Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Safety and immunogenicity of a protein subunit COVID-19 vaccine (ZF2001) in healthy children and adolescents aged 3–17 years in China: a randomised, double-blind, placebo-controlled, phase 1 trial and an open-label, non-randomised, non-inferiority, phase 2 trial

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Summary

Background ZF2001 is a recombinant protein subunit vaccine against SARS-CoV-2 that has been approved for use in China, Colombia, Indonesia, and Uzbekistan in adults aged 18 years or older, but not yet in children and adolescents younger than 18 years. We aimed to evaluate the safety and immunogenicity of ZF2001 in children and adolescents aged 3–17 years in China.

Methods The randomised, double-blind, placebo-controlled, phase 1 trial and the open-label, non-randomised, non-inferiority, phase 2 trial were done at the Xiangtan Center for Disease Control and Prevention (Hunan Province, China). Healthy children and adolescents aged 3–17 years, without a history of SARS-CoV-2 vaccination, without a history of COVID-19, without COVID-19 at the time of the study, and without contact with patients with confirmed or suspected COVID-19 were included in the phase 1 and phase 2 trials. In the phase 1 trial, participants were divided into three groups according to age (3–5 years, 6–11 years, and 12–17 years). Each group was randomly assigned (4:1), using block randomisation with five blocks, each with a block size of five, to receive three 25 μg doses of the vaccine, ZF2001, or placebo intramuscularly in the arm 30 days apart. The participants and investigators were masked to treatment allocation. In the phase 2 trial, participants received three 25 μg doses of ZF2001 30 days apart and remained stratified by age group. For phase 1, the primary endpoint was safety and the secondary endpoint was immunogenicity (humoral immune response on day 30 after the third vaccine dose: geometric mean titre [GMT] of prototype SARS-CoV-2 neutralising antibodies and seroconversion rate, and geometric mean concentration [GMC] of prototype SARS-CoV-2 receptor-binding domain [RBD]-binding IgG antibodies and seroconversion rate). For phase 2, the primary endpoint was the GMT of SARS-CoV-2 neutralising antibodies with seroconversion rate on day 14 after the third vaccine dose, and the secondary endpoints included the GMT of RBD-binding antibodies and seroconversion rate on day 14 after the third vaccine dose, the GMT of neutralising antibodies against the omicron BA.2 subvariant and seroconversion rate on day 14 after the third vaccine dose, and safety. Safety was analysed in participants who received at least one dose of the vaccine or placebo. Immunogenicity was analysed in the full-analysis set (ie, participants who received at least one dose and had antibody results) by intention to treat and in the per-protocol set (ie, participants who completed the whole vaccination course and had antibody results). Non-inferiority in the phase 2 trial (neutralising antibody titre of participants from this trial aged 3–17 years *vs* that of participants aged 18–59 years from a separate phase 3 trial) for clinical outcome assessment was based on the geometric mean ratio (GMR) and was considered met if the lower bound of the 95% CI for the GMR was 0·67 or greater. These trials are registered with ClinicalTrials.gov, NCT04961359 (phase 1) and NCT05109598 (phase 2).

Findings Between July 10 and Sept 4, 2021, 75 children and adolescents were randomly assigned to receive ZF2001 (n=60) or placebo (n=15) in the phase 1 trial and were included in safety and immunogenicity analyses. Between Nov 5, 2021, and Feb 14, 2022, 400 participants (130 aged 3–7 years, 210 aged 6–11 years, and 60 aged 12–17 years) were included in the phase 2 trial and were included in the safety analysis; six participants were excluded from the immunogenicity analyses. 25 (42%) of 60 participants in the ZF2001 group and seven (47%) of 15 participants in the placebo group in phase 1, and 179 (45%) of 400 participants in phase 2, had adverse events within 30 days after the third vaccination, without a significant difference between groups in phase 1. Most adverse events were grade 1 or 2 (73 [97%] of 75 in the phase 1 trial, and 391 [98%] of 400 in the phase 2 trial). One participant in the phase 1 trial and three in the phase 2 trial who received ZF2001 had serious adverse events. One serious adverse event (acute allergic dermatitis) in the phase 2 trial was possibly related to the vaccine. In the phase 1 trial, on day 30 after the third dose, in the ZF2001 group, seroconversion of neutralising antibodies against SARS-CoV-2 was observed in 56 (93%; 95% CI 84–98) of 60 participants, with a GMT of 176·5 (95% CI 118·6–262·8), and seroconversion of RBD-binding antibodies was observed in all 60 (100%; 95% CI 94–100) participants, with a GMC of 47·7 IU/mL (95% CI 40·1–56·6). In the

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For the Chinese translation of the abstract see Online for appendix 1

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phase 2 trial, on day 14 after the third dose, seroconversion of neutralising antibodies against SARS-CoV-2 was seen in 392 (99%; 95% CI 98–100) participants, with a GMT of 245·4 (95% CI 220·0–273·7), and seroconversion of RBD-binding antibodies was observed in all 394 (100%; 99–100) participants, with a GMT of 8021 (7366–8734). On day 14 after the third dose, seroconversion of neutralising antibodies against the omicron subvariant BA.2 was observed in 375 (95%; 95% CI 93–97) of 394 participants, with a GMT of 42·9 (95% CI 37·9–48·5). For the non-inferiority comparison of participants aged 3–17 years with those aged 18–59 years for SARS-CoV-2 neutralising antibodies, the adjusted GMR was 8·6 (95% CI 7·0–10·4), with the lower bound of the GMR greater than 0·67.

Interpretation ZF2001 is safe, well tolerated, and immunogenic in children and adolescents aged 3–17 years. Vaccine-elicited sera can neutralise the omicron BA.2 subvariant, but with reduced activity. The results support further studies of ZF2001 in children and adolescents.

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Introduction

The COVID-19 pandemic is ongoing, with omicron (B.1.1.529) variants of SARS-CoV-2 currently being the most prevalent.[1–3] Dozens of SARS-CoV-2 vaccines have been approved for use.[4–7] Although the constantly emerging SARS-CoV-2 variants, from alpha (B.1.1.7) to omicron, have decreased the sensitivity of sera in people vaccinated with prototype SARS-CoV-2-based vaccines, an additional booster of either homologous or heterologous prototype vaccine is still effective at reducing serious diseases caused by subsequent variants.[8,9]

On the basis of the prototype SARS-CoV-2 (HB-01 strain), we developed a SARS-CoV-2 vaccine, ZF2001, a protein subunit vaccine comprising the antigen of the tandem-repeat dimeric receptor-binding domain (RBD) of the spike protein.[10] Preclinical studies have shown the immunogenicity and protective efficacy of ZF2001 in rodents and macaques.[11] Phase 1 and 2 trials in adults aged 18–59 years showed that ZF2001 was safe, well tolerated, and immunogenic.[4] The data supported the use of a 25 μg vaccine dose in a three-dose regimen for adults.[4] In the phase 3 trial, which included 7359 participants (36 cases)

Research in context

Evidence before this study

We searched PubMed for research articles published up to Aug 24, 2022, with the search terms "COVID-19", "vaccine", and "clinical trial". No language and date restrictions were applied. We identified ten phase 1 and phase 2 studies on seven vaccines for participants younger than 18 years. These trials include four for inactivated vaccines (CoronaVac [Sinovac Biotech], BBIBP-CorV [Beijing Institute of Biological Products], and BBV152 [Bharat Biotech International]), two for viral vector vaccines (Ad5-nCoV [CanSino Biologics] and AZD1222 [Oxford–AstraZeneca]), and four for mRNA-based vaccines (BNT162b2 [Pfizer–BioNtech] and mRNA-1273 [Moderna]). These vaccines target the whole virus or the spike protein. On Sept 21, 2022, a phase 3 trial of a protein subunit vaccine, NVX-CoV2373 (Novavax), in adolescents aged 12–18 years was published on a preprint server. Yet, no clinical study of a protein subunit vaccine in children and adolescents aged 3–18 years has been reported. We previously assessed the safety, tolerability, immunogenicity, and efficacy of ZF2001, a recombinant, tandem-repeat, dimeric, receptor-binding domain (RBD)-based protein subunit COVID-19 vaccine, in adults aged 18 years or older. ZF2001 was approved in China, Colombia, Indonesia, and Uzbekistan in adults, with more than 300 million doses administered; however, its safety and immunogenicity in children and adolescents are yet to be determined.

Added value of this study

To the best of our knowledge, this study is the first report of clinical data for a protein subunit and an RBD-based COVID-19 vaccine in children and adolescents. We evaluated the safety, tolerability, and immunogenicity of ZF2001 in children and adolescents aged 3–17 years. Adverse events within 30 days after the third dose of ZF2001 occurred in 25 (42%) of 60 participants in the phase 1 trial and 179 (45%) of 400 participants in the phase 2 trial, with most being grade 1 or 2. In the phase 1 trial, seroconversion of neutralising antibodies against SARS-CoV-2 was observed in 56 (93%; 95% CI 84–98) of 60 participants, with a GMT of 176·5 (95% CI 118·6–262·8). In the phase 2 trial, seroconversion of neutralising antibodies against SARS-CoV-2 was seen in 392 (99%; 98–100) participants, with a GMT of 245·4 (220·0–273·7). The SARS-CoV-2 neutralising titre was higher in children and adolescents aged 3–17 years than in adults aged 18–59 years (adjusted geometric mean ratio 8·6 [95% CI 7·0–10·4]).

Implications of all the available evidence

Our findings suggest that ZF2001 is safe, well tolerated, and immunogenic in children and adolescents aged 3–17 years, and support further evaluations of ZF2001 in this age group.

in the ZF2001 group and 7322 (188 cases) in the placebo group, the protective efficacy of the vaccine against PCR-confirmed COVID-19 of any severity was 81·4% (95% CI 73·3–87·3) in the short-term follow-up (mean 50·4 days [SD 37·1] *vs* 50·6 days [37·1]) and 75·7% (71·0–79·8) in the long-term follow-up (178·6 days [56·9] *vs* 177·8 days [56·4]). The safety analysis proved in a larger population of 28 873 participants that ZF2001 was safe and well tolerated in adults aged 18 years or older.[12] As of Dec 31, 2022, more than 350 million doses of ZF2001 had been administered to adults in China, Colombia, Indonesia, and Uzbekistan.

Although a study regarding the application of a protein subunit vaccine, NVX-CoV2373, in adolescents aged 12–18 years has been reported in a preprint,[13] the safety and immunogenicity of protein subunit vaccines in younger children are still unknown. To determine the potential application of ZF2001 in children and adolescents, we aimed to evaluate the safety and immunogenicity of the ZF2001 vaccine first in participants aged 3–17 years and then again in a larger population of the same age, with the immunogenicity compared with that of a separate cohort of people aged 18–59 years.

Methods

Study design and participants

We did a randomised, double-blind, placebo-controlled, phase 1 trial and a non-randomised, open-label, non-inferiority, phase 2 trial at the Xiangtan Center for Disease Control and Prevention (Hunan Province, China). The Xiangtan Center for Disease Control and Prevention has 19 township health centres under its control. We identified several health centres with clinical trial recruitment experience and good compliance, and sent them the paper recruitment advertisements approved by ethics committee. The staff of health centres were responsible for recruiting local people and issued recruitment advertisements to legal guardians and participants. Healthy children aged 3–17 years with an axillary temperature of less than 37·3°C within 72 h of enrolment in adolescents older than 14 years and less than 37·5°C within 72 h of enrolment in children aged 14 years or younger were included in the phase 1 and phase 2 trials. Participants with a history of SARS-CoV-2 vaccination, a history of COVID-19, COVID-19 at the time of the study, or contact with patients with confirmed or suspected COVID-19 were excluded. The inclusion and exclusion criteria are listed in trial protocols (appendix 2 pp 48–49, 156–159).

Written informed consent for children aged 3–7 years was signed by their legal guardians. For participants aged 8–17 years, written informed consent was signed by both participants and their legal guardians before recruitment. We performed the phase 1 trial according to the Chinese Technical Guidelines for Clinical Trials of Vaccines issued by the China National Medical Products Administration. The two trials were approved by the China National Medical Products Administration (drug clinical trial approval number, 2020L00023) and the Ethics Committee of Hunan Provincial Center for Disease Control and Prevention (IRB-PJ2021007 for the phase 1 trial and IRB-PJ2021016 for the phase 2 trial).

Randomisation and masking

In the phase 1 trial, participants and investigational products were randomly assigned by statisticians. These statisticians were not allowed to participate in other aspects of the trial and were not allowed to disclose the masking code to others during the trial. Stratified randomisation and block randomisation were used for the assignment of participants. Participants were stratified into age groups of 3–5 years, 6–11 years, and 12–17 years. The participants in each age group were randomly assigned (4:1) to receive the vaccine or placebo, using block randomisation, with five blocks and each with a block size of five. Each participant obtained the study number according to the screening sequence and received the investigational product according to the study number. The participants, trial investigators, and laboratory team were masked to group allocation during the trial. In the phase 2 trial, randomisation and masking were not applicable, but participants were still allocated to the three age groups.

Procedures

ZF2001 is manufactured in Chinese hamster ovary (CHO) cells.[4] The vaccine used in these trials were 0·5 mL per vial and contained 25 μg recombinant RBD protein and 0·25 mg aluminum hydroxide adjuvant; the placebo had the same components as ZF2001, except for the dimeric RBD antigen, to keep the double-blinded status. In the phase 1 trial, participants received three doses of 25 μg ZF2001 or placebo intramuscularly in the arm. Participants aged 12–17 years received the first dose of vaccine or placebo, and then the data safety and monitoring board evaluated safety over 7 days. When the safety evaluation was complete, participants aged 6–11 years received the first dose of vaccine or placebo. After the safety evaluation of the vaccine 7 days after the second dose in participants aged 6–11 years and 12–17 years was complete, participants aged 3–5 years were given the first dose of vaccine or placebo. In the phase 2 trial, participants received three doses of 25 μg ZF2001 intramuscularly in the upper arm.

All participants underwent axillary temperature measurements at screening, before each vaccination, and within 7 days after each dose. They also underwent physical examination (skin check and pulmonary auscultation) at screening, before each vaccination, and on day 4 after the first and second doses. Participants aged 6–17 years in the phase 1 trial underwent laboratory tests for blood biochemistry, blood routine, blood coagulation, D-dimer, and urine routine at screening, on day 4 after the first dose, and on day 4 after the second dose. If the laboratory indicators of participants aged

See Online for appendix 2

6–17 years after the second vaccination passed the safety evaluation reviewed by the data safety and monitoring board, participants aged 3–5 years did not need to undergo tests for laboratory indicators.

After each intramuscular administration in the arm, participants were observed for emergent adverse events in the observation room for at least 30 min. All adverse events occurring between 0 and 7 days after each dose were recorded in a diary card by participants or their guardians and then evaluated by investigators. The adverse events between 8 days and 30 days were reported by participants or their guardians. Adverse events related to vaccination between 0 and 7 days were defined as either solicited or unsolicited. Solicited local vaccine-related adverse events included injection-site pain, swelling, induration, redness, rash, itching, and cellulitis (grade 2 or worse cellulitis in need of antibacterial, antifungal, or antiviral treatment). Solicited systemic vaccine-related adverse events included fever, cough, dyspnoea, diarrhoea, inappetence, nausea, vomiting, myalgia (away from vaccination site), headache, tiredness, fatigue, acute allergic reactions, irritability or depression, and malaise. The criteria for grading adverse events were based on the Guidelines for Grading Adverse Reactions in Clinical Trials of Vaccines for Preventive Use issued by the China National Medical Products Administration (number 102, 2019).

Sera from participants in the phase 1 trial were collected before the first dose and at 1 month, 3 months, and 6 months after the third dose. Samples were sent for immunogenicity measurement at the China National Institute for Food and Drug Control (Beijing, China). Sera from participants in the phase 2 trial were collected before the first dose, and at 14 days and 6 months after the third dose. Samples were sent for immunogenicity measurement at the Guangdong Provincial Institute of Public Health (Guangzhou, China). In addition, in a phase 3 trial[12] of adults aged 18–59 years who had been given three 25 μg doses of ZF2001, 375 serum samples were collected 14 days after the full vaccination schedule. These samples were also tested for immunogenicity at the Guangdong Provincial Institute of Public Health, and eligible samples were compared with the samples taken from the children and adolescents in the phase 2 trial. The sera in both the phase 1 and phase 2 trials were used to evaluate the binding responses to the RBD antigen and neutralising activity against live SARS-CoV-2. In this study, the sera collected before the first dose, at 30 days (phase 1), or at 14 days (phase 2) after the third dose were tested; sera at other timepoints have not yet been tested.

Outcomes

For the phase 1 trial, safety of ZF2001 was the primary outcome, and immunogenicity was the secondary outcome. For the phase 2 trial, the geometric mean titre (GMT) of prototype SARS-CoV-2 neutralising antibodies and seroconversion rate was the primary outcome, and the secondary outcomes included the GMT of prototype SARS-CoV-2 RBD-binding antibodies and seroconversion rate, the GMT of neutralising antibodies against the omicron BA.2 subvariant and seroconversion rate, and safety.

Safety outcomes, from the day of the first dose to day 30 after the third dose, included adverse events; adverse events related to the vaccine; grade 3 or worse adverse events; grade 3 or worse adverse events related to the vaccine; adverse events leading to withdrawal from the trial; adverse events leading to withdrawal from the trial and related to the vaccine; and clinically significant changes in laboratory test indicators after the first and second doses compared with before the first vaccination. Serious adverse events from the day of the first dose to 12 months after the third dose will be reported at a later date as data are still being collected; we report serious adverse events from the day of the first dose to day 30 after the third dose.

In phase 1, immunogenicity was the humoral response at day 30 after the third vaccine dose: the GMT of neutralising antibodies against SARS-CoV-2, detected using the cytopathic effect method (appendix 2 p 15), with seroconversion defined as the highest reciprocal dilution of serum higher than 4; and the geometric mean concentration (GMC) of RBD-binding antibodies, detected using the magnetic particle chemiluminescence method (appendix 2 p 15), with seroconversion defined as a concentration greater than 0·1 IU/mL. For serum samples under the detection limit, we divided the threshold values by 2, and derived the final value of 2 for the GMT or 0·05 IU/mL for the GMC. In phase 2, immunogenicity was the humoral response at day 14 after the third vaccine dose: the GMT of neutralising antibodies against SARS-CoV-2, detected using the cytopathic effect method, with seroconversion defined as a concentration greater than 0·1 IU/mL; and the GMT of RBD-binding antibodies, measured using ELISA (appendix 2 p 15), with seroconversion defined as the highest reciprocal dilution of serum greater than 11.

Statistical analysis

For the phase 1 trial, we recruited 25 volunteers in each age group without calculating the target sample size based on statistical power. For the phase 2 trial, the sample size calculation was based on the non-inferiority design. Assuming that the GMT of neutralising antibodies on day 14 after the whole course of immunisations would be equivalent between participants aged 3–17 years from this trial and those aged 18–59 years from the phase 3 trial,[12] the SD of $\log_{10}$-transformed neutralising antibody concentration after immunisation was 0·8, the non-inferiority margin was 0·67 (ie, the $\log_{10}$-transformed non-inferiority margin was –0·17609), and the level of significance was 0·025 (one-sided), 325 participants should be recruited in each group to draw a non-inferiority conclusion with 80% power. Considering a dropout rate of 10–20%, the

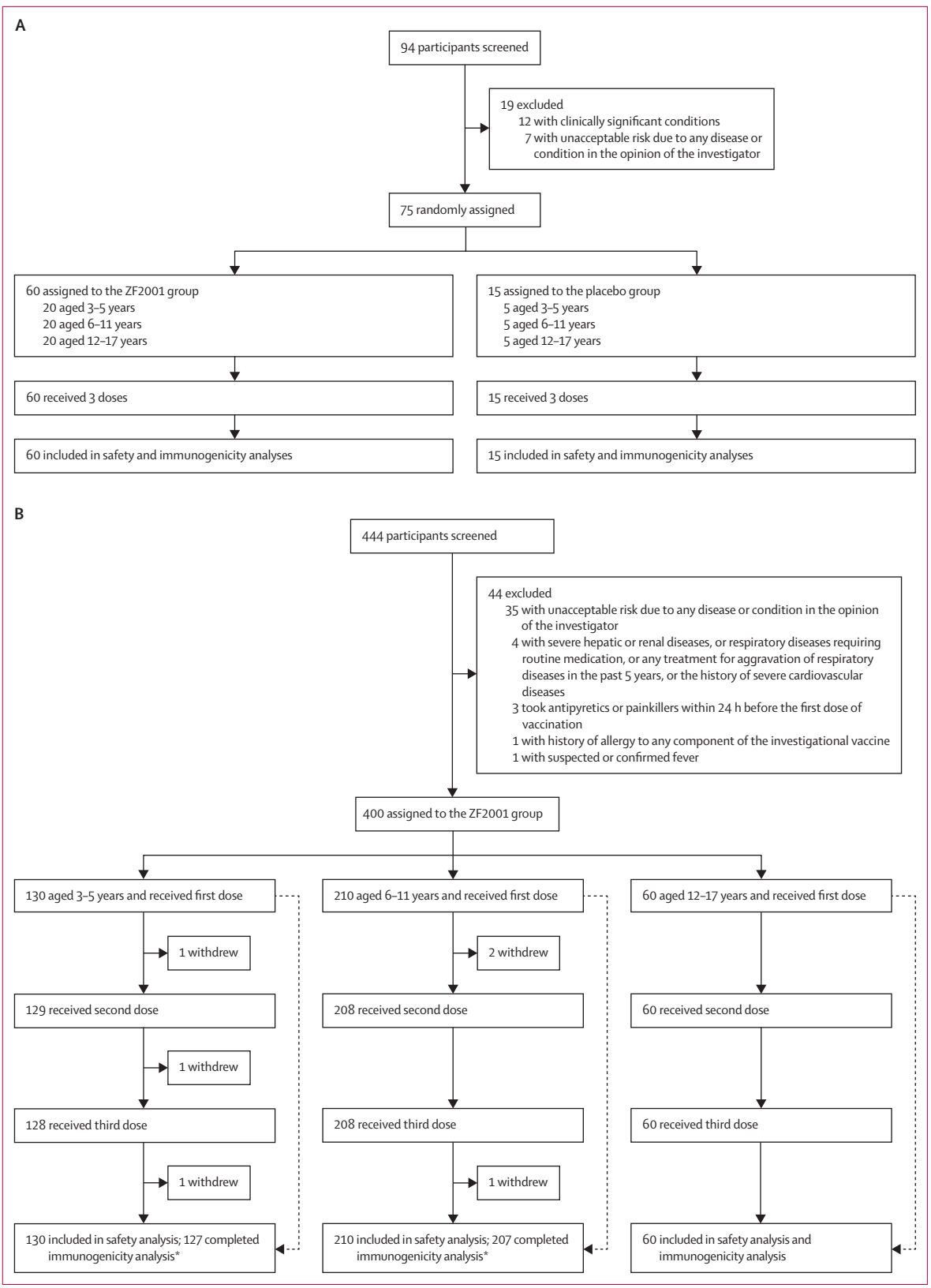

Figure 1: **Trial profiles**
(A) Phase 1 trial profile. (B) Phase 2 trial profile. *Six participants did not complete the follow-up for immunogenicity and withdrew from the trial.

	Phase 1		Phase 2
	Placebo group (n=15)	ZF2001 group (n=60)	ZF2001 group (n=400)
Age, years			
Mean	9·1 (3·9)	8·6 (4·0)	7·7 (3·1)
Median	10·0 (5·0–12·0)	8·0 (5·0–12·5)	8·0 (5·0–11·0)
Range	4·0–17·0	3·0–15·0	3·0–17·0
Weight, kg			
Mean	31·2 (12·8)	32·5 (16·3)	29·6 (12·6)
Median	27·0 (18·0–41·0)	25·0 (18·8–45·5)	27·0 (19·0–37·0)
Range	17·0–56·5	12·5–79·0	11·5–74·0
Height, cm			
Mean	134·7 (21·0)	131·4 (23·1)	127·0 (19·2)
Median	133·0 (115·0–152·0)	128·5 (111·0–152·3)	127·5 (110·5–143·0)
Range	105·0–170·0	94·0–173·0	87·0–174·0
Age stratification, years			
3–5	5 (33%)	20 (33%)	130 (33%)
6–11	5 (33%)	20 (33%)	210 (53%)
12–17	5 (33%)	20 (33%)	60 (15%)
Sex			
Female	8 (53%)	32 (53%)	202 (51%)
Male	7 (47%)	28 (47%)	198 (50%)
Ethnicity			
Han Chinese	15 (100%)	58 (97%)	393 (98%)
Other	0	2 (3%)	7 (2%)

Data are n (%), mean (SD), or median (IQR), unless otherwise stated. Percentages might not sum to 100 as a result of rounding.

Table 1: **Baseline demographic characteristics of participants**

sample size ranged from 362 to 407 for the non-inferiority comparison.

Safety and immunogenicity outcomes were determined in the ZF2001 and placebo groups, stratified by age group. Safety was analysed in participants who received at least one dose of ZF2001 or placebo. Immunogenicity was analysed in the full-analysis set and per-protocol set. The full-analysis set included participants who received at least one dose, had given blood samples before vaccination, and could provide antibody results, and were analysed by intention to treat. The per-protocol set included participants who completed the whole course of vaccination according to the protocol and had valid antibody data.

The Fisher's exact probability method was used to compare the safety differences and seroconversion rate differences between the groups. The Clopper-Pearson method was used to calculate 95% CIs. Non-inferiority (neutralising antibody titre of participants aged 3–17 years *vs* those aged 18–59 years from the phase 3 trial[12]) for clinical outcome assessment was based on the geometric mean ratio (GMR) and was considered met if the lower bound of the 95% CI for the GMR was 0·67 or greater. Non-inferiority was assessed in the per-protocol set. For the GMR calculation, the log-transformed titres were analysed using an ANCOVA model, adjusting for age group. The resulting least-squares means and 95% CIs are back-transformed to the original scale for presentation. SAS software (version 9.4) was used for the statistical analysis.

These trials are registered with ClinicalTrials.gov, NCT04961359 (phase 1) and NCT05109598 (phase 2).

Role of the funding source

As some authors are employed by or have received grant support from the funders, the funders had roles in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

Between July 10 and Sept 4, 2021, 94 children and adolescents were assessed for eligibility, and 75 were recruited in the phase 1 trial (60 were randomly assigned to the ZF2001 group and 15 to the placebo group). Participants were stratified into three age groups (3–5 years, 6–11 years, and 12–17 years), each of which comprised 25 participants, with 20 in each age group receiving ZF2001 and five in each age group receiving placebo (figure 1A). Participants in the ZF2001 group and the placebo group were similar in age, weight, and height, with even sex distribution (table 1). All participants completed the follow-up for safety and immunogenicity analyses (figure 1A). The safety data reported were collected from July 10, 2021, to Jan 17, 2022, for the phase 1 trial and from Nov 5, 2021, to May 11, 2022, for the phase 2 trial.

Between Nov 5, 2021, and Feb 14, 2022, 444 children and adolescents were recruited and screened for the phase 2 trial. After exclusion of 44 individuals, 400 participants, including 130 aged 3–7 years, 210 aged 6–11 years, and 60 aged 12–17 years, received at least one dose of 25 μg ZF2001 and were included in the safety analyses. Six participants did not complete the follow-up for immunogenicity and were therefore excluded from the immunogenicity analyses (figure 1B). The mean age of participants was 7·7 years (SD 3·1), with balanced sex distribution (table 1). 368 of 375 samples from participants aged 18–59 years from the phase 3 trial[12] were the comparative control for the neutralising assay; seven samples were excluded because of withdrawal of consent or protocol violations (appendix 2 p 1).

In the phase 1 trial, adverse events within 30 days after the third vaccination were recorded in 25 (42%) of 60 participants in the ZF2001 group and seven (47%) of 15 participants in the placebo group (table 2). 15 (25%) of 60 participants in the ZF2001 group and two (13%) of 15 participants in the placebo group had vaccine-related adverse events within 7 days after vaccination, and no vaccine-related adverse events occurred in 8–30 days after vaccination. Adverse events and vaccine-related adverse events did not differ significantly between the two groups when stratified by age (appendix 2 p 2). The most common solicited systemic vaccine-related adverse events were fever, inappetence, nausea, and fatigue. Three (5%) of 60 participants in the ZF2001 group reported at least one

solicited systemic vaccine-related adverse event (two reported fever, one reported inappetence, one reported nausea, and one reported fatigue) and one (7%) of 15 participants in the placebo group reported fever. The solicited local vaccine-related adverse events included injection-site pain, redness, swelling, and itching. 12 (20%) participants in the ZF2001 group reported at least one local vaccine-related adverse event (ten had redness, eight had injection-site pain, eight had swelling, and six had itching), and one participant (7%) in the placebo group reported injection-site pain. All vaccine-related adverse events were mild or moderate, and grade 3 vaccine-related adverse events were not noted (table 2). The abnormal indicators of laboratory tests for children and adolescents aged 6–17 years were included in the adverse events. One participant reported grade 1 elevated alanine aminotransferase and aspartate aminotransferase concentrations and recovered without treatment. In addition, there were no significant differences in the incidence of adverse events or vaccine-related adverse events after the second and third doses between the placebo and ZF2001 groups (appendix 2 p 4). The incidence of adverse events and vaccine-related adverse events within 30 min, 0–7 days, or 8–30 days after each vaccination showed no significant differences between the placebo and ZF2001 groups (appendix 2 p 6). One serious adverse event (acute haemorrhagic enteritis and acute mesenteric lymphadenitis) was reported in one participant from the ZF2001 group but was not considered to related to the vaccine by the investigator (appendix 2 p 8).

In the phase 2 trial, adverse events within 30 days after the third vaccination were recorded in 179 (45%) of 400 participants (table 2), and 89 (22%) participants had vaccine-related adverse events within 7 days. Most vaccine-related adverse events were mild or moderate (grade 1 or 2). The most common solicited systemic vaccine-related adverse events were cough, fever, and headache, and the most common solicited local vaccine-related adverse events were swelling, itching, redness, and injection-site pain with touch. Nine (2%) of 400 participants reported grade 3 adverse events, and three (cough, acute allergy, and injection-site pain with touch) were considered to be related to the vaccine (table 2). Notably, the rates of adverse events and vaccine-related adverse events were higher in participants aged 3–5 years than in those aged 6–17 years (appendix 2 pp 3, 5). The majority of adverse events and vaccine-related adverse events occurred within 7 days after each vaccination (appendix 2 p 7). Three serious adverse events from three participants were noted, and one with grade 3 allergic dermatitis was considered to be probably related to ZF2001. Two participants had adverse events that caused them to withdraw from the trial (table 2; appendix 2 pp 8–9). We monitored the risk of antibody-dependent enhancement caused by the vaccine from the beginning of the trial, and none of the participants were diagnosed with COVID-19 and no antibody-dependent enhancement case was reported.

	Phase 1 placebo group (n=15)		Phase 1 ZF2001 group (n=60)		Phase 2 ZF2001 group (n=400)	
	Events	Patients (%)	Events	Patients (%)	Events	Patients (%)
Overall adverse events	11	7 (47%)	70	25 (42%)	416	179 (45%)
Grade 3	0	0	5	2 (3%)	15	9 (2%)
Resulting in withdrawal from the trial	0	0	0	0	2	2 (1%)
Overall vaccine-related adverse events	3	2 (13%)	41	15 (25%)	234	89 (22%)
Grade 3	0	0	0	0	3	3 (1%)
Resulting in withdrawal from the trial	0	0	0	0	2	2 (1%)
Solicited (both local and systemic)						
Any	3	2 (13%)	39	14 (23%)	229	88 (22%)
Grade 3	0	0	0	0	3	3 (1%)
Systemic						
Any	1	1 (7%)	5	3 (5%)	51	34 (9%)
Grade 3	0	0	0	0	2	2 (1%)
Cough	0	0	0	0	19	18 (5%)
Grade 3	0	0	0	0	1	1 (<1%)
Fever	1	1 (7%)	2	2 (3%)	10	10 (3%)
Headache	0	0	0	0	8	7 (2%)
Vomiting	0	0	0	0	4	4 (1%)
Diarrhoea	0	0	0	0	3	3 (1%)
Inappetence	0	0	1	1 (2%)	2	2 (1%)
Nausea	0	0	1	1 (2%)	2	2 (1%)
Fatigue	0	0	1	1 (2%)	1	1 (<1%)
Muscle pain	0	0	0	0	1	1 (<1%)
Acute allergy (dermatitis)	0	0	0	0	1	1 (<1%)
Grade 3	0	0	0	0	1	1 (<1%)
Local						
Any	2	1 (7%)	34	12 (20%)	178	67 (17%)
Grade 3	0	0	0	0	1	1 (<1%)
Swelling	0	0	8	8 (13%)	48	41 (10%)
Itching	0	0	6	6 (10%)	41	37 (9%)
Redness	0	0	10	10 (17%)	35	29 (7%)
Injection-site pain	2	1 (7%)	10	8 (13%)	20	14 (4%)
Injection-site pain with touch	0	0	0	0	27	24 (6%)
Grade 3	0	0	0	0	1	1 (<1%)
Induration	0	0	0	0	5	5 (1%)
Rash	0	0	0	0	2	2 (1%)
Unsolicited	0	0	2	2 (3%)	4	3 (1%)

Data are n or n (%).

Table 2: **Adverse events and vaccine-related adverse events up to 30 days after the third dose**

The data of antibody responses were analysed in the full-analysis and per-protocol sets (appendix 2 pp 12, 13). In the phase 1 trial, the baselines of the RBD-binding antibodies and neutralising antibodies against SARS-CoV-2 were below the detection limit. In the full-analysis set, on day 30 after the third dose, seroconversion of neutralising antibodies in the ZF2001 group (ages 3–17 years) was seen in 56 (93%; 95% CI 84–98) participants, with a GMT of 176·5 (95% CI 118·6–262·8). The GMT was 197·5 (95% CI 92·0–425·3) in participants

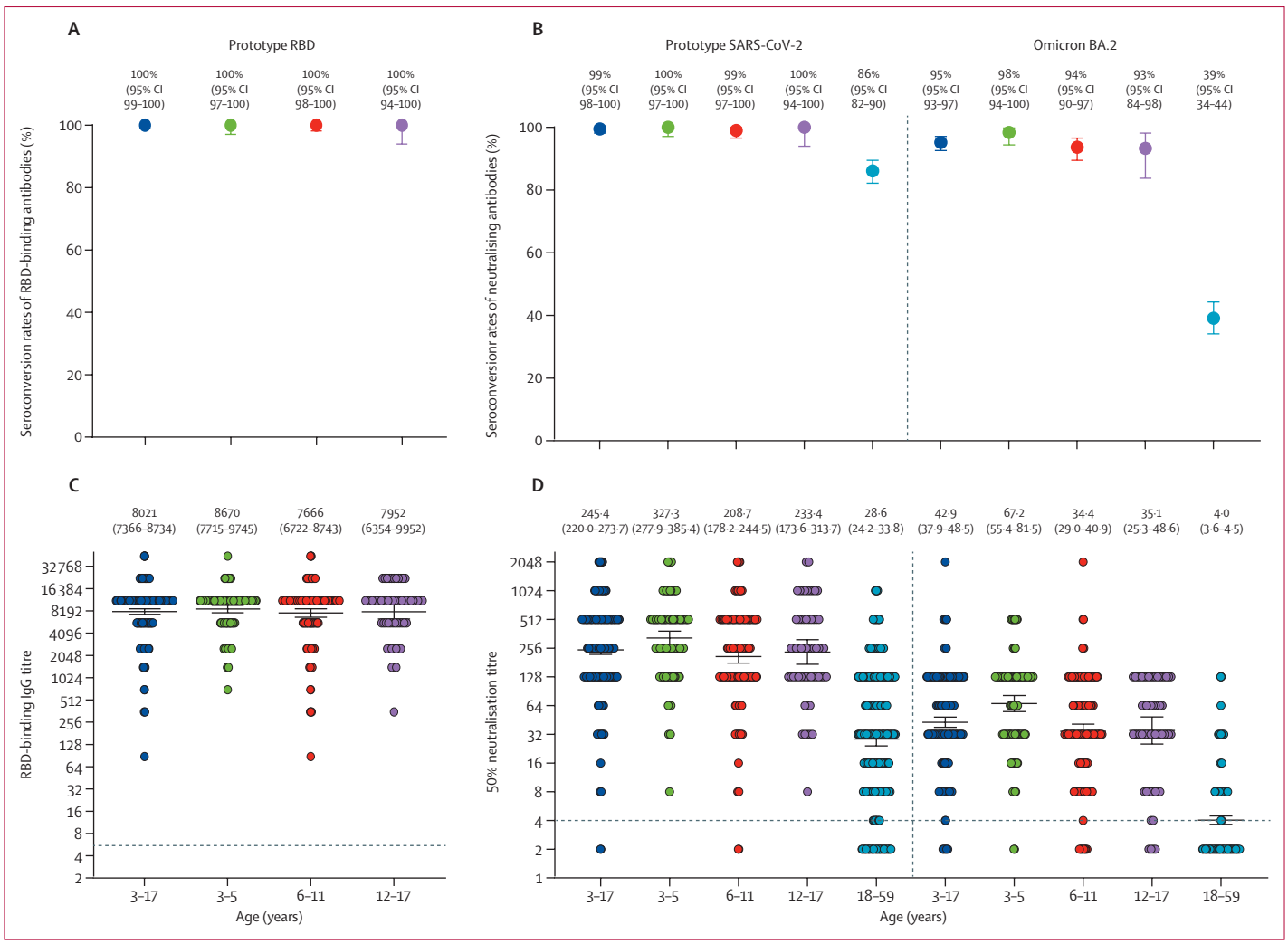

Figure 2: **Humoral immune response in the phase 2 trial**
Seroconversion rates (A) and GMTs (C) of prototype SARS-CoV-2 RBD-binding antibodies in the ZF2001 group on day 14 after the third dose. Seroconversion rates (B) and GMTs (D) of neutralising antibodies against prototype SARS-CoV-2 and omicron BA.2 subvariant in the ZF2001 group on day 14 after the third dose. Error bars represent 95% CIs. GMT=geometric mean titre. RBD=receptor-binding domain.

aged 3–5 years, 182·9 (98·4–339·8) in those aged 6–11 years, and 152·3 (68·8–337·4) in those aged 12–17 years. Seroconversion of RBD-binding antibodies in the ZF2001 group (ages 3–17 years) was observed in all 60 (100%; 95% CI 94–100) participants, with a GMC of 47·7 IU/mL (95% CI 40·1–56·6). The GMC was 52·0 IU/mL (95% CI 36·5–74·0) in participants aged 3–5 years, 44·4 IU/mL (34·1–58·0) in those aged 6–11 years, and 46·9 IU/mL (34·0–64·7) in those aged 12–17 years. By contrast, on day 30 after the final dose, the GMCs of RBD-binding antibodies and GMTs of neutralising antibodies in the placebo group were close to those before vaccination (appendix 2 pp 10–11).

In the phase 2 trial, the baselines of the prototype RBD-binding antibodies and neutralising antibodies against prototype SARS-CoV-2 and the omicron BA.2 subvariant are summarised in the appendix 2 (p 10). In the full-analysis set, on day 14 after the third dose, seroconversion of neutralising antibodies against SARS-CoV-2 was seen in 392 (99%; 95% CI 98–100) participants aged 3–17 years, with a GMT of 245·4 (95% CI 220·0–273·7; figure 2B, D), which was higher than in the comparative population aged 18–59 years, with seroconversion in 317 (86%; 82–90) of 368 participants and a GMT of 28·6 (24·2–33·8; figure 2B, D). The GMT was 327·3 (95% CI 277·9–385·4) in participants aged 3–5 years, 208·7 (178·2–244·5) in those aged 6–11 years, and 233·4 (173·6–313·7) in those aged 12–17 years. Seroconversion of RBD-binding antibodies in participants aged 3–17 years was observed in all 394 (100%; 95% CI 99–100) participants, with a GMT of 8021 (95% CI 7366–8734; figure 2A, C). The GMT was 8670 (95% CI 7715–9745) in participants aged 3–5 years, 7666 (6722–8743) in those aged 6–11 years, and 7952 (6354–9952) in those aged 12–17 years (figure 2C).

	Before immunisation	14 days after immunisation		Adjusted*geometric mean ratio (95% CI)	Met non-inferiority criteria
	GMT (95% CI)	GMT (95% CI)	Adjusted† GMT (95% CI)		
Prototype SARS-CoV-2					
Age 3–17 years *vs* 18–59 years	..	..	..	8·6 (7·0–10·4)	Yes
3–17 years (n=394)	2·0 (2·0–2·0)	245·4 (220·0–273·7)	245·4 (214·1–281·3)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	28·6 (24·2–33·8)	28·6 (24·9–33·0)	..	..
Age 3–5 years *vs* 18–59 years	..	..	..	11·4 (8·5–15·4)	Yes
3–5 years (n=127)	2·0 (2·0–2·0)	327·3 (277·9–385·4)	327·3 (253·0–423·4)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	28·6 (24·2–33·8)	28·6 (24·6–33·3)	..	..
Age 6–11 years *vs* 18–59 years	..	..	..	7·3 (5·7–9·4)	Yes
6–11 years (n=207)	2·0 (2·0–2·0)	208·7 (178·2–244·5)	208·7 (170·7–255·1)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	28·6 (24·2–33·8)	28·6 (24·6–33·3)	..	..
Age 12–17 years *vs* 18–59 years	..	..	..	8·2 (5·3–12·5)	Yes
12–17 years (n=60)	2·0 (2·0–2·0)	233·4 (173·6–313·7)	233·4 (156·9–347·2)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	28·6 (24·2–33·8)	28·6 (24·4–33·6)	..	..
Omicron BA.2 subvariant					
Age 3–17 years *vs* 18–59 years	..	..	..	10·6 (9·1–12·5)	Yes
3–17 years (n=394)	2·0 (2·0–2·0)	42·9 (37·9–48·5)	42·9 (38·3–47·9)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	4·0 (3·6–4·5)	4·0 (3·6–4·5)	..	..
Age 3–5 years *vs* 18–59 years	..	..	..	16·7 (13·6–20·5)	Yes
3–5 years (n=127)	2·0 (2·0–2·0)	67·2 (55·4–81·5)	67·2 (56·3–80·3)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	4·0 (3·6–4·5)	4·0 (3·6–4·5)	..	..
Age 6–11 years *vs* 18–59 years	..	..	..	8·5 (7·1–10·3)	Yes
6–11 years (n=207)	2·0 (2·0–2·0)	34·4 (29·0–40·9)	34·4 (29·7–40·0)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	4·0 (3·6–4·5)	4·0 (3·6–4·5)	..	..
Age 12–17 years *vs* 18–59 years	..	..	..	8·7 (6·6–11·6)	Yes
12–17 years (n=60)	2·0 (2·0–2·0)	35·1 (25·3–48·6)	35·1 (27·0–45·6)	..	..
18–59 years (n=368)	2·0 (2·0–2·0)	4·0 (3·6–4·5)	4·0 (3·6–4·5)	..	..

GMT=geometric mean titre. Samples from participants aged 18–59 years in a phase trial[12] were used as the comparator. *Adjusted for age group. †Adjusted for exclusion of the effect of GMT before immunisation; we balanced the GMT variance of the two groups when calculating and adjusting the GMT through least-squares means, hence giving slightly different GMT point estimates and 95% CIs for the 18–59 years age group.

Table 3: **Adjusted GMTs of neutralising antibodies for prototype SARS-CoV2 and omicron BA.2 subvariant by age group versus those aged 18–59 years**

Seroconversion of neutralising antibodies against the omicron BA.2 subvariant was seen in 375 (95%; 95% CI 93–97) of 394 participants aged 3–17 years, with a GMT of 42·9 (95% CI 37·9–48·5). The GMT was 67·2 (95% CI 55·4–81·5) in participants aged 3–5 years, 34·4 (29·0–40·9) in those aged 6–11 years, and 35·1 (25·3–48·6) in those aged 12–17 years. By contrast, seroconversion in people aged 18–59 years was seen in 144 (39%; 95% CI 34–44), with a GMT of 4·0 (95% CI 3·6–4·5; figure 2B, D). The results in the full-analysis set were similar to those in the per-protocol set (appendix 2 p 13).

For the non-inferiority comparison of participants aged 3–17 years to those aged 18–59 years for neutralising antibodies against SARS-CoV-2, the adjusted GMR was 8·6 (95% CI 7·0–10·4), with the lower bound of GMR more than 0·67 (table 3). The GMR was 11·4 (95% CI 8·5–15·4) in participants aged 3–5 years, 7·3 (5·7–9·4) in those aged 6–11 years, and 8·2 (5·3–12·5) in those aged 12–17 years (table 3). Similar results were also observed with the omicron BA.2 subvariant (table 3).

Discussion

The incidence of adverse events in participants aged 3–17 years was similar to the incidence seen in participants aged 18–59 years in our previous trial (about 40%);[4] however, it was higher than that seen in participants aged 60 years or older (about 29%).[12] Most adverse events in this trial were grade 1 or 2, similar to the results of the phase 1, 2, and 3 trials in adults older than 18 years.[4,12] The most common solicited adverse reactions in children and adolescents in these phase 1 and 2 trials and in adults in the phase 3 trial[12] were injection-site pain, swelling, itching, redness, fever, and headache. Therefore, ZF2001 showed a similar safety profile in participants aged 3–17 years to those aged 18 years or older.

One participant in the phase 1 trial and three participants in the phase 2 trial reported serious adverse events, most of which were deemed to be unrelated to ZF2001 by the investigators (appendix 2 pp 8–9). One serious adverse event in the phase 2 trial occurred 1 day after vaccination, diagnosed as grade 3 allergic dermatitis, and was

considered to be probably related to the vaccine. An allergic reaction is to be expected in a population aged 3–17 years, because it is one of the major adverse events in adults. According to the data from a phase 3 trial,[12] 0·8% of participants reported an allergic reaction and three of 14436 (about two per 10000) participants reported an allergic reaction of grade 3 or worse.

Neutralising antibody concentrations could be regarded as the surrogate index of protection efficacy, although there is no evidence indicating a linear relationship between the humoral immune response and efficacy.[7,14–17] In the phase 2 trial, we compared immunogenicity in response to ZF2001 in participants aged 3–17 years with those aged 18–59 years; we tested serum neutralisation of the same virus strains in the same laboratory, so the differences in neutralising titre between the groups are credible and comparable. The results showed that both seroconversion rates and GMTs of neutralising antibodies against both prototype SARS-CoV-2 and the omicron BA.2 subvariant were non-inferior and better in children and adolescents than in adults, suggesting a greater humoral response elicited by ZF2001 in this younger population. Although the serum samples for adults were from a phase 3 trial, which reported the efficacy of ZF2001 in providing protection against any severity of COVID-19,[12] the available data of neutralising antibodies from these representative sera are insufficient to calculate the efficacy of ZF2001 against any severity of COVID-19. However, neutralising antibodies elicited by a dimeric RBD-based vaccine led to effective protection against becoming infected with SARS-CoV-2 in mice.[18] Therefore, we infer that ZF2001 could provide protection for the population aged 3–17 years against COVID-19.

The GMT of neutralising antibodies in participants aged 3–5 years was higher in both phase 1 and phase 2 trials than in the other two older age groups, indicating that immunogenicity was better in younger participants. Similar results were also observed in trials of mRNA BNT162b2 (Pfizer–BioNtech) and inactivated vaccines.[19,20] We speculate that this phenomenon is related to the degradation and atrophy of the thymus during the onset of puberty, meaning reduced growth and differentiation of T cells, which leads to worse humoral immunity. Moreover, younger participants have fewer exogenous antigens compared with older participants and are less likely to be affected by pre-existing immunity, which might compromise the priming responses elicited by ZF2001.

These trials have several limitations. First, the study populations were not ethnically diverse with most participants being Han Chinese. Second, the methods, cells, and virus strains used in these two trials and those used in the phase 1 and 2 trials in adults were not identical; thus, we could not directly compare the magnitude of antibody concentrations between these trials. Third, ZF2001 was designed based on prototype SARS-CoV-2. Therefore, the humoral response was assessed mainly using the prototype antigens and early omicron subvariant BA.2. With the omicron subvariants BA.4 and BA.5 becoming the dominant subvariants, humoral responses against these subvariants need further evaluation. Fourth, cellular immunity was not evaluated in this trial because it was not prespecified in the trial protocol. Fifth, we used the same immunisation scheme in the children and adolescents as in adults, and the younger children, especially those aged 3–5 years, had more robust binding and neutralising antibodies, but had more frequent adverse events in the phase 2 trial. From our data, we cannot exclude the possibility that children might only need a two-dose series. Whether a lower dose of vaccine would elicit a similar response and the amount of vaccine needed (ie, <25 μg) should be studied further. Sixth, one of our study objectives for the phase 2 trial was to evaluate the immune persistence of ZF2001, and we collected the serum samples at indicated timepoints, as detailed in the protocol. However, the samples taken 1 month after the third dose were not tested because of the space limitation within the biosafety level 3 laboratory and shortage of workers, and hence the immunogenicity results at 1 month have not been reported. Finally, there were also methodological limitations, including measurement bias in intention-to-treat estimates, selection bias in per-protocol estimates, and selection bias due to missing outcome data.

ZF2001 has a promising safety profile, scalable and low-cost production, and convenient storage and transportation. Compared with the inactivated vaccine, which is manufactured with live virus in a biosafety level 3 laboratory, ZF2001 is manufactured with a base of CHO cells and is not restricted to being contained in a biosafety level 3 laboratory, and it contains the minimal dimeric RBD antigen as opposed to whole virus containing all proteins of SARS-CoV-2 in the inactivated vaccine. Compared with the mRNA vaccine, which is required to be stored below –20°C, ZF2001 is stored and transported at 2–8°C. Moreover, the mRNA and vector-based vaccines have a higher incidence of adverse reactions in children and adolescents.[21–26] However, the disadvantages of ZF2001 are its long-term process development and that one or two more doses are required to elicit a humoral response.

In conclusion, the ZF2001 vaccine is safe, well tolerated, and immunogenic for children and adolescents aged 3–17 years. Our data support further studies of a three-dose regimen of 25 μg ZF2001 in this age group.

Contributors

GFG and LD contributed to the conception and design of the vaccine. YL, HY, and LD contributed to the writing of the manuscript and the data analysis. LGa, TH, FL, SZ, DW, and GW contributed to the implementation and monitoring of the trial. PH, ZC, ZH, JS, ZY, and XZ contributed to the detection of immunogenicity. SY contributed to the trial design and conduct, LGo, FD, ML, XW, and LC participated in implementation of the trials. XZ, GFG, and LD contributed to the revision of the manuscript. All authors approved the final version of the manuscript. All authors had full access to, reviewed, and verified the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

YL, LD, and GFG are listed in the patent as inventors of the RBD dimer as a β-coronavirus vaccine. HY, SY, LGo, FD, ML, XW, LC, and XZ are employees of Anhui Zhifei Longcom Biopharmaceutica, and SY, FD, XW, LC, and XZ hold stock in the company. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article will be shared after de-identification (text, tables, figures, and appendices). These clinical trials are ongoing, and all individual participant-level data will not be available until after the immune persistence assessments have been done. The data will be made available immediately after publication and finalisation of the complete clinical study report, for at least 6 months. Researchers who provide a scientifically sound proposal will be allowed to access the de-identified individual participant data. Proposals should be sent to the corresponding author. These proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. To gain access, data requesters will need to sign a data access agreement.

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