Safety and immunogenicity of heterologous boost immunisation with an orally administered aerosolised Ad5-nCoV after two-dose priming with an inactivated SARS-CoV-2 vaccine in Chinese adults: a randomised, open-label, single-centre trial

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Summary

Background Due to waning immunity and protection against infection with SARS-CoV-2, a third dose of a homologous or heterologous COVID-19 vaccine has been proposed by health agencies for individuals who were previously primed with two doses of an inactivated COVID-19 vaccine.

Methods We did a randomised, open-label, controlled trial to evaluate the safety and immunogenicity of heterologous boost immunisation with an orally administered aerosolised adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in Chinese adults (≥18 years old) who had previously received two doses of an inactivated SARS-CoV-2 vaccine—Sinovac CoronaVac. Eligible participants were randomly assigned (1:1:1) to receive a heterologous booster vaccination with a low dose (1.0×10^{11} viral particles per mL; 0·1 mL; low dose group), or a high dose (1.0×10^{11} viral particles per mL; 0·2 mL; high dose group) aerosolised Ad5-nCoV, or a homologous intramuscular vaccination with CoronaVac (0·5 mL). Only laboratory staff were masked to group assignment. The primary endpoint for safety was the incidence of adverse reactions within 14 days after the booster dose. The primary endpoint for immunogenicity was the geometric mean titres (GMTs) of serum neutralising antibodies (NAbs) against live SARS-CoV-2 virus 14 days after the booster dose. This study was registered with ClinicalTrials.gov, NCT05043259.

Findings Between Sept 14 and 16, 2021, 420 participants were enrolled: 140 (33%) participants per group. Adverse reactions were reported by 26 (19%) participants in the low dose group and 33 (24%) in the high dose group within 14 days after the booster vaccination, significantly less than the 54 (39%) participants in the CoronaVac group (p<0·0001). The low dose group had a serum NAb GMT of 744·4 (95% CI 520·1–1065·6) and the high dose group had a GMT of 714·1 (479·4–1063·7) 14 days after booster dose, significantly higher than the GMT in the CoronaVac group (78·5 [60·5–101·7]; p<0·0001).

Interpretation We found that a heterologous booster vaccine with an orally administered aerosolised Ad5-nCoV is safe and highly immunogenic in adults who have previously received two doses of CoronaVac as the primary series vaccination.

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Introduction

The devastating health, economic, and social consequences of the COVID-19 pandemic have united researchers globally in their efforts to develop safe and effective COVID-19 vaccines. Several COVID-19 vaccines, including three inactivated vaccines (Sinovac CoronaVac, Sinopharm BBIBP-CorV, and Bharat Biotech BBV152), two mRNA COVID-19 vaccines (Pfizer-BioNTech BNT162b2 and Moderna mRNA-1273 CX-024414), two versions of adenovirus vectored vaccines (AstraZeneca-Oxford ChAdOx1 nCoV-19 and Janssen Ad26.COV2.S),

have been licensed or granted an emergency use listing by WHO and have been deployed globally.[1] Although these vaccines were found to provide persistent protection against severe COVID-19, waning of antibodies and a decrease in vaccine protection against mild-to-moderate symptomatic diseases over time were observed in real-world prospective studies.[2] Furthermore, the protection provided by vaccines could be compromised by new SARS-CoV-2 variants, which are able to escape from vaccine-induced immune responses, highlighting the need for a booster dose of COVID-19

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See Online for appendix

Research in context

Evidence before this study

We searched ClinicalTrials.gov and PubMed for studies of mucosal or intranasal SARS-CoV-2 vaccines in clinical trials or clinical trial reports with the search terms "COVID-19" or "SARS-CoV-2", "mucosal vaccine", or "intranasal vaccine", or "aerosolized vaccine" and "clinical trial" from the inception of each database to Jan 28, 2021, no language restrictions were applied. One open-label phase 1 clinical trial of an orally administered aerosolised adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) manufactured by CanSino in participants who had never had SARS-CoV-2 was found. Orally administered aerosolised Ad5-nCoV was well tolerated and immunogenic in this study. Two doses of aerosolised Ad5-nCoV elicited neutralising antibody responses, similar to one dose of intramuscular injection of Ad5-nCoV. The aerosolised booster vaccination given after an intramuscular injection with Ad5-nCoV induced a better neutralising antibody responses than did two doses of aerosolised Ad5-nCoV.

Added value of this study

This is the first report of a randomised trial to evaluate the safety and immunogenicity of an aerosolised Ad5-nCoV COVID-19 vaccine administered orally as a heterologous booster after two-dose priming with an inactivated SARS-CoV-2 vaccine, CoronaVac, versus homologous

immunisation of a third dose. The results showed that the heterologous booster regimen with aerosolised Ad5-nCoV was safe and significantly more immunogenic than a homologous boost with CoronaVac, showing lower occurrence of adverse reactions and 18·4–26·4-times higher concentration of live viral NAbs against SARS-CoV-2 28 days after the booster. Participants who received aerosolised Ad5-nCoV also had an 18·1–24·0-times higher cross-neutralising capability to the delta variant of SARS-CoV-2 compared with those who received a homologous boost with CoronaVac 28 days after booster vaccination.

Implications of all the available evidence

The heterologous prime-boost immunisation with an orally aerosolised Ad5-nCoV vaccine after two doses of CoronaVac has a good safety profile and could elicit high neutralising capability against both the wild-type and delta SARS-CoV-2 variants, which is significantly higher than that of WHO international standard (NIBSC code 20/136). These results indicate that the vaccination strategy—using an intramuscular vaccine to elicit a long-lived systemic IgG response, and followed by a mucosal booster that recruits memory B and T cells to form mucosal protection—could be highly efficient. Nevertheless, efficacy or effectiveness of this heterologous prime-boost immunisation still needs to be shown.

vaccine.[3,4] In Israel, a third dose of the BNT162b2 vaccine against COVID-19 was provided to people who were 60 years of age or older and who had been vaccinated at least 5 months earlier. The third dose reduced the risk of SARS-CoV-2 infection by 11·3 times and the rate of severe illness by 19·5 times.[5]

Although homologous boosting is generally considered standard practice, heterologous prime-boost regimen combining multiple vaccine platforms has been explored as a COVID-19 vaccine strategy, to reduce reactogenicity, increase immunogenicity, and enhance vaccine effectiveness.[6] Several reports on heterologous schedules with an adenoviral vectored vaccine (ChAdOx1 nCoV-19) and an mRNA vaccine (BNT162b2 or mRNA-1273) in randomised trials or cohort studies showed a heterologous prime-boost schedule can be more immunogenic than a homologous schedule.[4] The WHO strategic advisory group of experts on immunisation has suggested that a third dose of the homologous or heterologous vaccine should be offered to people aged 60 years and older as part of an extended primary series for inactivated vaccines, such as CoronaVac.[7] Previous studies showed, in healthy adults who were vaccinated with two doses of CoronaVac, significantly more robust antibody responses in participants who received BNT162b2 as the third dose compared with those who received a third dose of CoronaVac.[8,9] A randomised trial in adults who did not have COVID-19 also showed that an intramuscular injection of a AD5-nCOV booster—a replication-defective

adenovirus type-5 vectored (Ad5-vectored) COVID-19 vaccine containing 5×10^{10} viral particles per dose—after two doses of CoronaVac induced a significantly stronger neutralising antibody (NAbs) response than did a booster dose of CoronaVac.[10]

An orally administered aerosolised Ad5-nCoV vaccine that encodes the SARS-CoV-2 spike protein as the intramuscular vaccine has been developed by CanSino Biologics (Tianjin, China). In a previous phase 1 trial, the aerosolised Ad5-nCoV was well tolerated and elicited significant serum NAbs responses following one dose of intramuscular injection of Ad5-nCoV.[11] However, vaccinating with aerosolised Ad5-nCoV as a booster following two doses of inactivated SARS-CoV-2 vaccines has not yet been investigated.

We aimed to assess the safety and immunogenicity of a heterologous boost immunisation with an oral aerosolised Ad5-nCoV in Chinese adults aged 18 years or older who had completed the preliminary immunisation series with two doses of CoronaVac.

Methods

Study design and participants

We did a randomised, open-label, single-centre, controlled trial to evaluate the safety and immunogenicity of heterologous booster immunisation with an aerosolised Ad5-nCoV administered orally after two-dose priming with CoronaVac in adults. Participants were recruited at Donghai Center for Disease Control and

Prevention, Lianyungang, China. Healthy men and women (those without COVID-19 and other major diseases [eg, serious cardiovascular diseases and diseases with abnormal pulmonary function, including asthma, chronic obstructive pulmonary disease and pulmonary fibrosis]), aged 18 years or older, and who had two doses of CoronaVac in the previous 3–9 months were screened for eligibility. Key exclusion criteria included clinically confirmed or laboratory-confirmed COVID-19 or SARS-CoV-2 infection, nasal or oral diseases, known infection with HIV, and positive urine pregnancy test for women. A full list of inclusion and exclusion criteria are reported in the appendix (pp 1–2).

The trial protocol and informed consent were reviewed and approved by Research Ethics Committee of Jiangsu Provincial Center for Disease Control and Prevention. Written informed consent was obtained from each participant before inclusion. This trial was done following the principles of the Declaration of Helsinki, International Council for Harmonisation-Good Clinical Practice guidelines, and local guidelines.

Randomisation and masking

Eligible participants were randomly assigned (1:1:1) to receive a heterologous booster of low dose aerosolised Ad5-nCoV (low dose group) or high-dose aerosolised Ad5-nCoV (high dose group) by oral inhalation, or a homologous third dose of CoronaVac by intramuscular injection (CoronaVac group) with an interactive web response randomisation system. The randomisation lists were generated by an independent statistician using SAS software (version 9.4). Both the participants and investigators were not masked to treatment allocation. The laboratory staff, who were responsible for the sample testing, were masked to group allocation.

Procedures

The orally aerosolised Ad5-nCoV is a replication defective Ad5 vectored COVID-19 vaccine expressing the full-length spike gene of wild-type SARS-CoV-2, Wuhan-Hu-1.[12] The Ad5-nCoV vaccine was supplied in 1·5 mL per vial, at a concentration of $1·0 \times 10^{11}$ viral particles per mL as a liquid formulation, which was developed by Institute of Biotechnology (Beijing, China) and CanSino Biologics (Tianjin, China). We used a continuous vapouring system (which contained a vaporing unit [Aerogen, Galway, Ireland] integrated by Suzhou Weiqi Biological Technology [Suzhou City, China]) to aerosolise the Ad5-nCoV and pour the aerosolised droplets of vaccine into a disposable suction cup. Participants inhaled the aerosolised vaccine orally. Participants in the low-dose group inhaled 0·1 mL of the aerosolised vaccine droplets; participants in high-dose group inhaled 0·2 mL of the aerosolised vaccine droplets. The CoronaVac group received an inactivated COVID-19 vaccine CoronaVac (Vero cells), developed by Beijing Sinovac Research & Development, Beijing, which was administered intramuscularly at 0·5 mL per dose.

All participants were observed at the clinic for 30 min after the vaccination for any immediate vaccine-associated reactions, and then were instructed to keep a daily record of any solicited or unsolicited adverse events for the next 14 days. Severity of adverse events was graded according to the scale issued by the China State Food and Drug Administration (version 2019).[13] Serious adverse events reported by participants were documented throughout the study period after booster vaccination. Measurements of forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁), and peak expiratory flow rate (PEFR) were measured before receiving the booster dose and on days 7, 14, and 28 after the booster vaccination in the low-dose and high-dose groups, but not the control group. Blood samples were collected from all participants at baseline, before receiving the booster dose, and on days 14 and 28 after the booster dose. We isolated peripheral blood mononuclear cells (PBMCs) from blood samples of a cellular immune subgroup consisting of the first 40 participants in each group before receiving the booster dose and on days 7 and 14 after the booster vaccination.

Outcomes

The primary endpoint for safety was the incidence of adverse reactions within 14 days after the booster dose. The primary endpoint for immunogenicity was the serum geometric mean titres (GMTs) of NAbs against live SARS-CoV-2 virus at 14 days after the booster dose. NAbs against live SARS-CoV-2 virus were measured with cytopathic effect-based microneutralisation assay with a wild-type SARS-CoV-2 virus isolate BetaCoV/Jiangsu/JS02/2020 (GISAID EPI_ISL_411952) and a SARS-CoV-2 Delta (B.1.617.2) variant isolate hCoV-19/China/JS07/2021 (GISAID EPI_ISL_4515846) in Vero-E6 cells.[14,15] The serum dilution for microneutralisation assay ranged from 1:4 to 1:8192, and then mixed with the same volume of virus solution to achieve a 50% tissue culture infectious dose of 100 per well. The reported titre was the reciprocal of the highest sample dilution that protected at least 50% of cells from a cytopathic effect, which was observed under an inverted microscope. The seropositivity for NAbs was defined as a titre of 1:4 or more. Serum NAbs titres against the wild-type SARS-CoV-2 virus and delta variant were measured in a subgroup of the first 150 participants in the cohort. The WHO international standard (NIBSC code 20/136) was used as the reference for the cytopathic effect-based microneutralisation assay.

The prespecified secondary outcome for safety was the number of adverse events within 28 days of the booster dose and serious adverse events until 12 months after the booster dose. The secondary endpoints for immunogenicity were receptor-binding domain (RBD)-specific IgG response at days 14 and 28 after booster vaccination, which were measured by indirect ELISA assay with a cutoff titre of 1:10.[14] Anti-SARS-CoV-2 RBD-specific binding IgA antibodies in the serum before booster
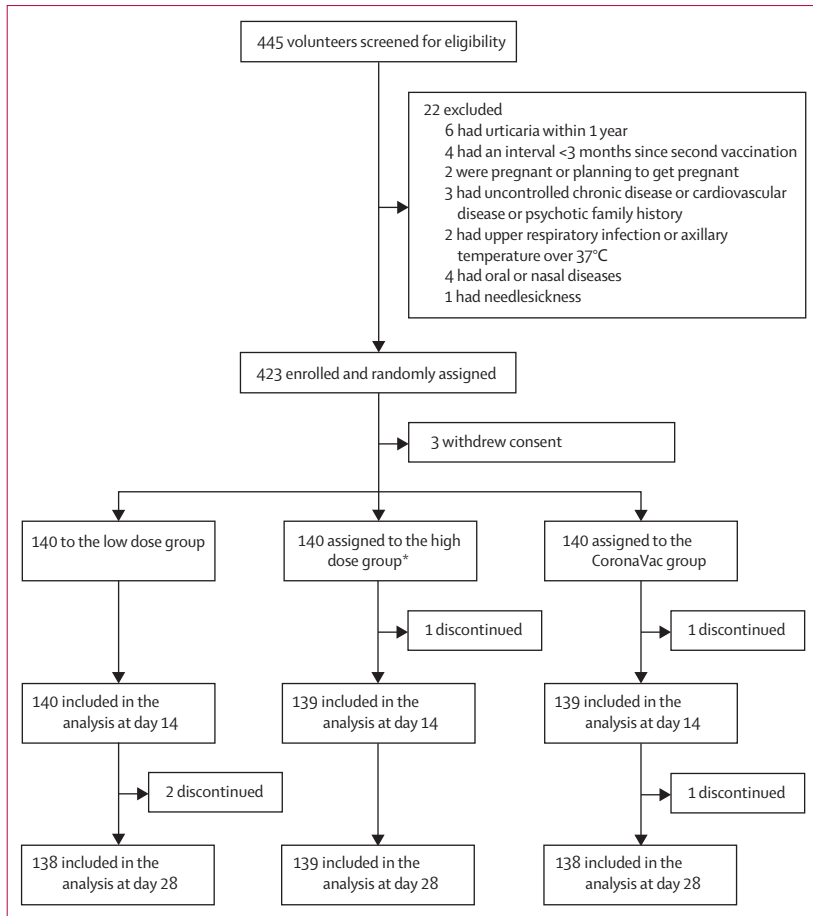
Figure 1: Trial profile

*One participant was randomly assigned to receive a booster vaccine with high-dose aerosolised Ad5-nCoV vaccine but was wrongly administrated with a third dose of the CoronaVac. This participant was involved in the intention-to-treat analysis and safety analysis in the originally assigned group.

	Low dose group (n=140)	High dose group (n=140)	CoronaVac group (n=140)
Age			
18–45 years	96 (69%)	93 (66%)	88 (63%)
≥46 years	44 (31%)	47 (34%)	52 (37%)
Mean age, years	40·1 (10·0)	41·0 (9·7)	41·2 (10·0)
Sex			
Male	59 (42%)	60 (43%)	60 (43%)
Female	81 (58%)	80 (57%)	80 (57%)
Time interval since the last priming dose of inactivated vaccine, months			
Median (IQR)	5·0 (5·0–5·0)	5·0 (5·0–5·0)	5·0 (5·0–5·0)
<5 months	22 (16%)	26 (19%)	25 (18%)
≥5 months	118 (84%)	114 (81%)	115 (82%)
Pre-existing adenovirus type-5 neutralising antibody			
Geometric mean titer (95% CI)	280·8 (191·3–412·0)	257·0 (173·0–381·9)	323·4 (219·8–475·8)
Participants with titre ≤1:200	52 (37%)	54 (39%)	47 (34%)
Participants with titre >1:200	88 (63%)	86 (61%)	93 (66%)

Data are n (%) or mean (SD), unless otherwise stated. The analysis was based on the intention-to-treat cohort, included all randomly participants who received the booster vaccination and had at least one evaluation data available.

Table: Baseline characteristics of the participants in the intention-to-treat cohort

vaccination and on days 14 and 28 after booster vaccination were also evaluated as an prespecified exploratory endpoint. The commercial Anti-SARS-CoV-2 RBD IgG/IgA ELISA kit (Vazyme Medical Technology, Nanjing, China) was used for detection. PBMCs were stimulated with a peptide pool covering the full length spike glycoprotein and cytokine secretions of T helper type 1 cells (Th1; IFN-γ and IL-2), and T helper type 2 cells (Th2; IL-13) were detected by enzyme-linked immunospot (ELISpot) assay (Mabtech, Stockholm, Sweden) as number of spot-forming cells per 10^6 PBMCs.[16] Pre-existing anti-Ad5 NAbs in serum at baseline in the participants were also measured, as described previously.[17]

Statistical analysis

Initially, we tested the non-inferior hypothesis that GMT of NAbs of heterologous boost group was not inferior to that of the homologous boost group. If that were true, we then tested the superior hypothesis that heterologous boost group is superior to that of the homologous boost group. The results of the non-inferior tests were presented as 95% CI of the GMT ratio in the appendix (p 8). The sample size calculation was based on the hypothesis that a booster vaccination with aerosolised Ad5-nCoV following the two doses of CoronaVac could elicit a non-inferior or superior serum NAbs response compared with using a third dose of CoronaVac as the booster. We estimated that a baseline NAbs GMT of 1:40 at 3–9 months before the booster vaccine would lead to a GMT of 1:160 after the aerosolised Ad5-nCoV-booster. The NAbs GMT in the CoronaVac group after the boosters would be around 1:80. An SD of 4 for GMT was estimated for both groups for the sample size calculation. A sample size of 43 individuals per group would provide at least 95% power to hold the non-inferiority hypothesis with a lower limit of 95% confidence of GMT ratio (heterologous boost group *vs* homologous boost group) over the non-inferiority margin of 0·67. For the safety evaluation, a sample size of 110 individuals was estimated to be able to provide a power of more than 80% to detect an increase of 5% adverse reactions of heterologous boost group (high dose and low dose groups) compared with that of the homologous boost group (CoronaVac group). Considering a dropout rate of about 20%, the sample size was adjusted to 140 per group.

The intent-to-treat (ITT) population and safety population included all randomly assigned participants who received the booster vaccination and had at least one
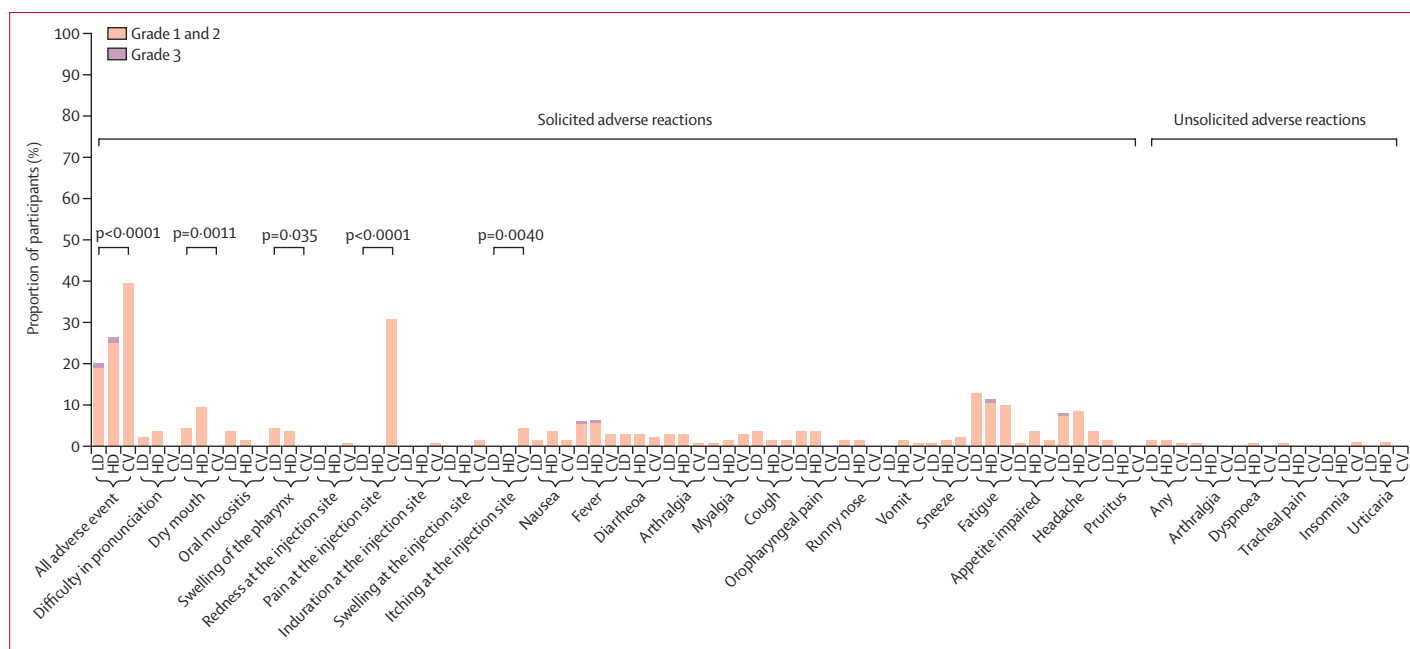
***Figure 2:* Solicited and unsolicited adverse reactions that occurred within 14 days after booster vaccination**
The analysis was based on the safety cohort, which included all randomly assigned participants who received the booster vaccination and had at least one evaluation datapoint available.
CV=CoronaVac group. HD=high dose group. LD=low dose group.

evaluable result. No imputation was made for missing data. The serum NAbs GMTs and GMT ratios of heterologous boost group versus homologous boost group were calculated with the two-sided 95% CIs, based on the t-distribution of the log-transformed titres, and were then back-transformed to the original scale. Geometric mean fold rises (GMFRs) of NAbs and RBD-specific binding antibodies in serum were also calculated. Seroconversion rate was defined as the proportion of participants with an increase in serum NAbs or RBD-specific binding antibodies of four times or more compared with the baseline level before the booster vaccine. Data below the detection limit were assigned a value half of the threshold; the data above the highest detection limit were assigned a value of that limit for calculation.

Categorical data, including incidence of adverse reactions and seroconversion, were analysed with the χ^2 test or Fisher's exact test when appropriate. Shapiro–Wilk test was used for normality determination of the data. Analysis of variance was used to compare normal distributed data across the groups, and the Wilcoxon rank-sum test was used for non-normal distributed data. Pearson correlation analysis of the association between log-transformed antibody neutralisation titres and RBD-specific binding IgG titres in serum were done. Post-hoc stratification analyses according to the pre-existing Ad5 neutralisation antibody titre (>200 *vs* ≤200), age (18–45 years *vs* ≥46 years) and sex (female *vs* male) were done in participants who received aerosolised Ad5-nCoV. Statistical analyses were done with SAS (version 9.4) or

GraphPad Prism (version 8.0.1). This trial is registered with ClinicalTrials.gov, NCT05043259.

Role of the funding source
The funders of the study were not involved in protocol design, data collection, statistical analysis, data interpretation, writing of the report and the decision to submit.

Results
Between Sept 14 and 16, 2021, 445 vaccinated volunteers were screened for eligibility. 423 (95%) participants were enrolled, of whom 140 (33%) were randomly assigned to the low dose group, 140 (33%) to the high-dose group, and 140 (33%) to the CoronaVac group (figure 1). Three (1%) individuals were not included in the ITT because they had discontinued before the baseline blood sample was taken and booster dose administration; the other 420 (99%) participants who received a booster dose were included in the safety analysis. One (1%) of 140 participants in the high dose group was administrated a dose of intramuscular CoronaVac by mistake. This participant was included in the ITT analysis in the high dose group for the safety and immunogenicity evaluation.

Demographic characteristics are reported in the table. The median time interval between the second dose and the booster dose was 5 months (IQR 5–5); the proportion of participants with an interval of 5 months or more was highest in the low dose group (table). 88 (63%) participants in the low dose group, 86 (61%) in the high dose group,
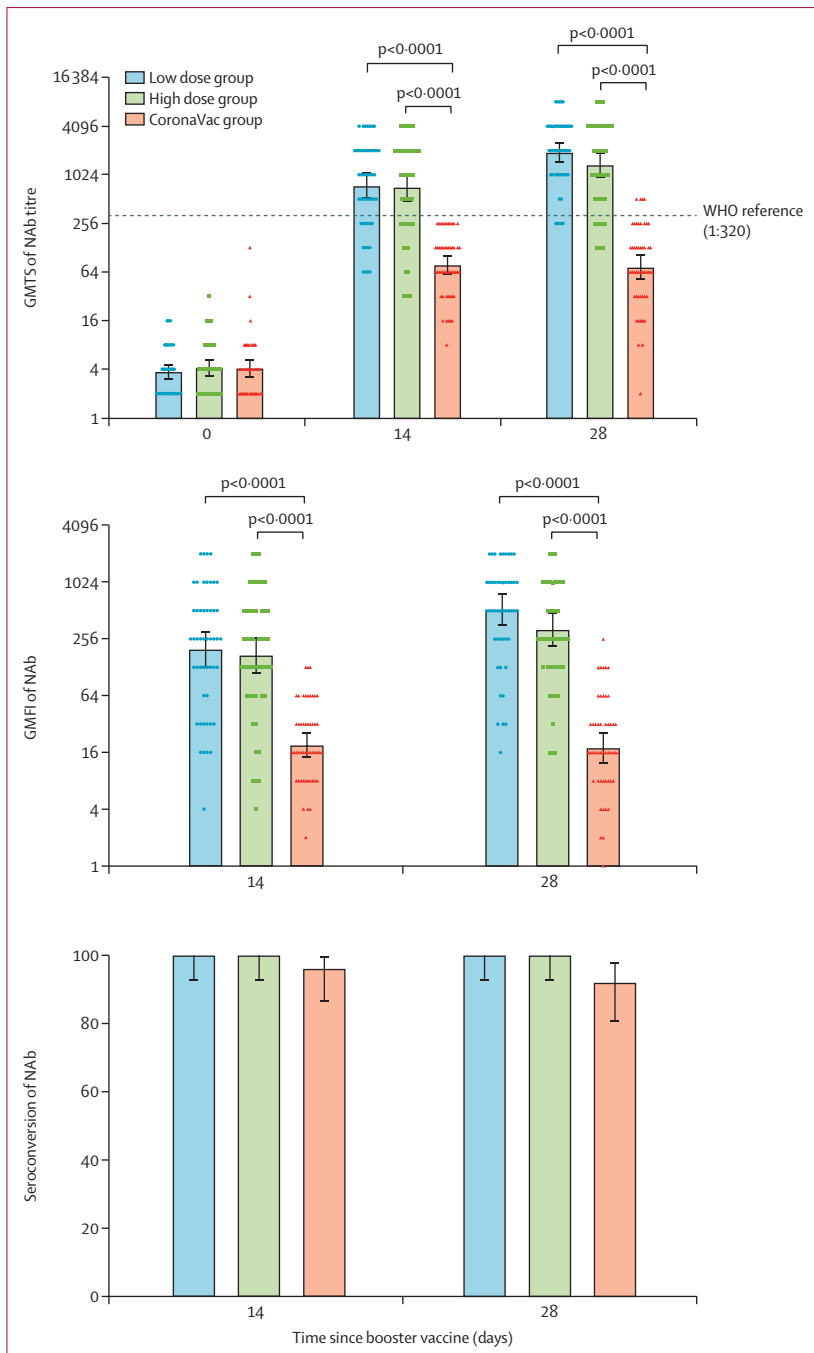
Figure 3: **Neutralising antibodies against wild-type SARS-CoV-2 before and after a booster vaccination**
Error bars are 95% CIs. The p values are the results of comparison between the three treatment groups. The WHO reference serum 1000 IU/mL equivalent to live viral neutralising antibody titre of 1:320 against the wild-type SARS-CoV-2. GMTS=geometric mean titre in serum. GMFI=geometric mean fold increase. NAb=neutralising antibody.

and 93 (66%) in the CoronaVac group had pre-existing Ad5 NAbs titre more than 1:200.

Adverse reactions were reported by 26 (19%) participants in the low-dose group, 33 (24%) in the high-dose group, and 54 (39%) participants in the CoronaVac

group within 14 days after the booster vaccination, showing significant differences across the treatment groups (p<0·0001; figure 2; appendix pp 3–5). Four grade 3 adverse reactions (two fever, one fatigue, and one headache) were observed in two (1%) participants in the low dose group and two (1%) participants in the high dose group, but no grade 3 adverse reactions were reported in the CoronaVac group. The most common adverse reaction in the CoronaVac group was injection-site pain, reported by 43 (31%) participants. The most common adverse reaction was fatigue in both the low dose group (18 [13%]) and high dose group (16 [11%]), followed by headache (11 [8%] in the low dose group and 12 [9%] in the high dose group), and fever (nine [6%] in the low dose group and nine [6%] in the high dose group). Occurrences of respiratory related adverse reactions—such as dry mouth (six [4%] in the low dose group and 13 [9%] in the high dose group *vs* no patients in the CoronaVac group) and pharyngeal swelling (six [4%] in the low dose group and five [4%] in the high dose group *vs* no patients in the CoronaVac group)— were more common in the participants who received aerosolised Ad5-nCoV compared with participants in the CoronaVac group (p=0·0011 compared with the low dose group; p=0·035 compared with the high dose group). In participants who received aerosolised Ad5-nCoV, the pre-existing Ad5 neutralisation antibody titre more than 1:200 was associated with a significantly lower occurrence of fever after vaccination (appendix p 6).

There were no clinically significant abnormalities in FVC, FEV_1, and PEFR at days 7, 14, and 28 after the booster vaccination in the low dose and high dose groups (appendix p 7). No severe adverse events were reported during the first 28 days after booster vaccination.

At baseline, serum NAbs against wild-type SARS-CoV-2 were measured in 150 participants (50 in the low dose group, 50 in the gigh dose group, and 51 in the CoronaVac group) and were similar in all three treatment groups: GMTs of 3·7 (95% CI 3·1–4·5) in the low dose group, 4·2 (3·3–5·2) in the high dose group, and 4·1 (3·3–5·2) in the CoronaVac group (figure 3; appendix p 8). At day 14, the low dose and high dose groups had significantly higher serum NAbs GMTs against wild-type SARS-CoV-2 than the CoronaVac group (744·4 [95% CI 520·1–1065·6] in the low dose group; 714·1 [479·4–1063·7] in the high dose group; 78·5 [60·5–101·7] the CoronaVac group; p<0·0001). Inhalation of aerosolised Ad5-nCoV at 0·1 mL or 0·2 mL elicited 6·7–10·7-times higher serum NAbs responses than that elicited by a homologous booster dose of CoronaVac between days 14 and 28. The NAb GMT peak was equivalent to 6054·1 (95% CI 4584·1–7995·0) NAbs units (IU)/mL in the low dose and 4221·3 (2976·9–5985·3) IU/mL in the high dose group at 28 days after the booster vaccination and using the WHO international standard. All participants in the low dose and high dose groups had seroconversion 14 days after

the booster vaccine, whereas 49 (96%) of 51 participants in the CoronaVac group had seroconversion. GMFIs of NAbs against wild-type SARS-CoV-2 were higher in the low dose group (199·5 [95% CI 129·5–307·2]) and the high dose group (171·3 [110·9–264·4]) compared with the CoronaVac group (19·1 [14·0–26·0]; p<0·0001). The serum GMTs of NAbs against wild-type SARS-CoV-2 increased to 1937·3 (95% CI 1466·9–2558·4) in the low dose group and 1350·8 (952·6–1915·3) in the high dose group at days 28 after the booster vaccine, compared with 73·5 (52·3–103·3) in the CoronaVac group at the same timepoint (p<0·0001).

The dose response relationships of the serum NAbs GMTs associated with the dosage of aerosolised Ad5-nCoV as a booster were not identified. Differences in post-vaccination antibody responses observed between the younger participants and the older participants were not statistically significant, and the antibody responses were generally consistent between sex subgroups within each aerosolised Ad5-nCoV group (appendix p 9). A post-hoc analysis showed that participants with low pre-existing anti-Ad5 immunity were more likely to have high antibody responses in both aerosolised Ad5-nCoV groups (appendix p 10).

Compared with GMTs of NAbs against the wild-type isolate in serum, NAbs for the Delta variant were around 6·2–7·0 times lower across the treatment groups (appendix pp 8, 11). At 28 days, the GMTs of serum NAbs titres against the Delta variant were similar in participants in the low-dose (278·2 [95% CI 200·1–386·9]) and high-dose (216·8 [153·7–305·7]) groups. GMTs were significantly higher in the two aerosolised groups than in the CoronaVac group (11·6 [8·5–16·0], p<0·0001; appendix pp 11–12).

After booster vaccination, the GMT of RBD-specific IgG antibodies in the two aerosolised groups were significantly higher than that in the CoronaVac group (4624·5 [95% CI 3513·3–6056·0] in the low dose group and 6175·1 [4858·3–7848·8] in the high dose group vs 670·0 [566·0–793·3] in the CoronaVac group at day 14, p<0·0001; 6217·0 [5106·5–7568·9] in the low dose group and 6578·2 [5472·0–7907·9] in the high dose group vs 449·4 [388·0–520·5] in the CoronaVac group at day 28, p<0·0001; appendix pp 13–14). Moderate correlations between the NAbs titres and the RBD-specific binding IgG antibodies were found in CoronaVac group after booster vaccination, with correlation coefficients of 0·74–0·80 (appendix p 15). Of note, the NAbs titres in the aerosolised Ad5-nCoV groups had numerically higher correlation coefficients (0·93 at day 14 and 0·83 at day 28 in the low dose group and 0·88 at day 14 and 0·87 at day 28 in the high dose group) with the RBD-specific binding IgG antibodies compared with the CoronaVac group.

The RBD-specific binding IgA antibodies in serum also increased significantly after the booster vaccine, with GMTs of 94·4 (95% CI 73·8–120·9) in the low dose
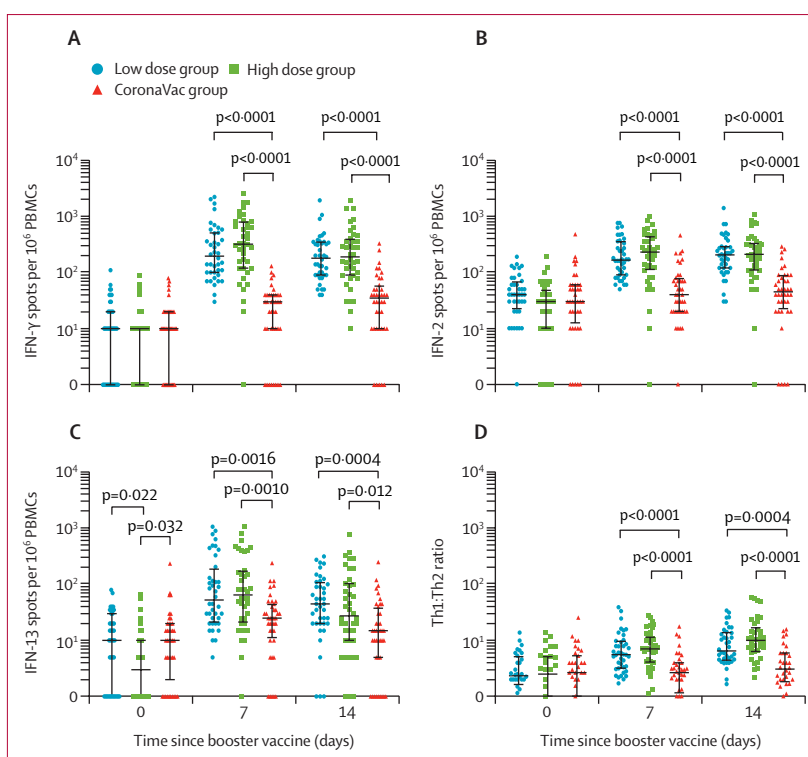
Figure 4: **SARS-CoV-2 spike-specific cytokine T cells responses before and after receiving a boost vaccination**
Cytokine T cells were background corrected for unstimulated cells and values lower than 0 were considered negative. Th1:Th2 ratio was calculated by the sum of IFN-γ plus IL-2 cytokine concentrations divided by the sum of IL-13 cytokine concentrations. Error bars are median (IQR). Samples of PBMCs were collected from the first 40 participants in the three treatment groups and included in the analysis. The discrepancies between the numbers of data points presented in the figures and the numbers of participants in the groups are due to the overlapping of the dots. PBMCs=peripheral blood mononuclear cells. TH=T helper cell.

group and 122·2 (97·0–154·0) in the high dose group at day 14, and 114·3 (92·9–140·5) in the low dose group and 116·3 (95·7–140·3) in the high dose group at days 28. The RBD-specific binding IgA antibodies in the CoronaVac group were 13·9 (12·1–16·1) at day 14 and 11·2 (9·8–12·8) at day 28, significantly lower than low dose and high dose groups with the heterologous boost (appendix p 16).

SARS-CoV-2 spike protein-specific IFN-γ and IL-2 ELISpot responses increased significantly 7 days after the booster vaccine in both aerosolised Ad5-nCoV groups; the responses were 6–10-times higher in the low dose group and 4–5-times higher in the high dose group compared with the CoronaVac group (p<0·0001; figure 4). A booster dose also enhanced the specific IL-13 responses in the aerosolised Ad5-nCoV groups, with a relatively mild increase (2·0–2·6-times) compared with the CoronaVac group at days 7 after the booster. Therefore, more Th1-skewed cellular immune responses were found in the aerosolised Ad5-nCoV groups. The sex and age of the participants did not alter the specific cellular immune responses to the booster dose, but pre-existing high concentrations of Ad5 NAbs in serum could compromise the increased concentrations of

IFN-γ and IL-2 expressed in cells after the vaccination in both aerosolised Ad5-nCoV groups (appendix pp 17–18).

Discussion

Overall, our results confirmed those of previous studies, which showed that a booster vaccination induces a substantial serum NAbs response against SARS-CoV-2 in adults who have received two doses of inactivated COVID-19 vaccine CoronaVac. Our results also suggest that a heterologous booster immunisation with an aerosolised Ad5-nCoV is safe and highly immunogenic. Inhalation of aerosolised Ad5-nCoV at 0·1 mL or 0·2 mL elicited much stronger serum NAbs responses. Both of which are substantially higher than that required for 50% vaccine efficacy against symptomatic SARS-CoV-2 infection provided by the two-dose regimen of CoronaVac reported previously.[18] The concentrations of serum NAbs in participants who received aerosolised Ad5-nCoV were higher compared with previous studies of other COVID-19 vaccines. For live-virus SARS-CoV-2, the NAbs was 1404·2 IU/mL after two doses of the mRNA-1273 and 928·8 IU/mL after two doses of BNT162b2 (two leading mRNA vaccines);[19] the NAbs was 408 IU/mL after two doses of MVC-COV1901 and 224 IU/mL after two doses of SCB-2019 (two recombinant protein vaccines adjuvanted with CpG1018-alum hydroxide) in participants who were SARS-CoV-2 naive;[19–21] and the NAbs for pseudotype virus was 955·7 IU/mL after a third dose of BNT162b2 and 1078·5 IU/mL after a third dose of mRNA1273 in individuals who were primed with two doses of BNT162b2.[4] Furthermore, the concentration of serum NAbs in participants who received aerosolised Ad5-nCoV were higher or similar to the highest concentration of NAbs induced by heterologous booster immunisation incorporating different COVID-19 vaccines, such as mRNA, viral-vector, and protein-adjuvant vaccines reported previously.[4,22]

Several studies have found that heterologous prime-boost regimens with COVID-19 vaccines from multiple platforms could be more effective than two doses of a single platform vaccine.[23,24] Our results not only showed that a heterologous booster with aerosolised Ad5-nCoV in those previously vaccinated with two doses of CoronaVac could induce stronger NAbs responses than a third dose of CoronaVac, but also triggered stronger NAbs responses compared with the response generated by intramuscular injection of AD5-nCOV, a recombinant Ad5-vectored COVID-19 vaccine containing 5×10^{10} viral particles per dose, as a heterologous booster dose in vaccinated adults (617 IU/mL).[14] These results suggest that oral inhalation could enhance the immunogenicity of the aerosolised Ad5-vectored vaccine compared with the intramuscular injection of this vaccine as a booster. Compared with intramuscular vaccines, airway mucosal vaccine-elicited IgA and resident memory B and T cells in the respiratory mucosa provide an effective barrier to infection at those sites.[25] Resident memory B and T cells,

which encounter the antigen early and respond more quickly than systemic memory cells, impede viral replication and reduce viral shedding and transmission.[26]

Other SARS-CoV-2 vaccines delivered through the mucosa are currently in clinical trials,[27] but they are all intranasal vaccine candidates. The aerosolised Ad5-nCoV vaccine we used is the only COVID-19 vaccine candidate in clinical trials administrated by oral inhalation. Evidence from non-human primate studies showed that delivering an aerosolised vaccine via the mouth could induce a more effective circulating humoral response than intranasal delivery.[28] Results showed that orally aerosolised Ad5-nCoV vaccine with a quarter of the dose of intranasal Ad5-nCoV vaccine produced anti-SARS-CoV-2 spike IgG antibodies with ten-times higher GMT than that produced by intranasal administration, and also higher IgA antibody concentrations in the bronchoalveolar lavage fluid.[28] Although the exact mechanism is unclear, the orally administered aerosolised Ad5-nCoV might target lymphoid tissues of both the upper and lower of the respiratory tract increasing the efficiency of entry of the viral vectors or prolonging the expression of viral antigen by the vaccine vectors.

The responses boosted by the heterologous aerosolised Ad5-nCoV in this study also showed a high cross-neutralising capability to the Delta variant of SARS-CoV-2, which was about three-times higher than the WHO international standard (NIBSC code 20/136), whereas the neutralising capability against the Delta variant elicited by homologous booster with CoronaVac was less than 20% of that for WHO international standard as reference. The higher correlation coefficients between NAbs titres and the RBD-specific binding antibodies in serum in participants who received aerosolised Ad5-nCoV might reflect a higher ratio of NAbs to total antibodies than that in participants who received CoronaVac.

Higher cellular immunity is another important advantage of the immune responses induced by aerosolised Ad5-nCoV compared with inactivated vaccines, which is crucial in the elimination of the SARS-CoV-2 virus-infected cells.[29] The inhaled immunisation with low dose or high dose aerosolised Ad5-nCoV booster vaccine could induce a Th1-skewed cell-mediated immunity.

Orally administered aerosolised Ad5-nCoV showed an excellent safety profile as a booster dose after two-dose priming with CoronaVac. We observed lower occurrences of adverse reactions in participants who received the aerosolised Ad5-nCoV than in participants who received the CoronaVac booster, which were also significantly lower than that following the one-dose regimen of AD5-nCOV.[12,16] Immunisation with aerosolised Ad5-nCoV by inhaling and exhaling through the mouth used only one-fifth to two-fifths of the volume of one dose of AD5-nCOV, and was associated with a reduced occurrence and severity of adverse reactions following the vaccination.[14,16] No immune thrombocytopenia and thrombosis were found in individuals who received

aerosolised Ad5-nCoV by 14 days after the booster dose. However, these results were collected over a short time period: until 28 days after booster vaccination. Long-term follow-up data are therefore required to better characterise the long-term persistence of immunogenicity and long-term safety.

As an interim analysis, our trial has some limitations. First, we did not report the elicited mucosal immunities in individuals who received aerosolised Ad5-nCoV. Although antibody-secreting cells that produce IgA were expected and t was planned in the protocol to measure secreting IgA in saliva, the establishment and validation of the methods for these tests are not yet complete. Second, we only measured the serum NAbs titres against the Delta variant but not other variants of concern, including the Omicron (B.1.1.529) variant. Considerable escape of the Omicron variant to antibody neutralisation induced by vaccination has been reported,[30,31] and newly emerging SARS-CoV-2 variants have also raised concerns about the antibody-mediated efficacy of COVID-19 vaccines. Third, we only evaluated immunogenicity and not the efficacy of the heterologous boost immunisation with the aerosolised Ad5-nCoV in vaccinated individuals. The high concentration of serum NAbs and IgA following the booster is encouraging, but might not guarantee a higher efficacy against symptomatic COVID-19 and community transmission of SARS-CoV-2 compared with boosting with other types of vaccines. Finally, the sample size of this study is relatively small, with a relatively young mean age and short follow-up period. To confirm the clinical significance of the immunogenicity reported here and longer-term safety, additional studies are required.

In conclusion, our results showed that a heterologous boost immunisation with an aerosolised Ad5-nCoV in previously vaccinated adults is safe and highly immunogenic, and elicited significantly higher NAbs concentrations than a homologous third dose of CoronaVac. A multicentre, randomised, double-blind, parallel-controlled efficacy trial of aerosolised Ad5-nCoV as a booster dose following a primary series of immunisation with AD5-nCOV is plannedin Mexico (NCT05124561).

Contributors
J-XL was the principal investigator. J-XL, S-PW, and RT designed the trials and the study protocol. J-XL drafted the manuscript. F-CZ and WC critically reviewed and revised the report. J-XL, S-PW, and RT interpreted the data and revised the manuscript. X-LG led the laboratory analyses. B-YH, L-HH, W-XS, YC, X-YX, W-JT, and L-BC did the laboratory tests. TZ and H-XP supervised the study. X-QC, H-TH, and JZ led and participated in the site work, including the recruitment, follow-up, and data collection. Z-PL and X-YZ did the statistical analysis. J-BG, XW, and X-LZ monitored the trial. All authors had full accessed to and verified the data. F-CZ and WC were responsible for the decision to submit the manuscript. All the authors had final responsibility for the decision to submit for publication.

Declaration of interests
H-TH, J-BG, W-XS, XW, X-LZ, and TZ are employees of CanSino Biologics. All the other authors declare no competing interests.

Data sharing
The individual participant data that underlie the results reported in this Article, after de-identification will be shared. Because this clinical trial is

ongoing, the data will be available 1 month after the completion of the study. The study protocol will be available immediately following publication for at least 1 year. Researchers who provide a scientifically sound proposal will be allowed to access to the de-identified individual participant data. Proposals should be directed to the corresponding authors.

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