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GRAPHICAL ABSTRACT

HIGHLIGHTS

- **XBB.1.5-containing bivalent generated superior immunogenicity against XBB lineages compared to BA.2/BA.5-containing bivalent.**
- **The efficacy of booster dose against XBB lineages waned after 2-3 months.**
- **Newer variant-matched vaccine elicits an enhanced immune response against newly emerged variants.**

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Safety, immunogenicity, and preliminary efficacy of a randomized clinical trial of omicron XBB.1.5-containing bivalent mRNA vaccine

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ABSTRACT

Periodically updating coronavirus disease 19 (COVID-19) vaccines that offer broad-spectrum protection is needed given the strong immune evasion by the circulating omicron sublineages. The effectiveness of prototype and BA.4/5-containing bivalent mRNA vaccines is reduced when XBB subvariants predominate. We initiated an observer-blinded, three-arms study in 376 patients in Chinese individuals aged from 18 to 55 years old who had previously received three doses COVID-19 vaccine. Immunogenicity in terms of neutralizing antibodies elicited by a 30-μg dose of XBB.1.5-containing bivalent vaccine (RQ3027), a 30-μg dose of BA.2/BA.5-Alpha/Beta bivalent vaccine (RQ3025) and their precedent 30-μg Alpha/Beta (combined mutations) monovalent mRNA vaccine (RQ3013) and safety are primary and secondary endpoints, respectively. We recorded prescribed COVID-19 cases to explore the preliminary efficacy of three vaccines. RQ3027 and RQ3025 boosters elicited superior neutralizing antibodies (NAbs) against XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1 compared to RQ3013 at day 14 in participants without SARS-CoV-2 infection. All study vaccines were well-tolerated without serious adverse reactions identified. The incidence rates per 1000 person-years of COVID-19 cases during the 2nd-19th week after randomization were lowest in RQ3027. Overall, our data show that XBB.1.5-containing bivalent booster generated superior immunogenicity and better protection against newer severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants compared to BA.2/BA.5-containing bivalent and Alpha/Beta monovalent with no new safety concerns.

KEYWORDS severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); JN.1; XBB lineages; coronavirus disease 19 (COVID-19) vaccine; mRNA bivalent vaccine; randomized clinical trial

INTRODUCTION

The global spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in extensive morbidity and mortality, placing immense pressure on healthcare systems worldwide [1]. Though the number of new coronavirus disease-19 (COVID-19) cases has declined notably, the SARS-CoV-2 could still be concerning when the virus has further evolved or the immunity of the population has waned further [2–4]. Multiple component-updated COVID-19 vaccines have been developed and authorized for emergency use or approved for full use in various countries worldwide [5,6]. These vaccines have played a crucial role in mitigating the impact of the COVID-19 pandemic by preventing severe illness, hospitalizations, and deaths [7–9]. However, the spectrum of antibodies induced by SARS-CoV-2

variant-containing booster vaccines could be influenced synergistically by the antigen design and the "immune imprinting" as determined by the immune history of the host [10–12]. In China, most people have received three doses of the prototype-based vaccine prior to a wave of infection with two main subvariant lineages BA.5.2 and BF.7 nationwide in December 2022 [13].

Previous studies demonstrated that RQ3013 (a monovalent mRNA vaccine encoding a chimeric Spike protein containing mutations from both B.1.1.7 and B.1.351 variants) has higher geometric mean ratios (GMRs) of 12.6, 14.7, and 31.3 against the wild-type, the delta variant and the omicron variant, respectively, compared to inactivated vaccine (CoronaVac) at day 14 post-vaccination [14]. Variant-containing bivalent boosters comprised of mRNAs encoding the Alpha/Beta plus omicron XBB.1.5 (RQ3027) or omicron BA.2/BA.5 (RQ3025) strains have been developed to mitigate immune evasion caused by recent SARS-CoV-2 variants of concern (VOCs). However, randomized active-controlled studies of the contemporaneous administration of the omicron-containing bivalent and original vaccines have not been undertaken.

Here, we aim to describe the safety and immunogenicity of the omicron XBB.1.5-containing bivalent (RQ3027, Alpha/Beta, and omicron XBB.1.5 spike mRNAs) and omicron BA.2/BA.5-containing bivalent (RQ3025, Alpha/Beta and omicron BA.2/BA.5 spike mRNAs) boosters in Chinese healthy adults who had completed three vaccination schedules. Incidence rates of COVID-19 (breakthrough infection) post-vaccination are also summarized to explore the effectiveness of RQ3027 and RQ3025.

RESULTS

Participants

Pre-screening, recruitment, and vaccinations were done between April 21, 2023, and May 27, 2023. The last participant's fourth visit (day 14) took place on June 10, 2023. For the exploratory analysis of the protection level, all data collected from the first participant's third visit (day 7) until the last participant's last visit (seventh visit, day 180) were considered.

We screened a total of 396 participants. 376 of them enrolled and received the investigational vaccines as follows: 126 participants received RQ3027, 124 participants received RQ3025, and 126 participants received RQ3013. The safety data set included 376 participants: 126 in the RQ3027 group, 124 in the RQ3025 group, and 126 in the RQ3013 group. The baseline characteristics were well-balanced among three groups (Table 1). The median ages were 32.0, 29.0, and 29.0 in the RQ3027, RQ3025 and RQ3013 group, respectively. Most of the participants (97.9%) received ancestral strain-inactivated vaccines as primary series. The median interval since the last dose was 177 (interquartile range [IQR], 162.0–469.0), 155 (IQR, 146.5–437.5), and 177 (IQR, 154.0–473.5) days in the RQ3027, RQ3025 and RQ3013 group. For the immunogenicity analysis, participants who had SARS-CoV-2 infection within day 90 post-vaccination were excluded from the seronegative immunogenicity data set, thus, 100 participants of the RQ3027 group, 96 participants of the RQ3025 group, 101 participants of the RQ3013 group were included in seronegative immunogenicity analysis (Figure 1). The breakthrough infections were stratified and analyzed. All 376 participants' COVID-19 cases was followed up until the last visit and the analysis of the immunogenicity at day 180 is ongoing.

Safety and Reactogenicity of RQ3027, RQ3025 and RQ3013

The vaccines were safe and generally well tolerated. Solicited local and systemic reactogenicities were predominantly mild (grade 1) or moderate (grade 2) (Figure 2). The most common solicited local and systemic adverse reactions were injection site pain (374 [99.5%] of 376) and fatigue (311 [82.7%] of 376), severe (grade 3) injection site pain was observed in less than 4% RQ3027 recipients and less than 2% RQ3025 and RQ3013 recipients. Participants receiving RQ3027 and RQ3025 reported fever less frequently ($P < 0.05$) than RQ3013, severe fever (temperature, 38.5–39.5 °C) was reported in less than 8% of each vaccine recipients and typically resolved within 1–2 days. However, redness and joint pain were more frequently ($P < 0.05$) reported by the RQ3027 group than the RQ3013 group (Table S1). Severe systemic reactions including joint pain, muscular pain, headache, chill, and fatigue were noted during the initial 1–2 days following vaccination and resolved quickly afterward. All other solicited adverse reactions reported within 14 days after vaccination were comparable across three vaccines, suggesting the two variant-modified mRNA vaccines were as safe as the original vaccine. No serious adverse reactions or safety pauses occurred during the trial.

Immunogenicity of Seronegative Participants

Regarding immunogenicity, we measured the neutralizing antibodies (NAbs) against various SARS-CoV-2 variants at day 14 post-booster using a pseudovirus-based assay (Figure 3A) [15]. The baseline NAbs against prototype, Alpha, Beta, BA.4/5, BF.7, BQ.1.1, XBB.1.5, XBB.1.16, and XBB.1.9.1 were comparable between three vaccine groups (Table 1). For participants without breakthrough infection, we detected the highest NAbs increase against XBB.1.5 in RQ3027 with a geometric mean fold rise (GMFR) of 56.1 (95% confidence interval [CI], 44.5–70.9) followed by RQ3025 with a GMFR of 23.6 (95% CI, 18.5–30.2) at day 14 after vaccination (Figures 3A and S2). Consistently, the NAbs increase at day 14 in RQ3027 was the highest among three groups for XBB.1.9.1 cross-neutralization, with a GMFR of 36.6 (95% CI, 29.6–45.2) (Figures 3A and S2, Table S2).

The observed geometric mean titer (GMT) against XBB.1.5 was 6356.1 (95% CI, 4918.5–8213.9) at day 14 for RQ3027 and 4626.9 (95% CI, 3624.3–5906.8) for RQ3025, both higher than RQ3013 group (950.2 [95% CI, 764.1–1181.7]). The ANCOVA-modelled GMR against XBB.1.5 at day 14 was 7.2 (95% CI, 5.5–9.6) for RQ3027 versus RQ3013 and 4.0 (95% CI, 3.0–5.3) for RQ3025 versus RQ3013, meeting the pre-specified criterion for superiority (lower bound of 95% CI > 1) (Figure 3B, Table S2). We observed a similar trend for the NAbs against XBB.1.9.1. The GMT at day 14 for RQ3027 (5639.4 [95% CI, 4299.6–7396.8]) and RQ3025 (4726.9 [95% CI, 3591.0–6222.0]) were significantly higher than that of RQ3013 (701.5 [95% CI, 570.1–863.1]) with a GMR of 9.3 (95% CI,

Table 1. Demographics of participants in the mITT population

Characteristics *n* (%)	RQ3027 (*n* = 126)	RQ3025 (*n* = 124)	RQ3013 (*n* = 126)
Age, years			
Mean, SD	32.5 ± 6.9	30.4 ± 6.4	30.4 ± 6.8
Median, IQR	32.0 (26.0, 37.0)	29.0 (25.0, 36.0)	29.0 (25.0, 35.0)
Sex			
Female	53 (42.1)	59 (47.6)	47 (37.3)
Male	73 (57.9)	65 (52.4)	79 (62.7)
Body-mass index, *n* (mean)			
<18.5: underweight	9 (17.4)	14 (17.6)	11 (17.4)
18.5–24.9: healthy weight	84 (22.2)	76 (21.4)	81 (21.8)
25.0–29.9: overweight	29 (27.5)	31 (27.5)	30 (26.5)
≥30.0: obesity	4 (35.8)	3 (33.2)	4 (31.0)
Prior vaccination received (primary series)			
Inactivated vaccines (ancestral strain)	125 (99.2)	121 (97.6)	122 (96.8)
Protein subunit vaccines	0	1 (0.01)	0
mRNA vaccines	1 (0.01)	2 (0.02)	4 (0.03)
Days since last dose			
Median, IQR	177 (162.0, 469.0)	155 (146.5, 437.5)	177 (154.0, 473.5)
Neutralizing antibodies against SARS-CoV-2 variants pre-boosters, GMT (95% CI)			
Prototype	3098.8 (2577.8–3725.1)	3812.7 (3177.2–4575.2)	3816.6 (3100.3–4698.3)
Alpha	4363.9 (3584.4–5313.0)	5333.4 (4369.4–6510.1)	4959.9 (4015.5–6126.5)
Beta	2668.2 (2127.5–3346.4)	3076.5 (2533.8–3735.5)	3305.6 (2621.6–4168.2)
BA.4/5	1056.7 (851.7–1310.9)	1369.6 (1135.0–1652.8)	1344.9 (1091.9–1656.6)
BF.7	927.5 (762.4–1128.3)	1095.7 (913.2–1314.7)	1147.8 (932.3–1413.2)
BQ.1.1	155.0 (133.8–179.5)	198.0 (164.7–237.9)	166.5 (140.3–197.5)
XBB.1.5	108.6 (86.2–136.7)	177.7 (141.3–223.5)	121.9 (97.2–152.9)
XBB.1.16	254.0 (213.8–301.7)	390.6 (331.1–460.7)	363.4 (307.0–430.2)
XBB.1.9.1	141.0 (112.8–176.2)	249.6 (201.6–309.0)	187.4 (151.1–232.5)

Percentages are based on the number of participants in the modified intention-to-treat population. BMI classification is interpreted according to the U.S. Centers for Disease Control and Prevention.

Abbreviations: mITT, modified intention-to-treat; SD, standard deviation; IQR, interquartile range; GMT, geometric mean titer; CI, confidence interval; BMI, body-mass index.

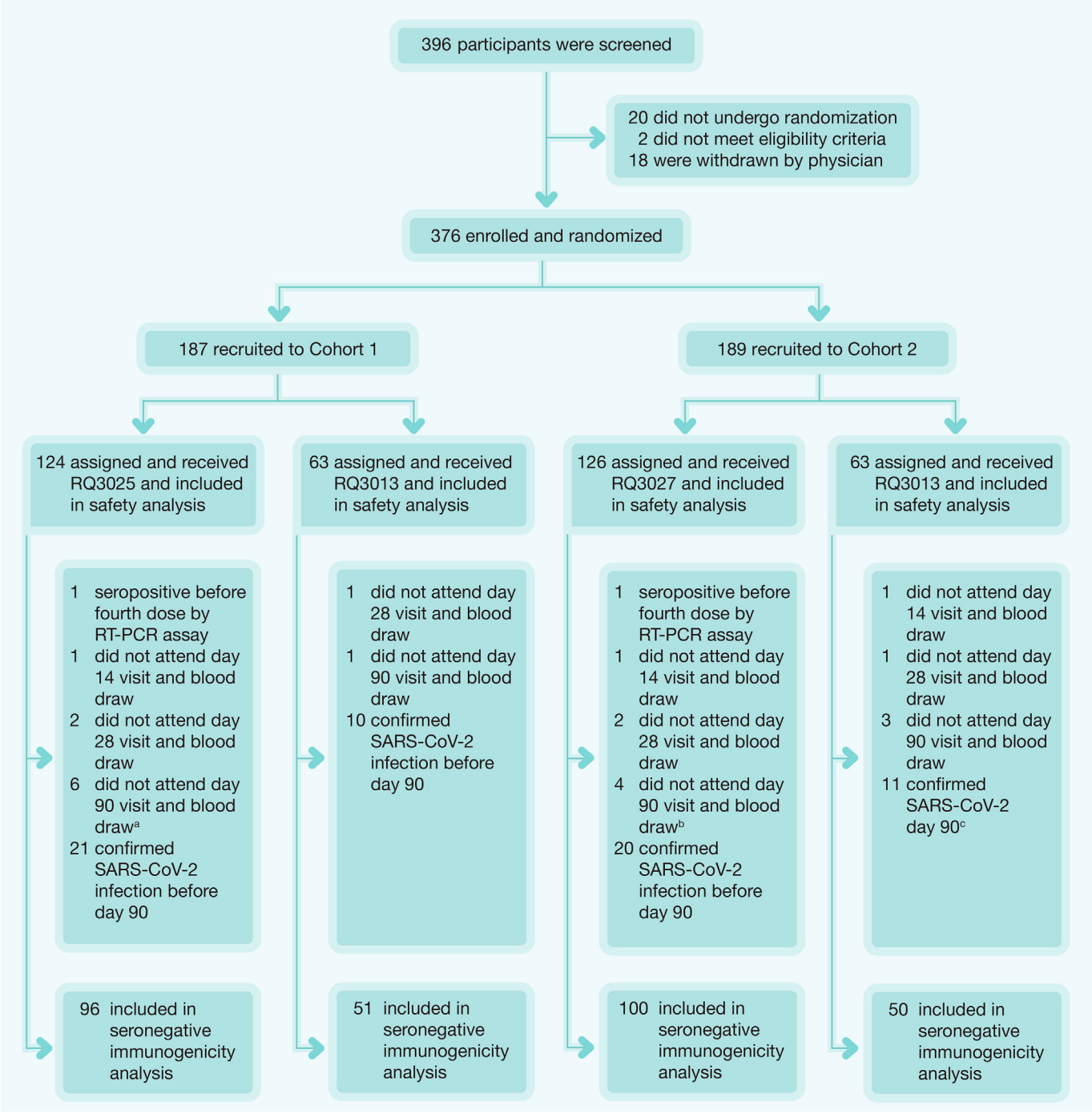

Figure 1. Consort flow diagram of the trial
The modified intention-to-treat population consists of all participants who met the inclusion/exclusion criteria then took the booster vaccination and had at least one post-dose immunogenicity data. The safety data set was used in the safety analysis. The immunogenicity data set without SARS-CoV-2 infection before day 90 was used in the seronegative immunogenicity analysis. [a]One participant who received RQ3025 did not attend the day 14, 28, and 90 visits. [b]Two participants who received RQ3027 did not attend the day 28, 90 visits, and confirmed SARS-CoV-2 infection before the day 90 visit. [c]Three participants who received RQ3013 did not attend the day 90 visit and confirmed SARS-CoV-2 infection before the day 90 visit. Abbreviations: RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

7.1–12.4) and 5.6 (95% CI, 4.2–7.4), respectively (Figure 3B, Table S2). Thus, XBB.1.5-containing and BA.2.4.5-containing bivalent vaccines demonstrated superior cross-neutralization against the newer variants than the monovalent vaccine encoding the ancestral strain. Interestingly, these two bivalent vaccines showed a much weaker superiority than the monovalent vaccine on the NAbs against XBB.1.16, with a GMR of 1.9 (95% CI, 1.6–2.2) and 1.4 (95% CI, 1.2–1.7) for RQ3027 versus RQ3013 and RQ3025 versus RQ3013 respectively (Figure 3B, Table S2). Furthermore, JN.1 showed robust resistance to

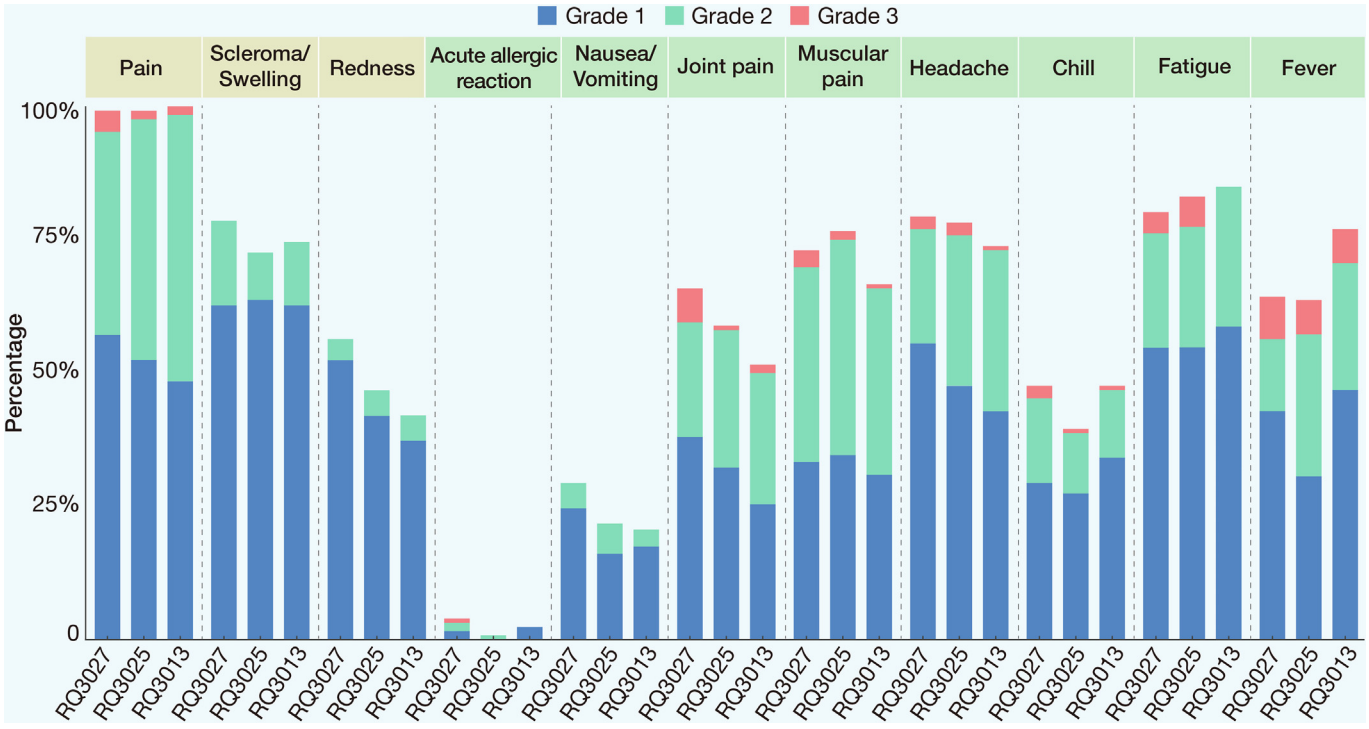

Figure 2. Solicited adverse reactions within 14 days after receipt of RQ3027, RQ3025, and RQ3013
The frequency of reported local/systemic adverse reactions presented. We counted the maximum severity of respective solicited adverse reactions recorded for each participant within 14 days after receipt of RQ3027, RQ3025, and RQ3013.

three investigational vaccines. XBB.1.5-containing bivalent still exhibited non-inferior immunogenicity of JN.1 than BA.2/BA.5-containing bivalent and corresponding superiority than Alpha/Beta monovalent. Additionally, the superiority of the neutralization at day 14 against omicron BA.4/5, BF.7, BQ.1.1 for RQ3027 and RQ3025 compared with RQ3013 booster were demonstrated (lower bound of CI > 1), but with small GMR estimates (Table S2).

Despite the superiority against newer SARS-CoV-2 variants (refers to BA.4/5, BF.7, BQ.1.1, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1), XBB.1.5-containing bivalent was non-inferior on the NAbs against previous variants (refers to prototype, Alpha, Beta) compared to Alpha/Beta monovalent. The NAbs against prototype was 16,617.0 (95% CI, 13,627.2–20,262.8) for RQ3027 and 16,820.7 (95% CI, 14,051.8–20,135.3) for RQ3013 at day 14, with a GMR of 1.0 (95% CI, 0.8–1.3) that met the criterion for non-inferiority (lower bound of 95% CI > 0.67) (Figure 3B, Table S2). The NAbs following RQ3027 and RQ3013 against Alpha and Beta strains at day 14 were similar to the prototype with GMRs of RQ3027 versus RQ3013 meeting the non-inferiority conditions (Table S2).

Comparing two bivalent vaccines, the better variant-matched vaccine RQ3027 was superior to its precedent bivalent vaccine RQ3025 against XBB.1.5 ($GMR_{RQ3027/RQ3025}$ = 1.8 [95% CI, 1.4–2.4]), XBB.1.9.1 ($GMR_{RQ3027/RQ3025}$ = 1.7 [95% CI, 1.2–2.2]) and XBB.1.16 ($GMR_{RQ3027/RQ3025}$ = 1.3 [95% CI, 1.1–1.6]) at day 14 (Table S2).

We then used serum neutralization results at day 14 post-booster to generate antigenic maps to reflect the antigenic associations in three investigational vaccines against SARS-CoV-2 variants. Most of the serum samples were closely distributed around the prototype and were far from BQ and XBB lineages pre-booster (Figure S3). After vaccination, the RQ3013 cohort was distributed closer to previous SARS-CoV-2 variants, whereas the RQ3027 cohort posited in approximation to newer variants and most of the RQ3025 serum cohort were distributed in the middle of the other two investigational vaccines (Figure S3). In addition, the antigenic maps revealed a central cluster of SARS-CoV-2 variants, which grouped based on mutual spike mutations [16]. These early variants are antigenically similar, distributing closer to each other in antigenic space. However, XBB.1.16 and JN.1 have evolved as distinct antigenic outliers post-booster (Figures S3 and S4). The estimated antigenic distances could denote the average divergence from each variant in the population of a certain immune background. The antigenic distance from all sera to XBB.1.5 in RQ3013 recipients was 5.0, which decreased to 2.3 and 2.8 in RQ3027 and RQ3025 recipients, respectively. Similar to XBB.1.5, the antigenic distances to XBB.1.16 and XBB.1.9.1 were shortened after receiving RQ3027 (3.9 and 2.3) and RQ3025 (4.2 and 2.7) compared to RQ3013 (4.5 and 5.4) (Figure 3C, Table S3). These data suggest a broader spectrum of cross-neutralization elicited by the XBB.1.5-containing bivalent vaccine.

Neutralizing Antibodies of XBB Breakthrough Infection Participants

For participants who had XBB breakthrough infection after receipt of three investigational vaccines (Table S4), the

neutralizing antibodies were significantly higher against BA.4/5 ($P < 0.05$), BF.7 ($P < 0.01$), XBB.1.5 ($P < 0.0001$), XBB.1.16 ($P < 0.0001$) and XBB.1.9.1 ($P < 0.0001$) compared to those who did not confirm SARS-CoV-2 infection (Figure 4A). In the XBB infection cohort, NAbs against XBB.1.16 were significantly lower compared to prototype ($P < 0.01$), XBB.1.5 ($P < 0.01$), and XBB.1.9.1 ($P < 0.05$) (Figure 4B). Nevertheless, when stratified by booster vaccines, XBB.1.5-containing bivalent vaccine showed much less immune escape by XBB.1.16 than Alpha/Beta monovalent and BA.2/BA.5-containing bivalent vaccines after XBB breakthrough infection (Figure 4C).

Cumulative Incidence of COVID-19 Cases in Modified Intention-to-Treat Population

As a planned exploratory endpoint, this study also evaluated the protection against infection by SARS-CoV-2 antigen rapid test and saliva-based PCR-confirmed COVID-19 cases. A total of 60 COVID-19 cases occurred from at least 7 days post-vaccination to the data cutoff date. From the 4th to 9th week post-vaccination, a noticeably lowered incidence rate of infection was observed in RQ3027 compared to the other two vaccines. However, the difference in risk of infection between vaccine recipients gradually diminished from the 9th week until the 16th week when incidence rates became comparable across three vaccines (Figure 5A). To date extraction cut off in the 19th week post-vaccination, the total overall person-years were 52.8 in the RQ3027 group, 51.9 in the RQ3025 group, and 53.0 in the RQ3013 group, per the primary case definition for COVID-19. The incidence rates per 1000 person-years of COVID-19 cases 7 days or more after randomization were 341.4 (95% CI, 202.0–538.7) for RQ3027, 404.7 (95% CI, 250.4–618.3) for RQ3025, and 396.1 (95% CI, 245.2–605.5) for RQ3013. RQ3027 recipients demonstrated a hazard ratio (HR) of 0.9 (95% CI, 0.5–1.6) compared to RQ3013, whereas RQ3025 demonstrated a HR of 1.0 (95% CI, 0.6–1.9) compared to RQ3013 (Figure 5A). No severe COVID-19 cases or related hospitalizations were reported in this study.

In line with the observed trend in the cumulative infection rates, RQ3027 demonstrated significant NAbs superiority against XBB lineages at day 28 post-booster (Figure 5B). These variants conferred the majority of COVID-19-confirmed cases (Table S4). However, by day 90, the superiority of RQ3027-elicited NAbs against these VOCs had drastically degenerated.

DISCUSSION

This randomized, observer-blinded, parallel-controlled clinical trial compared omicron XBB.1.5-containing or BA.2/BA.5-containing bivalent booster vaccines head-to-head with its precedent Alpha/Beta monovalent vaccine. Our results indicated that the XBB.1.5-containing bivalent vaccine and BA.2/BA.5-containing bivalent vaccine had a similar reactogenicity profile to the Alpha/Beta monovalent vaccine when administered as booster doses. Overall, the variant-updated bivalent mRNA vaccines RQ3027 and RQ3025 are as safe as their precedent monovalent mRNA vaccine RQ3013 among people with three prior doses administered and with an interval of at least 3 months from the prior dose.

In this study, we used a validated pseudotyped virus assay to evaluate the NAbs against SARS-CoV-2 variants elicited by three mRNA vaccines [15]. Our results showed that the XBB.1.5-containing bivalent (RQ3027) elicited NAbs responses which were superior against omicron BA.4/5, BF.7, BQ.1.1, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1 and non-inferior against prototype, Alpha and Beta to Alpha/Beta monovalent vaccine (RQ3013) at day 14 post-booster. Besides, XBB.1.5-containing bivalent has superiority of NAbs against the recently prevalent variants XBB.1.16 and XBB.1.9.1 than BA.2/BA.5-containing bivalent up to 90 days after booster. For the currently circulating JN.1 variant (up to Jan 20th, 2024) [17], the strongest immune escape was observed in all three investigational vaccines. BA.2/BA.5-containing bivalent elicited equivalent immunogenicity to XBB.1.5-containing bivalent, likely attributed to the closer antigenic distance between BA.2 and JN.1. The mRNA-1273 bivalent (original and omicron BA.4/BA.5) vaccine has shown effectiveness against COVID-19 when BA.5 was the predominant circulating strain [18], but the neutralization against XBB.1.5 was considerably lower [19]. Besides, preprint clinical data suggested an XBB-containing monovalent vaccine was more immunogenic against XBB variants than the authorized BA.4/BA.5 vaccine which was consistent with our findings [20]. These data suggested that better variant-matched vaccines could induce better cross-neutralization against newer variants.

We found a robust antibody response following three investigational vaccines, however, vaccine-induced antibodies peaked at different times (Figure S2). Most of the antibodies induced by RQ3027 and RQ3013 against the detected variants peaked on day 14 and then declined whereas RQ3025 peaked on day 7. The distinct immune dynamic response is likely shaped by different vaccine-immune histories [21,22]. For example, vaccine composition better matching the recipients' most recent infection/vaccination strain might induce a more rapid antibody response.

Using antigenic cartography, we found that pre-omicron variants all grouped into the same antigenic cluster before vaccination with investigational vaccines, in line with previous data on human sera [23]. Given the infection by BA.5.2 and BF.7 substrains, since the authority lifted zero COVID-19 policies in December 2022, the position of BA.4/5 and BF.7 was close to pre-omicron variants compared to XBB lineages in the antigenic map, reflecting the immune history of participants. In the map by Wilks et al., there was about a 4-fold larger distance between D614G and Beta, and a 6-fold larger distance between D614G and omicron BA.2 [23]. This might be caused by low titers against more immune-evasive variants dropped off more that lead to relatively large antigenic distances [16].

Based on the NAbs of individuals who had XBB lineages breakthrough infection post-booster, we found this cohort produced higher NAbs against the omicron variants than those who did not have breakthrough infections, which is consistent with the work of Yisimayi et al. that repeated omicron infection mitigates the immune imprinting by ancestral SARS-CoV-2 [24]. Neutralizing antibody titers have been used as predictors to assess the effectiveness of COVID-19 vaccines [25,26]. We consistently observed the superior performance of RQ3027

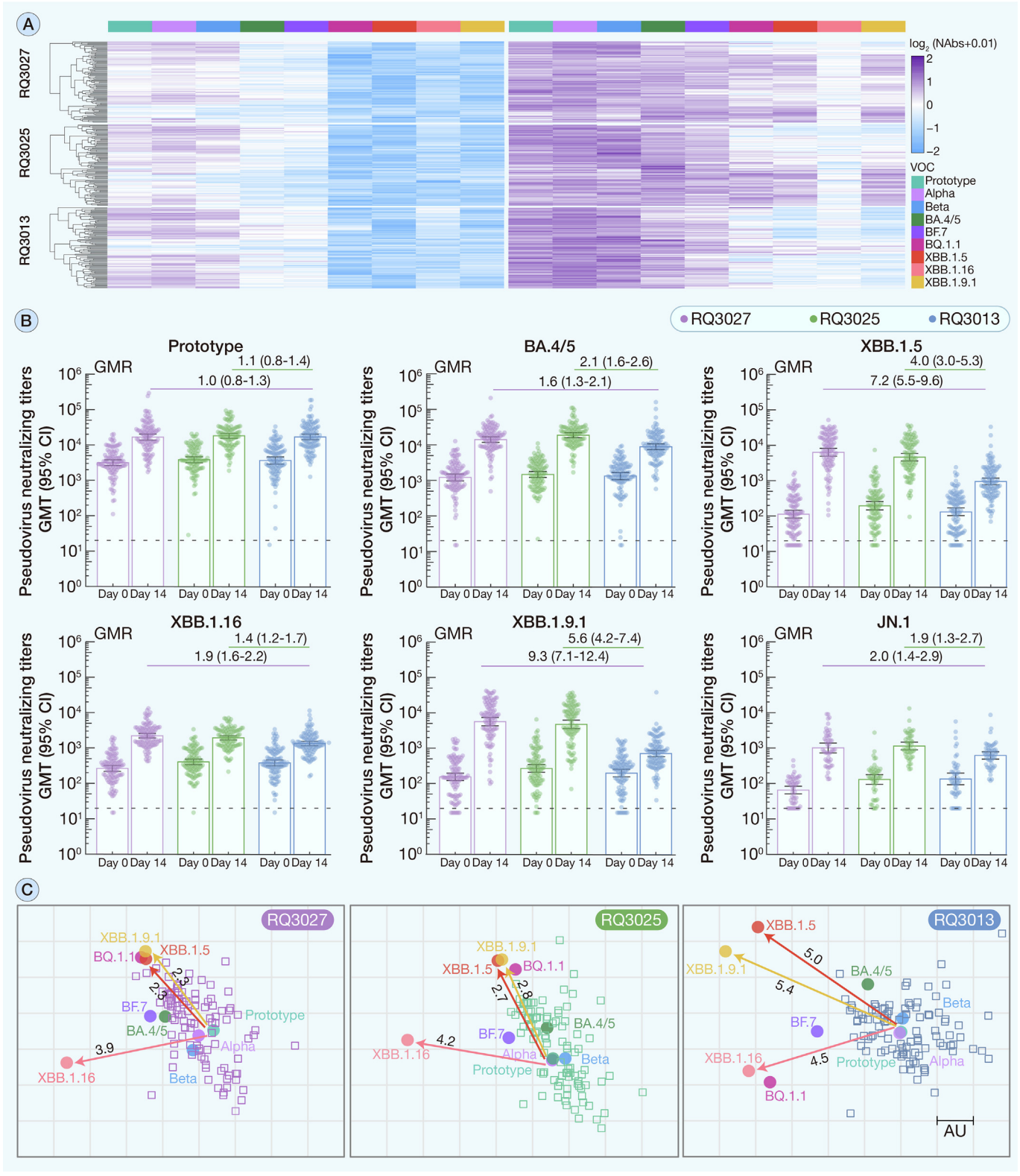

Figure 3. Pseudovirus neutralization antibodies to multiple variants before and after receipt of RQ3027, RQ3025, and RQ3013 vaccines in seronegative participants

(A) Heatmap comparisons of NAbs at baseline and 14 days after booster dose. Color intensity indicates normalization of log2(NAbs+0.01) for each specific SARS-CoV-2 variant tested. Total neutralization titers against prototype, Alpha, Beta, BA.4/5, BF.7, BQ.1.1, XBB.1.5, XBB.1.16, and XBB.1.9.1 (RQ3027, *n* = 100; RQ3025, *n* = 96; RQ3013, *n* = 100). (B) Pseudovirus neutralizing titers against prototype, BA.4/5, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1 at day 14 following vaccination are presented as the GMTs with 95% CI. Each dot represents the NAbs of each participant. The GMR in titers of the RQ3027 and RQ3025 versus RQ3013 are shown above the graph. The dashed line indicates the detection limit (20-fold). * For JN.1, RQ3027 *n* = 48, RQ3025 *n* = 47, RQ3013 *n* = 49. (C) An antigenic map was created using neutralization data from various serum cohorts as indicated. The prototype acts as the central reference for all serum cohorts, and the antigenic distances are

(legend continued on next page)

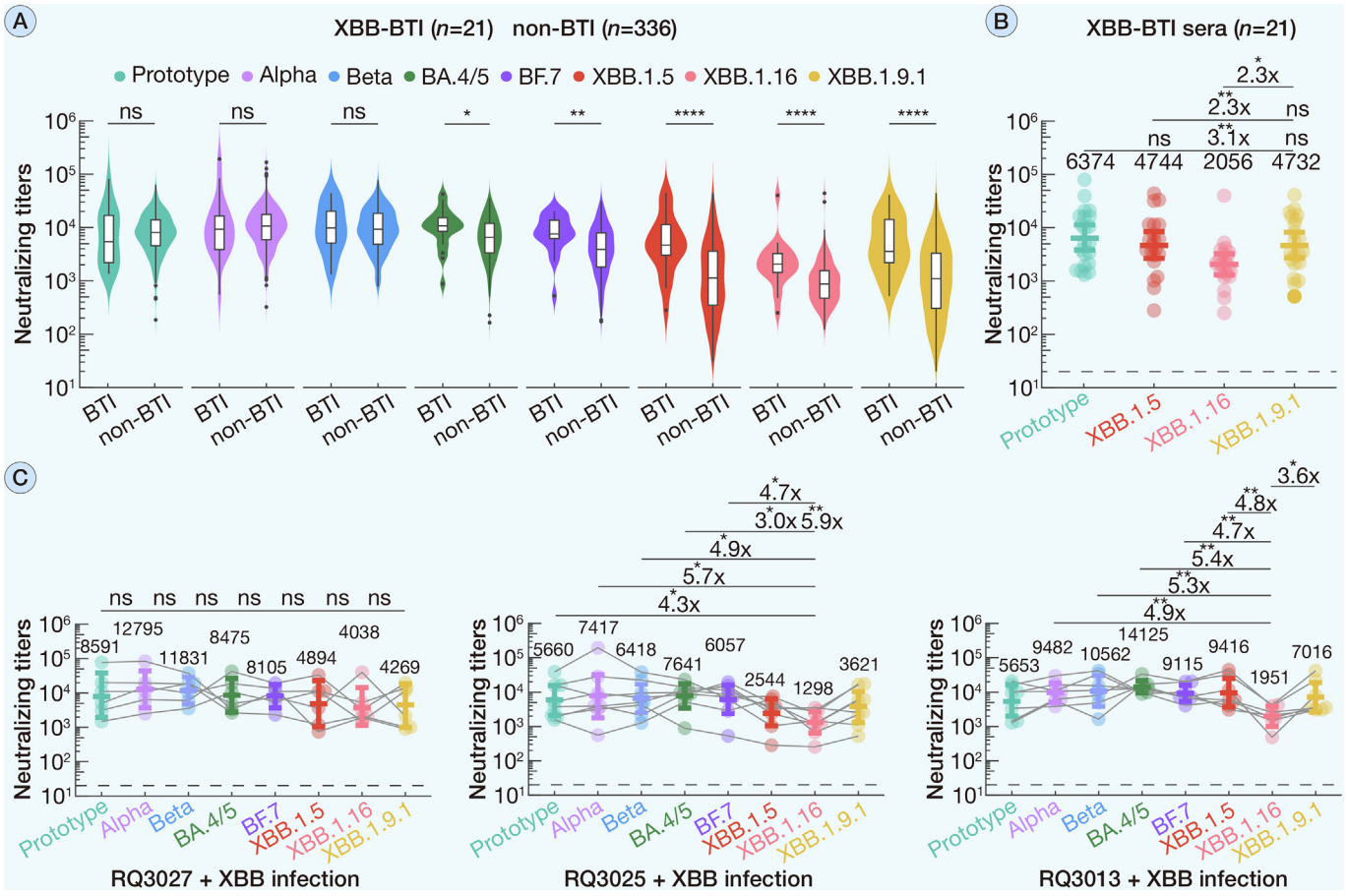

Figure 4. Neutralizing antibodies of XBB breakthrough infection participants
(A) NAbs against prototype, Alpha, Beta, omicron BA.4/5, BF.7, XBB.1.5, XBB.1.16, and XBB.1.9.1 of XBB infection participants and participants without SARS-CoV-2 infection. Statistical tests were determined using two-tailed Wilcoxon rank-sum tests. Boxes display the 25th percentile, median, and 75th percentile. Violin plots show kernel density estimation curves of the distribution. The numbers of the two cohorts are labeled above the violin plots. *P < 0.05, **P <0.01, ****P < 0.0001 and ns P > 0.05. (B–C) NAbs against prototype, omicron XBB.1.5, XBB.1.16, and XBB.1.9.1 in XBB cohorts after receiving of RQ3027, RQ3025 and RQ3013. Each dot represents the NAbs of each participant. The GMTs and 95% CI are labeled. The dashed line indicates the detection limit (20-fold). Statistical tests were determined using two-tailed Wilcoxon rank-sum tests. The fold rise is labeled. *P < 0.05, **P < 0.01 and NS P > 0.05. Abbreviations: NAbs, neutralizing antibodies; ns, not significant; GMT, geometric mean titer; CI, confidence interval.

compared to the other two vaccines, with respect to robust NAbs against the XBB lineages and the reduced incidence rates of infections by the XBB lineages. These findings suggest that a better antigen-matched vaccine, which elicits higher NAbs against circulating variants [27], likely contributes to enhanced efficacy until the NAbs wane or escape occurs.

There are some limitations to this clinical trial. The inclusion criteria related to past SARS-CoV-2 infections may be influenced by undetected asymptomatic infections during study screening. The primary objective of the trial was to evaluate the immunogenicity of three investigational vaccines, and it was not adequately powered to compare efficacy across them. However, the exploratory efficacy analyses still offered valuable insights into the potential effectiveness of these vaccines in protecting against infection. The newly emerged JN.1 and HV.1 variants were not sequenced by our COVID-19 breakthrough cases, so the investigation of protection of composition updated vaccine against newly emerged variants (e.g., JN.1, EG.5.1, HV.1) is warranted.

The U.S. FDA has shifted from a bivalent to a monovalent COVID-19 vaccine that aligns with the XBB.1.5 variant because the original vaccine is not as potent in enhancing immunity against Omicron strains and the original strain of SARS-CoV-2 has not been in circulation since 2021 [28]. Our results illustrated in Figure 3 indicate that the Alpha/Beta monovalent does not significantly increase the immune response to Omicron variants. In fact, continued use of the original vaccine could influence the immune imprinting of these variants. As a result, a monovalent vaccine tailored to the specific variant sequences is now preferred.

calculated to represent the average deviation from each variant. Each small square symbolizes serum from one participant, while each small circle represents different SARS-CoV-2 variants. One AU corresponds to an approximately two fold change in NAbs. Abbreviations: VOC, variant of concern; NAbs, neutralizing antibodies; GMTs, geometric mean titers; CI, confidence intervals; GMR, geometric mean ratio; AU, antigenic unit.

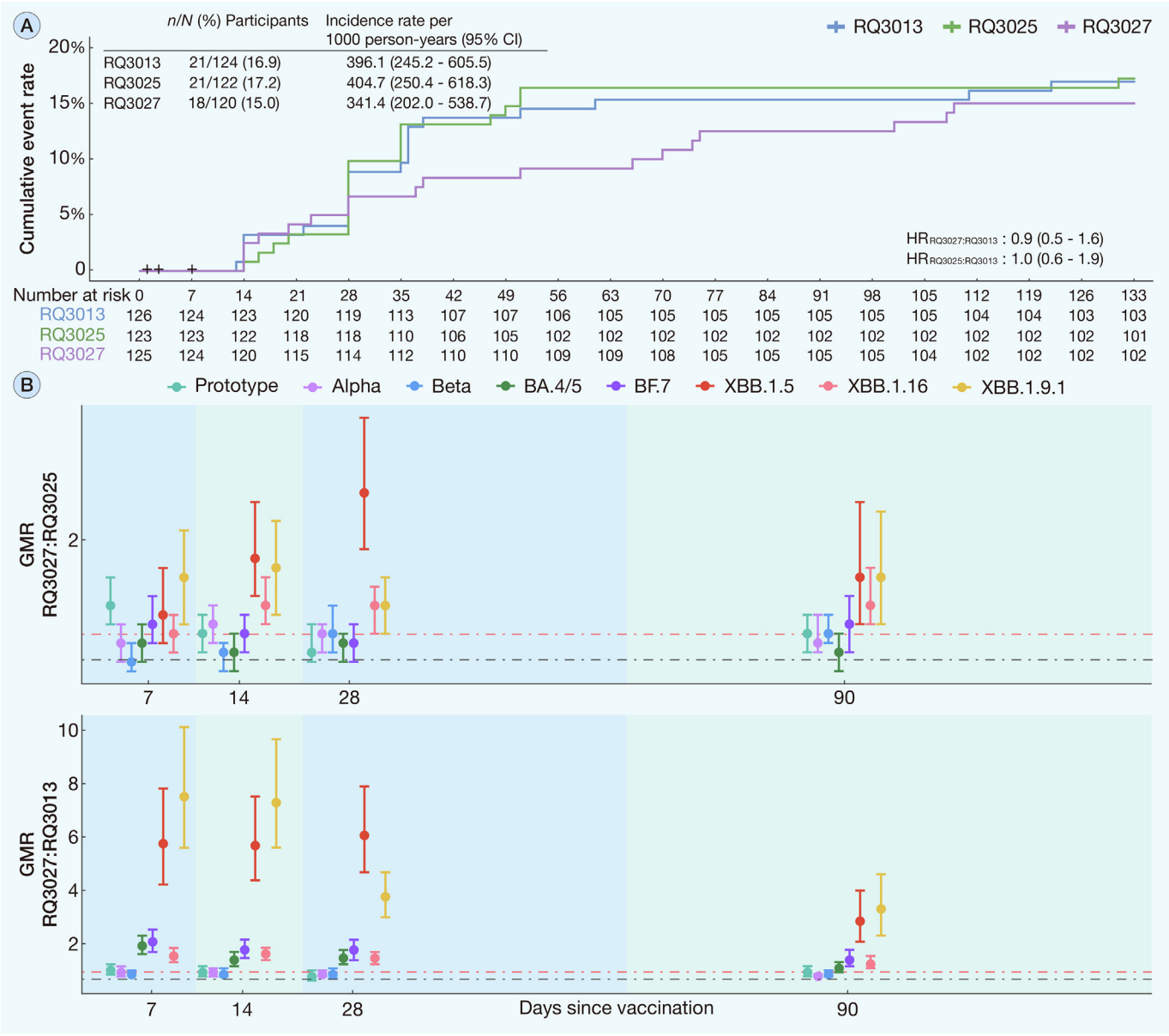

Figure 5. Cumulative incidence of COVID-19 cases in mITT population and GMR of neutralization antibodies against different variants after receipt of RQ3027, RQ3025, and RQ3013

(A) Cumulative event rates of COVID-19 per primary case definition in the trial protocol for RQ3027, RQ3025, and RQ3013 based on assessment starting 7 days after randomization in the per-protocol efficacy population detected during May 18th - September 20th, 2023 in positive SARS-CoV-2 cases. Tick marks indicate censored data. (B) The projection of GMR at days 7, 14, 28, and 90 after receiving three vaccines. Each dot represents the GMR of different groups, and 95% CI is labeled. The red dotdash line represents GMR equal to 1. The black dotdash line represents GMR equal to 0.67. Abbreviations: mITT, modified intention-to-treat; GMR, geometric mean ratio; CI, confidence interval.

In summary, our work quantified changes in cross-reactivity and response breadth after receipt of omicron XBB.1.5-containing bivalent and BA.2/BA.5-containing bivalent booster.

MATERIALS AND METHODS

Study Design

This randomized, active-controlled, observer-blinded trial was done in a single center (The Affiliated Hospital of Yunnan University, Yunnan, China), assessing bivalent mRNA vaccines RQ3027, RQ3025, and monovalent mRNA vaccine RQ3013 in three doses of COVID-19 vaccine-experienced Chinese healthy adults. This trial was reviewed and approved by the Scientific Review Committee and the Ethics Committee of the Affiliated Hospital of Yunnan University (ID: 2023113). ClinicalTrials.gov identifier NCT05907044. The study protocol is available with the full text of this article (Trial Protocol in Supplementary Material). All the enrolled participants have provided written informed consents. The trial was conducted in accordance with the Declaration of Helsinki, International Council for Harmonization's Good Clinical Practice, and local guidelines.

Participants

Healthy participants (determined by medical history and physical examination) aged from 18 to 55 years old and fully vaccinated with 3 doses of COVID-19 vaccine according to the national recommendations were included in this study. The main exclusion criteria were last prior COVID-19 vaccination within 90 days or COVID-19 infection within 28 days before screening, pregnancy, or breastfeeding, and unwillingness to practice effective birth control and enrolling in or planning to participate in other interventional clinical trials. Receiving of systemic immunoglobulins or blood products within 3 months before screening and a history of anaphylaxis, urticaria, or other severe adverse reactions requiring medical intervention after receipt of a vaccine led to exclusion. The exhaustive list of eligibility criteria is available in the trial protocol.

Randomization and Masking

We used the SAS (version 9.4) to generate a randomization list (allocation ratio 1:1:1, block size = 6). The vaccine number is the same as the randomization number which was assigned by the order that participants enrolled. The unmasked statistician oversaw recording each participant's vaccine number in the electronic Case Report Form (eCRF) (Deipai EDC, Beijing, China).

In this observer-blinded study, participants, observers (doctors, nurses, investigators, and laboratory testers) who were involved in the observation of the participants post-boosters, or any further evaluation of safety and immunogenicity were kept masked for vaccine randomization. To facilitate rapid review of data, principal investigators will not be blinded. The unmasked investigators were responsible for vaccine storage preparation and transportation. Vaccine blinding was performed by statisticians, that is, the printed vaccine label was affixed to the designated position of each vaccine. The masked nurses from the investigational center were in charge of the injection of vaccines.

Procedures

The active-control RQ3013 is a lipid-nanoparticle-embedded modified-mRNA-based vaccine encoding a chimeric Spike protein containing mutations from both B.1.1.7 (Alpha) and B.1.351 (Beta) variants, which is jointly developed by Fudan University, Shanghai Rnacure Biopharma Co., Ltd. and Walvax Biotechnology Co., Ltd. [29]. RQ3027 is an immunogen-updated bivalent mRNA-based vaccine composed of XBB.1.5 and Alpha/Beta variants using the same platform as RQ3013. RQ3025 is an updated bivalent mRNA-based vaccine composite of BA.2/BA.5 (combined mutation of BA.2 and BA.4/5) and Alpha/Beta variants Spike. All the investigational vaccines containing 30 μg mRNA were administered intramuscularly at 0.15 mL per dose (Figure S1).

After recruiting eligible potential participants, they were enrolled if they met the inclusion criteria and didn't meet the exclusion criteria. Nasopharyngeal swabs were collected for polymerase-chain-reaction (PCR) confirmation of SARS-CoV-2 infection before vaccination. COVID-19 cases (defined according to World Health Organization COVID-19 cases definitions) were recorded from day 7 up to day 180 after vaccination. Material for COVID-19 rapid lateral flow tests (Vazyme, Jiangsu, China) was provided to participants. Participants presenting with COVID-19-like illness were requested to perform the test at home [30], nasopharyngeal swab was done by the investigator if COVID-19-like illness and positive lateral flow test were reported in eCRF whenever possible.

Safety monitoring included a 30-minute post-vaccination observation period and self-reported daily record of any solicited and unsolicited adverse reactions on the eCRF for 14 days post-vaccination. Adverse events and serious adverse events of special interest were monitored each week after 14 days up to 6 months post-vaccination. Participants reported the severity of their adverse reactions as Grade 1–4 according to the China National Medical Products Administration guidelines [31].

Blood samples (16 mL of heparinized whole blood to isolate PBMCs and 8 mL of blood to harvest serum) were collected in the intention-to-treat (ITT) population pre-booster (day 0) and days 1, 7, 14, 28, 90, 180 after vaccination to explore the cellular and humoral immunity. Neutralizing antibody against VOCs was measured using the pseudotyped virus assay [15].

Outcomes

The analysis of safety, immunogenicity, and exploratory endpoints within 6 months post-vaccination were planned in the trial protocol and the follow-up was completed on November 25th, 2023. Here, we report all the data collected within 90 days post-vaccination. The primary safety endpoint was the adverse event rates from 30 min to 14 days post-vaccination. The secondary safety endpoint was the serious adverse reactions and pregnancy events within 180 days post-vaccination.

The primary immunogenicity endpoints evaluated the neutralizing antibody titers against SARS-CoV-2 prototype, Alpha, Beta, BA.4/5, BF.7, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1 variants by the pseudotyped virus-based assay at day 14 post-vaccination. The secondary immunogenicity endpoint evaluated the NAbs against SARS-CoV-2 prototype, Alpha, Beta, BA.5, BF.7, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1 pseudotyped virus at day 1, 7, 28, 90, 180 post-vaccination.

The main exploratory endpoint was the level of protection induced by RQ3027, RQ3025, and RQ3013 as assessed by confirmed COVID-19 cases from 7 to 180 days post-vaccination. A complete list of outcomes and endpoints can be found in the trial protocol of the supplementary material.

Pseudovirus Production

The plasmids of SARS-CoV-2 Spike and vesicular stomatitis virus (VSV-G) were constructed using the pcDNA3.1 vector. We used the Lipofectamine Kit (Thermo Fisher, Massachusetts, USA) to produce the SARS-CoV-2 pseudovirus, and transferred the prepared mix to the supernatant of the HEK-293T cells in a 10 cm dish slowly. Placed the dishes in 5% CO_2 incubator at 37 °C for 6–8 h, added 7.0 × 10^4 $TCID_{50}$/mL VSV-ΔG pseudovirus (G*ΔG – VSV pseudovirus) for 6–8 h until the cells were rounded. Then, we used PBS to wash the dishes and added DMEM with 10 mL 10% FBS (Thermo Fisher, Massachusetts, USA) for 24 h. The G*ΔG – VSV pseudovirus (or VSV-SARS-CoV-2 pseudovirus) was harvested by centrifuging and filtering the supernatant and stored at −80 °C up to 1 year after distributing aliquots of the mixture into 2 mL microtubes.

VSV-SARS-CoV-2 Pseudovirus Neutralization Assay

All serum samples were heat-inactivated at 56 °C for 30 min and serially diluted, starting from wells B4 to B10, in the complete DMEM medium (BassIMedia, Shanghai, China). The initial dilutions for wells B3 to G3 were set at 1:20 or 1:200, using a 96-well white flat-bottom culture plate (BeyoGoldT, Fujian, China). Various VSV-SARS-CoV-2 pseudovirus lineages (prototype, Alpha, Beta, and Omicron sub-lineages BA.4/5, BF.7, BQ.1.1, XBB.1.5, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1) were standardized to the same TCID50/mL in complete DMEM medium. A volume of 100 μL of these diluted pseudoviruses was added to each well containing 100 μL of serum, followed by incubation in a 5% CO_2 incubator at 37 °C for 1 h. This excluded wells B11-G11, which served as positive controls, and B2-G2, which served as negative controls. Subsequently, 100 μL of HEK293T-ACE2 cells at a concentration of 2×10^5 cells/mL was added to each well, and the plate was returned to the 5% CO_2 incubator at 37 °C for an additional 24 h. Relative light unit (RLU) values for each well were measured using diluted Renilla Luciferase reagent (Beyotime Biotechnology, Shanghai, China), incubated for 3–5 min, and read using a microplate reader (PerkinElmer, Massachusetts, USA). The 50% neutralizing antibody titers (NC50) and geometric mean titers were calculated based on a VSV neutralization assay, as previously described [15].

Antigenic Map

We used an established multidimensional scaling algorithm to construct antigenic maps from the neutralization data [32]. The visualization was created using the Racmacs package (v.1.1.4, https://acorg.github.io/Racmacs/) in R, with 5000 optimization steps and the minimum column basis parameter set to 'none'. The 'mapDistances' function was used to calculate antigenic distances between each serum sample and variant, and the average distances from all sera to each variant determined the final representation.

Statistical Analysis

The sample size was estimated based on the immunogenicity outcome in this trial. The primary analysis of this study will be a superiority test by comparing the GMTs of neutralizing antibodies against the SARS-CoV-2 VOCs between investigational vaccines. We assumed the standard deviation of the neutralizing titers on a log scale (base 10) was 0.36 based on immunogenicity available data, the minimum clinical difference to detect was 1.2 folds difference in NAbs GMT against SARS-CoV-2 VOCs on a log scale (base 10). Based on the above assumptions, the study needs to recruit at least 126 participants in each group to achieve 80% power with a two-sided significance level of 0.05. Considering a drop-out rate of 10%, a sample size of 140 participants in each group was required. Meanwhile, to ensure the safety assessment 100 participants per arm are usually required in the phase II clinical trials. Therefore, a sample size of 140 per group was finally chosen and the targeted sample size in total was 420.

We assessed immunogenicity data in the immunogenicity data set (known as per-protocol population). Participants in this data set complied with inclusion criteria, received the investigational vaccine, and had available primary immunogenicity outcomes. A summary of baseline characteristics is presented in the modified intention-to-treat (mITT) population (Table 1). The NAbs against the SARS-CoV-2 prototype, Alpha, Beta, BA.5, BF.7, XBB.1.5, XBB.1.16, XBB.1.9.1, and JN.1 were reported as GMT and 95% CI. The GMT was calculated as the mean of the assay results after making the logarithm transformation and exponentiating the mean to express the representative results. Comparison of NAbs between each vaccine or each visit was represented as GMR and GMFR. The GMR was analyzed using the ANCOVA model with the vaccine group or SARS-CoV-2 variants as a fixed effect, adjusting for baseline neutralizing titers and comparison group. GMFR was defined as the ratio of the result after vaccination to the result before vaccination. The secondary immunogenicity objective is to assess the superiority with respect to neutralizing titers of the anti-omicron BA.4/5, BF.7, XBB.1.5, XBB.1.16, and XBB.1.9.1 immune response induced by RQ3027 relative to the anti-omicron immune response elicited by RQ3025. Superiority based on GMR (day 14) was declared if the lower limit of the two-sided 95% CI for the GMR is greater than 1. The noninferiority with respect to neutralizing titers of the anti-prototype immune response induced by RQ3027 and RQ3025 relative to that elicited by RQ3013 based on the GMR (day 14) was declared if the lower limit of the two-sided 95% CI for the GMR was greater than 0.67. The statistical methods are exhaustively described in statistical consideration (Trial protocol in Supplementary Material).

Safety analysis was evaluated in the safety data set, which included all participants who received vaccines after randomization. Percentages of participants with confirmed COVID-19 cases 7 days or more after vaccination are summarized. We also provided cumulative event rates (Kaplan–Meier method) adjusting for person-time with 95% CI (Poisson distribution) starting 7 days post-booster. Breakthrough infection sera were collected and tested by saliva-based PCR screening at The Affiliated Hospital of Yunnan University. The SARS-CoV-2 genome sequencing was performed by BGI Co. Ltd. (Guangdong, China).

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DECLARATION OF COMPETING INTEREST

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

ZJZ served as a principle investigator in a phase 4 clinical study sponsored by Sinovac Biotech Ltd. The funder has no

role in study design, implementation and manuscript writing in this study. The other authors declare that they have no competing interests.

ETHICS APPROVAL

This trial was reviewed and approved by the Scientific Review Committee and the Ethics Committee of the Affiliated Hospital of Yunnan University (ID: 2023113). All participants enrolled have provided written informed consent.

DATA AVAILABILITY

Access to de-identified data by qualified external researchers may be available from the corresponding author Z.J.Z. (zijiezhang@ynu.edu.cn) upon request.

AUTHOR CONTRIBUTIONS

Xuanjing Yu: writing – original draft, visualization, validation, supervision, software, resources, project administration, methodology, investigation, formal analysis, conceptualization. Wei Yang: supervision, methodology. Wei Li: supervision, investigation. Na Wan: supervision, methodology. Guanghong Yan: software, formal analysis. Zumi Zhou: validation, methodology. Xiao Zhu: validation, methodology. Wei Su: supervision, methodology. Yani Li: supervision, investigation. Chenyu Xing: supervision, investigation. Sifan Duan: supervision, investigation. Houze Yu: supervision, methodology. Xinshuai Zhao: supervision, methodology. Chunmei Li: supervision, funding acquisition. Taicheng Zhou: supervision, investigation, conceptualization. Dingyun You: supervision, investigation, data curation, conceptualization. Jia Wei: funding acquisition, conceptualization. Zijie Zhang: writing – review & editing, supervision, project administration, methodology, investigation, funding acquisition, conceptualization.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hlife.2024.01.005.

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