Safety and immunogenicity of a recombinant adenovirus type-5-vectored COVID-19 vaccine with a homologous prime-boost regimen in healthy participants aged 6 years and above: a randomised, double-blind, placebo-controlled, phase 2b trial

Fengcai Zhu[1,2,a], Pengfei Jin[1,a], Tao Zhu[3,a], Wenjuan Wang[1,a], Huayue Ye[4,a], Hongxing Pan[1], Lihua Hou[5], Jingxin Li[1,2], Xue Wang[3], Shipo Wu[5], Ying Wang[3], Jinbo Gou[3], Haitao Huang[3], Hongbin Wu[4], Xuewen Wang[6], Wei Chen[5]

[1] Vaccine Clinical Evaluation Department, Jiangsu Province Center for Disease Control and Prevention, Nanjing, China

[2] NHC Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Province Center for Disease Control and Prevention, Nanjing, China

[3] CanSino Biologics, Tianjin, China

[4] Taizhou Center for Vaccine Clinical Research, Taizhou, China

[5] Beijing Institute of Biotechnology, Beijing, China

[6] Shanghai Canming Medical Technology, Shanghai, China

F.Z., P. J., T. Z., W. W., H. Y. contributed equally to this article.

Corresponding author: Wei Chen, Beijing Institute of Biotechnology, Beijing 100071, China (cw0226@foxmail.com)

Summary：Ad5-vectored COVID-19 vaccine with a single dose was safe and tolerated, and induced robust immune responses in children and adolescents aged 6-17 years. The boosting effect of the homologous prime-boost regime apart 56 days on immune responses was limited.

Abstract

Background. We assessed the safety and immunogenicity of a recombinant adenovirus type-5 (Ad5)-vectored COVID-19 vaccine with homologous prime-boost regimens in healthy participants aged 6 years and above.

Methods. In this randomised, double-blind, placebo-controlled trial, participants received low-dose vaccine, middle-dose vaccine or placebo. Prime-booster regimens were given intramuscularly 56 days apart. ELISA antibodies to the receptor binding domain (RBD) and pseudovirus neutralising antibodies were detected. Adverse events were monitored for 28 days following each vaccination.

Results. A total of 430 participants were enrolled in the study, with 30 participants aged 18-55 years (MID cohort), 250 participants aged 56 years and older (OLD cohort), and 150 participants aged 6-17 years (MIN cohort). Ad5-vectored COVID-19 vaccine induced significant RBD-specific ELISA antibodies which decreased with increasing age, with geometric mean titres (GMTs) of 1037.5 in MIN cohort, 647.2 in MID cohort, and 338.0 in OLD cohort receiving 5×10^{10} viral particles on day 28 following boost vaccination. Pseudovirus neutralising antibodies showed a similar pattern, with GMTs of 168.0 in MIN cohort, 76.8 in MID cohort, and 79.7 in OLD cohort. A single dose in children and adolescents induced higher antibody responses than that elicited by two doses in adults, with

GMTs of 1091.6 and 96.6 in ELISA antibody and neutralising antibody, respectively. Homologous prime-boost vaccination was safety and tolerable.

Conclusions. Ad5-vectored COVID-19 vaccine with a single dose was safe and induced robust immune responses in children and adolescents aged 6-17 years. A prime-boost regimen needs further exploration for Ad5-vectored COVID-19 vaccine.

Clinical Trials Registration. NCT04566770

Key words: SARS-CoV-2; adenovirus type-5-vectored COVID-19 vaccine; phase 2b trial; homologous prime-boost

As of Jun 29, 2021, the coronavirus disease 2019 (COVID-19) pandemic has caused more than 181 million infections and over 3.9 million death globally [1]. To bring this pandemic to an end, widespread vaccination programs have commenced in some countries [2]. Although children and adolescents generally have milder COVID-19 than adults, severe illness can occur in this population, especially in those with underlying medical conditions. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can lead to a serious complication called multisystem inflammatory syndrome in children [3, 4]. In addition, children and adolescents play an important role in SARS-CoV-2 transmission, and their vaccination may contribute to herd immunity [5, 6]. Currently, only three COVID-19 vaccines have been authorized for emergency use in children and adolescents, with two inactivated SARS-CoV-2 vaccines (Corona Vac, BBIBP-CorV) and BNT162b2 mRNA vaccine [7].

Ad5-vectored COVID-19 vaccine is a replication defective Ad5 vectored vaccine expressing the spike glycoprotein of SARS-CoV-2, which induced significant immune responses in adults aged 18 years or older after a single vaccination with 5×10^{10} viral particles [8]. The interim analysis data of the phase III clinical trial ongoing showed that a single vaccination could provide vaccine efficacy of 65.3% and 90.1% at preventing symptomatic and severe COVID-19, respectively [9]. Prime-boost immunization might be a potential solution to provide enhancement of immune responses with longer duration, according to our previous experience with an Ad5 vector-based Ebola vaccine [10]. Notably, pre-existing immunity against vaccine vector could reduce the immunogenicity of candidate vaccine. Considering that the potential effect of pre-existing anti-Ad5 neutralising antibodies on the immunogenicity and the need to enhance immune responses, we selected a homologous prime-boost regimen with a 56-day interval.

Here we present the safety and immunogenicity results of Ad5-vectored COVID-19 vaccine with a homologous prime-boost regimen in healthy participants aged 6 years and above.

Method

Study design and participants

We did a single-center, randomised, double-blind, placebo-controlled, phase 2b trial of the Ad5-vectored COVID-19 vaccine in Taizhou, Jiangsu province, China. Healthy subjects aged 6 years and above were enrolled in an age-sequential manner, into younger adults aged 18-55 years cohort (MID cohort), and older adults aged 56 years and older cohort (OLD cohort), and children and adolescents aged 6-17 years cohort (MIN cohort).

The participants were enrolled in four stages: Stage 1, participants in MID cohort; Stage 2, sentinel participants aged 56 years and older, and aged 13-17 years; Stage 3, nonsentinel participants for stage 2 cohorts, and sentinel participants age 6-12 years; Stage 4, nonsentinel participants age 6-12 years. A minimum of 2 weeks of safety data was reviewed by the Data Safety Monitoring Board (DSMB) before enrollment to each successive stage. 28 October 2020, safety data review in Stage 2 indicated that 5 (16.7%), 3 (10.0%) of 30 sentinel participants aged 13-17 years developed Grade 2 fever and headache. To raise the tolerability of Ad5-vectored COVID-19 vaccine in children and adolescents, DSMB recommended that the dosage of vaccine decreased from 0.5 mL to 0.3 mL in children and adolescents not yet enrolled.

The inclusion and exclusion criteria are listed in the Supplementary Table 1. The protocol and informed consent were approved by the institutional review board of the Jiangsu Provincial Center of Disease Control and Prevention. Written informed consent was obtained

from all participants before screening. The trial is being done in accordance with the Declaration of Helsinki and Good Clinical Practice.

Randomisation and masking

In MIN and MID cohorts, participants were randomly assigned (2:1) to low-dose group or placebo group. For OLD cohort, participants were randomly assigned (2:2:1) to middle-dose group, low-dose group or placebo group. The placebo contained the same excipients as the vaccine, with no viral particles. The experimental vaccines and placebos had identical packaging with a randomisation number on each vial as the only identifiers.

Randomisation lists, using block randomisationstratified by study cohort, was generated by an independent statistician using SAS software (version 9.4). Block sizes were chosen to align with the study cohort sizes. Individuals involved in randomisation and masking had no involvement in the rest of the trial. Investigators, participants, and laboratory staff were masked to treatment assignment.

Procedures

Ad5-vectored COVID-19 vaccine was produced as previously described [11], and provided in prefilled syringes containing 5×10^{10} viruses particles per 0.5 mL. Prime-booster regimens were given intramuscularly 56 days apart. In MIN and MID cohorts, a single shot was allocated with one viral of vaccine or placebo in the arm. The participants in OLD cohort received a double-shot regimen, with one viral of vaccine in each arm in middle-dose group, one viral of vaccine in one arm and one viral of placebo in other arm in low-dose group, and one viral of placebo in each arm in placebo group.

Participants were monitored for 30 min post vaccination for any immediate adverse reactions. Participants were instructed to record any injection site or systemic adverse events

within 14 days after each dose on paper diary cards. We followed up and verified adverse events on day 14 and 28 post each vaccination on trial site. For sentinel participants in each cohort, laboratory safety tests were measured before enrollment and on day 4 post each dose to assess any toxic effects. Serious adverse events self-reported by participants were documented throughout the study. We graded adverse events and abnormal changes in laboratory tests according to the scale issued by the China State Food and Drug Administration (version 2019). If a subject develops fever accompanied by respiratory symptoms, he/she will be instructed to seek medical attention and notify study staff. For the suspected COVID-19, the participant will have a nasal/throat swab taken for PCR test.

Blood samples were taken from participants for serology tests at Day 0 (immediately before the prime dose), Day 28, Day 56 (immediately before the boost dose), Day 84 (day 28 after the boost dose), and at month 6 post boost vaccination. Peripheral blood mononuclear cells were isolated form whole blood at Day 0, and at 28 days after each vaccinations. The detailed methods of the assays have been reported previously [11]. We assessed binding antibody responses against the receptor binding domain (RBD) using ELISA kits (Beijing Wantai BioPharm, Beijing, China), and the neutralising antibody responses using pseudovirus neutralisation test (a vesicular stomatitis virus pseudovirus system expressing the spike glycoprotein) [12]. The cellular immune responses of the expression of interferon (IFN) γ, interleukin-2 (IL-2), IL-4, IL-5 and IL-13 stimulated by the overlapping peptide pool of spike glycoprotein were detected by enzyme-linked immunospot (ELISpot) assay (Mabtech, Stockholm, Sweden). Anti-Ad5 neutralising antibody titres were measured with the serum neutralisation assay [13].

Outcomes

The primary endpoints were the incidence of adverse reactions within 14 days after each vaccination, and the GMTs of RBD-specific ELISA antibodies and pseudovirus neutralising antibodies on day 28 after boost vaccination. The secondary endpoints included: (1) Adverse events within 28 days after each vaccination, and serious adverse events reported up to 6 months, and any abnormal changes in laboratory measures at 4 days after each vaccination for sentinel participants; (2) GMT and seroconversion rates of RBD specific ELISA antibody and pseudovirus neutralising antibody at day 28 after prime vaccination, and at month 6 post boost vaccination, and specific T-cell responses at 28 days after prime and boost vaccinations.

Statistical analysis

We assumed that antibody responses induced by Ad5-vectored COVID-19 vaccine in children and adolescents, and older adults were no lower than that in younger adults. Non-inferiority margin was set to 0.5 for the geometric mean tire ratio. We calculated that 86 participants receiving vaccine would be needed with power of 90%, assuming an SD (log10 units) of 0.6 for the geometric mean titres. Give the possibility of dropouts, the sample size in MIN and OLD cohort was 150 and 250, respectively.

The primary immunogenicity analysis was done in the pre-protocol cohort, including all participants who received two dose of vaccine and donated at least a blood sample post prime-boost vaccination, and the safety analysis set included all enrolled participants who received at least one dose of vaccine. We used the χ^2 test or Fisher's exact test to analyze categorical data, ANOVA to analyze the log transformed antibody titres. Multiple comparisons were done if a significant difference across the treatment groups was noted,

using Student Newman-Keuls test or Bonferroni adjusted α value when relevant. Statistical tests were two-sided with an α value of 0·05, and analyzed using SAS version 9.4.

Results

Between September 24 and November 28, 2020, 666 volunteers were recruited and screened for eligibility (Figure 1). 430 participants were enrolled in the study and randomly assigned to vaccine or placebo group: 30 participants in MID cohort, 250 participants in OLD cohort, and 150 participants in MIN cohort. Among 100 participants in vaccine group of MIN cohort, 20 participants received 5×10^{10} virus particles dose of vaccine, and 80 participants received 3×10^{10} virus particles dose of vaccine. All participants were randomly assigned to receive the prime vaccination. Among them, 2 participants (low-dose group in OLD cohort) did not continue to receive the boost dose and were excluded from immunology analyses. Baseline characteristics of the participants are shown in Table 1.

Safety

Within 14 days after prime and boost vaccination with low-dose Ad5-vectored COVID-19 vaccine, at least one adverse reaction (AR) was reported by 69 (69%) of 100 participants in MIN cohort, 14 (70.0%) of 20 participants in MID cohort, and 39 (39.0%) of 100 participants in OLD cohort. 40 (40.0%) of 100 participants in OLD cohort receiving the middle-dose vaccine reported at least one AR, similar to those who received the low-dose vaccine (Figure 2). In each cohort, the rate of AR in vaccine group was significantly higher than that in the placebo group (MIN cohort, 22.0% (11/50), $p<0.001$; MID cohort 10.0% (1/10), $p=0.002$; OLD cohort, 16.0% (8/50), $p=0.008$). Most of the reported ARs was mild or moderate in severity. The most common ARs were injection-site pain, fever, headache and fatigue. Grade 3 fever was the only severe AR, and was reported in 4% (4/100) participants of MIN cohort,

10% (2/20) participants of MID cohort, and 1.5% (3/200) participants of OLD cohort. Fewer ARs were reported following boost vaccination than prime vaccination. (Supplementary Table 2-4).

Overall, 82 (82.0%) participants in MIN cohort, 15 (75.0%) participants in MID cohort, and 107 (53.5%) participants in LOD cohort experienced at least one or more adverse event within 28 days after vaccination. As of 25 June, 2021, 9 serious adverse events occurred during the study period, one of which was related with vaccine consisting of a diagnosis of gastrointestinal disorder. The participant aged 11 years old developed Grade 2 abdominal pain on day 2 after boost vaccination and required hospitalization due to exacerbation symptom on day 8. The participant recovered after hospitalization for 6 days and was clinically well throughout the study. All these observed abnormalities in laboratory measures were asymptomatic and clinically irrelevant to the vaccine (Supplementary Table 5). There was no COVID-19 outbreak in trial site during the study, and no cases of SARS-CoV-2 infection were reported.

Immunogenicity

At day 28 following prime vaccination with low-dose Ad5-vectored COVID-19 vaccine, RBD-binding ELISA titres increased significantly, with GMTs of 1091.6 (95% CI: 873.7, 1363.7) in MIN cohort, and 607.8 (373.9, 987.9) in MID cohort, and 169.8 (130.1, 221.7) in OLD cohort. (Figure 3A; Supplementary Table 6). A slight decrease of RBD-binding ELISA titres was seen at day 56 in each age cohort. At day 28 after boost injection, participants in both MIN and MID cohort had similar RBD-binding ELISA titres as the prime vaccination, with GMTs of 1037.5 (889.3-1210.5) and 647.2 (399.3-1049.0), respectively. In OLD cohort, boost vaccination led to a significant increase in RBD-binding ELISA titres, with GMT of 338.0 (263.0, 434.4) on day 84.

In general, neutralising antibodies to pseudovirus showed a similar pattern of RBD-binding ELISA antibodies, and decreased with increasing age (Figure 3B). Compared with the prime vaccination, boost vaccination elicited higher neutralising antibody (boost *vs* prime dose: MIN cohort, 168.0 (95% CI: 143.3, 197.1) *vs* 96.6 (76.8, 121.4); MID cohort, 76.8 (52.4, 112.7) *vs* 45.1 (24.8, 81.8); OLD cohort, 79.7 (64.9, 98.0) *vs* 35.8 (28.4, 45.1)). Seroconversion rate post boost injection was range 88.8 to 100.0% and 86.7 to 98.0% for RBD-binding antibody and pseudovirus neutralising antibody responses, respectively (Table 2). Within OLD cohort, no significant differences were observed between middle-dose and low-dose group in antibody responses post boost vaccination. Participants with low pre-existing anti-Ad5 immunity had antibody responses to SARS-CoV-2 that were approximately two-times higher than those with high pre-existing anti-Ad5 immunity (Supplementary table 7). Ad5 neutralising antibody titres immediately before boost vaccination were negatively correlated with RBD-binding antibody and pseudovirus neutralising antibody titres 28 days after boost vaccination (Supplementary Table 8-9).

Ad5-vectored COVID-19 vaccine induced significant specific T -cell responses measured by ELISpot at day 28 post prime vaccination that reflected mainly in Th1 cell responses (Figure 4). Of those, a median of 120.0 (IQR: 50.0, 300.0) spot-forming cells with secretion of IFN-γ per 1×10^6 peripheral blood mononuclear cells (PBMCs) in MIN cohort, 220 (150.0, 640.0) in MID cohort and 80.0 (40.0, 210.0) in low-dose group of OLD cohort , respectively, were observed at day 28 (Supplementary Table 11). In participants receiving the low-dose vaccine, 151 (69%), 95 (44%), 41 (19%), 39 (18%)and 20 (9%) of 218 participants across all age cohort showed positive IFN-γ, IL-2, IL-4, IL-5, and IL-13 ELISpot responses on day 28, respectively (Supplementary Figure 2). ELISpot T cell responses were not significantly different between low-dose and middle-dose group in OLD cohort. Additionally, ELISpot T-cell responses did not further increase after boost vaccination.

Discussion

To our knowledge, this is the first report on the safety and immunogenicity of adenovirus vectored COVID-19 vaccine in children and adolescents. Our findings show that Ad5-vectored COVID-19 vaccine was safe and tolerated in children and adolescents as well as adults. Older adults had a lower side-effect profile than younger adults. The most common adverse reactions were injection-site pain, fever and headache, in line with the results of our phase 1 and 2 study [8, 11]. In general, fewer adverse reactions were reported after boost vaccination than after prime vaccination. In this study, a suspected unexpected serious adverse reaction (SUSAR), diagnosed as gastrointestinal disorder, was reported in participants aged 6 to 13 years. In addition, vaccine-induced immune thrombotic thrombocytopaenia (VITT) is a rare adverse effect, which has been reported after COVID-19 adenoviral vector vaccination [14,15]. Hence, we need to carefully monitor SUSARs and rare but serious adverse events to ensure the safety of Ad5-vectored COVID-19 vaccine in the phase 3 trial and post-marketing safety surveillance.

Ad5-vectored COVID-19 vaccine induced specific antibody responses to SARS-CoV-2 after prime vaccination across all age cohorts, with a decrease of antibody titres on day 56. Boost vaccination elicited increased antibody levels regardless of age, especially for older adults. Prime-boost regime of Ad5-vectored COVID-19 vaccine was able to elicit a seroconversion in binding antibodies to RBD in 89-100% of participants, and in neutralising antibodies to pseudovirus in 87-98% of participants. Nonetheless, the ability of a boost vaccination apart 56 days to induce increased immune responses was limited. Pre-existing anti-adenovirus immunity is the biggest obstacle for the adenovirus-vectored vaccines to overcome, especially for Ad5 eliciting widespread pre-existing immunity in the human population. In this study, the prime dose increased anti-Ad5 neutralising antibodies by 2.4-7.5

times at day 56 post vaccination (before boost vaccination) (Supplementary Figure 1). The boosting effect with a homologous vaccine could be affected by high anti-Ad5 antibodiy elicited by the prime dose. In order to minimize the negative effect of pre-existing anti-Ad5 antibody, a wider prime-boost interval needs to be considered. ChAdOx1 nCoV-19 has also shown that a longer prime-boost interval (≥12 weeks) provided higher protective efficacy than a short interval (＜6 weeks) [16]. Heterologous prime-boost regimens consisting of COVID-19 vaccines from different platforms seemed to be more effective to minimize the negative effect of the response to the vetcors. We are currently implementing trials to assess the safety and immunogenicity of Ad5-vectored COVID-19 vaccine with other COVID-19 vaccines (ClinicalTrials.gov Identifier: NCT04833101; NCT04892459).

We found that immunity responses elicited by Ad5-vectored COVID-19 vaccine decreased with aging, which has also been observed in other COVID-19 vaccines. The inactivated SARS-CoV-2 vaccine (Corona Vac) and BNT162b2 have both shown higher antibody titres in children and adolescents than adults and elderly [17-19]. In addition, antibody responses elicited by a single dose in children and adolescents were higher than that elicited by two doses in adults, which indicated that a single dose of Ad5-vectored COVID-19 vaccine was adequate. Interestingly, antibody titres elicited by 0.3 mL of vaccine were numerically higher than 0.5 mL of vaccine, with overlapping 95% confidence intervals (Spplementary Table 10), and further studies with a larger sample size are warranted.

Specific T-cell responses play an important role in controlling natural infection and reducing severity of COVID-19 diseases [20-21]. In addition, the CD4 T-cell responses are critical for the cytotoxic T-cell response and the maturating of neutralising antibodies. Therefore, generation of a robust cellular immune response is a desirable attribute for a successful COVID-19 vaccine. Here, Ad5-vectored COVID-19 vaccine elicited specific T-

cell responses after the prime vaccination. However, a boost in cellular responses was not observed following boost vaccination. This is consistent with the findings on ChAdOx1 nCoV-19 vaccine given as part of a homologous prime-boost regimen [22-23]. Therefore, the relatively weak boosting effect of T-cell responses is a major concern about the homologous boosting regimen. Theoretical concerns have been raised that some SARS-CoV-2 vaccines may potentially cause enhanced disease. Poor neutralizing potency of humoral immunity and Th2-skewed cellular immune responses were suggested to be related to this safety concern. Ad5-vectored COVID-19 vaccine induced spike-specific CD4 T-cell responses with a predominantly Th1 profile regardless of age and can reduce the risk of vaccine-enhanced disease.

Our study has some limitations. First, we did not measure neutralising antibody to live virus due to limitations of settings and resource. Neutralising antibody targeting spike protein has been suggested as a potential correlate of protection for COVID-19 [24-25], but the protective antibody titres is not defined. The results of phase 2 trial for Ad5-vectored COVID-19 vaccine showed that both ELISA antibody titres to RBD and pseudovirus neutralising antibody titres were significantly correlated with neutralising antibody titres to live virus [11]. Second, the sample size of two dose group in MIN cohort was relatively small, which limited the statistical power for subgroup analyses. It is needed to be validated in a large sample size. Third, the selection of older adults, with a range age of 56-75 years and few comorbidities, might not be representative of the general older population. Fourth, we do not present data about the durability of the vaccine-induced immunity due to unavailable at the time of publication.

In conclusion, Ad5-vectored COVID-19 vaccine with a single dose was well tolerated and induced robust humoral and cellular immune responses in children and adolescents aged 6-17 years, which could support the further study and use of this vaccine in this population. A prime-boost regimen needs further exploration given the duration of immune responses elicited by Ad5-vectored COVID-19 vaccine.

Notes

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Author contributions

F.Z. is the principal investigator of this trial. F.Z., W.C., T.Z., L.H. and W.W. designed the trial and the study protocol. P.J. drafted the manuscript. W.C. and F.Z. contributed to the critical review and revision of the report. F.Z. and P.J. contributed to the data interpretation and revising the report. H.P., J.L., H.Y., W.W., P.J. and H.W. led and participated in the site work, including the recruitment, follow-up, and data collection. X.W., Y.W. and H.H. contributed to study supervision. J.G. and S.W. monitored the trial. X.W. was responsible for the statistical analysis. All authors reviewed and approved the final version of the report.

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Conflict of Interest

W.C. reports grants from National Key R&D Program of China (2020YFC0849800). T.Z., X.W., Y.W., J.G. and H.H. are the employees of CanSino Biologics. All other authors declare no competing interests.

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Table 1. Baseline characteristics

	MIN cohort		MID cohort		OLD cohort		
	Low-dose group (N=100)	**Placebo group (N=50)**	**Low-dose group (N=20)**	**Placebo group (N=10)**	**Middle-dose group (N=100)**	**Low-dose group (N=100)**	**Placebo group (N=50)**
Age, years, mean (SD)	11.2 (3.1)	11.6 (2.6)	46.1 (5.6)	41.2 (10.5)	63.8 (5.1)	63.5 (5.5)	64.6 (5.3)
Gender							
Male	54 (54.0%)	25 (50.0%)	7 (35.0%)	5 (50.0%)	66 (66.0%)	59 (59.0%)	35 (70.0%)
Female	46 (46.0%)	25 (50.0%)	13 (65.0%)	5 (50.0%)	34 (34.0%)	41 (41.0%)	15 (30.0%)
BMI, kg/m^2, mean (SD)	20.0 (4.3)	20.7 (4.3)	25.0 (3.2)	25.0 (4.9)	24.3 (3.0)	25.1 (4.0)	24.1 (2.9)

Pre-existing adenovirus type-5 neutralising antibody							
GMT, mean (95% CI)	184.1 (103.0, 329.4)	134.0 (58.4,307.2)	303.3 (93.3,986.6)	56.3 (10.3,308.8)	222.1 (143.7,343.4)	303.1 (197.0,466.3)	390.2 (219.0,695.3)
≤1:200, n (%)	43 (43.0%)	25 (50.0%)	8 (40.0%)	7 (70.0%)	42 (42.0%)	38 (38.0%)	17 (34.0%)
＞1:200, n (%)	57 (57.0%)	25 (50.0%)	12 (60.0%)	3 (30.0%)	58 (58.0%)	62 (62.0%)	33 (66.0%)

Data are mean (SD), number of participants (%), or GMT (95% CI).

SD=standard deviation; BMI=body-mass index; GMT=geometirc mean titre; 95% CI=95% confidence interval

Table 2. Seroconversion rates of RBD-binding ELISA antibodies and neutralizing antibodies to pseudovirus post-vaccination

Timepoints	MIN cohort		MID cohort		OLD cohort		
	Low-dose group (N=100)	Placebo group (N=50)	Low-dose group (N=20)	Placebo group (N=10)	Middle-dose group (N=100)	Low-dose group (N=98)	Placebo group (N=50)
RBD-binding ELISA antibody							
Day 28	98.0% (93.0, 99.5)	0	95.0% (76.4, 99.1)	0	79.0% (70.0, 85.8)	66.3% (56.5, 74.9)	0
Day 56	97.0% (91.6, 99.0)	0	95.0% (76.4, 99.1)	0	73.0% (63.6, 80.7)	50.0% (40.3, 59.7)	0
Day 84	100.0% (96.3, 100.0)	0	100.0% (83.9, 100.0)	0	89.0% (81.4, 93.8)	88.8% (81.0, 93.6)	0
Neutralising antibody to pseudovirus							
Day 28	88.0% (80.2,93.0)	6.0% (2.1, 16.2)	75.0% (53.1, 88.8)	10.0% （1.8, 40.4）	83.0% (74.5, 89.1)	65.3% (55.5, 74.0)	0
Day 56	85.0% (76.7, 90.7)	6.0% (2.1, 16.2)	60.0% (38.7, 78.1)	0	65.0% (55.3, 73.6)	41.8% (32.6, 51.7)	4.0% (1.1, 13.5)
Day 84	98.0% (93.0, 99.5)	4.0% (1.1, 13.5)	95.0% (76.4, 99.1)	0	98.0% (93.0, 99.4)	86.7% (78.6, 92.1)	2.0% (0.4, 10.5)

Data are the percentage of participants with seroconversion (95% CI). Seroconversion was defined as an increase in post-vaccination titre of at least four-times baseline. Timepoints refer to the number of days since the prime vaccination. RBD=receptor binding domain; 95% CI=95% confidence interval.

FIGURE LEGENDS

Figure 1. Trial Profile

Footnote: *Two participants excluded from immunogenicity analyses, due to did not received boost vaccination. Reasons for not receiving boost dose included withdrawal due to severe adverse event or other reasons.

Figure 2. Common Adverse Reactions within 14 days following prime and boost vaccination

Data are the percentage of participants with adverse reaction within 14 days following prime and boost vaccination.

Figure 3. Antibody titres of RBD-binding ELISA antibodies and neutralising antibodies to pseudovirus post prime vaccination

A. RBD-binding IgG antibody titres; B. Neutralising antibody titres to pseudovirus. The detection limit for the RBD-specific ELISA antibody test and the neutralizing antibody test to pseudovirus was 1:40 and 1:10, respectively. Undetectable antibody titres in serum were assigned values of half the detection limits for calculation. The error bars indicate the 95% CI of the GMT. GMT=geometric mean antibody titre; RBD=receptor binding domain.

Figure 4. Specific T-cell response measured by ELISpot at baseline and at day 28 and 84 post prime vaccination

Data are the spot-forming cells with secretion of cytokines per 1×10^6 PBMCs in participants who received vaccine, including IFN-γ, IL-2, IL-4, IL-5 and IL-13. Th1/Th2 ratio was calculated by the sum of IFN-γ plus IL-2 cytokine levels divided by the sum of IL-4, and IL-5 plus IL-13 cytokine level. Horizontal bars show the median and error bars show the interquartile range. ELISpot= enzyme-linked immunospot; IFN=interferon; IL=interleukin; PBMCs= peripheral blood mononuclear cells.

Figure 1

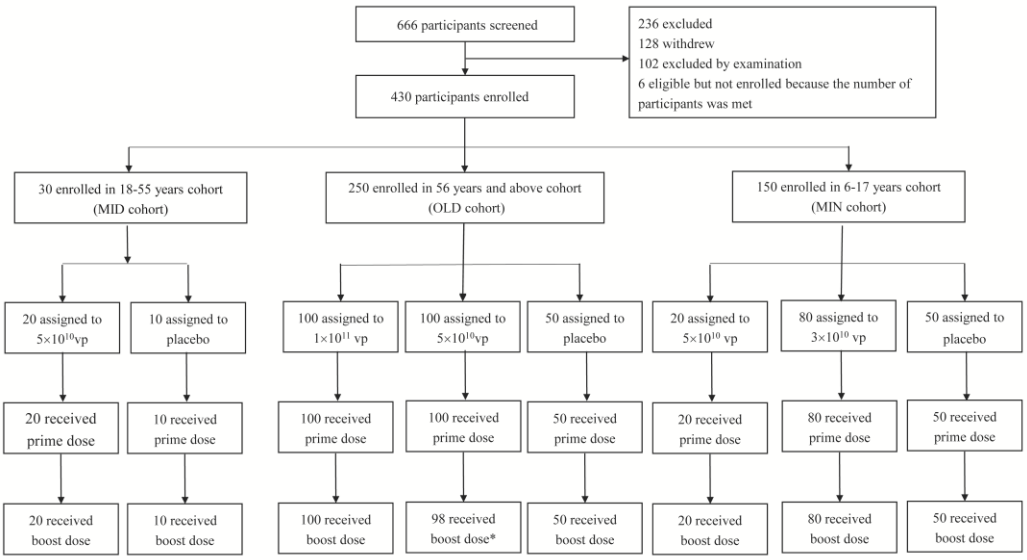


Figure 2

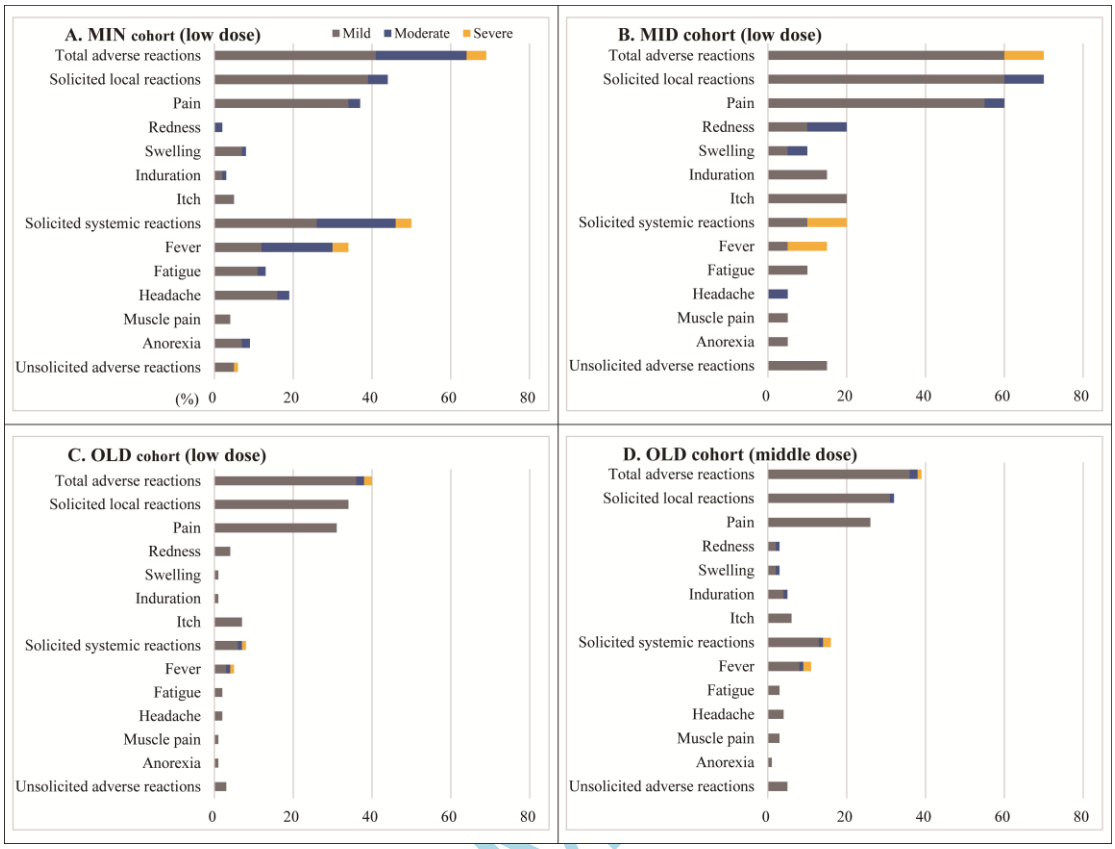


Figure 3

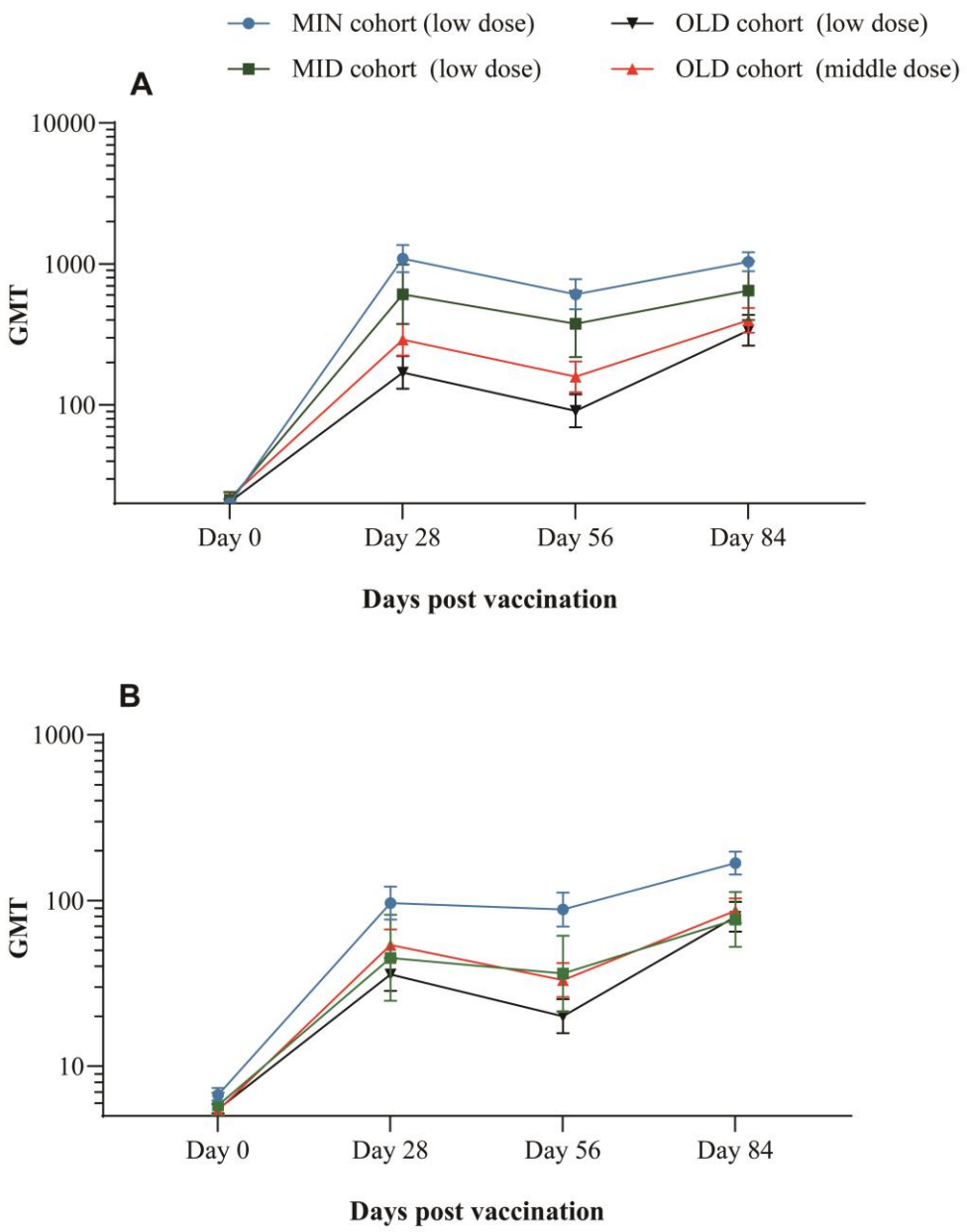
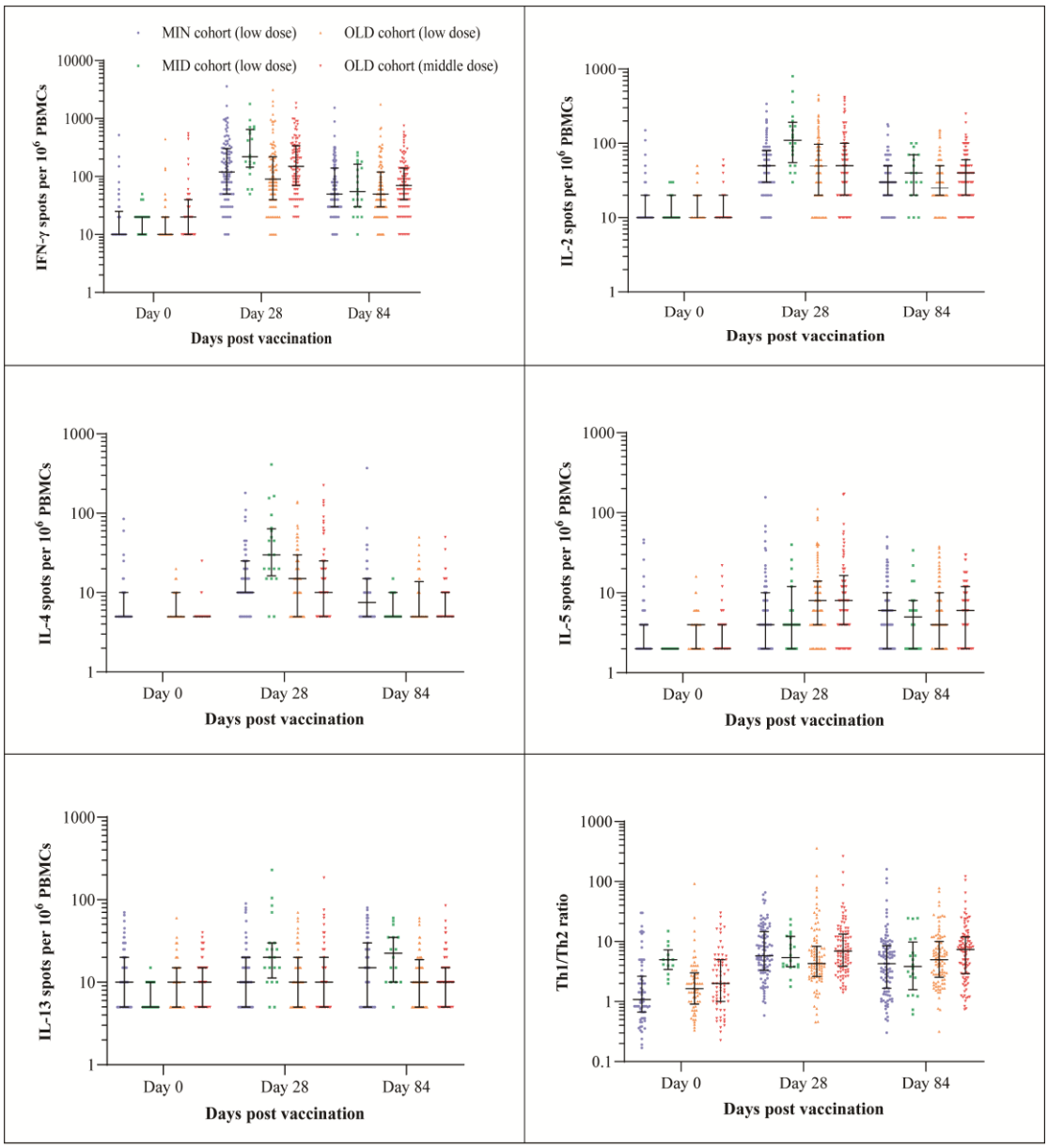

MIN cohort (low dose)
MID cohort (low dose)
OLD cohort (low dose)
OLD cohort (middle dose)
A
10000
1000
100
GMT
Day 0
Day 28
Day 56
Day 84
Days post vaccination
B
1000
100
10
GMT
Day 0
Day 28
Day 56
Day 84
Days post vaccination

Figure 4


MIN cohort (low dose)
MID cohort (low dose)
OLD cohort (low dose)
OLD cohort (middle dose)
IFN-γ spots per 10^6 PBMCs
IL-2 spots per 10^6 PBMCs
IL-4 spots per 10^6 PBMCs
IL-5 spots per 10^6 PBMCs
IL-13 spots per 10^6 PBMCs
Th1/Th2 ratio
Day 0
Day 28
Day 84
Days post vaccination