Safety and immunogenicity of heterologous boosting with orally aerosolised or intramuscular Ad5-nCoV vaccine and homologous boosting with inactivated vaccines (BBIBP-CorV or CoronaVac) in children and adolescents: a randomised, open-label, parallel-controlled, non-inferiority, single-centre study

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Summary

Background Heterologous booster immunisation with orally administered aerosolised Ad5-nCoV vaccine (AAd5) has been shown to be safe and highly immunogenic in adults. Here, we aimed to assess the safety and immunogenicity of heterologous booster immunisation with orally administered AAd5 in children and adolescents aged 6–17 years who had received two doses of inactivated vaccine (BBIBP-CorV or CoronaVac).

Methods We did a randomised, open-label, parallel-controlled, non-inferiority study to assess the safety and immunogenicity of heterologous booster immunisation with AAd5 (0·1 mL) or intramuscular Ad5-nCoV vaccine (IMAd5; 0·3 mL) and homologous booster immunisation with inactivated vaccine (BBIBP-CorV or CoronaVac; 0·5 mL) in children (aged 6–12 years) and adolescents (aged 13–17 years) who had received two doses of inactivated vaccine at least 3 months earlier in Hunan, China. Children and adolescents who were previously immunised with two-dose BBIBP-CorV or CoronaVac were recruited for eligibility screening at least 3 months after the second dose. A stratified block method was used for randomisation, and participants were stratified by age and randomly assigned (3:1:1) to receive AAd5, IMAd5, or inactivated vaccine. The study staff and participants were not masked to treatment allocation. Laboratory and statistical staff were masked during the study. In this interim analysis, adverse events within 14 days and geometric mean titre (GMT) of serum neutralising antibodies on day 28 after the booster vaccination, based on the per-protocol population, were used as the primary outcomes. The analysis of non-inferiority was based on comparison using a one-sided 97·5% CI with a non-inferiority margin of 0·67. This study was registered at ClinicalTrials.gov, NCT05330871, and is ongoing.

Findings Between April 17 and May 28, 2022, 436 participants were screened and 360 were enrolled: 220 received AAd5, 70 received IMAd5, and 70 received inactivated vaccine. Within 14 days after booster vaccination, vaccine-related adverse reactions were reported: 35 adverse events (in 13 [12%] of 110 children and 22 [20%] of 110 adolescents) in 220 individuals in the AAd5 group, 35 (in 18 [51%] of 35 children and 17 [49%] of 35 adolescents) in 70 individuals in the IMAd5 group, and 13 (in five [14%] of 35 children and eight [23%] of 35 adolescents) in 70 individuals in the inactivated vaccine group. Solicited adverse reactions were also reported: 34 (13 [12%] of 110 children and 21 [10%] of 110 adolescents) in 220 individuals in the AAd5 group, 34 (17 [49%] of 35 children and 17 [49%] of 35 adolescents) in 70 individuals in the IMAd5 group, and 12 (five [14%] of 35 children and seven [20%] of 35 adolescents) in 70 individuals in the inactivated vaccine group. The GMTs of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 (Pango lineage B) in the AAd5 group were significantly higher than the GMTs in the inactivated vaccine group (adjusted GMT ratio 10·2 [95% CI 8·0–13·1]; p<0·0001).

Interpretation Our study shows that a heterologous booster with AAd5 is safe and highly immunogenic against ancestral SARS-CoV-2 Wuhan-Hu-1 in children and adolescents.

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Research in context

Evidence before this study

We searched ClinicalTrials.gov and PubMed for clinical trials of mucosal or intranasal COVID-19 vaccines in children and adolescents, from database inception to Nov 15, 2022, using the terms "(COVID-19 OR SARS-CoV-2) AND (mucosal vaccine or intranasal vaccine) AND (children OR adolescents) AND (clinical trial)" with no language restrictions. No trials of COVID-19 vaccination by the mucosal immune pathway in the paediatric population were identified in PubMed. Only one phase 1 clinical trial .is evaluating the safety and immunogenicity of CVXGA1–001, the intranasal parainfluenza virus 5 COVID-19 vaccine expressing SARS-CoV-2 spike protein, in adolescents and adults (aged 12–55 years). This trial started on Aug 6, 2021, and is estimated to be completed by Jan 31, 2024.

Added value of this study

Our results showed that a heterologous booster with aerosolised Ad5-nCoV vaccine (AAd5) was safe and had fewer adverse reactions than did intramuscular inactivated vaccines. Although AAd5, intramuscular Ad5-nCoV (IMAd5), and inactivated vaccine booster induced increased neutralising antibodies and receptor-binding domain-specific IgG antibodies compared with antibody titres before booster vaccination in both children and adolescents 28 days after immunisation, heterologous AAd5 and IMAd5 immunisation elicited a higher antibody response than did homologous inactivated vaccine immunisation. Booster vaccinated children and adolescents produced similar neutralising antibody and RBD-specific IgG antibody responses. However, adolescents produced a more robust T-cell response than did children. In particular, AAd5 showed better immunogenicity against SARS-CoV-2 omicron BA.4 and BA.5 than that of inactivated vaccine. The lower dose, lower rates of adverse events, and good compliance with AAd5 in children and adolescents suggest that use of this vaccine might be a promising approach for booster vaccination in children and adolescents.

Implications of all the available evidence

Our results support the benefit of heterologous booster vaccination with AAd5 after a primary series of inactivated and Ad5-nCoV vaccines. The advantage of good compliance with AAd5 for the child and adolescent population could be one reason to recommend this booster strategy. AAd5 vaccine also showed good immunogenicity against omicron BA.4 and BA.5.

Introduction

The current COVID-19 vaccines, including mRNA, adenovirus vector, recombinant protein, and inactivated vaccines, have effectively protected against hospitalisation, severe illness, and death.[1–3] However, the continuous emergence of SARS-CoV-2 variants of concern, which can escape neutralisation elicited by infection or vaccination, remains a considerable challenge to public health and has caused increasing numbers of breakthrough infections and new waves of COVID-19 worldwide.[4–6]

Due to the waning of immunity induced by primary series vaccination, booster vaccination (including heterologous and homologous vaccination) has been shown to be safe and highly immunogenic, and has been implemented globally. A phase 2/3 clinical trial of booster vaccination with BNT162b2 mRNA COVID-19 vaccine in children (aged 5–12 years) reported on safety and immunogenicity, showed mostly mild reactogenicity, and was expected to confer protection against COVID-19, including against omicron.[7] Notably, heterologous booster vaccination is more immunogenic than homologous booster vaccination.[8–11] The current COVID-19 vaccines are intramuscular injections that can effectively stimulate the systemic immune response; however, despite the additional protection against SARS-CoV-2 omicron variants and mild reactogenicity of booster vaccination, the mucosal immune response is weak.[12] Because SARS-CoV-2 initially infects the upper respiratory tract, developing a nasal spray or aerosolised COVID-19 vaccine that elicits high mucosal immunity in the respiratory tract might be a key strategy to restrict viral replication and the clearance of SARS-CoV-2.[13]

In October, 2022, an orally administered aerosolised Ad5-nCoV vaccine (AAd5) that encodes the SARS-CoV-2 spike protein, developed by CanSino Biologics (Tianjin, China),[14,15] was authorised for emergency use for booster vaccination in adults in China. A clinical trial has shown that heterologous booster vaccination with AAd5 is safe and highly immunogenic in adults.[14–16] However, the safety and immunogenicity of heterologous booster immunisation with AAd5 in children and adolescents after two doses of inactivated vaccine are unknown. Therefore, we did a randomised, open-label, parallel-controlled, non-inferiority study to assess the safety and immunogenicity of heterologous booster immunisation with AAd5 vaccine in children aged 6–12 years and adolescents aged 13–17 years who had received two doses of inactivated vaccine. Additionally, we did an exploratory comparison of safety and immunogenicity with AAd5 and intramuscular Ad5-nCoV vaccine (IMAd5).

Methods

Study design and participants

We did a randomised, open-label, parallel-controlled, non-inferiority study to assess the safety and immunogenicity of heterologous booster immunisation with orally administered AAd5 (CanSino, Tianjin, China), IMAd5 (CanSino, Tianjin, China), or homologous booster immunisation with the inactivated vaccine BBIBP-CorV

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(Sinopharm, Beijing, China) or CoronaVac (Sinovac, Beijing, China) in children aged 6–12 years and adolescents aged 13–17 years who had received two doses of BBIBP-CorV or CoronaVac in Hunan, China. Children and adolescents who were previously immunised with two-dose BBIBP-CorV or CoronaVac were recruited for eligibility screening at least 3 months after the second dose. Children and adolescents were recruited by posters, and by conferences at local schools and township health centres, to the Luxi County Center for Disease Control and Prevention in Hunan, China. Details of inclusion and exclusion criteria are shown in the appendix (pp 1–2). Data on patient sex were collected by self-report. The study protocol (appendix p 28) and informed consent were reviewed and approved by the Ethics Committee of the Seventh Medical Center of PLA General Hospital (S2021–001–02) and Hunan Provincial Center for Disease Control and Prevention (IRB-PJ2022017), China. Written informed consent was obtained from all parents or guardians of children and adolescents before the psychological, clinical, and laboratory evaluation. No data monitoring board or safety monitoring board was set up for this study.

See Online for appendix

Randomisation and masking

The first five participants in the 13–17-year-old age group (adolescents) and 6–12-year-old age group (children) were assigned to two sentinel groups; participants aged 13–17 years were enrolled before those aged 6–12 years. The participants of the sentinel groups were allocated to receive AAd5 by the order of signing of informed consent and were monitored for safety before the rest of the enrolment process. A stratified block randomisation method was used for the other 350 participants. 175 participants in the adolescent group and 175 participants in the child group who met the eligibility criteria were randomly assigned (3:1:1), by a randomisation statistician using SAS statistical software (version 9.4), to receive AAd5 (n=105), IMAd5 (n=35), or inactivated vaccine (n=35) in each age group. An independent statistician generated the randomisation lists with SAS software (version 9.4). Excluding the sentinel groups, the first 50 participants in each age group who had been allocated to one of the three treatment groups were enrolled in a safety group; the next 51–125 participants were enrolled in an immune persistence group, and the next 126–175 participants were enrolled in a cellular immunity group (appendix p 17). The study staff and participants were not masked to treatment allocation. Laboratory and statistical staff who did immunological experiments and statistical analysis were masked to group allocation during the whole study; they identified samples or groups by serial numbers.

Procedures

Orally administered AAd5 and IMAd5, recombinant adenovirus type-5 (Ad5)-vectored vaccines expressing the full-length spike gene of ancestral SARS-CoV-2 Wuhan-Hu-1 (accession number NC_045512.2),[17] were manufactured at CanSino Biologics (Tianjin, China). AAd5 was supplied as a liquid formulation of 1·5 mL per vial at a concentration of $1·0\times10^{11}$ viral particles per mL, and 0·1 mL per dose was administered. IMAd5 is a single-dose vaccine containing $1·0\times10^{11}$ viral particles per mL, and 0·3 mL per dose was administered. BBIBP-CorV (Sinopharm; Beijing, China) and CoronaVac (Sinovac; Beijing, China) vaccines are two inactivated whole-virion vaccines with aluminium hydroxide as the adjuvant, and these vaccines were administered intramuscularly at 0·5 mL per dose.

The follow-up time of the full study was 12 months; here, we report the interim analysis with endpoints up to 28 days after booster vaccination. Participants were monitored on site for 30 min for any immediate vaccine-associated reactions after vaccination and were instructed to keep a daily record of any solicited or unsolicited adverse events for the next 28 days. All reactions were reported by participants via telephone. Medical history, current medical status, adverse events, and serious adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 25.0). Serious adverse events reported by participants were documented throughout the study. Adverse reactions were defined as unexpected or damaging reactions that occurred during vaccination at the prescribed doses and procedures, and were generally associated with vaccination. Blood samples for laboratory testing (eg, complete blood count, blood biochemistry, conventional coagulation examinations) were collected before (day 0) and at day 4 after the booster dose in the sentinel group and safety group. Blood samples for antibody measurements (neutralising antibodies against the ancestral SARS-CoV-2 Wuhan-Hu-1, receptor-binding domain [RBD]-specific IgG antibodies, and RBD-specific binding IgA) were collected from all participants at baseline (day 0) and at day 28 after the booster dose. For measurement of T-cell responses, peripheral blood mononuclear cells were collected from blood samples of participants in the cellular immunity group and assessed before (day 0) and at day 14 after the booster vaccination. Details of antibody measurements and SARS-CoV-2 spike protein-specific T-cell response are specified in the appendix (pp 23–24).

Outcomes

The primary endpoint for safety (assessed in the safety population: all participants who received booster vaccine) was the incidence of adverse reactions within 14 days after booster vaccination. Adverse reactions included solicited local and systemic reactions, and all adverse events are listed in the appendix (pp 2–10). The secondary endpoints for safety (assessed in the safety population) were incidence of adverse reactions and events 30 min after immunisation, incidence of adverse reactions and events within days 0–28 after immunisation, incidence

of serious adverse events within 12 months after immunisation, and changes in laboratory test indicators (eg, white blood cell count, lymphocyte count, eosinophils, neutrophils, platelets, haemoglobin, alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatinine, and activated partial thromboplastin time) and respiratory rate on day 4 after each dose in the sentinel group and safety group. The primary endpoint for immunogenicity (assessed in the per-protocol population: all participants who did not violate the inclusion or exclusion criteria, underwent randomisation, had complete immunisation, had complete blood collection for immunogenicity evaluation before immunisation and at corresponding timepoints, and had antibody test results) was the geometric mean titre (GMT) of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 on day 28 after the booster dose. The secondary endpoint for immunogenicity (assessed in the per-protocol population) included seroconversion rates and geometric mean fold increase (GMI) of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1, as well as geometric mean concentration (GMC), seroconversion rates, and GMI of RBD-specific IgG antibodies on day 28 after the booster dose. Other secondary endpoints were baseline concentration of anti-Ad5-specific neutralising antibodies in participants, and stratified analysis based on baseline concentration of anti-Ad5-specific neutralising antibodies (Ad5 titre >200 and Ad5 titre ≤200). The exploratory endpoints (assessed in the full analysis set: all patients who followed the principle of intention-to-treat, were randomly assigned, received at least one dose of vaccine, completed pre-vaccination blood collection, and had antibody test results) were immunogenicity, including GMC, GMI of antibodies, and seroconversion rate of IgA antibodies on day 28 after immunisation; and seroconversion rate and response of interferon (IFN)-γ before immunisation and on day 14 after immunisation. Seroconversion was defined as a titre equal to or greater than a four-fold increase on day 28 compared with titre before booster vaccination. The post-hoc outcomes (assessed in the full analysis set) were GMT, GMI, and seroconversion rate of neutralising antibodies against SARS-CoV-2 omicron (BA.4 and BA.5).

Statistical analysis

The sample size was calculated using PASS (version 16; PASS, Kaysville, UT, USA) and non-inferiority testing for the ratio of two means. Because there were no immunogenicity data on booster AAd5 use for children and adolescents, the immunogenicity data of GMT (150·287 [SD 2·1519]) of neutralising antibodies 28 days after the booster dose in adults were used as a reference.[16] Considering the possible large variability of AAd5 in children and adolescents, we assumed that the SD was 2·73 and the coefficient of variation of GMT was 1·32. The non-inferiority margin was set to 0·67, and the GMT ratio was assumed to be 1·0. The AAd5 and inactivated vaccine groups were allocated at a ratio of 3:1 and, taking into account the dropout rate of approximately 5%, the final sample size was 211 and 71, respectively. We also added 70 participants (in the IMAd5 group) to explore the immunogenicity of IMAd5.

Data were analysed by SAS (version 9.4). The analysis of non-inferiority was based on comparison using a one-sided 97·5% CI with a non-inferiority margin of 0·67. Continuous variables were summarised as the mean (SD) or median (IQR), and categorical variables were summarised as frequencies and proportions. In the population aged 6–17 years, analysis of covariance of GMT of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 and GMT ratio 28 days after immunisation were adjusted for confounders, including treatment group (AAd5, IMAd5, and inactivated vaccine), age group (6–12 years and 13–17 years), and baseline anti-Ad5-specific neutralising antibodies (Ad5 titre >200and Ad5 titre ≤200). The seroconversion rate and rate difference of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 were calculated by fitting with the logistic regression model adjusted for the aforementioned confounders. In the child and adolescent cohorts, the Wald method was used to construct the 95% CI of the mean GMT (or GMC). The Wilson score method, which inverts the related score test with null rather than estimated SE, yields coverage probabilities close to nominal confidence levels, even for very small sample sizes,[18] and was used to calculate the 95% CI of the seroconversion rate of the antibody titre. The χ^2 test or Fisher's exact test was used to compare the difference in the seroconversion rate between the three subgroups in the child and adolescent cohorts. The difference in seroconversion rate was estimated with 95% CI and p value, using the Cochran–Mantel–Haenszel χ^2 test. The Shapiro–Wilk test was used to test for normal distribution and the Levene test for variance homogeneity. The independent sample *t* test was used on the basis of logarithmic transformation to compare GMTs and calculate the GMT ratio between each vaccination type and between anti-Ad5 concentrations in the child and adolescent cohorts. The Kruskal–Wallis test with the false discovery rate method was used for multiple comparisons of antibody titres by vaccine type. The Mann–Whitney U test was used to compare T-cell response differences before and after the booster dose. The per-protocol population was used for analysis of the primary endpoint for immunogenicity and the safety population was used for the primary endpoint for safety.

This study was registered at ClinicalTrials.gov, NCT05330871.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between April 17 and May 28, 2022, 436 children and adolescents were screened. 360 were enrolled, of whom 180 were children aged 6–12 years and 180 were adolescents aged 13–17 years. Five adolescents and five children were allocated to the sentinel group to receive AAd5, and the other adolescents and children were randomly assigned to receive AAd5 (n=105), IMAd5 (n=35), or inactivated vaccine (n=35), respectively. By 28 days after booster immunisation, 19 participants were withdrawn from the study; five were lost to follow-up, three were outside of the visitation window, and 11 had unsuccessful assays. The 19 participants who withdrew were not included in the per-protocol population (figure 1; appendix pp 9–10). The number of participants that were included in the safety population (for safety analysis, laboratory test indicators, and respiratory rate), per-protocol population (for analysis of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 and RBD-specific binding IgG antibodies), and full analysis set (for analysis of RBD-specific binding IgA antibodies, neutralising antibodies against omicron, and IFN-γ) are shown in the appendix (p 20). The demographic characteristics of children and adolescents are shown in table 1.

Within 14 days after booster vaccination, vaccine-related adverse reactions were reported: 35 adverse events (in 13 [12%] of 110 children and 22 [20%] of 110 adolescents) in 220 individuals in the AAd5 group, 35 (in 18 [51%] of 35 children and 17 [49%] of 35 adolescents) in 70 individuals in the IMAd5 group, and 13 (in five [14%] of 35 children and eight [23%] of 35 adolescents) in 70 individuals in the inactivated vaccine group. Solicited adverse reactions were also reported: 34 (13 [12%] of 110 children and 21 [10%] of 110 adolescents) in 220 individuals in the AAd5 group, 34 (17 [49%] of 35 children and 17 [49%] of 35 adolescents) in 70 individuals in the IMAd5 group, and 12 (five [14%] of 35 children and seven [20%] of 35 adolescents) in 70 individuals in the inactivated vaccine group. For the children and adolescents who received AAd5, the most frequent adverse event was xerostomia, and a few participants (two [2%] children and seven [6%] adolescents) had a fever (≥37·3°C). A higher proportion of participants reported fever in the IMAd5 group (12 [34%] of 35 children and six [17%] of 35 adolescents) than in the AAd5 and inactivated vaccine groups (p<0·0001). The common adverse reactions of the injection site were pain, swelling, itching, sclerosis, and redness in children and adolescents who received IMAd5 and inactivated vaccine. Respiratory-related adverse reactions included cough and runny nose, and there were no significant differences among the three groups (table 2).

Generally, results for all participants aged 6–17 years in the per-protocol population showed that the lower limit of the 95% CI of the GMT ratio of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 28 days after immunisation in the AAd5 group compared with the inactivated vaccine group was higher than 0·67 (appendix

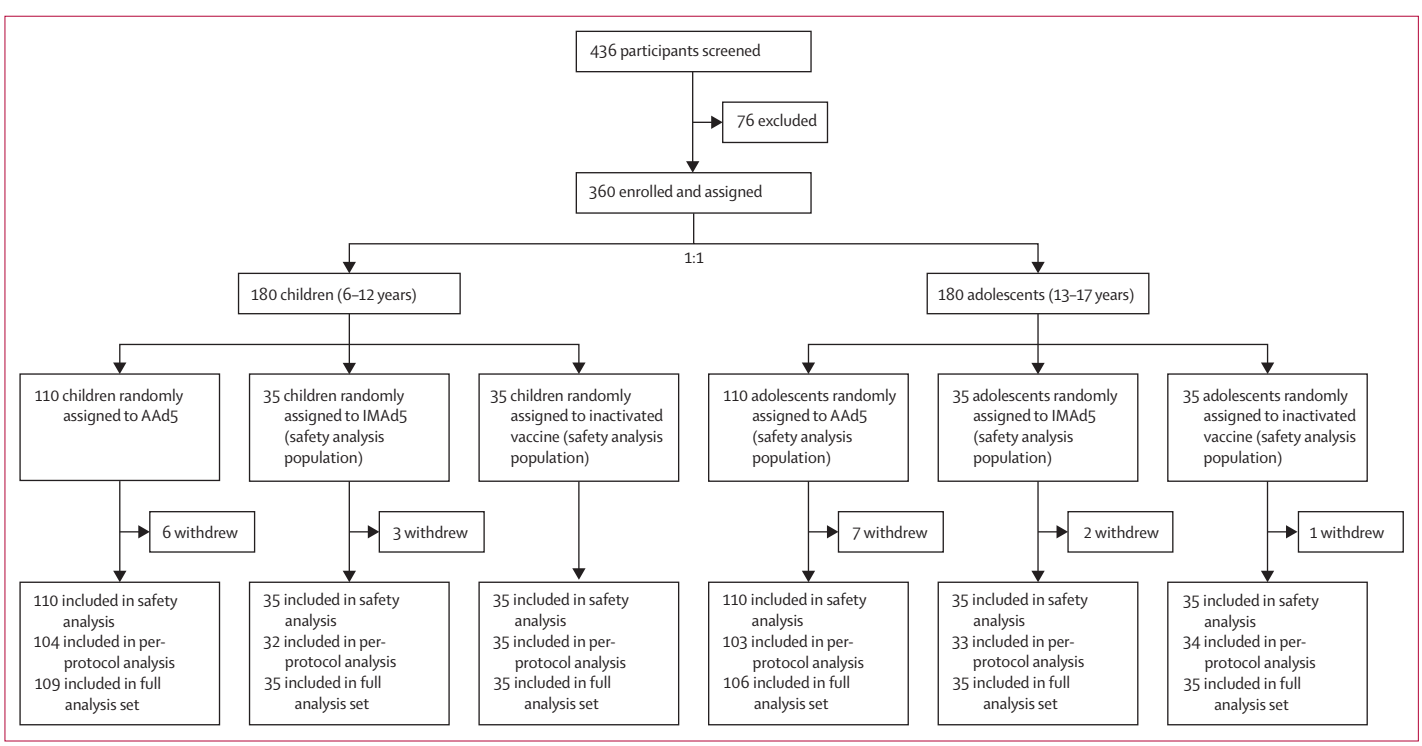

Figure 1: **Trial profile**
For details of group allocation for analysis, see appendix (p 10). Ad5=aerosolised Ad5-nCoV vaccine. IMAd5=intramuscular Ad5-nCoV vaccine.

	Children			Adolescents		
	AAd5 (n=110)	IMAd5 (n=35)	Inactivated vaccine (n=35)	AAd5 (n=110)	IMAd5 (n=35)	Inactivated vaccine (n=35)
Age, years	9·5 (1·7)	8·9 (1·5)	9·5 (1·7)	14·5 (1·2)	14·4 (1·2)	14·3 (1·3)
Sex						
Male	48 (44%)	17 (49%)	16 (46%)	62 (56%)	17 (49%)	18 (51·%)
Female	62 (56%)	18 (51%)	19 (54%)	48 (44%)	18 (51%)	17 (49%)
Weight, kg	32·4 (8·7)	30·9 (8·2)	34·4 (10·0)	56·0 (12·9)	52·2 (12·3)	52·2 (8·6)
Height, cm	137·9 (12·1)	134·9 (11·3)	140·1 (12·2)	163·4 (9·3)	161·3 (8·1)	162·0 (6·8)
Temperature, °C	36·3 (0·3)	36·3 (0·3)	36·3 (0·3)	36·4 (0·3)	36·3 (0·3)	36·5 (0·3)
Respiration rate, breaths per min*						
Number of participants who tested	35 (32%)	10 (29%)	10 (29%)	35 (32%)	10 (29%)	10 (29%)
Mean (SD)	15·5 (0·8)	15·4 (1·3)	15·7 (0·5)	15·2 (0·9)	14·9 (1·0)	15·1 (1·1)
Participants with an abnormality in the oral cavity or nasal cavity that could affect the trial	0	0	0	0	0	0
Pre-existing Ad5 neutralising antibody†						
Titre	793·4 (1620·4)	950·5 (2116·8)	700·7 (1685·0)	1038·9 (1393·7)	979·1 (1770·3)	835·2 (1037·7)
Participants with titre ≤1:200	59 (54%)	19 (54%)	24 (69%)	43 (39%)	21 (60%)	14 (40%)
Participants with titre >1:200	51 (46%)	16 (46%)	11 (31%)	67 (61%)	14 (40%)	21 (60%)

Data are n (%) or mean (SD). The analysis was based on the intention-to-treat population (ie, all participants who followed the principle of intention-to-treat and were randomly assigned) for neutralising antibodies. AAd5=aerosolised Ad5-nCoV vaccine. IMAd5=intramuscular Ad5-nCoV vaccine. *Only the sentinel group (n=5) and the safety group (n=30 in AAd5; n=10 in IMAd5; n=10 in the inactivated vaccine group were evaluated. †Comparison results after logarithmic transformation.

Table 1: **Demographics and baseline characteristics of the intention-to-treat population**

p 16), suggesting that the GMT of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 in participants who received AAd5 28 days after immunisation was not inferior to that in those who received inactivated vaccine. Further statistical testing was done to evaluate whether AAd5 was superior to inactivated vaccine. The lower limit of the 95% CI of the GMT ratio of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 28 days after immunisation in the AAd5 group compared with the inactivated vaccine group was higher than 1, suggesting that the GMT of neutralising antibodies in the AAd5 group 28 days after immunisation was superior to that of the inactivated vaccine group (appendix p 16).

In the population aged 6–17 years, the GMTs of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 were increased 28 days after booster in the AAd5 (461·2 [95% CI 401·0–530·4]), the IMAd5 (349·3 [290·8–419·5]), and the inactivated vaccine (47·5 [37·8–59·7]) groups compared with the GMTs of 4·5 (4·3–4·7), 4·2 (4·0–4·4), and 4·4 (4·1–4·7) in the same groups on day 0 (figure 2A). The adjusted GMT ratio of AAd5 to inactivated vaccine was 10·2 (95% CI 8·0–13·1; p<0·0001). These results showed that the immunogenicity of AAd5 was better than that of inactivated vaccine (figure 2; appendix p 16).

No adverse reactions were reported between day 14 and day 28. There were no abnormal laboratory test indicator results reported at day 4 after booster vaccination, with the exception of one adolescent in the AAd5 group who reported an increased white blood cell count. No statistically significant difference in respiratory rate on day 4 compared with that on day 0 after booster vaccination was reported (appendix pp 14–15).

In the child cohort 4 weeks after booster vaccination, the GMTs of neutralising antibodies against ancestral SARS-CoV-2 Wuhan-Hu-1 had increased in the AAd5 (446·9 [95% CI 361·5–552·6]), IMAd5 (372·9 [297·0–468·2]), and inactivated vaccine (51·6 [36·6–72·7]) groups compared with the measures of 4·7 (4·4–5·1), 4·2 (3·9–4·6), and 4·5 (4·1–5·0) in the same groups before booster vaccination (figure 2B), with 95·0-times (76·6–117·9), 88·1-times (69·9–111·1), and 11·4-times (8·0–16·2) increases in GMT, respectively. In line with the child cohort, the GMTs of neutralising antibodies in the adolescent cohort significantly increased in the AAd5, the IMAd5, and the inactivated vaccine groups 4 weeks after the booster vaccination compared with the measures before vaccination (figure 2C), with GMTs of 476·0 (395·4–573·1) for AAd5, 327·8 (243·7–441·0) for IMAd5, and 43·6 (31·7–59·9) for inactivated vaccine; in the respective treatment groups, there were 111·3-times (91·4–135·7), 79·3-times (58·9–106·8), and 10·3-times (7·3–14·4) increases in GMT compared with measures before the booster vaccination.

No statistically significant differences in neutralising antibody seroconversion rates occurred between the three treatment groups in the child or adolescent cohorts, except that the seroconversion rate for adolescents in the AAd5 group was 7·9% (95% CI –1·9 to 17·6) higher than that of adolescents in the inactivated vaccine group (p=0·019). However, in both age cohorts, the GMT of

	Children with AAd5 (n=110)	Children with IMAd5 (n=35)	Children with inactivated vaccine (n=35)	p value*	Adolescents with AAd5 (n=110)	Adolescents with IMAd5 (n=35)	Adolescents with inactivated vaccine (n=35)	p value*
Adverse events	13 (12%)	18 (51%)	5 (14%)	p<0·0001	22 (20%)	17 (49%)	8 (23%)	p=0·006
Solicited adverse reactions within 14 days†	13 (12%)	17 (49%)	5 (14%)	p<0·0001	21 (19%)	17 (49%)	7 (20%)	p=0·003
Local adverse reactions	9 (8%)	8 (23%)	4 (11%)	p=0·065	13 (12%)	14 (40%)	5 (14%)	p=0·001
Xerostomia	9 (8%)	NA	NA	NA	10 (9%)	NA	NA	NA
Hoarseness	0	NA	NA	NA	0	NA	NA	NA
Oral mucositis	0	NA	NA	NA	0	NA	NA	NA
Pharyngeal swelling	0	NA	NA	NA	1 (<1%)	NA	NA	NA
Pharyngalgia	1 (<1%)	NA	NA	NA	4 (4%)	NA	NA	NA
Pain	NA	5 (14%)	2 (6%)	p=0·428	NA	13 (37%)	5 (14%)	p=0·054
Induration	NA	0	0	NA	NA	1 (3%)	0	p=1·000
Swelling	NA	2 (6%)	1 (3%)	p=1·000	NA	3 (9%)	0	p=0·239
Tetter	NA	0	0	NA	NA	0	0	NA
Redness	NA	2 (6%)	1 (3%)	p=1·000	NA	3 (9%)	0	p=0·239
Itch	NA	0	0	NA	NA	2 (6%)	0	p=0·493
Cellulitis	NA	0	0	NA	NA	0	0	NA
Systemic adverse reactions								
Pharyngalgia	NA	1 (3%)	0	p=1·000	NA	0	1 (3%)	p=1·000
Fever	2 (2%)	12 (34%)	1 (3%)	p<0·0001	7 (6%)	6 (17%)	0	p=0·018
Diarrhoea	0	0	0	NA	1 (<1%)	0	1 (3%)	p=0·628
Fatigue	0	0	0	NA	3 (3%)	1 (3%)	1 (3%)	p=1·000
Nausea	0	0	0	NA	0	0	0	NA
Loss of appetite to food	0	0	0	NA	0	0	0	NA
Vomiting	0	0	0	NA	0	0	0	NA
Headache	1 (<1%)	3 (9%)	0	p=0·056	2 (2%)	3 (9%)	1 (3%)	p=0·111
Cough	0	0	0	NA	0	1 (3%)	0	p=0·389
Joint pain	1 (<1%)	0	0	p=1·000	1 (<1%)	1 (3%)	0	p=0·628
Chest pain	0	0	0	NA	0	0	0	NA
Muscle pain	1 (<1%)	0	0	p=1·000	1 (<1%)	0	0	p=1·000
Pruritus at non-inoculated sites (no skin damage)	0	1 (3%)	0	p=0·389	0	0	0	NA
Abnormal skin and mucous membrane	1 (<1%)	1 (3%)	0	p=0·628	1 (<1%)	0	0	p=1·000
Runny nose	0	0	0	NA	1 (<1%)	0	1 (3%)	p=0·628
Sneezing	0	0	0	NA	0	0	0	NA
Unsolicited adverse reactions†								
Total	2 (2%)	4 (11%)	0	p=0·028	4 (4%)	4 (11%)	1 (3%)	p=0·177
Dizzy	1 (<1%)	2 (6%)	0	p=0·194	0	3 (9%)	0	p=0·014
Anorexia	0	1 (3%)	0	p=0·389	0	0	0	NA
Allergic dermatitis	0	1 (3%)	0	p=0·389	0	0	0	NA
Altered mental status	0	1 (3%)	0	p=0·389	0	0	0	NA

Data are n (%), unless otherwise specified. AAd5=aerosolised Ad5-nCoV vaccine. IMAd5=intramuscular Ad5-nCoV vaccine. NA=not applicable. *p values were calculated by Fisher's exact test. p values show IMAd5 and inactivated vaccine groups *vs* the AAd5 group. †Unsolicited adverse events were coded with MedDRA version 25.0, and solicited adverse events were listed according to the name in the protocol.

Table 2: **Vaccine-related adverse events that occurred within 14 days after booster vaccination (safety population)**

neutralising antibodies at day 28 was significantly higher in the heterologous booster immunisation groups than in the homologous booster immunisation groups. In children, the ratio of GMT at day 28 after immunisation in the AAd5 group, compared with that of the inactivated vaccine group, was 8·7 (95% CI 5·7–13·1; *t* test p<0·0001); for the IMAd5 group versus the inactivated vaccine group the value was 7·2 (4·8–10·9; *t* test p<0·0001); and for the AAd5 group versus the IMAd5 group the value was 1·2 (0·8–1·8; *t* test p=0·374). In adolescents, the ratio of GMT at day 28 after immunisation in the AAd5 group compared with the inactivated vaccine group was 10·9 (7·6–15·8;

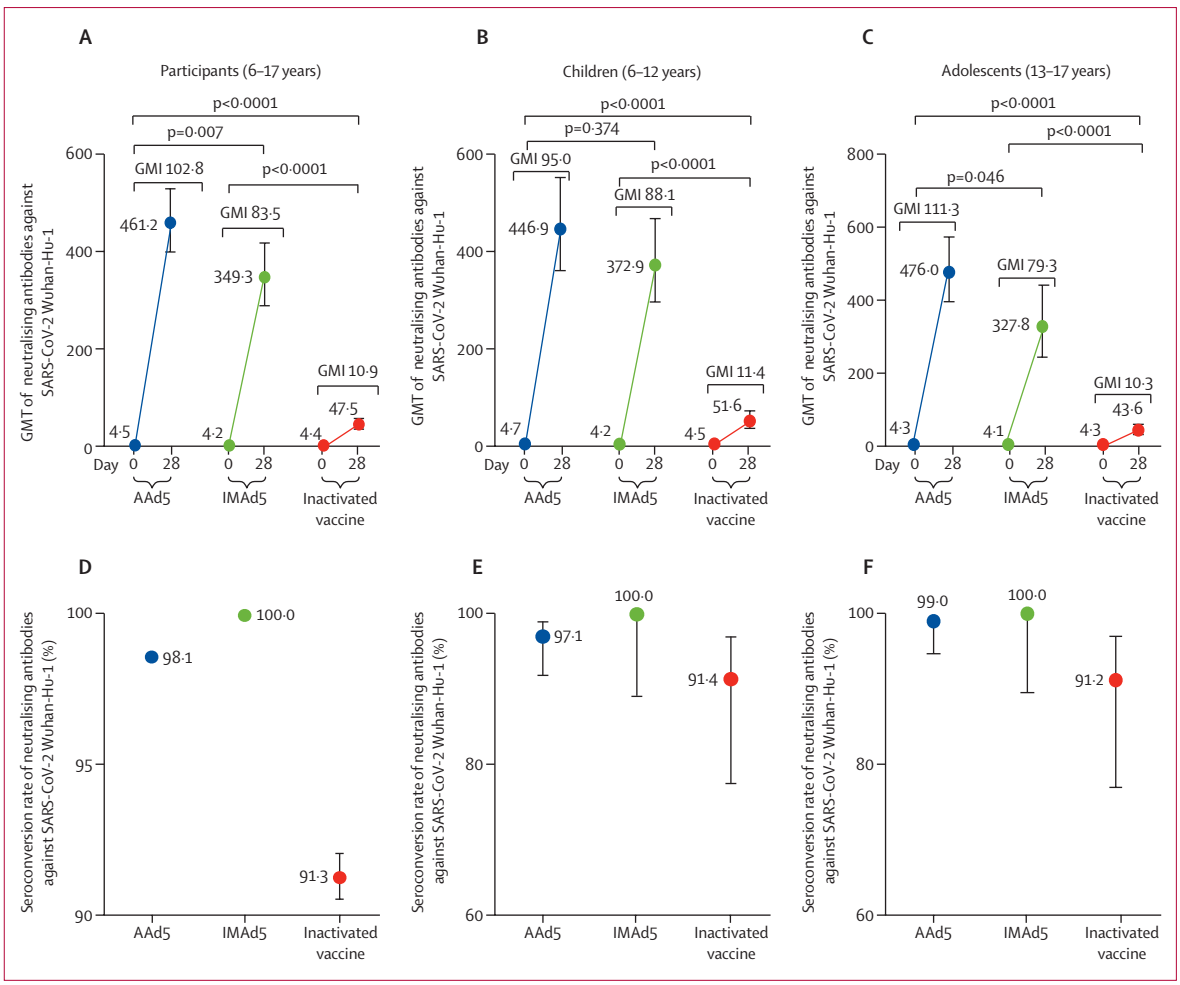

Figure 2: **Neutralising antibody titres against ancestral SARS-CoV-2 Wuhan-Hu-1 at baseline (day 0) and day 28 after booster vaccination and seroconversion rates at day 28**

Neutralising antibody titres against ancestral SARS-CoV-2 Wuhan-Hu-1 in all participants aged 6–17 years (A), children aged 6–12 years (B), and adolescents aged 13–17 years (C) who received AAd5, IMAd5, or inactivated booster vaccines. Seroconversion rates of neutralising antibodies against ancestral SARS-CoV-2 in all participants aged 6–17 years (D), children aged 6–12 years (E), and adolescents aged 13–17 years (F) who received AAd5, IMAd5, or inactivated booster vaccines at day 28. Error bars show 95% CIs. AAd5=aerosolised Ad5-nCoV vaccine. GMI=geometric mean fold increase. GMT=geometric mean titre. IMAd5=intramuscular Ad5-nCoV vaccine.

t test p<0·0001); for the IMAd5 group versus the inactivated vaccine group the value was 7·5 (4·9–11·5; *t* test p<0·0001); and for the AAd5 group versus the IMAd5 group the value was 1·46 (1·0–2·1; *t* test p=0·046; appendix p 21).

The GMI of neutralising antibodies against ancestral SARS-CoV2 Wuhan-Hu-1 was higher in children with an Ad5 titre of less than or equal to 200 (158·4 [95% CI 134·9–186·0]) than in children with an Ad5 titre of more than 200 (52·3 [36·2–75·5]) in the AAd5 group (p<0·0001); by contrast, GMIs were similar in children with an Ad5 titre of at least 200 compared with those with an Ad5 titre of less than 200 in the IMAd5 and inactivated vaccine groups. Similar findings were found in adolescents (appendix pp 24–26).

In children, RBD-specific binding IgG antibody concentrations were higher on day 28 after booster immunisation than on day 0 (figure 3; appendix p 18); the GMC in children in the AAd5, the IMAd5, and the inactivated vaccine groups increased by 121·6-times (95% CI 93·3–158·5), 102·9-times (80·2–132·0), and 12·0-times (8·2–17·4), respectively. Similar results were found in adolescents (figure 3; appendix p 18). 103 (95%) of 108 children in the AAd5 group, 34 (100) of 34 children in the IMAd5 group, and 32 (91%) of 35 children in the inactivated vaccine group, as well as 105 (99%) of 106 adolescents in the AAd5 group, 34 (100%) of 34 adolescents in the IMAd5 group, and 33 (94%) of 34 adolescents in the inactivated vaccine group, had seroconversion on day 28 after booster vaccination (figure 3).

Similar to neutralising antibodies and RBD-specific IgG antibodies, RBD-specific binding IgA antibody concentrations also increased 28 days after booster

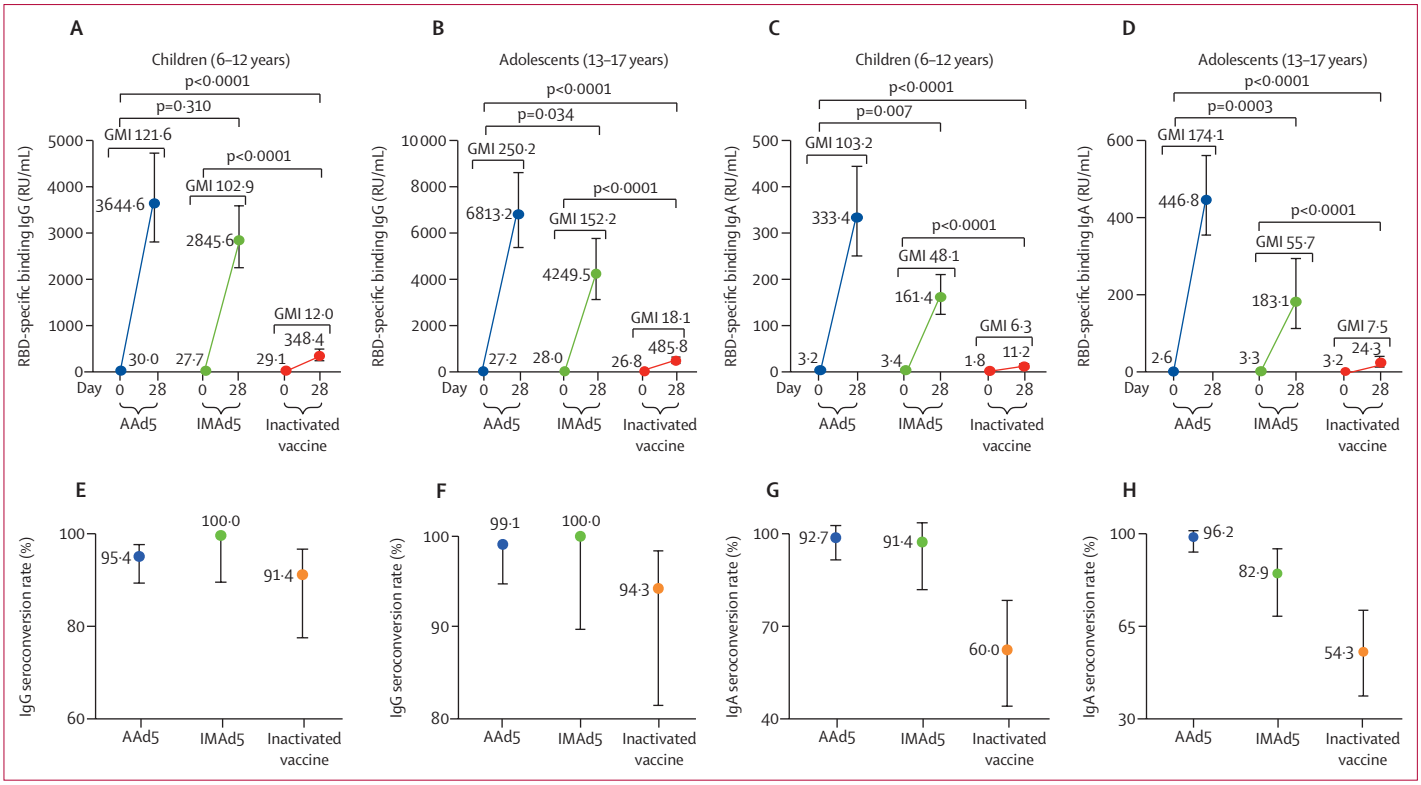

***Figure 3*: RBD-specific binding IgG and IgA antibodies at baseline (day 0) and day 28 after booster vaccination**

SARS-CoV-2 spike RBD-specific binding IgG antibody in children aged 6–12 years (A) and adolescents aged 13–17 years (B) who received AAd5, IMAd5, or inactivated booster vaccines. Seroconversion rates of RBD-specific binding IgG in children aged 6–12 years (C) and adolescents aged 13–17 years (D) who received AAd5, IMAd5, or inactivated boosters. SARS-CoV-2 spike RBD-specific binding IgA antibodies in children aged 6–12 years (E) and adolescents aged 13–17 years (F) who received AAd5, IMAd5, or inactivated boosters. Seroconversion rate of RBD-specific binding IgA in children (G) and adolescents (H) who received AAd5, IMAd5, or inactivated boosters. Error bars show 95% CIs. AAd5=aerosolised Ad5-nCoV vaccine. GMI=geometric mean fold increase. IMAd5=intramuscular Ad5-nCoV vaccine. RBD=receptor-binding domain.

vaccination, and participants in the AAd5 and the IMAd5 groups had higher concentrations of RBD-specific binding IgA antibodies than did participants in the inactivated vaccine group (figure 3). However, only 21 (60%) of 35 children in the inactivated vaccine group and 19 (54%) of 35 adolescents in the inactivated vaccine group had seroconversion in RBD-specific binding IgA antibodies, whereas 101 (93%) of 109 children in the AAd5 group, 32 (91%) of 35 children in the IMAd5 group, 29 (83%) of 35 adolescents in the IMAd5 group, and 102 (96%) of 106 adolescents in the AAd5 group had seroconversion in RBD-specific binding IgA antibodies (appendix p 19).

In the child cohort, those who had AAd5 and inactivated vaccine had undetectable IFN-γ-secreting T cells before the booster vaccination. Moreover, only three children in the AAd5 group and one child in the inactivated vaccine group had increased counts after the booster vaccination (appendix p 27). Although there were two children in the IMAd5 group who had detectable IFN-γ-secreting T cells before the booster vaccination, none of them had an increase in IFN-γ-secreting T cells, and in both cases IFN-γ-secreting T cells became undetectable after the booster vaccination. By contrast, IFN-γ-secreting T cells in adolescents in the AAd5 group at day 28, with a median of 259 spots (95% CI 57–462), were significantly increased compared with IFN-γ-secreting T cells before booster vaccination (appendix p 27). However, there was a small increase in IFN-γ-secreting T cells in adolescents in the IMAd5 and inactivated vaccine groups, similar to the increase in IFN-γ-secreting T cells reported in children in the respective groups.

On day 28 after immunisation, the GMT of neutralising antibodies against omicron variants BA.4 and BA.5 increased in children in the AAd5, the IMAd5, and the inactivated vaccine groups, with 21·9-times (95% CI 12·8–37·6), 15·8-times (11·5–21·7), and 2·5-times (2·0–3·2) increases in GMT, respectively. The GMT ratio for children in the AAd5 group versus the inactivated vaccine group was 6·1 (95% CI 3·5–10·5; p<0·0001); for the IMAd5 group versus the inactivated vaccine group the value was 5·4 (3·8–7·8; p<0·0001); and for the AAd5 group versus the IMAd5 group the value was 1·1 (0·6–2·0; p=0·677). Similar results for neutralising antibodies against the omicron variants BA.4 and BA.5 were observed in the adolescent cohort (appendix pp 23, 27). Specifically, adolescents boosted with AAd5 had higher

neutralising antibody concentrations than did those boosted with IMAd5 (GMT ratio 2·2 [1·2–4·1]; p=0·0057). 24 (80%) of 30 children and 27 (90%) of 30 adolescents in the AAd5 group, and 29 (97%) of 30 children and 27 (90%) of 30 adolescents in the IMAd5 group, underwent seroconversion. By contrast, only a few children (five [17%] of 30 children) and adolescents (10 [33%] of 30 adolescents) in the inactivated vaccine group had seroconversion of neutralising antibodies against omicron variants BA.4 and BA.5 (appendix pp 22, 28).

Discussion

We systematically assessed the safety and immunogenicity after booster vaccination in this randomised trial of heterologous boosting with AAd5 and IMAd5, and homologous boosting with inactivated vaccine. The results showed that a heterologous booster with AAd5 was safe and had better immunogenic properties against ancestral SARS-CoV-2 Wuhan-Hu-1 than did homologous boosting with inactivated vaccine.

In our study, heterologous boosting with AAd5 or inactivated vaccine was well tolerated, with no serious adverse events observed in children or adolescents, which is consistent with booster AAd5 in adults.[14] Xerostomia or injection site pain was the most common adverse event observed in participants who had AAd5 or inactivated vaccine. By contrast, adverse events were more frequently reported in children and adolescents who had IMAd5, including injection site pain, swelling, fever, and headache, and 3% (one of 35 participants with grade 3 fever) of boosted children reported severe adverse events. Compared with the phase 2/3 clinical trial of boosting with BNT162b2,[7] in which 73·9% of the children had pain at the injection site, 45·6% had fatigue, 34·0% had headache, 18·3% had muscle pain, 10·5% had chill, and 6·7% had fever, for children who boosted with AAd5 in our study, the rates were lower (0%, 0%, <1%, <1%, 0%, and 2%, respectively). These data suggest that orally administered AAd5 is well tolerated as a booster immunisation in children and adolescents and has a similar safety profile to inactivated vaccines in booster vaccination.

We found that heterologous booster vaccination with AAd5 that contained one-third of the dose of IMAd5 elicited high neutralising and RBD-IgG antibodies in both children and adolescents, and that the antibody titres were similar to those of IMAd5 booster but substantially higher than homologous booster vaccination with inactivated vaccine, which is consistent with AAd5 booster vaccination in adults and children.[7,14,16] Moreover, AAd5 booster immunisation induced a significantly higher serum RBD-IgA antibody titre than did IMAd5 or inactivated vaccine. A previous study showed that AAd5-immunised rhesus macaques could induce higher robust mucosal S-RBD-specific IgA and IgM antibodies in the bronchoalveolar lavage fluid.[19] Although we did not measure secretory IgA antibodies in this study, serum circulating dimeric and monomeric IgA might mediate isotype-specific function independent of localisation.[20–22] Collectively, these results indicate that booster vaccination with AAd5 could induce a strong humoral immune response, including serum RBD-IgA antibodies. The evaluation of mucosal immunity after vaccination with AAd5 and its correlation with serum IgA antibodies should be further studied. In particular, AAd5 showed better immunogenicity against SARS-CoV-2 omicron BA.4 and BA.5 than that of inactivated vaccines.

Compared with the strong antibody response after AAd5 in both children and adolescents, booster vaccination with AAd5 induced a low T-cell response in children but a significantly increased T-cell response in adolescents. By contrast, both IMAd5 and inactivated vaccines elicited low T-cell responses in both children and adolescents. Although the reason for children generating a lower T-cell response is unclear, previous studies have shown that children and adolescents developed a lower T-cell response after SARS-CoV-2 infection than did adults,[23–26] which suggests that immune system development varies according to age.[27] Moreover, children with influenza A virus infection also developed a T-cell response lower than that of young adults (aged 20–28 years).[28] This finding indicates that there might be differences in T-cell response to pathogens between children and adults.[23] Therefore, the basis of lower T-cell responses after vaccination in children should be further studied and could provide insights into the development of vaccines to elicit strong T-cell responses.

Our study has several limitations. First, we did not measure mucosal immunity, such as secretory IgA in oral secretory fluids or saliva in children and adolescents, which is crucial for evaluating mucosal immunity after vaccination with AAd5. Second, we did not assess the B-cell response, which could provide further insights into the mechanisms underlying the enhanced antibody responses after AAd5. Third, we measured the antibody response and T-cell response before and 28 days after vaccination, and the durability of immunity remains to be determined beyond 1 month after booster vaccination. Fourth, unmeasured random (comparison between treatment groups) and structural (comparison between levels of the stratification variable, children, and adolescents) confounding in between-group comparisons and regression to the mean in within-group comparison were not taken into account in the analysis within the child and adolescent cohorts. Finally, our findings might not be definitive enough for us to claim the safety of the AAd5 booster vaccine given that the sample size was relatively small, and further studies with a larger sample size of the potentially rare adverse events are needed.

In conclusion, our results show that heterologous booster immunisation with AAd5 is well tolerated in previously vaccinated children and adolescents, and elicits a strong antibody response. Moreover, a lower

dose—at one-third of the dose used for intramuscular Ad5-nCoV—and good compliance with AAd5 would be beneficial for improving the vaccination rate in children. Together with previously reported studies, our findings suggest that AAd5 would be a good option for booster programmes in children and adolescents in China under the condition that most children and adolescents in China have only received two doses of inactivated vaccine from the end of 2021 to the beginning of 2022.

Contributors

Z-CF, L-DG, L-HH, and J-BG conceived the trial. Z-CF and TH were the principal investigators. Z-CF, TH, and SZ designed the trial and the study protocol. TH, SZ, and D-FD led the implementation of the study. B-SW and LZ designed and conducted the statistical analysis. Z-FW led the laboratory analyses. TH, SZ, LZ, W-DZ, Y-PZ, and X-YJ drafted the report. J-SZ, S-PW, Q-PL, X-LY, and Z-HZ contributed to the recruitment, follow-up, and data collection. H-TH, XW, and HL conducted vaccine inoculation training and monitored the trial. All authors had full access to and verified the data. All the authors had final responsibility for the decision to submit for publication.

Declaration of interests

J-BG, H-TH, XW, and HL are employees of CanSino Biologics. The other authors declare no competing interests.

Data sharing

Individual participant data after de-identification will be made available when the study is complete, in response to reasonable requests made to the corresponding author; data can be shared through secure online platforms after proposals are approved. Researchers who provide a scientifically sound proposal will be allowed to access the de-identified individual participant data.

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