. Solicited adverse reactions were followed up within the first 7 days, and unsolicited adverse events were recorded up to day 28 after the booster vaccination. Additionally, a self-report system for the serious adverse events was implemented during the whole study period from the priming vaccination at day 0 to the end of the booster study.

Besides the five visits between day 0 and day 28, which have already been reported in the previous preliminary report,[7] participants received another five visits in the following study, including visits at day 56, 112, and 168 after the priming, and visits at day 28 and month 12 after the boosting. Blood samples were donated for antibody measurement at each visit. We assessed Ebola-specific antibody responses against the vaccine-matched glycoprotein with ELISA in terms of background subtracted ELISA 90% effective concentration (ELISA EC_{90}),[3] and the antigen-specified T-cell responses with intracellular cytokine staining assay and Enzyme-Linked ImmunoSpot (ELISpot). The neutralising antibody titres against human adenovirus type-5 were measured by using a serum neutralisation assay at the specified time points both before and after vaccination.[10]

Outcomes

Primary outcomes were Ebola glycoprotein-specific ELISA antibody responses 28 days post-boost and the occurrences of adverse reactions post-boost. The safety outcomes were measured by the incidence of solicited adverse reactions within 7 days, unsolicited adverse events within 28 days after the booster vaccination, and any occurrence of serious adverse events during the whole follow-up period. We did severity grading of the adverse events according to the standard guidelines issued by China state Food and Drug Administration.[11] Laboratory analyses of blood were performed at day 56 post-vaccination for blood routine tests, biochemical tests, and coagulation function tests for analysis of safety outcomes.

Immunogenicity outcomes included the percentage of vaccine responders and the magnitude of the humoral and cellular immune responses against Ebola glycoprotein at specific timepoints both after the priming and boosting vaccinations. The durations of specific immune responses were assessed up to 6 months after the prime vaccination, and 12 months after the booster injection.

Statistical analysis

We estimated a sample size of 40 per group would produce preliminary data for the incidence of adverse reactions and allow us to identify a 50% higher proportion of positive immune response post-vaccination in at least one vaccine group than in the placebo group with a power of 90%.

We analysed the safety endpoints based on the intention-to-treat cohort, in which all participants who received an injection were included, and the immunogenicity based on a per-protocol cohort in which those who did not complete all required visits or

For the **protocol** see http://www.jscdc.cn/jgzn/zzjg/ymlcpjs/ymlcpjs_gzdt/201612/P020161220410594669984.pdf

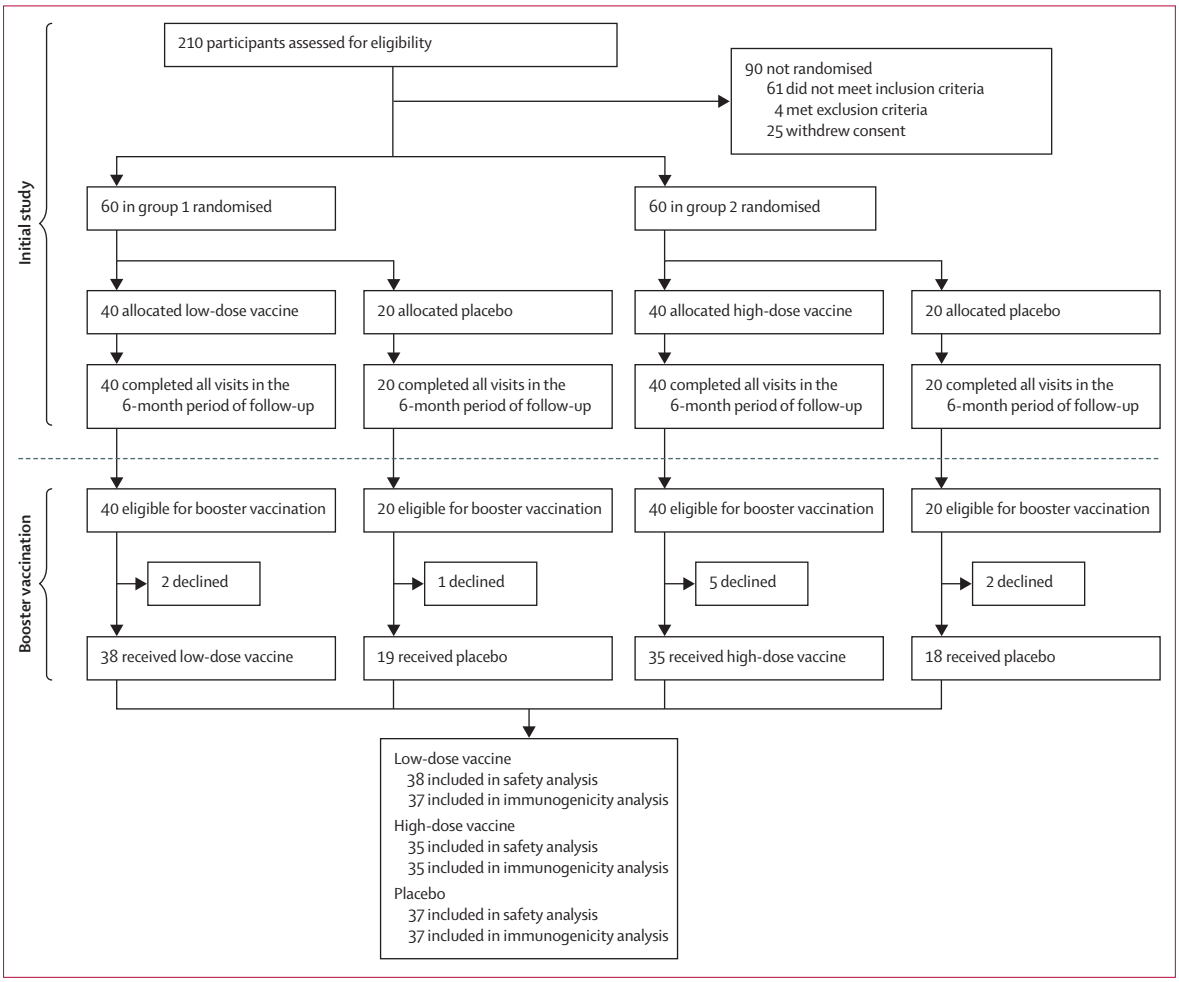

***Figure 1*: Trial profiles of the initial study and the booster study.**
High-dose vaccine= adenovirus type-5 Ebola virus vaccines of $1{\cdot}6 \times 10^{11}$ viral particles. Low-dose vaccine=adenovirus type-5 Ebola virus vaccines of $4{\cdot}0 \times 10^{10}$ viral particles.

	Placebo (n=37)	Low dose: 4×10^{10} VP (n=38)	High dose: $1{\cdot}6 \times 10^{11}$ VP (n=35)
Mean age (years)	43·9, SD 11·3	44·0, SD 9·6	44·1, SD 9·2
Sex			
Male	17	19	17
Female	20	19	18
BMI (kg/m²)	24·2, SD 2·3	23·7, SD 2·3	24·3, SD 2·9
Adenovirus type-5 antibody titres before prime			
>1:200	21	20	21
≤1:200	16	18	14
Adenovirus type-5 antibody titres before boost*			
>1:200	21	26	30
≤1:200	16	12	5

Data are n unless otherwise specified. VP=viral particles. BMI=body-mass index. *Significant difference of the adenovirus type-5 antibody titres at the time before boost was noted across the treatment groups.

***Table 1*: Demography characteristics and adenovirus type-5 neutralising antibody titres in participants involved in the booster study**

blood tests were excluded. The stratified analysis of the immune responses after the prime dose up to day 168 were done according to the baseline adenovirus type-5 neutralising antibody titres of participants (low or negative ≤1:200 or high >1:200), while the boosting responses were stratified based on the adenovirus type-5 neutralising antibody titres on day 168 before the boost injection (low or negative ≤1:200 or high >1:200). T-cell intracellular cytokine staining data of different treatment groups were analysed and displayed by using SPICE (version 5.3.5). Chi-square or Fisher's exact test was used for categorical data, and the multiple comparisons were performed based on a Bonferroni-adjusted alpha when there was a significant difference across the treatment groups. Analysis of variance was used for the log-transformed antibody titres, while Wilcoxon rank-sum test was used for abnormal data. Student-Newman-Keuls test was used for multiple comparisons between paired treatment groups when relevant. All statistical tests were two-sided with $\alpha=0{\cdot}05$ and performed by an independent statistician using

	Placebo group (n=37)	Low-dose group (n=38)	High-dose group (n=35)	P value*
Solicited adverse reactions within 0–7 days				
Any	16	29	33	<0·0001†
Grade 1	13	24	24	0·0085‡
Grade 2	2	4	9	0·0338§
Grade 3	1	1	0	1·0000
Injection-site adverse reactions				
Any	11	27	30	<0·0001‖
Pain				
Grade 1	4	19	29	<0·0001¶
Grade 2	1	3	1	0·6166
Induration				
Grade 1	0	0	8	0·0001**
Redness				
Grade 1	5	4	9	0·1825
Grade 2	1	0	2	0·2074
Grade 3	0	1	0	1·0000
Swelling				
Grade 1	0	3	4	0·1082
Grade 2	1	1	0	1·0000
Grade 3	1	0	0	0·6545
Itch				
Grade 1	0	1	0	1·0000
Any	8	10	15	0·1202
Fever				
Grade 1	7	7	8	0·8760
Grade 2	0	0	6	0·0008**
Headache				
Grade 1	0	1	0	1·0000
Grade 2	0	0	1	0·3182
Fatigue				
Grade 1	0	0	3	0·0303**
Grade 2	0	1	1	0·7655
(Table 2 continues in next column)				
(Continued from previous column)				
Vomiting				
Grade 1	1	0	0	0·6545
Diarrhoea				
Grade 1	0	1	1	0·7655
Muscle pain				
Grade 1	0	1	0	1·0000
Grade 2	0	0	1	0·3182
Joint pain				
Grade 2	0	1	0	1·0000
Throat pain				
Grade 1	0	1	1	0·7655
Cough				
Grade 1	0	1	1	0·7655
Unsolicited adverse events within 0–28 days				
Any	12	9	5	0·1938
Grade 1	8	6	2	0·1546
Grade 2	4	2	3	0·6934
Grade 3	0	1	0	1·0000

Data are n=number of participants. Grade 1 was a mild reaction, grade 2 was a moderate reaction, and grade 3 was a severe reaction (ie, prevented activity). *p values were generated from the comparison across the three groups. When significant difference across treatment groups was found, we further applied pairwise comparisons on the basis of an adjusted α=0·017. †Both the high-dose and low-dose groups showed higher incidences than did placebo group. ‡Both the high-dose and low-dose groups showed higher incidences than did placebo group. §The high-dose group had a higher incidence than did the placebo group; ‖Both the high-dose and low-dose groups showed higher incidences than did placebo group. ¶The high-dose group showed a higher incidence than did the low-dose group, while both the high-dose and low-dose groups showed higher incidences than did placebo group. **The high-dose group showed a higher incidence than did the low-dose group and the placebo group.

***Table 2*: Solicited adverse reactions and unsolicited adverse events reported after the booster immunisation**

SAS (version 9.3). A five-member external data and safety monitoring board was built for the safety data monitoring of this trial (see protocol).

The initial trial and the added boosting study were registered with ClinicalTrials.gov, numbers NCT02326194 and NCT02533791, respectively.

Role of the funding source

The funders of the study were involved in protocol design, but had no role in clinical data collection, safety monitoring, statistical analysis, data interpretation, or writing of the report. All the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Dec 28, 2014, and Jan 9, 2015, we enrolled 210 volunteers. 90 participants were not randomised due to not meeting inclusion criteria (61), meeting exclusion criteria (4), or withdrawal of consent (25). 120 participants received either adenovirus type-5 Ebola virus vaccine of $4·0\times10^{10}$ viral particles, adenovirus type-5 Ebola virus vaccine of $1·6\times10^{11}$ of viral particles, or placebo as prime immunisation at day 0, in the initial study, with 40 participants in each treatment group. The compliance of participants with the visits following vaccination was perfect up to the end of 6-month follow-up at day 168 (figure 1). In July, 2015, we invited all the 120 participants for a booster study 6 months after prime vaccination, and 110 agreed to participate. Ten participants declined to receive booster injection, with two in the low-dose group, five in the high-dose group, and three in the placebo group. Baseline characteristics of participants who were involved in the booster study are described in table 1. Among them, 109 participants donated blood samples at day 28 after booster injection, and 102 participants donated blood samples at month 12 after the booster injection. Another four participants

	Overall			Pre-existing adenovirus type-5 antibody titres ≤1:200*			Pre-existing adenovirus type-5 antibody titres >1:200*		
	n	GMT	Responders	n	GMT	Responders	n	GMT	Responders
Day 56									
Placebo group	40	5·0	0	16	5·0	0	24	5·0	0
Low-dose group	40	489·8 (295·3–812·3)	37	18	1163·3 (303·0–5283·0)	18	22	241·3 (5·0–3465·0)	19
High-dose group	40	1282·9 (998·6–1648·3)	40	15	2290·1 (1268·0–7220·0)	15	25	906·2 (305·4–5802·0)	25
p value		<0·0001†	<0·0001‡		<0·0001†	<0·0001†		<0·0001†	<0·0001‡
Day 112									
Placebo group	40	5·0	0	16	5·0	0	24	5·0	0
Low-dose group	40	386·4 (252·7–591·0)	38	18	780·3 (189·9–4227·0)	18	22	217·4 (5·0–3159·0)	20
High-dose group	40	856·8 (662·9–1107·4)	40	15	1538·2 (720·5–6010·0)	15	25	603·1 (142·4–4576·0)	25
p value		<0·0001†	<0·0001‡		<0·0001†	<0·0001†		<0·0001†	<0·0001‡
Day 168									
Placebo group	40	5·0	0	16	5·0	0	24	5·0	0
Low-dose group	40	197·9 (107·9–362·7)	34	18	539·2 (262·5–1107·8)	17	22	87·1 (38·4–197·4)	17
High-dose group	40	575·5 (394·8–838·8)	39	15	1216·2 (978·6–1511·4)	15	25	367·3 (217·7–619·7)	24
p value		<0·0001†	<0·0001†		<0·0001†	<0·0001‡		<0·0001†	<0·0001‡
Day 28 after boost									
Placebo group	37	5·0	0	16	5·0	0	21	5·0	0
Low-dose group	37	6110·3 (4705·4–7934·6)	37	17	7553·3 (5090·6–11207·4)	17	20	5102·7 (3558·6–7316·7)	20
High-dose group	35	11824·9 (8903·6–15704·8)	35	14	15680·0 (11041·5–22267·0)	14	21	9797·1 (6475·8–14821·9)	21
p value		<0·0001†	<0·0001†		<0·0001†	<0·0001†		<0·0001†	<0·0001†
Month 12 after boost									
Placebo group	34	5·0	0	14	5·0	0	20	5·0	0
Low-dose group	33	674·1 (505·9–898·2)	33	16	840·1 (555·5–1270·4)	16	17	547·9 (361·8–829·8)	17
High-dose group	35	856·8 (573·8–1279·2)	34	14	1314·5 (1022·3–1690·2)	14	21	644·0 (339·6–1221·5)	20
p value		<0·0001‡	<0·0001‡		<0·0001†	<0·0001†		<0·0001‡	<0·0001‡

GMT=geometric mean titre. n=number of participants. Ebola antibody EC_{90} measured by ELISA, according to treatment groups and timepoints. Optical density was read at 450 nm. A positive Ebola specific antibody response was defined as the EC_{90} 10 or more. For Ebola ELISA antibody EC_{90} less than 10, a value of 5 was used for GMT calculation. *The stratified analyses of the immune responses were performed according to the baseline adenovirus type-5 neutralising antibody titres of participants before the prime injection. †The pairwise comparisons showed a significantly higher response level in the high-dose group compared with that in the low-dose group, and both the high-dose and low-dose groups showed a significantly higher responses than did the placebo group. ‡Both the high-dose and low-dose groups showed significantly higher responses than did the placebo group, but no significant difference was noted between the high-dose and low-dose groups.

***Table 3*: ELISA antibody responses to the Ebola glycoprotein after initial and boost vaccination**

(one in placebo group and three in the high-dose vaccine group) did not receive the booster injection but donated blood samples at the last visit (18 months after the prime vaccination).

Laboratory adverse events were detected in 7 participants at day 56, with 4 in the low-dose group and 3 in the placebo group, which were all mild and clinically insignificant (appendix p 1). Among the 110 participants who received the booster dose, 78 (71%) reported at least one solicited adverse reaction within the first 7 days, with a similar overall incidence to that observed after initial vaccination.[7] The occurrence of solicited adverse reactions differed significantly between treatment groups with p<0·0001 (table 2). Both the high-dose and low-dose vaccine groups showed significantly higher incidences of mild or moderate solicited adverse reactions than did the placebo group. The most common injection-site reaction was pain, while the most common systemic adverse reaction was fever. Participants who received high-dose vaccine reported higher incidences of pain, induration, fever, and fatigue, than did those who received placebo. All 6 moderate fever events were found in the high-dose group. However, the occurrence of severe adverse

See Online for appendix

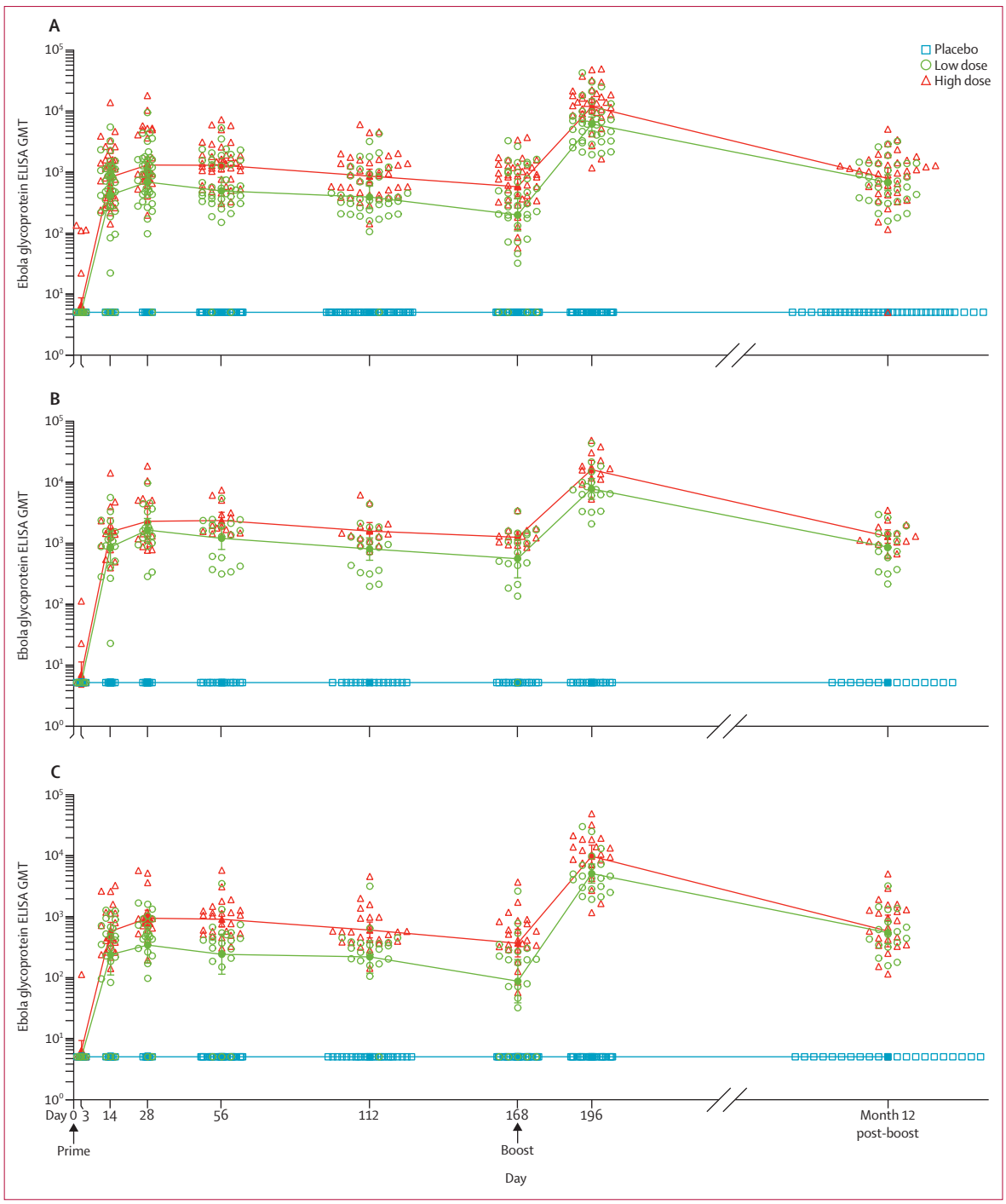

***Figure 2*: Ebola-specific ELISA EC_{50} titres to glycoprotein**
GMT= geometric mean titre (A) all participants, (B) patients with baseline adenovirus type-5 neutralising antibody titres , (C) patients with baseline adenovirus type-5 neutralising antibody titres >1:200.

reactions was not significantly different across groups. Two severe adverse reactions (grade 3) were noted, one swelling at injection site in the high dose group and one with redness at injection site in the low dose group (table 2). The incidences of unsolicited adverse events after boost vaccine were similar between treatment groups in the 28 day follow-up period after boost. No safety concerns were found associated with the anti-adenovirus type-5 neutralising antibodies at the time of boost (appendix p 2).

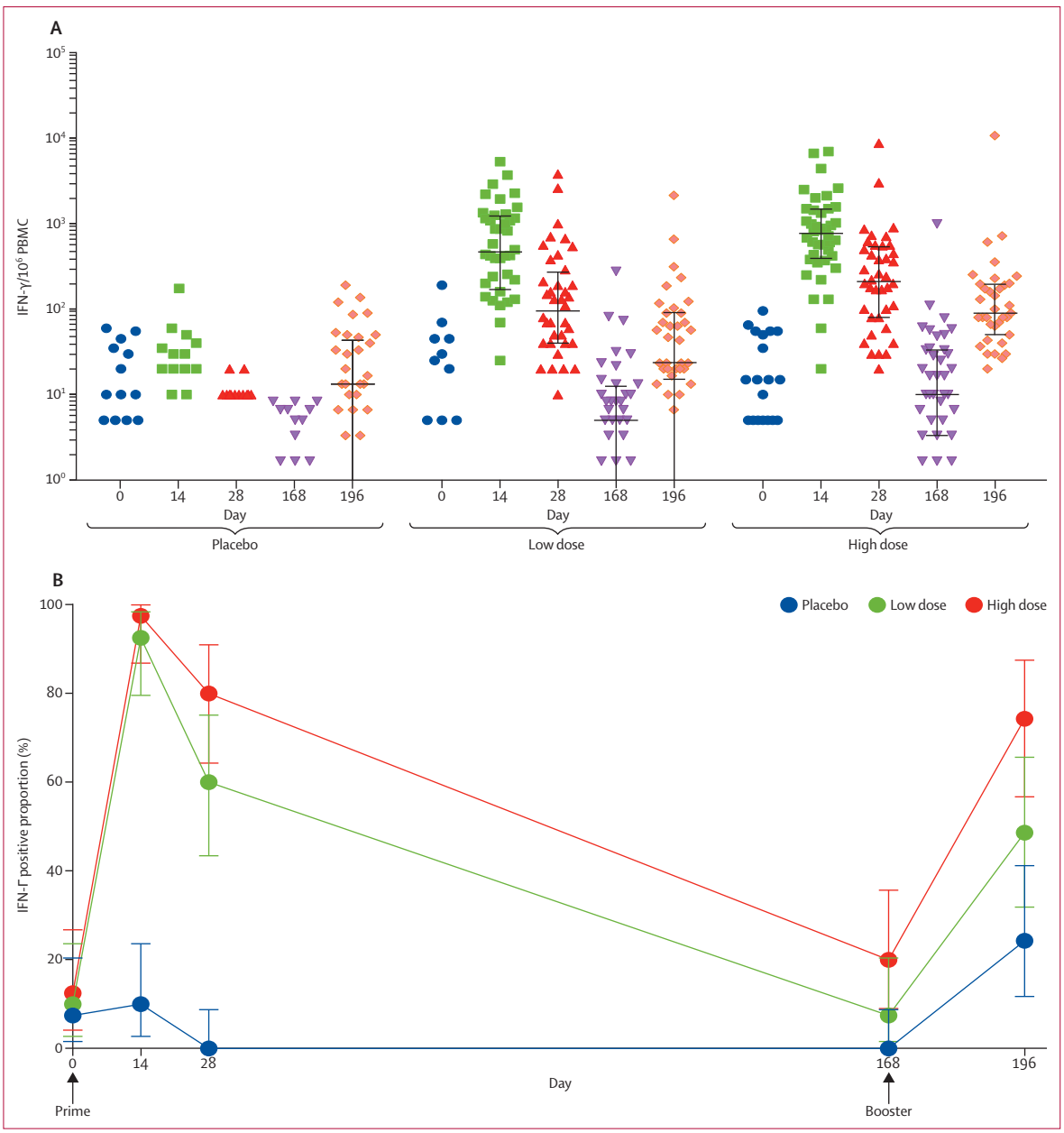

***Figure 3*: Glycoprotein-specific T-cell response measured by Enzyme-Linked ImmunoSpot at different time points before and after prime and boost vaccination**

(A) IFN-γ expressing T cells per 10^6 PBMC. (B) proportion of participants with a positive response of IFN-γ expressing T cells. IFN=interferon. PBMC=peripheral blood mononuclear cells.

Two serious adverse events were reported during the study period, including one case of pneumonia that occurred in the high-dose vaccine group 5 months after the prime vaccination and one case of duodenal ampulla ulcers in the low-dose vaccine group reported 2 weeks after the booster injection. Both events were cured after hospital treatment and deemed unrelated to the vaccination.

Ebola glycoprotein-specific ELISA titres are shown in table 3 and figure 2. After prime vaccination with adenovirus type-5 Ebola virus vaccines, we found that ELISA EC_{90} titres increased significantly at day 14 and peaked at day 28 in both high-dose and low-dose vaccine groups (682·7 [95% CI 424·3–1098·5] in the low-dose vaccine group and 1305·7 [970·1–1757·2] in the high-dose vaccine group at day 28), whereas no specific response was recorded in the placebo group. The data at day 14 and 28 after prime vaccination was reported in the preliminary report.[7] ELISA EC_{90} titres of the low-dose and high-dose vaccine groups fell gradually, with a geometric mean titre (GMT) of 489·8 (95% CI 295·3–812·3) and 1282·9

(998·6–1648·3) at day 56, 386·4 (252·7–591·0) and 856·8 (662·9–1107·4) at day 112, and 197·9 (107·9–362·7) and 575·5 (394·8–838·8) at day 168. No Ebola glycoprotein-specific response was observed in the placebo group. The GMT of the ELISA EC90 titres for the placebo group was 5·0 throughout the whole study period. The high-dose group showed significantly higher and more sustainable ELISA EC_{90} titres in terms of GMTs than did the low-dose group throughout the 6-month follow-up period. However, no difference in the proportion of the positive responders was found between the high-dose and low-dose groups. Presence of high pre-existing adenovirus type-5 neutralising antibodies not only weakened the vaccine-elicited glycoprotein-specific antibody responses, but also accelerated the decay of ELISA EC_{90} titres, especially in the participants receiving the low dose. On the contrary, participants with naive or low baseline anti-adenovirus type-5 neutralising antibodies showed only a slight waning of antibody titres, indicating a good durability of the antibody response after a single-dose vaccination. The Ebola glycoprotein-specific ELISA titres observed after the high-dose vaccination in the individuals with high baseline anti-adenovirus type-5 neutralising antibodies were similar to those observed after the low dose in those individuals with low baseline anti-adenovirus type-5 neutralising antibodies.

Positive responses of specific CD4 and CD8 T cells in participants receiving vaccines peaked at day 14 after prime vaccination, and then declined gradually during the following period of study (appendix p 3). No significant difference of the CD4 and CD8 T-cell responses was noted between the vaccine group and the placebo group at day 168. The medians of background subtracted spot-forming cells per 1×10^6 cells were weak, with only 5 (IQR 0·0–11·7) in the low-dose vaccine group and 10 (3·3–33·3) in the high-dose vaccine group at day 168 (figure 3). Double or triple cytokine positive specific CD4 and CD8 T-cell responses also decreased, but remained detectable in both vaccine groups up to 168 days after the prime vaccination (appendix p 4).

A boosting vaccination with homologous vaccine at month 6 after the prime immunisation elicited strong humoral immune responses to Ebola glycoprotein regardless of the pre-existing immunity to the adenovirus type-5 at the time of boost (figure 2 and appendix p 5). The glycoprotein specific antibodies to the boost dose were superior to those induced by a single shot of priming vaccination alone. At day 28 after boost vaccination, the ELISA EC_{90} titres rapidly rose by over 30 times to 6110 (95% CI 4705–7935) in the low-dose group and 20 times to 11 825 (8904–15 705) in the high-dose group compared with pre-boost (table 3). No Ebola glycoprotein-specific response was observed in the placebo group. The GMT of the ELISA EC90 titres for placebo group was 5.0 throughout the whole study period. The recipients of high-dose vaccine of $1·6\times10^{11}$ viral particles showed stronger postboost antibody titres than the recipients of low-dose vaccine did. However, Ebola glycoprotein-specific ELISA EC_{90} titres declined gradually after the peak, with GMTs of 674·1 (95% CI 505·9–898·2) in the low-dose vaccine group and 856·8 (573·8–1279·2) in the high-dose vaccine group at month 12 after boost, which were significantly higher levels than those at month 6 after initial vaccination. Contrarily, three high-dose recipients in the initial prime study who did not receive the booster injection showed ELISA EC_{90} titres ranging from 152 to 610 with an average of 285·5 at the same timepoint.

The specific T-cell responses quantified by interferon-γ (IFN-γ) showed a moderate increase after the booster injection, with 23·3 spot-forming cells (IQR 16·7–90·0) in the low-dose group and 95·0 spot-forming cells (63·3–196·7) in the high-dose group, which was significantly higher than those in the placebo group (figure 3). Although the booster elicited greater IFN-γ expression by T cells than that showed at day 168 before boosting, the magnitude of responses was significantly lower than that observed at day 28 after initial vaccination.

The adenovirus type-5 neutralising antibody titres against the vaccine vector peaked at day 14 after the priming vaccination, and then waned gradually during the next 6 months. Although the boost immunisation with a homologous vaccine also boosted the anti-adenovirus type-5 neutralising antibody titres, antibody titres after the boost were not as high as those after the initial dose and declined quickly during the following 12 months (appendix p 6–7).

Discussion

Our results suggest that the adenovirus type-5 Ebola virus vaccine of $1·6\times10^{11}$ viral particles was highly immunogenic for all participants, including those with a positive adenovirus type-5 baseline in healthy adults in China. Although a higher incidence of injection-site reactions was associated with the higher dose of the adenovirus 5-vectored Ebola vaccine, no severe safety concerns were raised. In a preclinical challenge study with non-human primates (NHPs) immunised with adenovirus 5-vectored Ebola vaccine, a titre of 1000 or higher showed 77% protection against death.[12] Assuming that the value of 1000 in the ELISA is the level of protection for human beings, the high-dose adenovirus type-5 Ebola vaccine of $1·6\times10^{11}$ viral particles was able to induce high glycoprotein-specific antibody response, indicating a significant protection to vaccinated human beings, at least for a short time (2–3 months based on these data).

The concerns ober the duration of protection against Ebola virus conferred by the vaccine were also first raised in NHPs challenge studies, which showed that the humoral and cellular immunity waned gradually over time, and protection was lost.[12–14] In our trials, we observed a significant waning of the glycoprotein-specific antibodies in vaccine recipients within 6 months after the initial vaccination. The vaccine-elicited immune response

against Ebola virus could also be compromised by the pre-existing neutralising antibodies against the vaccine vector of adenovirus type-5. Although a single-dose regimen of protective vaccine would be much preferable for reactive immunisation against Ebola outbreaks and easier to administer in outbreak regions, a boost immunisation strategy is recommended to prolong the protection. Three studies adopted heterologous prime-boost regimens, including two using chimpanzee adenovirus 3-vectored Ebola vaccine (ChAd3-EBO-Z) as prime and then boosted with modified vaccinia Ankara-vectored Ebola (MVA-BN-Filo) vaccine at the different dosages, and one using adenovirus type–26 vectored Ebola vaccine (Ad26.ZEBOV) or MVA-BN-Filo as prime and then boosted with the alternative vaccine.[15–17] Results from these studies showed that both heterologous vector vaccines regimens of ChAd3-EBO-Z priming and Ad26.ZEBOV priming could elicit strong immune responses to Ebola virus that were superior to those induced by single dose prime vaccination alone, but a relatively low response to MVA-BN-Filo prime schedule with Ad26.ZEBOV boost was noted.

We adopted a homologous prime-boost regimen in this booster study simply because the adenovirus type-5 Ebola virus vaccine is the only approved Ebola vaccine candidate for clinical trials in China. Considering that the boosting effect with a homologous vaccine could be affected by high anti-adenovirus type-5 neutralising antibodies elicited by the prime dose, the booster dose was administered at a 6-month interval to avoid the peak of antibodies against the vaccine vector induced by initial vaccination. After the booster vaccination, significant immunological memory responses with a large enhancement of glycoprotein-specific antibodies were found, which were significantly higher than non-boosted responses. However, the boosting effects to T-cell immunogenicity were relatively few.

Compared with the results from previous studies, heterologous prime-boost regimens consisting of two different viral vectors seemed to be more effective to minimise the negative effect on the response to the vectors and can elicit stronger cellular immune responses to the gene inserts than homologous regimens.[18–20] Therefore, the relatively weak boosting effect of T-cell response is a major concern about the homologous boosting regimen with adenovirus type-5 Ebola virus vaccine applied in our study. Although rodents and NHPs challenge studies showed that induction of both antibodies and CD8 T-cell responses was potentially protective against Ebola virus disease,[1,21] later studies found that the humoral immune response without CD8 T-cell response still provided high survival after Ebola virus challenge, suggesting Ebola glycoprotein-specific antibodies alone might be sufficient for protection.[22–24] Therefore, the magnitude of humoral immune responses enhanced by the homologous adenovirus 5-vectored booster in our study was quite encouraging. Additionally, the protective level of antibody needed for most human beings might not be as high as that in NHP challenging models, because human beings are likely to be infected with smaller quantities of virus via different routes in natural exposure to the Ebola virus. A lower antibody response might therefore still be protective for human beings. A vaccine might not be able to offer 100% protection, but protection around 50–70% might be sufficient to help control an Ebola outbreak.

A limitation of this study is that adenovirus type-5 vector vaccine platform could be compromised by the pre-existing immunity against the vector, since a large proportion of adults have pre-existing adenovirus type-5 immunity worldwide.[22–24] The presence of pre-existing anti-adenovirus type 5 neutralising antibodies could not only significantly blunt the vaccine-elicited specific humoral and cell response against Ebola glycoprotein, but also weaken the persistence of the vaccine-elicited immunity. However, our results suggested that the negative effect of pre-existing immunity to adenovirus type-5 vector could be partly relieved by increasing the dose of the adenovirus type-5 vector Ebola virus vaccine administered.

Another limitation of this study is that homologous prime-boost regimen is generally considered as less effective as a heterologous prime-boost regimen. Our absence of access to other Ebola virus vaccine candidates besides the adenovirus type-5 Ebola virus vaccine prevented us from studying the immunogenicity of a heterologous prime-boost regimen. Nevertheless, the homologous prime-boost regimen with the adenovirus type-5 Ebola virus vaccines seemed powerfully immunogenic in our study, showing ability to induce high and durable specific humoral responses, but not the cellular immunity responses. However, the 6-month interval between prime and boost might not be optimal, since the Ebola glycoprotein-specific antibodies measured in the vaccine recipients already declined to less than 1000 at day 112. These results might suggest a demand for a better immunisation regimen with a shorter prime-boost interval, but the effect of pre-existing adenovirus type-5 neutralising antibodies at different boosting timepoints should be considered. Although a longer prime-boost interval was associated with a higher boosting response, such a long-interval regimen might not facilitate vaccine deployment.[4] Therefore, the booster vaccination at other prime-boost intervals or with a heterologous Ebola vaccine such as DNA vaccine or recombinant subunit vaccine needs to be investigated in future studies.

Although the data presented here suggest acceptable safety and good immunogenicity of the adenovirus 5-vectored Ebola vaccine and the potential benefits from the boosting regimen as compared with single dose priming alone, results from this phase 1 study in a few healthy adults from a small area in China could be limited in generalisability. Since the safety and immunogenicity of this vaccine in the Ebola-risk population in Africa are more crucial, a phase 2 study of the adenovirus 5-vectored Ebola vaccine in Sierra Leone began in October, 2014.[25]

Contributors

F-CZ is principal investigator of this study. F-CZ designed the trials and the study protocols, and contributed to the critical review and revision of the report. J-XL contributed to the study design, data interpretation, drafting the manuscript, and revising the report. L-HH contributed to lead the laboratory analyses, data interpretation, and revising the report. F-YM, Y-MH, QL, KC, W-JW, RT, and J-LH participated in the site work, including the recruitment, follow-up, and data collection. S-PW, J-JX, and ZZ contributed to participate in the laboratory analyses, data interpretation, and literature search. RJ contributed to the vaccine management. WC supervised the whole process of study and took the responsibility for all the data both from the sites and laboratories. PL and LL contributed to the statistical analysis. All authors reviewed and approved the final version of the report.

Declaration of interests

Rong Jiang is an employee of Tianjin CanSino Biotechnology. All other authors declare no competing interests.

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References

1 WHO. Ebola virus disease outbreak. http://www.who.int/csr/disease/ebola/en/ (accessed Nov 22, 2016).

2 Henao-Restrepo AM, Longini IM, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet* 2015; **386:** 857–66.

3 Ledgerwood JE, Dezure AD, Stanley DA, et al. Chimpanzee adenovirus vector Ebola vaccine—preliminary report. *N Eng J Med* 2015; **373:** 775–76.

4 Martins KA, Jahrling PB, Bavari S, Kuhn JH. Ebola Virus Disease Candidate Vaccines Under Evaluation in Clinical Trials. *Expert Review of Vaccines* 2016; **15:** 1101–12.

5 Agnandji ST, Huttner A, Zinser ME, et al. Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe—Preliminary Report. *N Eng J Med* 2015; **374:** 1647–60.

6 De SO, Audran R, Pothin E, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. *Lancet Infect Dis* 2015; **16:** 311–20.

7 Zhu FC, Hou LH, Li JX, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet* 2015; **385:** 2272–79.

8 Pratt WD, Wang D, Nichols DK, et al. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vacc Immunol Cvi* 2010; **17:** 572–81.

9 Marzi A, Falzarano D. An updated Ebola vaccine: immunogenic, but will it protect? *Lancet* 2015; **385:** 2229–30.

10 Sprangers MC, Lakhai W, Koudstaal W, et al. Quantifying Adenovirus-Neutralizing Antibodies by Luciferase Transgene Detection: Addressing Preexisting Immunity to Vaccine and Gene Therapy Vectors. *J Clin Microbiol* 2003; **41:** 5046–52.

11 CFDA. The standard guidelines for adverse reactions grading of vaccine clinical trials. http://www.sda.gov.cn/WS01/CL0844/9350_5.html (accessed Dec 27, 2014).

12 Stanley DA, Honko AN, Asiedu C, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nature Med* 2014; **20:** 1126–29.

13 Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 2003; **424:** 681–84.

14 Sullivan NJ, Hensley L, Asiedu C, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. *Nature Med* 2011; **17:** 1128–31.

15 Ewer K, Rampling T, Venkatraman N, et al. A monovalent chimpanzee adenovirus Ebola vaccine boosted with MVA. *N Eng J Med* 2016; **374:** 1635–46.

16 Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia Ankara-vectored Ebola vaccines: a randomized clinical trial. *JAMA* 2016; **315:** 1610–23.

17 Tapia MD, Sow SO, Lyke KE, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis* 2016; **16:** 31–42.

18 Honda M, Wang R, Kong WP, et al. Different vaccine vectors delivering the same antigen elicit CD8+ T cell responses with distinct clonotype and epitope specificity. *J Immunol* 2009; **183:** 2425–34.

19 Rattokim S, Currier JR, Cox JH, et al. Heterologous prime-boost regimens using rAd35 and rMVA vectors elicit stronger cellular immune responses to HIV proteins than homologous regimens. *Plos One* 2012; **7:** e45840.

20 Reyessandoval A, Berthoud T, Alder N, et al. Prime-boost immunization with adenoviral and modified vaccinia virus Ankara vectors enhances the durability and polyfunctionality of protective malaria CD8+ T-cell responses. *Infect Immun* 2010; **78:** 145–53.

21 Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. *Nature Rev Microbiol* 2009; **7:** 393–400.

22 Dye JM, Herbert AS, Kuehne AI, et al. Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. *Proc Natl Acad Sci* 2012; **109:** 5034–39.

23 Qiu X, Audet J, Wong G, et al. Successful treatment of Ebola virus–infected Cynomolgus Macaques with monoclonal antibodies. *Sci Transl Med* 2012; **4:** 2824–32.

24 Wong G, Richardson JS, Pillet S, et al. Immune parameters correlate with protection against ebola virus infection in rodents and nonhuman primates. *Sci Transl Med* 2012; **4:** 158ra46.

25 Zhu FC, Alie HW, Hou LH, et al. Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2016; published online Dec 22. http://dx.doi.org/10.1016/S0140-6736(16)32617-4.