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ORIGINAL RESEARCH

Safety and Immunogenicity of Homologous Recombinant Adenovirus Type 5-Vectored COVID-19 Vaccine Booster Dose in Healthy Adults Aged 18–60 Years: a Single-Center, Open-Label Trial

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ABSTRACT

Introduction: The waning antibody levels several months after prime vaccination and the persistent epidemics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) around the world have generated great interest in the evaluation of a booster dose. We aimed to assess the safety and immunogenicity of a homologous booster dose of the recombinant adenovirus type 5-vectored coronavirus disease 2019 (COVID-19) vaccine (Ad5-nCoV).

Methods: In this trial, we recruited healthy adults aged 18–60 years who had received one dose of Ad5-nCoV vaccine (low, middle, or high dose) in the previous phase 1 trial approximately 6 months earlier, and then all participants received a booster dose of 5×10^{10} viral particles (low dose) intramuscularly. The primary outcome was the incidence of adverse reactions within 14 days after booster vaccination. The specific binding antibodies were measured by enzyme-linked immunosorbent assay and the neutralizing antibody responses were assessed with live SARS-CoV-2 and pseudovirus neutralization assay. The cellular immune responses were analyzed by enzyme-linked immunospot assay and intracellular cytokine staining.

Results: From September 26 to 28, 2020, 108 volunteers were recruited and 89 eligible participants (52% male) were enrolled and received a booster dose of Ad5-nCoV vaccine: 28 (31%) had received a low prime dose, 30 (34%) a middle prime dose, and 31 (35%) a high prime dose in the previous phase 1 trial. All participants were included in the safety analysis and immunogenicity was assessed in 88 (99%) participants. Twenty-three (82%) participants in the low prime dose group, 23 (77%) participants in the middle prime dose group, and 26 (84%) participants in the high prime dose group reported at least one adverse reaction within the first 14 days post booster. Pain at the injection site and fatigue

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were the most common adverse reactions. Most adverse reactions were mild or moderate in all groups and no vaccine-related severe adverse event was noted within 12 months after booster vaccination. Neutralizing antibodies increased moderately at day 14 and peaked at 28 days post booster. T cell responses were also boosted at 14 days after vaccination.

Conclusions: A homologous booster of Ad5-nCoV vaccine is well tolerated and immunogenic in healthy adults aged 18–60 years who had received a priming dose of Ad5-nCoV 6 months previously. The neutralizing antibodies against SARS-CoV-2 peaked at day 28 and specific T cell responses were noted at day 14 after booster. Ad5-nCoV vaccine can be considered as a homologous booster 6 months after a priming dose.

Trial registration: ClinicalTrials.gov, NCT04568811.

Keywords: SASR-CoV-2; Adenovirus-vectored vaccine; Homologous booster

Key Summary Points

Why carry out this study?

Waning antibody titers were observed over time compared to peak titers at 28 days after a single dose of the recombinant adenovirus type 5-vectored coronavirus disease 2019 (COVID-19) vaccine (Ad5-nCoV) in previous trials. Besides, persistent epidemics of SARS-CoV-2 around the world have generated great interest in the evaluation of a booster dose.

This study was designed to evaluate the safety and immunogenicity of a homologous booster in persons who received a single primary dose of Ad5-nCoV vaccine approximately 6 months earlier.

What was learned from the study?

A homologous booster of Ad5-nCoV vaccine, given at 6 months after the priming dose, was safe and well tolerated and effectively boosted the immune responses, including SARS-CoV-2-specific antibody titers and T cell responses, which probably provides a longer protection against SARS-CoV-2.

INTRODUCTION

The recombinant adenovirus type 5-vectored coronavirus disease 2019 (COVID-19) vaccine (Ad5-nCoV), with a single-shot immunization regimen, is effective against mild-to-severe COVID-19 [1]. Notably, waning antibody titers were observed over time compared to peak titers at 28 days after a single dose of Ad5-nCoV in previous trials, which might indicate a waning protection [2, 3]. In addition, the continuous epidemics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the emergence of SARS-CoV-2 variants have generated interest in assessment of a booster dose. In the recent study, a booster immunization (the third vaccination) of ChAdOx1 nCoV-19 vaccine was well tolerated and induced a strong boost to immune responses and antibody activity against variants [4]. Homologous boosters after Ad26.COV2.S priming had an acceptable safety profile and increased binding antibody levels and T cell responses [5]. A booster (the third dose) of BNT162b2 vaccine could prolong protection and further increase the breadth of protection [6]. mRNA-1273 boosters were safe and increased neutralization titers against key variants, including B.1.351, P.1, and B.1.617.2 [7]. However, the durability of protection and potential need for a homologous booster of Ad5-nCoV are unclear. In March 2020, we conducted the phase 1 trial of Ad5-nCoV, which was the world's first in-human COVID-19 vaccine clinical trial. The phase 1 trial involved 108 participants and included three dose groups. Results of the phase 1 trial showed Ad5-nCoV is tolerable and immunogenic in healthy adults aged 18–60 years. Based on this, a single-center, open-label trial was performed to evaluate the safety and immunogenicity of a homologous booster in persons who received a single primary dose of Ad5-nCoV vaccine approximately 6 months earlier [2].

METHODS

Study Design and participants

This open-label trial of Ad5-nCoV boosters was conducted in a single center in Wuhan (Hubei province, China). This trial was registered with ClinicalTrials.gov (NCT04568811).

Inclusion criteria include participants aged 18–60 years, who have received an Ad5-nCoV low/middle/high prime dose in the previous phase 1 trial (NCT04313127) approximately 6 months earlier [2], willing to complete the entire scheduled study procedures, and able to provide informed written consent prior to enrollment. Furthermore, all participants must be in general good health as established by physical examination (e.g., fingertip blood test negative for HIV antibodies, urine pregnancy test negative in women of childbearing age, axillary temperature ≤ 37 °C. People with major congenital defects, psychiatric disorders, serious cardiovascular diseases, or poorly controlled chronic illnesses were excluded. Women who were pregnant or breastfeeding were also excluded.

Ethical Approval

The trial protocol and informed consent form were reviewed and approved by the institutional review board of the Jiangsu Provincial Center of Disease Control and Prevention (JSJK2020-A055-01). This trial was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice.

Vaccine Regimen and Procedures

The Ad5-nCoV vaccine was developed by Beijing Institute of Biotechnology and CanSino Biologics Inc. The vaccine contained a non-replicating Ad5 vector expressing the full-length spike protein based on Wuhan-Hu-1 strain (RefSeq: YP_009724390). The Ad5-nCoV vaccine was manufactured as a liquid formulation containing 5×10^{10} viral particles (vp) per 0.5 mL in a vial.

All participants received a booster dose of 5×10^{10} vp by injection into the deltoid muscle and remained under observation for immediate adverse reactions within 30 min post vaccination. In the following 28 days, any injection site or systemic adverse events were self-recorded by vaccine recipients in the diary cards. The first to fifth visits were conducted at day 1, day 14, day 28, month 6, and month 12 after vaccination. At day 1, intravenous blood was collected for routine blood test and blood biochemical index examination to evaluate safety. Serious adverse events were documented throughout the study.

Blood samples were collected for immunogenicity detection at day 0 pre vaccination and at day 14, day 28, month 6, and month 12 post vaccination. Specific IgG antibody responses against SARS-CoV-2 receptor binding domain (RBD) were determined by using an enzyme-linked immunosorbent assay (ELISA) kit (Beijing Wantai BioPharm, Beijing, China) [2]. Neutralizing antibody responses were detected by using infectious SARS-CoV-2 virus (Wuhan-Hu-1, GenBank: MT291831.1) neutralization tests [8]. Pseudovirus neutralization antibodies were measured via a vesicular stomatitis virus pseudovirus system expressing the spike glycoprotein [9]. The levels of anti-Ad5 antibodies were analyzed by serum neutralization assay [10].

Blood samples from participants were drawn by venipuncture and collected in EDTA blood collection tubes. Peripheral blood mononuclear cells (PBMCs) were isolated for cellular immune response testing prior to vaccination (baseline) and 14 days after administration of the booster dose. An enzyme-linked immunospot assay (ELISpot) was used to assess specific cellular interferon gamma (IFNγ) responses with PBMC stimulated by overlapping peptide pools of spike glycoprotein. Results were reported as the number of spot-forming cells (SFC) per 10^5 PBMCs [11]. The cytokine profiles (including IFNγ and tumor necrosis factor alpha, TNFα) of $CD4^+$ and $CD8^+$ T cells were detected by spike-specific stimulation and intracellular cytokine staining (ICS). Results were displayed as the proportion of the specific event out of parent events [12].

Outcomes

The major objective was to assess the safety of an Ad5-nCoV vaccine booster in healthy adults aged 18–60 years and the main outcome was the incidence of adverse reactions within 14 days after booster vaccination. The secondary immunogenicity outcome was the evaluation of the levels of the specific antibodies at each visit and the assessment of the specific cellular responses at day 14 post booster. Geometric mean titer (GMT), geometric mean increase (GMI), and seroconversion rate of RBD-specific IgG antibody (ELISA) and neutralizing antibody were analyzed. Seroconversion was defined as an increase of the antibody titer post vaccination at least four times compared to baseline. The levels of Ad5 neutralizing antibody were assessed. Stratified analysis of immunogenicity was conducted on the basis of the pre-existing Ad5 neutralizing antibody titers (baseline): baseline $\leq$ 1:200 was defined as negative or low pre-existing Ad5 immunity and baseline > 1:200 as high pre-existing Ad5 immunity. The secondary endpoint for safety included the occurrence of adverse events within 28 days after booster, and serious adverse events reported up to 12 months. Adverse reactions and events were graded according to the scale issued by the China State Food and Drug Administration [13].

Statistical Analysis

The safety endpoints were assessed in the safety population, which included all participants who had received one dose of the vaccine. We assessed the immunogenicity endpoints in the full analysis population, which included all participants who received one dose of the vaccine and had at least one antibody titer after vaccination available.

Statistical analysis was performed using SAS version 9.4. For all statistical comparisons, a two-sided test was used. The significance level was set at $p < 0.05$. The descriptive statistics of the incidence of the adverse events/reactions were provided to assess the safety of the vaccine. Chi-square test or Fisher exact test was used to analyze categorical data. The levels of the antibodies were reported as geometric GMTs with 95% confidence intervals (CIs) and analysis of variance (ANOVA) was used to analyze the log-transformed antibody titers. The cellular immune responses were presented as the proportion of positive responders and Wilcoxon rank sum test was used for data with non-normal distribution. Repeated measurement analysis was used for multiple comparisons.

RESULTS

From September 26 to 28, 2020, 108 volunteers, who received an Ad5-nCoV prime dose 6 months earlier in the previous phase 1 trial, were recruited and screened for enrollment (Fig. S1). A total of 89 eligible participants, including low prime dose group (5×10^{10} vp, $n = 28$), middle prime dose group (1×10^{11} vp, $n = 30$), and high prime dose group (1.5×10^{11} vp, $n = 31$), were enrolled. The mean age of the participants was 38.0 years (SD 10.8), with 82 (92%) individuals aged 18–-55 years and 7 (8%) aged 56–60 years (Table 1). Forty-six (52%) of the 89 participants were male. Baseline characteristics did not differ considerably across the prime dose groups. The proportion of participants with high pre-existing Ad5 immunity was 64% (18/28) in the low prime dose group, followed by 50% (15/30) in the middle prime dose group, and 45% (14/31) in the high prime dose group, but there was no significant difference among the three prime dose groups ($p = 0.316$). All the participants received the booster dose and completed the scheduled safety observation within 28 days post vaccination. Eighty-eight (99%) individuals completed blood sample collection at day 14 and 28.

Seventy-two (81%) of the 89 participants reported at least one adverse reaction within the first 14 days after booster: 23 (82%) in the low prime dose group, 23 (77%) in the middle prime dose group, and 26 (84%) in the high prime dose group (Table 2). No significant difference in the number of adverse reactions within 14 days ($p > 0.05$) across the three prime groups was observed, nor in the overall number of

Table 1 Baseline characteristics

	Low prime dose group (n = 28)	Medium prime dose group (n = 30)	High prime dose group (n = 31)	Total (n = 89)
Age, years				
18–55	26 (93%)	27 (90%)	29 (94%)	82 (92%)
56–60	2 (7%)	3 (10%)	2 (6%)	7 (8%)
Mean	38.9 (10.8)	38.7 (11.4)	36.6 (10.5)	38.0 (10.8)
Sex				
Male	15 (54%)	17 (57%)	14 (45%)	46 (52%)
Female	13 (46%)	13 (43%)	17 (55%)	43 (48%)
Height, cm	165.6 (9.1)	166.4 (8.5)	165.9 (7.5)	166.0 (8.3)
Weight, kg	64.9 (10.8)	66.8 (11.2)	66.5 (12.1)	66.1 (11.3)
BMI, kg/m^2	23.6 (2.5)	24.0 (2.9)	24.1 (3.3)	23.9 (2.9)
Pre-exiting Ad5 neutralizing antibodies				
Mean GMT	208.1 (15.0)	134.0 (10.1)	113.9 (15.2)	145.5 (13.0)
≤ 200, titer	10 (36%)	15 (50%)	17 (55%)	42 (47%)
> 200, titer	18 (64%)	15 (50%)	14 (45%)	47 (53%)

Data are mean (SD) or number of participants (%)
BMI body mass index, *GMT* geometric mean titer, *Ad5* adenovirus type 5

adverse events within 28 days (p = 0.837). In all groups, most adverse reactions (> 60% grade 1) were mild or moderate: pain at the injection site and fatigue were the most common adverse reactions. Injection site induration in the individuals with prior high dose was the most prevalent (p = 0.008). No clinically significant abnormal changes in hematological indexes were observed at day 1. Two pregnancy events occurred within 12 months after booster, and no abnormal pregnancy outcome was observed. Besides, one severe adverse event was reported but not considered vaccine related, which was hospitalization for erysipelas in the right lower limb over 8 months after the booster dose, and this participant finally recovered after symptomatic treatment.

Specific binding antibody responses to RBD were determined via ELISA (Table 3). At day 28, the participants in the prior low dose group had a higher GMT (1124.7, 95% CI 762.8–1658.2) of binding antibody, compared with 543.5 (384.5–768.1) for the middle and 639.0 (476.1–857.6) for the high prime dose group. Meanwhile, 28 (100%) individuals in the prior low dose group, 28 (97%) in the prior middle dose group, and 30 (97%) in the high prime dose group had at least a fourfold increase (seroconversion) in binding antibody titers at day 28. No significant difference in

Table 2 Adverse reactions within 14 days and overall adverse events within 28 days after vaccination

	Low prime dose group (n = 28)	Medium prime dose group (n = 30)	High prime dose group (n = 31)	Total (n = 89)	p value
All adverse reactions within 0–14 days					
Any	23 (82%)	23 (77%)	26 (84%)	72 (81%)	> 0.05
Grade 1	21 (75%)	19 (63%)	23 (74%)	63 (71%)	0.543
Grade 2	2 (7%)	4 (13%)	3 (10%)	9 (10%)	0.830
Grade 3	0	0	0	0	–
Injection site adverse reactions within 0–14 days					
Any	21 (75%)	22 (73%)	24 (77%)	67 (75%)	0.933
Pain	18 (64%)	21 (70%)	20 (65%)	59 (66%)	0.87
Swelling	3 (11%)	3 (10%)	7 (23%)	13 (15%)	0.362
Induration	2 (7%)	0	7 (23%)	9 (10%)	0.008
Redness	3 (11%)	1 (3%)	2 (6%)	6 (7%)	0.514
Itch	1 (4%)	1 (3%)	2 (6%)	4 (4%)	> 0.999
Discomfort	0	1 (3%)	0	1 (1%)	0.652
Muscular weakness	1 (4%)	0	0	1 (1%)	0.315
Systemic adverse reactions within 0–14 days					
Any	18 (64%)	12 (40%)	12 (39%)	42 (47%)	0.091
Fatigue	13 (46%)	8 (27%)	6 (19%)	27 (30%)	0.068
Headache	6 (21%)	5 (17%)	2 (6%)	13 (15%)	0.235
Fever	3 (11%)	2 (7%)	5 (16%)	10 (11%)	0.543
Myalgia	3 (11%)	2 (7%)	2 (6%)	7 (8%)	0.789
Nausea	1 (4%)	1 (3%)	2 (6%)	4 (4%)	> 0.999
Appetite impaired	1 (4%)	2 (7%)	1 (3%)	4 (4%)	0.84
Diarrhea	1 (4%)	2 (7%)	0	3 (3%)	0.414
Oropharyngeal pain	0	1 (3%)	2 (6%)	3 (3%)	0.771
Cough	0	1 (3%)	0	1 (1%)	0.652
Dizziness	1 (4%)	0	0	1 (1%)	0.315
Pruritus	0	0	1 (3%)	1 (1%)	> 0.999
Overall adverse events within 28 days					
Any	24 (86%)	24 (80%)	26 (84%)	74 (83%)	0.837

Table 2 continued

	Low prime dose group (n = 28)	Medium prime dose group (n = 30)	High prime dose group (n = 31)	Total (n = 89)	p value
Grade 3	0	0	0	0	-

Data are number of participants (%). Any refers to all the participants with any grade adverse reactions or events. Adverse reactions and events were graded according to the scale issued by the China State Food and Drug Administration. The p value was for the differences across all prime dose groups

seroconversion rate ($p > 0.999$) was shown across the three groups. Neutralizing antibodies against live SARS-CoV-2 had a moderate increase at day 14 compared to baseline and peaked at 28 days after booster. The GMTs of neutralizing antibody were noted as 101.8 (65.7–157.7), 42.5 (30.2–59.8), and 58.9 (41.3–84.0) in the low, middle, and high prime dose group at day 28, respectively. Eighty (91%) of 88 individuals experienced at least a fourfold increase of neutralizing antibody titers: all 28 recipients (100%) in the low prime dose group, 24 (83%) of 29 in the middle prime dose group, and 28 (90%) of 31 in the high prime dose group on day 28. For neutralizing antibodies against pseudovirus, all three groups had 100% seroconversion both at days 14 and 28. ELISA binding antibodies to RBD showed positive correlation of 0.766 with neutralizing antibodies against live virus and the association between binding antibodies and neutralizing antibodies to pseudovirus was 0.845 at day 28 (Fig. S2).

Interestingly, all participants (100%) with low pre-existing Ad5 immunity prior to vaccination had a fourfold increase in neutralizing antibody titers at 28 days after booster, whereas 18 individuals (100%) in the low prime dose group, 9 (64%) of 14 in the middle prime dose group, and 11 (79%) of 14 in the high prime dose group, who had high pre-existing Ad5 immunity prior to vaccination, had at least a fourfold increase in neutralizing antibody titers at 28 days after booster. This showed that high pre-existing anti-Ad5 vector immunity moderately impaired the effectiveness of antibody responses, albeit to a fairly acceptable level (Table S1). Moreover, the levels of Ad5 neutralizing antibodies significantly improved post booster (Table S2).

IFNγ of specific T cell responses was determined with ELISpot as the number of SFCs per 10^5 cells. The proportions of ELISpot-positive responses ranged from 86% to 94% across the three prime dose groups at 14 days post booster with a median number of SFCs per 10^5 cells of 17.5 (interquartile range (IQR) 10.0–29.8) in the prior low dose group, 15.0 (7.0–21.0) in the prior middle dose group, and 14.0 (6.0–22.0) in the prior high dose group (Fig. 1). No significant difference was shown across the three groups ($p = 0.604$). Compared to those with low Ad5 neutralizing antibodies at baseline, high pre-existing Ad5 immunity slightly reduced the proportion of ELISpot-positive responders after booster: 16 (89%) of 18 participants in the low prime dose group, 11 (79%) of 14 in the middle prime dose group, and 13 (93%) of 14 in the high prime dose group, who had high Ad5 neutralizing antibody titers prior to vaccination, were identified as positive responders at day 14.

IFNγ and TNFα expression was detected by ICS. The booster vaccination at day 14 enhanced the specific IFNγ and TNFα responses in $CD4^+$ T cells in all groups, but no significant difference was found with an overall p value of 0.357 for IFNγ responses (0.634 for TNFα) across the three prime dose groups (Fig. 2). Similar $CD8^+$ T cell responses were observed. The further stratified analysis showed that the pre-existing anti-Ad5 neutralizing antibody had an acceptable effect on IFNγ and TNFα responses in $CD4^+$ and $CD8^+$ T cells (Fig. S3).

Besides, because COVID-19 cases were sporadic in China at that time, no natural SARS-

Table 3 Specific antibody responses to RBD, neutralizing antibodies against live SARS-CoV-2 and pseudovirus-based neutralizing antibodies

	Day 14				Day 28			
	Low prime dose group (n = 28)	Medium prime dose group (n = 29)	High prime dose group (n = 31)	p value	Low prime dose group (n = 28)	Medium prime dose group (n = 29)	High prime dose group (n = 31)	p value
Binding antibodies to RBD								
GMT	1557.2 (951.3–2549.0)	627.1 (446.3–881.1)	866.2 (625.3–1200.0)	0.005	1124.7 (762.8–1658.2)	543.5 (384.5–768.1)	639.0 (476.1–857.6)	0.008
≥ Fourfold increase	28 (100%)	28 (97%)	30 (97%)	> 0.999	28 (100%)	28 (97%)	30 (97%)	> 0.999
Neutralizing antibodies against live SARS-CoV-2								
GMT	74.4 (46.8–118.0)	36.2 (25.9–50.6)	52.9 (38.0–73.7)	0.028	101.8 (65.7–157.7)	42.5 (30.2–59.8)	58.9 (41.3–84.0)	0.005
≥ Fourfold increase	24 (86%)	23 (79%)	27 (87%)	0.761	28 (100%)	24 (83%)	28 (90%)	0.071
Neutralizing antibodies against pseudovirus								
GMT	519.5 (344.8–782.7)	227.9 (165.5–313.7)	306.6 (222.7–422.2)	0.004	426.1 (298.2–608.9)	207.3 (152.7–281.4)	255.0 (188.5–345.0)	0.006
≥ Fourfold increase	28 (100%)	29 (100%)	31 (100%)	–	28 (100%)	29 (100%)	31 (100%)	–

*R*Data are geometric mean (95% CI) or number of participants (%). The p value was for the differences across all prime dose groups
BD receptor binding domain, *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2, *GMT* geometric mean titer

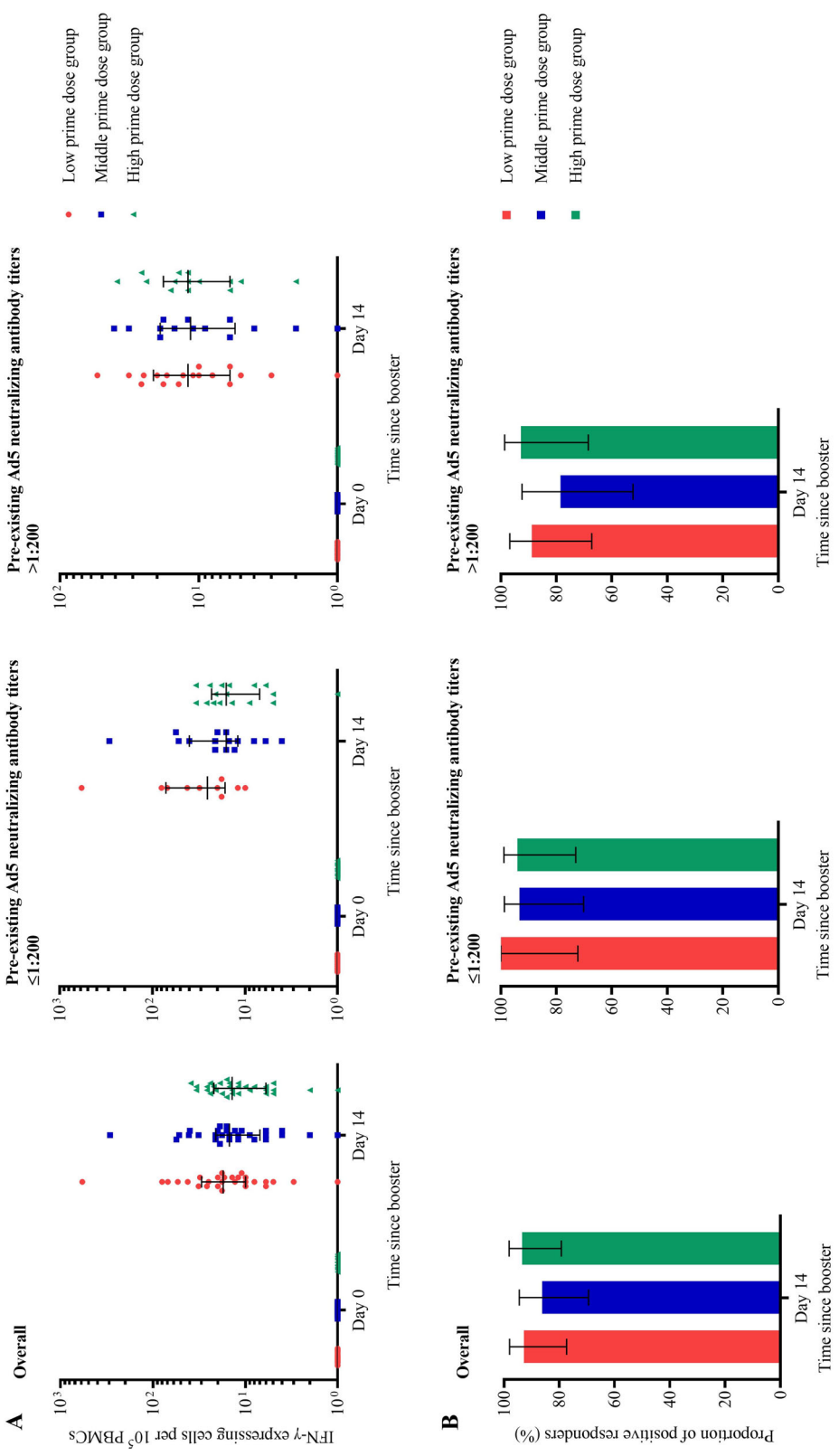

Fig. 1 IFNγ of specific T cell responses measured by ELISpot. **a** Number of IFNγ-secreting cells per 10^5 PBMCs at day 0 and 14 in all participants and stratified by the pre-existing anti-Ad5 neutralizing antibody titers. **b** Proportion of positive responders at day 14 post booster in all participants and stratified by the pre-existing anti-Ad5 neutralizing antibody titers. Error bars are interquartile ranges (IQRs). *ELISpot* enzyme-linked immunospot assay, *IFN*γ interferon gamma, *PBMCs* peripheral blood mononuclear cells, *Ad5* adenovirus type 5

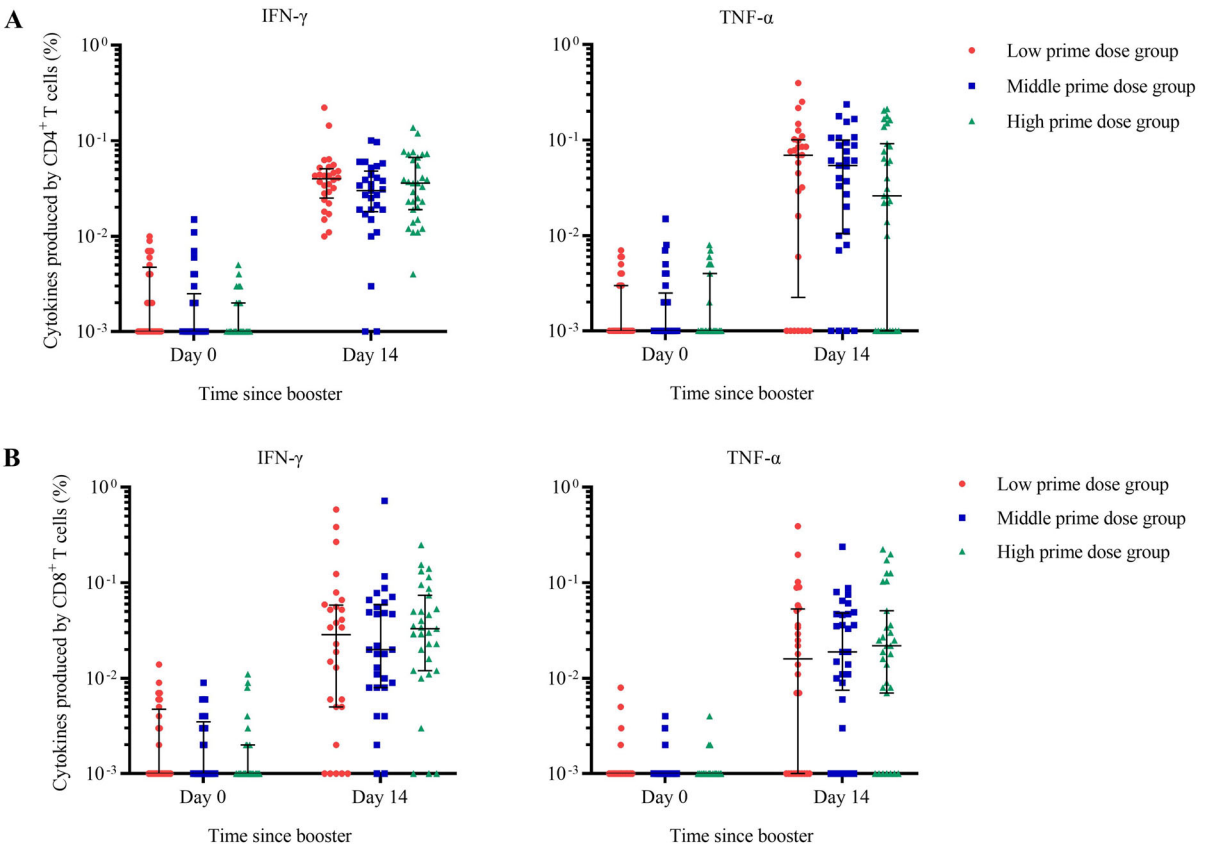

Fig. 2 Spike protein-specific IFNγ and TNFα responses in $CD4^+$ and $CD8^+$ T cells detected by ICS. **a** Percentage of $CD4^+$ T cells producing IFNγ and TNFα at day 0 and 14. **b** Percentage of $CD8^+$ T cells producing IFNγ and TNFα at day 0 and 14. Error bars are interquartile ranges (IQRs). *ICS* intracellular cytokine staining, *IFNγ* interferon gamma, *TNFα* tumor necrosis factor alpha

CoV-2 infection was found during the study period.

DISCUSSION

In this trial, we examined the safety and immunogenicity of homologous boosters in persons aged 18–60 years who had been vaccinated about 6 months previously with the authorized dose of Ad5-nCoV. The outcomes were evaluated post booster and indicated that local adverse reactions and systemic events, similar to those after the prime dose, were predominantly mild to moderate. However, the safety of the booster vaccination should be evaluated after several booster doses in long-term trials or real-world studies. Homologous boosters of Ad5-nCoV led to an increase in spike-specific binding antibodies, neutralizing antibodies against infectious SARS-CoV-2 and pseudovirus by 28 days after vaccination, which were higher than those within the corresponding period after the first dose, due to the maintained and effective responses of antigen-specific immunological memory [2]. These results were similar to the performances of the booster vaccinations of another replication-deficient recombinant adenovirus type 26-vectored SARS-CoV-2 vaccine Ad26.COV2.S [5]. In addition, T cells kill the infected cells, control the inflammatory milieu, and facilitate the humoral immune responses through a variety of effector mechanisms [14]. High proportions of positive T cell responders were observed across the three prime dose groups after booster.

Spike-specific $CD4^+$ and $CD8^+$ T cell responses were also activated. The effect of the pre-existing anti-Ad5 neutralizing antibodies on T cell responses was weak.

Overall, pre-existing Ad5 immunity had a moderate effect on the immune responses to booster administration, which are consistent with the results of the priming dose vaccination [2]. Both in participants with low and high pre-existing anti-Ad5 neutralizing antibody titers, the low prime dose group showed the strongest SARS-CoV-2-specific antibody responses along with the highest induced anti-Ad5 antibodies post booster across the three prime dose groups, which is probably because low primary dose vaccination had induced the lowest Ad5 immunity responses after the first injection and therefore the expression of Ad5-vectored spike antigen after booster was impaired at a minimum. The inhibitory effect of pre-existing Ad5 immunity on the immune responses of booster is worth continuing to explore.

A potential limitation of our trial is that the boosters were administered 6 months after the primary dose, as obviously waning antibody levels in prior trials and pre-existing Ad5 immunity usually decline significantly 6 months after the latest vaccination, but the optimum prime–boost interval remains to be determined. The results of trials of ChAdOx1 nCoV-19 and Ad26.COV2.S vaccines showed that longer dose intervals might induce a higher responses than shorter intervals [4, 5]. Thus, further evaluations of the booster vaccination with longer intervals should be considered in trials with longer follow-up. Furthermore, data indicated effective neutralizing antibodies against wild-type SARS-CoV-2 after booster, but a paucity of data of neutralizing antibodies to variants of concern (VOCs) and variants of interest (VOIs) which were predominant after the inception of our trial. Reported booster vaccinations of ChAdOx1 nCoV-19, BNT162b2, and mRNA-1273 vaccines induced some degree of neutralizing antibody titers against different VOCs and VOIs, which might play a key role in cross-protection [4, 6, 7]. Data evaluating effective neutralizing antibodies against VOCs or VOIs after Ad5-nCoV booster should be analyzed in future studies. Finally, as the sample size of our trial is relatively small, the conclusions drawn from our trial need to be further verified in future studies with larger sample size.

CONCLUSIONS

A homologous booster of Ad5-nCoV vaccine, given at 6 months after the priming dose, was safe and well tolerated and effectively boosted the immune responses, including SARS-CoV-2-specific antibody titers and T cell responses, which probably provides a longer protection against SARS-CoV-2. Ad5-nCoV can be considered as a homologous booster 6 months after a priming dose.

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Data Availability. Researchers who provide a scientifically sound proposal are allowed to access to the de-identified individual participant data. Individual participant data can be obtained with a request to wangwj@jscdc.cn, houlihua@sina.com, or jszfc@vip.sina.com.

Declarations

Conflict of Interest. Xue Wang, Ge Guo, Ying Wang, and Jingxuan Wan are employees of CanSino Biologics. Siyue Jia, Jinlong Zhang, Zhe Zhang, Busen Wang, Jun Zhang, Hudachuan Jiang, Wenjuan Wang, Lihua Hou and Fengcai Zhu have no competiting interests.

Ethical Approval. The trial protocol and informed consent form were reviewed and approved by the institutional review board of the Jiangsu Provincial Center of Disease Control and Prevention (JSJK2020-A055-01). This trial was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice.

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