Safety, immunogenicity, and efficacy of an mRNA COVID-19 vaccine (RQ3013) given as the fourth booster following three doses of inactivated vaccines: a double-blinded, randomised, controlled, phase 3b trial

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Summary

Background Heterologous vaccine schedules have been recommended to provide superior immunity and protection against emergent SARS-CoV-2 variants of concern. We aimed to evaluate the safety, immunogenicity, and efficacy of an mRNA COVID-19 vaccine RQ3013 compared with adenoviral vectored vaccine Ad5-nCoV and protein subunit vaccine ZF2001 as the fourth dose in adults primed with three doses of inactivated vaccines in China.

Methods We conducted a double-blinded, randomised, controlled, phase 3b trial among healthy Chinese adults at Lancang County, Yunnan, China. Adults who had received three doses of inactivated COVID-19 vaccines at least 6 months prior were randomly allocated (3:1:1) to receive heterologous boosters with RQ3013, Ad5-nCoV, or ZF2001. We assessed safety within 28 days post boost and the serum geometric mean titres (GMTs) of neutralising antibodies (NAbs) against the live SARS-CoV-2 omicron variant BA.5 on day 14 post-boost. We used Poisson regression to assess the vaccine efficacy against the first episode of virologically confirmed symptomatic COVID-19 occurring at least 7 days post boost. Subgroup analyses categorized by age and sex were also performed for safety and immunogenicity outcomes. This trial has been registered with the Chinese Clinical Trial Registry (ChiCTR2200065281) and is now complete.

Findings Between December 12 and December 18, 2022, a total of 1382 adults were screened, and 1250 were enrolled and randomly assigned to receive one dose of RQ3013 (n = 750), Ad5-nCoV (n = 250), or ZF2001 (n = 250). Although solicited adverse reactions within 28 days post boost were more frequent in the RQ3013 group (175 [23.3%]) compared to the control groups (24 [9.6%] in both the Ad5-nCOV and ZF2001 groups, $P < 0.05$), incidences of Grade 3 events were low (9 [0.7%]) and comparable across three groups ($P > 0.05$). On day 14 post-boost, RQ3013 (GMT 69.14, 95% CI 47.90–99.81) elicited 4.8-fold and 5.6-fold higher concentrations of NAbs against BA.5 than did Ad5-nCoV (14.37, 7.78–26.56) and ZF2001 (12.21, 5.13–29.06), respectively. On day 28 post-boost, RQ3013 demonstrated a relative efficacy of 62.2% (95% CI 13.7–83.1, $P = 0.02$) compared to Ad5-nCoV, and of 69.0% (33.5–85.7, $P = 0.002$) compared to ZF2001.

Interpretation The administrations of all the three heterologous boosters were well tolerated. The heterologous prime-boost regimen with RQ3013 elicited superior immune responses and demonstrated better protection against

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symptomatic SARS-CoV-2 infections compared with Ad5-nCoV or ZF2001, supporting the use of RQ3013 as a booster vaccination in adults.

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Keywords: COVID-19 vaccine; Phase 3 trial; Safety; Immunogenicity; Efficacy

Research in context

Evidence before this study

We searched PubMed for related studies from database inception to July 14, 2023, using a combination of the search terms "COVID-19 or SARS-CoV-2", "inactivated vaccine", "heterologous booster", and "trial". We found heterologous boosting of inactivated vaccines with ChAdOx1 nCoV-19 (AstraZeneca Gaithersburg, MD, USA), AD5-nCOV (Zhifei, China), AWcorna (Walvax, China) or BNT162b2 (Pfizer–BioNTech Mainz, Germany) produced greater neutralising antibody titres than did homologous boosting.

Added value of this study

We report a comprehensive analysis of the safety, immunogenicity, and efficacy of the mRNA COVID-19 vaccine RQ3013 versus that of adenoviral vectored vaccine (Ad5-nCoV) and protein subunit vaccine (ZF2001) as a heterologous booster in adults primed with three doses of inactivated vaccines at least 6 months ago. We observe that boosting with mRNA vaccine RQ3013 had an acceptable safety profile and superior immunogenicity of neutralising antibodies to the omicron variants BA.5 and XBB.1.9.1. The relative efficacy for RQ3013 was 62.2% (95% CI 13.7–83.1) versus Ad5-nCoV and 69.0% (33.5–85.7) versus ZF2001.

Implications of all the available evidence

These findings indicate that heterologous boosting with mRNA vaccine RQ3013 following three doses of inactivated COVID-19 vaccines leads to superior enhancement of immune responses and better protection against symptomatic SARS-CoV-2 infection, compared to boosting with Ad5-nCoV or ZF2001.

Introduction

To date, several COVID-19 vaccines based on different platforms have been licensed or granted emergency use.[1] These vaccines have been approved worldwide to build herd immunity, suppress severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission, and decrease severity after infection.[2,3] In the pandemic of omicron variants, well-designed mRNA vaccines are proven as the most effective option worldwide.[4] Till July 2022, more than 90% of the Chinese population had received at least one dose of COVID-19 vaccination, some 76% of people aged 60 or older, and 42% of those aged 80 or more, have received three doses of COVID-19 vaccine, which were primary inactivated vaccines.[5] Heterologous vaccine schedules have been recommended to provide greater immunity and protection against variants of concern,[6,7] though the safety, immunogenicity, and efficacy of booster mRNA vaccines on participants primed with inactivated vaccines are unclear yet.

The broad-spectrum RQ3013 (Walvax) vaccine is designed to contain pseudouridine-modified messenger RNAs encoding the spike protein harboring a combination of mutations responsible for immune evasion of SARS-CoV-2 variants of concern. The preclinical study of RQ3013 provided robust data on the safety, immunogenicity, and protective efficacy in rodents and macaques.[8] The phase 2 clinical trials showed that the RQ3013 vaccine was safe and robustly immunogenic in healthy adult participants.[9]

Here, we reported the results of a double-blinded, randomised, controlled, phase 3b trial to evaluate the safety, immunogenicity, and efficacy of RQ3013 compared with two widely used vaccines in China, i.e., Ad5-nCoV[10] and ZF2001,[11] as the fourth dose in adults primed with three doses of inactivated vaccines in China. This trial was initiated during a period when the omicron BA.5 and BA.7 variants were prevalent, and China just started to lift nationwide Zero-COVID restriction policies.

Methods

Study design and participants

This double-blinded, randomised, controlled, phase 3b trial was conducted at Lancang County, Yunnan, China. Adults aged at least 18 years and had previously received three doses of inactivated vaccination (CoronaVac [CanSino, China] or BBIBP-CorV [Sinopharm, China]) at least 6 months prior were eligible for enrollment. Participants with documented RT-PCR-confirmed COVID-19 and a prior known history of SARS-CoV, SARS-CoV-2, or Middle East respiratory syndrome infection (via on-site inquiry) were excluded. Additional key exclusion criteria included: 1) presence of abnormal pulse or blood pressure, 2) use of any preventive

medicine for COVID-19 except for 3 doses of inactivated vaccine, 3) with a temperature higher than 37.4 °C within the 72 h before the planned dose of study vaccine, and 4) with a history of allergic reactions to vaccinations. Full inclusion and exclusion criteria are detailed in the Appendix (p 2).

The trial protocol was reviewed and approved by the Ethics Committee of the First People's Hospital of Anning City. Written informed consent was obtained from all participants before screening. This study has been registered with the Chinese Clinical Trial Registry (ChiCTR2200065281) and was conducted following the principles of the Declaration of Helsinki and local guidelines.

Randomisation and masking

Participants were randomly assigned to the vaccine candidate group receiving RQ3013 or two control groups receiving Ad5-nCoV (CanSino Biologics, China) or ZF2001 (Anhui Zhifei Longcom Biopharmaceutical, China) in a 3:1:1 ratio (the RQ3013 group n = 750, the Ad5-nCoV group n = 250, and the ZF2001 group n = 250). The computer-generated randomisation list was created by an independent statistician using SAS (version 9.4) with a block size of 10. The concealed random grouping allocation and blind codes were kept in signed and sealed envelopes and were blinded to the sponsor, investigators, and participants.

Procedures

RQ3013 (Walvax, China) is an mRNA vaccine designed to contain pseudouridine-modified messenger RNAs encoding the spike protein harboring a combination of mutations responsible for immune evasion of SARS-CoV-2 variants of concern, which was administered intramuscularly 30 μg mRNA in 0.15 mL per dose. The Ad5-nCoV vaccine is a replication-defective Ad5-vectored vaccine expressing the full-length spike gene of wild-type SARS-CoV-2 (Wuhan-Hu-1). The ZF2001 vaccine is a protein subunit vaccine with tandem-repeat dimeric RBD of the SARS-CoV-2 spike protein (from Wuhan-Hu-1 strain) as the antigen. Both Ad5-nCoV and ZF2001 were administered intramuscularly at 0.5 mL per dose. After receiving a booster dose, participants were monitored for at least 30 min for any acute reactions and were provided with a thermometer and diary cards to record any post-vaccination adverse events (both solicited and unsolicited) for up to 28 days post boost. Blood samples were collected prior to the booster dose on day 0 and subsequently on days 14 and 28 post-boost for immunological assessments.

Outcomes

The primary outcomes included the incidence of vaccine-related adverse reactions occurring within 28 days post boost and the serum geometric mean titres (GMTs) of neutralising antibodies (NAbs) against the SARS-CoV-2 live virus omicron variant BA.5 on day 14 post-boost. The secondary safety outcome was the incidence of any serious adverse events within 28 days post boost. The secondary immunogenicity outcomes included the serum GMTs of NAbs against the live omicron BA.5 virus on day 28 post-boost; and the serum GMTs SARS-CoV-2 anti-spike IgG titres on days 14 and 28 post-boost. NAbs against the omicron XBB.1.9.1 variant was further measured on day 28 post-boost in a subset of participants. The secondary efficacy endpoint was the incidence of the first episode of virologically confirmed symptomatic COVID-19 of any severity, occurring at least 7 days post boost.

The severity of adverse reactions was graded according to a modified US Food and Drug Administration toxicity scale (Appendix p 3). An independent data and safety monitoring board was responsible to review the safety data. For each adverse event, causality was determined based on the criteria of reasonable possibility, temporal relationship, and alternate causes to ascertain whether it was related or unrelated to the vaccination. Adverse reactions were defined as adverse events that were either directly related or potentially related to the vaccination.

Serum titres of NAbs against the live SARS-CoV-2 omicron variants BA.5 (GDCPP.2.00303, Guangzhou Center for Disease Control and Prevention) and XBB.1.9.1 (IQTC-IM2396943, Center for Guangzhou Customs) were measured with cytopathic effect based microneutralisation assay, following a previously established protocol.[12] Briefly, the two-fold serial dilutions were tested in duplicate wells for NAbs presence. A total of 100 units of the 50% Tissue Culture Infective Dose (TCID50) of the virus in 50 μl per well was incubated with an equal volume of serum in 96-well plates for 2 h. Vero E6 cells were trypsinized and resuspended in Dulbecco's Modified Eagle Medium (DMEM) containing 4% fetal bovine serum and 1% penicillin/streptomycin. Subsequently, 1.2×10^4 cells in 100 μl media were added to one well of the 96-well plates, followed by incubation at 37 °C, 5% CO_2 for 4 days. Neutralisation was determined by observing the CPE in images captured with a Celigo Image Cytometer on day 4 post-infection. The NAbs titre was defined as the reciprocal of the highest sample dilution that protected cells in one well from CPE. Serum titres of SARS-CoV-2 anti-spike IgG were measured using a commercial IgG ELISA kit (Vazyme, China) with the WHO international standard for anti-SARS-CoV-2 antibody (NIBSC code 20/136) as a reference for calibration.[13] The neutralisation antibody titres below the detection limit (1:8 dilution) were set as 4.

Participants were contacted using follow-up telephone calls every two weeks post-boost to identify any suspected symptomatic cases of COVID-19. In the event of reported respiratory symptoms, such as cough, shortness of breath, difficulty breathing, or other COVID-19 related symptoms including chills, malaise/

fatigue, headache, and muscle pain, nasopharyngeal swabs were collected on-site from the participants and RT-PCR test was performed to confirm the presence of infection.

Statistical analysis

The population for safety analysis included all participants who were randomly assigned and completed all visits (n = 1250). Safety analyses are presented as counts, percentages, and corresponding Clopper–Pearson 95% confidence intervals (CI) for local reactions, systemic events, and any adverse events following vaccination in each group.

Sample size calculation for immunogenicity analyses was based on the hypothesis that the GMTs of antibody response would be at least twice as high using RQ3013 as the booster following three doses of inactivated vaccines on day 14 post-boost, compared to using Ad5-nCoV or ZF2001 as the booster (equivalent to 0.301 after $\log_{10}$ transformation). The superiority margin was set at 1 (equivalent to 0 after $\log_{10}$ transformation), indicating a superior antibody response of RQ3013 over the other two boosters. The standard deviation of antibody titres post boost was expected to be no greater than 0.8 in all the three groups. Based on the parameters above, a group sample size of 300 for the RQ3013 group and 100 for each control group was established using PASS 15.0. Consequently, the first randomised 500 participants were enrolled in the immunogenicity subgroup for the analysis of NAbs against BA.5 and anti-spike IgG, which were allocated to the RQ3013 group, Ad5-nCoV group, and ZF2001 group in a 3:1:1 ratio. The exploratory analysis of NAbs against XBB.1.9.1 was performed in a random subset of 80 participants receiving RQ3013 or ZF2001 (the RQ3013 group n = 60, the ZF2001 group n = 20).

Immunogenicity data were presented as GMT and 95% CI, and values below the detection limit were replaced by a value equal to the lowest limit divided by two. Immunogenicity data were $\log_{10}$ transformed for the following analysis. Between-group comparisons of immunological outcomes were evaluated using linear regression models adjusting for age, sex, and baseline body-mass index (BMI). The geometric mean ratios (GMR) and 99% CIs between RQ3013 and control groups were also reported. The GMR was calculated as the antilogarithm of the difference in the means of $\log_{10}$-transformed titres between two groups during the same time period. Correlations between normalized immunological outcomes were evaluated using Pearson correlation coefficients.

The analysis of efficacy was performed among enrolled participants demonstrated no evidence of infection within 7 days post boost and had no major protocol deviations. A Poisson regression model was used to calculate vaccine relative efficacy and its corresponding 95% CI, based on the number of cases per person-year, with adjustment for age, sex, and baseline BMI. Following the previous studies,[14] we assumed the observed numbers of COVID-19 cases in the RQ3013 group and control groups followed Poisson distributions.

In an exploratory analysis, subgroup analyses were performed for safety and immunogenicity outcomes, categorized by age (<60 years and ≥60 years) and sex (male and female).

All analyses were conducted using R (version 4.3) and SAS software (version 9.4).

Role of the funding source

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

Results

Between December 12 and December 18, 2022, 1382 adults were screened, of which 1250 were enrolled and randomly assigned to receive one dose of RQ3013 (n = 750), Ad5-nCoV (n = 250), or ZF2001 (n = 250). At the data cut-off date of March 10, 2023, all participants had at least 28 days of safety data available post boost and met the criteria for the per-protocol population for safety and efficacy analyses. A subset of the population (RQ3013 group n = 300, Ad5-nCoV group n = 100, and ZF2001 group n = 100) were included in the immunogenicity analyses (Fig. 1).

Demographics and baseline characteristics were well-balanced among groups (Table 1), with 632 (50.6%) participants being female. Among the participants, 329 (26.3%) were aged 60 years and older, and the median age for all participants was 52 years (interquartile range 40–60). Approximately 22% of the participants had at least one coexisting condition identified as a risk factor for severe COVID-19, including hypertension, diabetes, and obesity (BMI [the weight in kilograms divided by the square of the height in meters] of at least 30.0). The average time since the last administration of inactivated vaccine (57 weeks) exceeded one year for all vaccination groups.

Within 28 days post boost, solicited adverse reactions were more frequent in the RQ3013 group (23.3%) compared to the control groups (9.6% for both Ad5-nCOV and ZF2001), while unsolicited adverse reactions showed no significant differences among the three groups (Appendix p 7). Very low proportions of Grade 3 events were observed across groups (0.8% for RQ3013, 0.4% for Ad5-nCoV, and 0.8% for ZF2001) (Fig. 2). Pyrexia was the most frequently reported systemic adverse reaction across all groups, with a higher incidence in RQ3013 (10.1%) compared with that in Ad5-nCoV (2.4%) and ZF2001 (5.2%) (Fig. 2). Injection-site pain was the most frequently reported local adverse reaction in all groups, also exhibiting a higher prevalence in the RQ3013 group (10.9%) than in the other

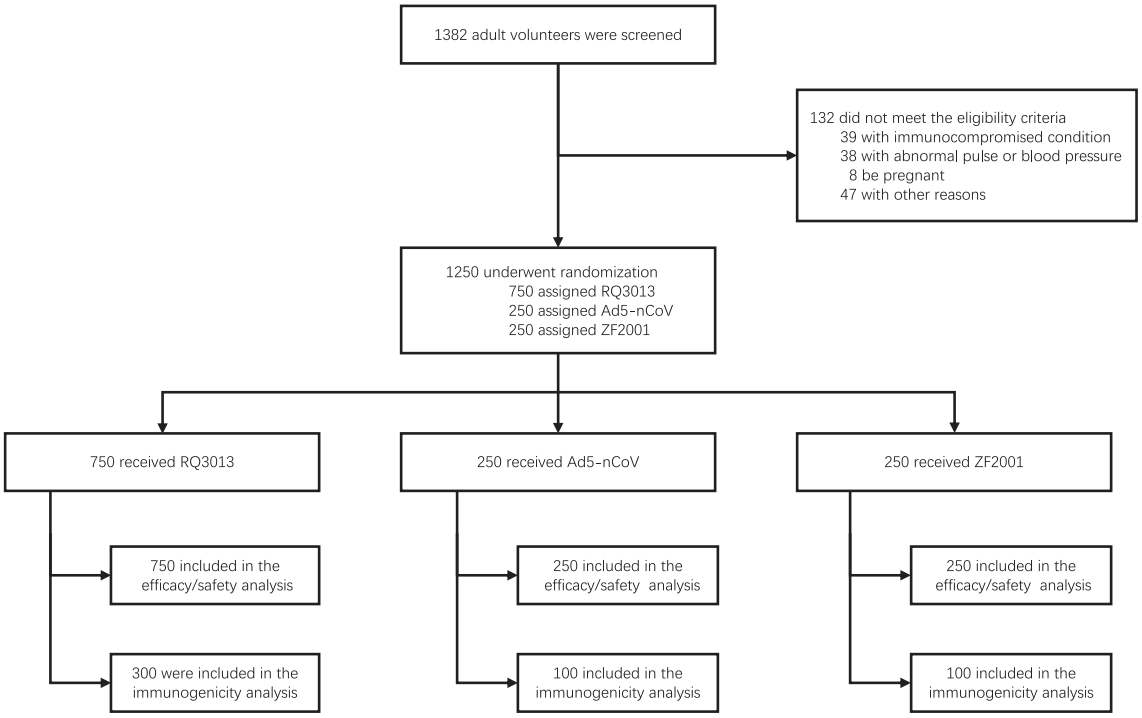

Fig. 1: **Trial profile.**

two control groups (3.2% for both Ad5-nCoV and ZF2001). Nevertheless, incidences of Grade 3 events were low (9 [0.7%]) and comparable across three groups (Fig. 2). In subgroup assessments, such observations from the overall population were also seen in participants aged 18–59 years. However, in the older population (≥60 years) the incidence of adverse reactions was comparable among all three groups, and no Grade 3 event was documented in the RQ3013 group (Appendix p 14). The incidence of adverse reactions was consistent across both sex subgroups (Appendix p 15). Two cases of serious adverse events were documented, and none was considered possibly associated with vaccine administration (Appendix p 8).

On day 0, all participants showed very low serum titres of NAbs against BA.5 and SARS-CoV-2 anti-spike IgG, with no difference in their GMTs among the three groups (Fig. 3). On day 14 post-boost, the GMT of NAbs against BA.5 in the RQ3013 group (69.14, 95% CI 47.9–99.81) was 4.8-fold and 5.6-fold higher than that in Ad5-nCoV group (14.37, 7.78–26.56) and ZF2001 group (12.21, 5.13–29.06) (both $P < 0.05$, Fig. 3A, Appendix p 16). The GMT of SARS-CoV-2 anti-spike IgG on day 14 post-boost in the RQ3013 group (247,597.03 BAU/mL, 95% CI 198,610.71–308,665.57) was 7.2-fold higher than those in the Ad5-nCoV group (34,435.83 BAU/mL, 19,268.61–61,541.87) and 23.3-fold higher than those in the ZF2001 group (10,934.88 BAU/mL, 2840.35–42,097.46) (both $P < 0.001$, Fig. 3B, Appendix p 16). On day 28 post-boost, higher titres of NAbs against BA.5 and SARS-CoV-2 anti-spike IgG were still observed in the RQ3013 group compared with the control groups (Appendix p 10). Notably, in a random subset of 80 participants, the GMT of NAbs against XBB.1.9.1 in the

	Ad5-nCoV (N = 250)	ZF2001 (N = 250)	RQ3013 (N = 750)	Total (N = 1250)
Age, yr				
Mean ± SD	50.6 ± 13.0	51.4 ± 12.8	50.2 ± 13.1	50.5 ± 13.0
Median (range)	52 (21–81)	52 (20–85)	51 (18–87)	52 (18–87)
Distribution, no. (%)				
<60 yr	183 (73.2)	181 (72.4)	557 (74.3)	921 (73.7)
≥60 yr	67 (26.8)	69 (27.6)	193 (25.7)	329 (26.3)
Sex, no. (%)				
Female	122 (48.8)	123 (49.2)	387 (51.6)	632 (50.6)
Male	128 (51.2)	127 (50.8)	363 (48.4)	618 (49.4)
Body mass index, kg/m^2	24.1 ± 3.6	24.1 ± 3.6	24.1 ± 3.6	24.3 ± 3.7
Comorbidities, no. (%)				
Hypertension	40 (16.0)	37 (14.8)	110 (14.7)	187 (15.0)
Diabetes	8 (3.2)	5 (2.0)	25 (3.3)	38 (3.0)
Obese	39 (15.6)	33 (13.2)	126 (16.8)	92 (7.4)
Time since the last vaccination, weeks	57.1 ± 2.2	57.1 ± 2.2	57.1 ± 2.2	57.10 ± 2.2

Results are for the safety/efficacy population. The data are shown as mean ± SD or no. (%). The body mass index is the weight in kilograms divided by the square of the height in meters.

Table 1: **Basic characteristics of all participants at baseline.**

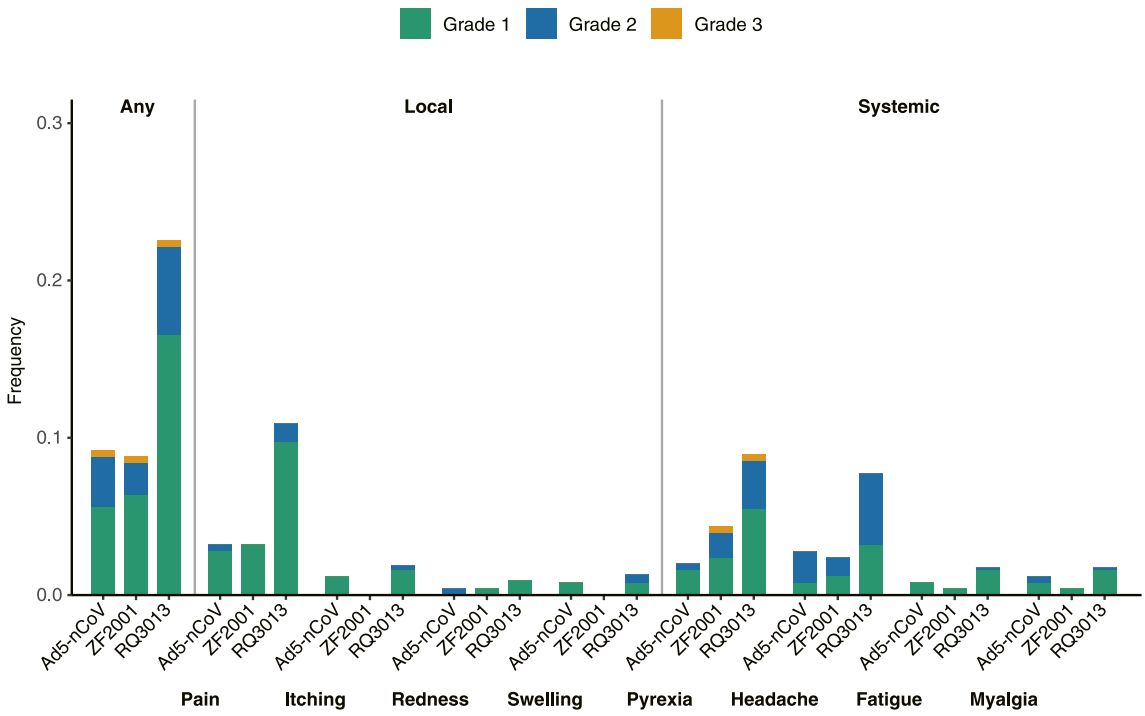

***Fig. 2:* Solicited local and systemic adverse reactions.** The percentage of participants who had solicited local and systemic adverse reactions within 28 days post boost is plotted according to the maximum toxicity grade.

RQ3013 group (57.22, 95% CI 42.39–77.23) was 9.1-fold higher than that in the ZF2001 group (6.31, 3.34–11.95) (P < 0.001, Appendix p 13). Consistent patterns of GMRs were observed in the stratified analyses based on age and sex (Appendix p 10), as well as in the sensitivity analysis that excluded participants with a COVID-19 diagnosis within 28 days post boost (n = 25) (Appendix p 12). Notably, subgroup analyses revealed that the superior immune enhancement effect of RQ3013 compared to Ad5-nCoV was more pronounced among older participants than younger participants (P-interaction <0.05, Appendix p 16). The NAbs were strongly and positively correlated with the SARS-CoV-2 anti-spike IgG titres in each vaccination group (all P < 0.001, Fig. 3C).

Among the 38 COVID-19 cases diagnosed between day 7 and day 28 post-boost, 13 cases were documented in the RQ3013 group, and 25 cases in the control groups (11 cases in the Ad5-nCoV group and 14 cases in the ZF2001 group), which corresponded to a relative efficacy for RQ3013 of 62.2% (95% CI 13.7–83.1, P = 0.02) compared to Ad5-nCoV and of 69.0% (33.5–85.7, P = 0.002) compared to ZF2001 (Fig. 4). All recorded COVID-19 cases were mild, with no hospitalizations or deaths among the vaccine recipients. In the corresponding analysis conducted for the onset of COVID-19 at least 14 days post boost, the relative efficacy for RQ3013 was statistically insignificant compared to Ad5-nCoV and was 74.8% (27.3–91.7, P = 0.01) compared to ZF2001, respectively (Fig. 4). Among all the enrolled participants, 17 cases of COVID-19 were documented in the RQ3013 group and 27 in the control groups (12 cases in the Ad5-nCoV group and 15 in the ZF2001 group), which corresponded to a relative vaccine efficacy for RQ3013 of 54.2% (95% CI 1.6–78.0, P = 0.04) compared to Ad5-nCoV and of 62.6% (24.1–81.4, P = 0.006) compared to ZF2001, respectively (Fig. 4).

Discussion

In our study, the heterologous prime-boost regimen, consisting of a single dose of RQ3013 administrated more than 6 months after three doses of the inactivated SARS-CoV-2 vaccine, demonstrated superior immunogenicity, general safety, and better protection against COVID-19 infection of RQ3013 compared to two control boosters for adults aged 18 years and older.

Our findings echo previous research that demonstrated significant enhancement of humoral immune responses following heterologous booster vaccinations against COVID-19.[9,15] Specifically, RQ3013 exhibited superior immunogenicity compared to either Ad5-nCoV (adenoviral vectored) or ZF2001 (protein subunit) as the fourth dose, among participants primed with three doses of inactivated vaccines. Our observation is

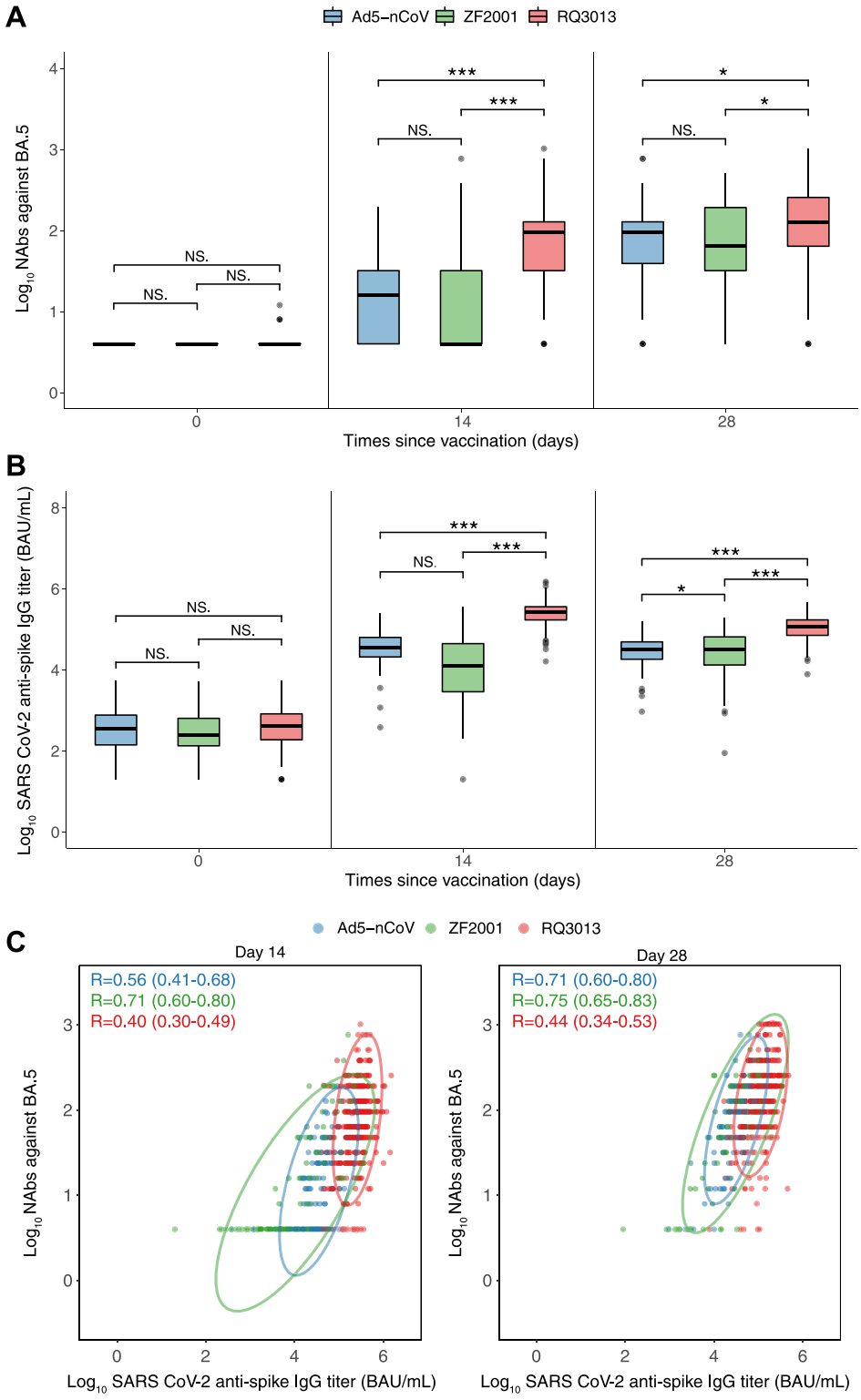

Fig. 3: **Neutralisation responses and antibody titres post-boost.** Neutralising antibodies (A) and SARS CoV-2 anti-spike IgG titres (B) measured in both the RQ3013 and control groups on days 0, 7, and 14 post-boost. Between-group comparisons of immunological outcomes were evaluated using linear regression models adjusting for age, sex, and baseline BMI. (C) Correlation between serum NAbs titre and SARS CoV-2 anti-spike IgG titres. BMI, body mass index; NAbs, neutralising antibody; N.S., not significant; *$P < 0.05$; ***$P < 0.001$.

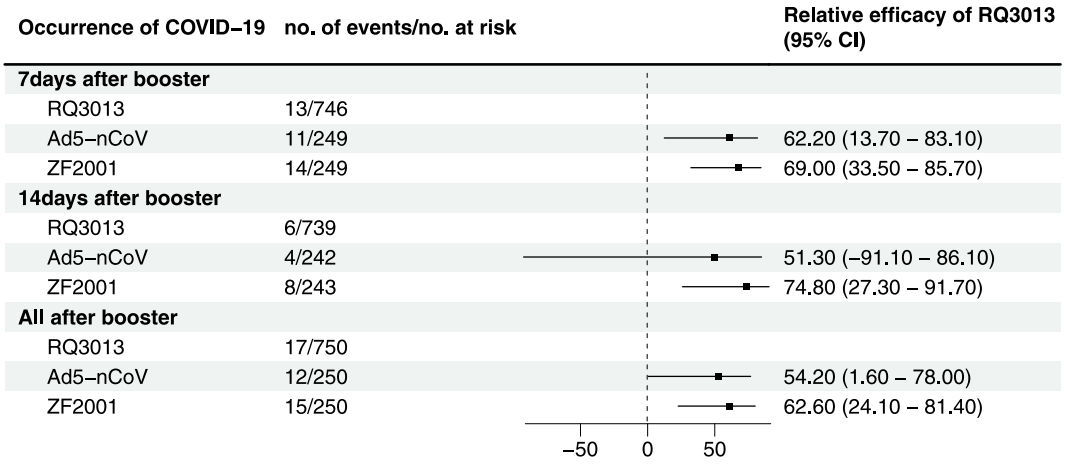

Fig. 4: **Relative vaccine efficacy of RQ3013 versus Ad5-nCoV or ZF2001 as the booster vaccination.** Shown is the relative efficacy for RQ3013 versus Ad5-nCoV or ZF2001 against COVID-19 infections in the per-protocol population with three previous doses of inactivated vaccines. Relative vaccine efficacy and 95% CI were derived with the use of the Poisson regression.

consistent with previous results that reported more robust immune responses of mRNA boosters than vaccines of other platforms among recipients of inactivated vaccines.[16–20] The superior immunogenicity of RQ3013 could be attributed to its capacity to deliver genetic material into cells and shorter dosing interval as mRNA vaccine,[21] which is more effective at stimulating immune response compared to traditional vaccines. RQ3013 is designed to effectively counteract the immune evasion mechanisms employed by variants of concern, making it a promising candidate for broad protection against COVID-19.[8] The vaccine encodes the spike protein with a combination of mutations specifically chosen to provide cross-protection against a range of SARS-CoV-2 variants, including B.1.1.7, B.1.351, and the newly emerged omicron variants. In addition to the high efficacy of RQ3013 in this trial, during which the predominant variants were BA.5.2 and BF.7,[22,23] we also observed an enhanced immune response of RQ3013 against XBB.1.9.1, a recently prevalent variant in China and around the world. Taken together, these pieces of evidence suggested the potential of RQ3013 as a broadly protective vaccine candidate for both prevalent and emerging variants.

In our study, the frequency of adverse reactions in the RQ3013 group was comparable to that of previous mRNA boosters,[24,25] while distinct reactogenicity characteristics were observed for RQ3013. It seems that participants who received RQ3013 as a booster were more likely to report pyrexia but less prone to report fatigue and myalgia compared to those who received BNT162b2 or mRNA-1273 as a booster.[15,17] Consistent with previous findings of other COVID-19 vaccines,[26,27] the incidence of adverse reactions after vaccination in our study was lower in participants aged 60 years and older compared with the younger participants. Fatigue and myalgia were commonly reported side effects of mRNA vaccines and might be influenced by subjective factors and demographic factors.[28,29] Differences in study population, dosing schedule, and assessment methods could also contribute to the observed differences in side effect profiles between RQ3013 and other mRNA vaccines.

In this trial, the relative vaccine efficacy of the mRNA booster RQ3013 ranged from 62.2% to 69.0% compared with two other vaccines (adenoviral vectored vaccine Ad5-nCoV and protein subunit vaccine ZF2001), which is higher than the relative efficacy (compared with the inactivated vaccine) of a widely used mRNA booster BNT162b2 (53.4%)[30] and a recent reported mRNA booster CS-2034 (38.0%),[16,30] and even close to the absolute efficacy of other mRNA boosters (about 84% 7–59 days after a boost dose).[31] However, the sample size for the this efficacy analysis was relatively small, and the results should be explained with caution. We are currently conducting a formal phase 3 trial to primary evaluate the efficacy of heterologous prime-boost vaccination with RQ3013 in a larger population (ChiCTR2200067184) and will update the results in the near future.

The current study was performed among Chinese population, approximately 18% of the global population, most of which received a three-dose inactivated vaccine regimen. Given the emergence of new variants, public health and social measures remain essential components of the COVID-19 prevention strategy. Efforts to introduce booster doses should depend on solid evidence and target the highest risk populations, particularly the elderly.[7] Our study adds valuable evidence to the ongoing discussion on the use of heterologous booster doses, such as RQ3013, in preventing COVID-19 outcomes, especially in the elderly population.

There are several limitations in our study. First, as mentioned above, the sample size for efficacy analysis was relatively small and the results of efficacy should be interpreted with caution. Second, we did not investigate the cellular responses or mechanisms underlying the enhanced immune responses observed following heterologous prime-boost regimens. Third, due to the short follow-up period in the current analysis, the long-term safety profile and durability of the immune response following the boost are unclear. However, it has been reported that the immunity provided by other mRNA boosters against omicron variants can last for up to 4 months.[32] To better understand the public health implications of RQ3013 as a booster, we are following study participants for at least six months to monitor immunity and evidence of vaccine failures, and plan to report on humoral immunity assays in forthcoming studies. Fourth, the generalizability of our conclusion may be constrained due to the high prevalence of prior SARS-CoV-2 infections in the current population. Due to the unavailability of other mRNA vaccines, especially the new bivalent vaccines developed by Pfizer and Moderna that contain both the original COVID-19 strain and the omicron strain, we were currently not able to compare RQ3013 with other mRNA boosters. Further trial are warranted to collect the comparative data between RQ3013 and these bivalent vaccines.

In conclusion, the heterologous prime-boost regimen with RQ3013 is immunogenic and safe. The strong enhancement of antibody titres after heterologous boosting is encouraging, though the durability and the neutralising activity of these antibodies against other variants of concern need further investigation. Our findings support the use of RQ3013 as a booster vaccination for adults primed with inactivated vaccines.

Contributors

X-QL is the principal investigator of this trial. LY, J-JC, L-HM, Z-YX, X-MY, and WL provided supervision. X-QL, YZ (Yunnan Center for Disease Control and Prevention), and Y-XZ curated the data. J-ZL, Z-FW, and X-YY did the laboratory work. Z-HS did the statistical analysis. YZ (Fudan University), J-ZL, Z-HS, H-JY, Q-HW, H-LS, JL, HZ, Y-PW, and FQ wrote, reviewed, and edited the manuscript. All authors critically reviewed and approved the final version. All authors confirm that they had full access to all the data in the study and verified the data.

Data sharing statement

The study protocol will be available immediately following publication for at least 1 year. The individual participant data that underlie the results reported in this article will be shared after de-identification. Researchers who provide a scientifically sound proposal will be allowed to access to the de-identified individual participant data. Proposals should be directed to the corresponding authors.

Declaration of interests

This trial was sponsored by Walvax Biotechnology. RQ3013 was co-developed by Walvax and RNACure. LY, J-JC, Z-YX, X-MY, and WL are employees of Walvax. JL, HZ, and Y-PW are employees of RNACure. H-JY was supported by the National Natural Science Foundation of China, outside the submitted work. The other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102231.

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