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# Heterologous RBMRNA-405 mRNA booster enhances humoral immunity post-inactivated COVID-19 vaccination: a randomized clinical trial in adults and older through 12 months

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# Title

Heterologous RBMRNA-405 mRNA Booster Enhances Humoral Immunity Post-Inactivated COVID-19 Vaccination: A Randomized Clinical Trial in Adults and Older Through 12 Months

## Running Title

Safety and immunogenicity of SARS-CoV-2 mRNA vaccine

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## Abstract

To address the urgent need for effective immunization strategies against SARS-CoV-2 variants, particularly in older adults who are more vulnerable to severe disease, we evaluated the safety and immunogenicity of the bivalent COVID-19 mRNA vaccine RBMRNA-405 as a third-dose booster in adults aged 18 years and older previously vaccinated with two doses of inactivated vaccine. In a single-center, randomized, positive-controlled phase I study in China, 60 healthy participants were assigned (2:1) to receive either a heterologous booster (RBMRNA-405) or a homologous booster (CoronaVac). Primary endpoints assessed solicited local and systemic adverse events within 14 days post-vaccination, while secondary endpoints evaluated long-term safety and humoral immunogenicity via anti-Spike IgG ELISA and live-virus neutralization. Local pain at the injection site was the most common adverse event, primarily mild-to-moderate, with no serious vaccine-related adverse events reported. RBMRNA-405 induced 3.30-fold higher neutralizing antibodies against Omicron BA.1 and 17.66-fold higher anti-Spike IgG titers compared to CoronaVac on day 14 post-boost ($P<0.0001$), with robust responses in older adults, where age-related immune decline necessitates tailored immunization strategies.

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## Trial Registration

Clinical Trial Main ID: NCT05897190
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## Keywords

- COVID-19 vaccine
- mRNA vaccine
- Heterologous boost
- Safety
- Immunogenicity
- Older Adults

## Introduction

As of November 2023, more than 13.5 billion vaccine doses have been administered globally against COVID-19[1], where inactivated vaccines were utilized mainly in low- and middle-income countries (LMICs)[2]. CoronaVac, developed by Sinovac Life Sciences, is an inactivated whole-virion SARS-CoV-2 vaccine that employs β-propiolactone for viral inactivation and aluminum hydroxide as an adjuvant to enhance immune response. This formulation targets the original Wuhan strain of the virus, providing a traditional platform for inducing humoral immunity through exposure to the complete inactivated pathogen[3]. Globally, CoronaVac has been widely deployed in primary vaccination series, with over 2.4 billion doses administered primarily in low- and middle-income countries as part of emergency use authorizations and national campaigns. Its extensive use underscores its role in accessible COVID-19 immunization strategies worldwide[4]. Despite the implementation of different vaccine platforms, including inactivated virus, viral vector, protein subunit and mRNA[5,6], vaccine-induced immunity wanes over time[7,8]. The emergence of breakthrough infections, driven by waning immunity and new variants, poses a significant challenge to the effectiveness of COVID-19 vaccination strategies[9,10]. RNA-based modalities, such as mRNA and saRNA vaccines, have been intensely developed against COVID-19[11]. While primary series vaccinations have reduced hospitalizations and mortality, their long-term effectiveness against emerging variants of concern are being re-evaluated[12]. Heterologous boosting appeared as a potential solution to enhance immune responses against COVID-19[13,14]. Mounting evidence indicates that heterologous schedules utilizing mRNA boosters may provide broaden protection and enhanced immunity compared to homologous inactivated booster schedules[15-19]. Despite increasing evidence for heterologous boosting, data on older adults previously vaccinated with inactivated vaccines, the mainstay in many LMICs, remain scarce. Further investigation in the safety and immunogenicity of mRNA booster vaccines in different age populations primed with inactivated vaccines is crucial to inform optimal booster strategies and elucidate the long-term safety profile of mRNA boosters, especially for those aged 60 years and above.

RBMRNA-405 is a bivalent mRNA vaccine containing a 1:1 mix of pseudouridine-modified messenger RNAs encoding Omicron-derived and Delta-derived spike proteins to provide protection against emerging variants. Notably, the thermostability profile of RBMRNA vaccines are found optimal at 2-8˚C and can also be stored at room temperature for seven days. Preclinical studies of RBMRNA vaccines in rodents and non-human primates (NHP) revealed favorable safety, immunogenicity, and protection[20,21].

This Phase I study reports on the safety and immunogenicity of RBMRNA-405 as a third-dose booster in participants who received two doses of inactivated vaccine compared to those of homologous booster, initiated in May 2022 during the Omicron predominance period.

## Results

### Participant Enrollment and Baseline Characteristics

From 16 May 2022, 90 participants were screened, of which, 60 adults were enrolled and stratified into two age groups: aged 18 to 59 years (n=30) and aged 60 and above (n=30), whom were then randomly assigned to RBMRNA-405 or CoronaVac booster vaccine groups (Fig. 1.) Baseline characteristics were balanced between groups, with 31 (51.7%) participants being male. The median ages of the adult groups were 27.0 and 27.5 years; the median ages of the older adult groups were 64.0 and 67.5 years (Table 1.)

### Safety Profile from Booster Vaccination

Regarding the primary safety endpoint, the frequency of AEs following RBMRNA-405 or CoronaVac booster through Day 360 is outlined in the Supplementary Appendix (Supplementary Table 1). Notably, all vaccine-related adverse events occurred within the first 14 days post-vaccination, with no new vaccine-related AEs emerging between Day 14 and Day 360. The cumulative proportion of participants experiencing vaccine-related AEs remained stable at 65.0% for RBMRNA-405 and 25.0% for CoronaVac from Day 14 through Day 360, indicating that reactogenicity was confined to the immediate post-vaccination period with favorable long-term safety. Statistical comparisons using Fisher's exact test confirmed significantly higher rates of local reactogenicity in the RBMRNA-405 group ($p<0.0001$), driven primarily by injection site pain. However, no significant differences were observed in systemic adverse events including fever ($p=0.65$), fatigue ($p=0.17$), headache ($p=1.00$), myalgia ($p=1.00$), or cough ($p=0.10$) (Supplementary Table 1). Additionally, no instances of vaccine-related SAEs, serious unexpected suspected adverse reactions (SUSARs), AESI, fatalities or AE leading to withdrawal were reported in either group (Supplementary Table 1). The most frequently reported solicited local adverse event was pain at injection site, with a higher incidence in RBMRNA-405 (62.5%) compared to CoronaVac (5.0%) ($p<0.0001$; Fig.2). The most frequently reported solicited systemic adverse event was fever (≥37.3˚C, axillary temperature) in both groups, with an incidence of 12.5% in RBMRNA-405 and 5.0% in CoronaVac (Fig.2). In terms of severity of solicited AEs, most were grade 1 or 2 events, both RBMRNA-405 and CoronVac groups each reported one case of grade 3 fever (2.5% and 5.0%; Fig.2), whom both recovered (Supplementary Table 2).

In the analysis of age subgroups, adverse event incidences in the 18–59-year cohort were similar between the 0–14-day and >14-day periods, while a lower incidence was evident in participants aged ≥60 years across both groups. However, only pain at injection site frequencies were higher in RBMRNA-405 compared to CoronaVac (Fig. 3A & 3B) for both adult (18-59 years) and older (≥60 years) groups.

### Neutralizing Antibody Response Against Omicron BA.1

At baseline (Day 0), serum neutralizing antibody (NAb) titers against Omicron BA.1 were minimal to undetectable across all participants, with no differences in geometric mean titers (GMTs) between the RBMRNA-405 and CoronaVac groups (Fig. 4). By Day 14 post-booster, the RBMRNA-405 group exhibited a significantly higher GMT of 333.65 (95% CI: 241.42, 466.66) compared to 101.84 (95% CI: 63.90, 162.30) in the CoronaVac group, reflecting a 3.30-fold increase ($p<0.0001$). This superiority persisted at Day 28, with GMTs of 22.16 (95% CI: 12.42, 39.51) in RBMRNA-405 versus 1.77 (95% CI: 0.78, 4.00) in CoronaVac, a 12.55-fold increase ($p<0.0001$) (Fig. 4).

### Age-Dependent Neutralizing Antibody Dynamics

Across both adult (18–59 years) and older adult (≥60 years) cohorts, the RBMRNA-405 group demonstrated significantly higher NAb GMTs against Omicron BA.1 at Days 14 and 28 compared to the CoronaVac group (Supplementary Table 3). In adults, the RBMRNA-405 group achieved a GMT of 476.63 (95% CI: 285.34, 796.17) at Day 14, compared to 99.75 (95% CI: 48.28, 206.07) in the CoronaVac group, a 4.78-fold increase ($p<0.01$). At Day 28, the RBMRNA-405 group maintained a 13.06-fold higher GMT [40.74 (95% CI: 18.40, 90.18)] versus 3.12 (95% CI: 1.01, 9.59) in the CoronaVac group ($p<0.001$) (Fig. 5A). In older adults, RBMRNA-405 elicited a GMT of 236.37 (95% CI: 153.63, 363.66) at Day 14, a 2.27-fold increase over 103.98 (95% CI: 56.54, 191.23) in the CoronaVac group ($p<0.05$). By Day 28, despite declines in both groups, RBMRNA-405 retained a 12.05-fold higher GMT ($p<0.01$) (Fig. 5B).

### Anti-Spike IgG Response Post-Booster

Baseline anti-spike IgG GMTs were detectable but low, at 200.00 for RBMRNA-405 and 186.61 for CoronaVac. Following booster administration, RBMRNA-405 induced a substantial 529.10-fold increase from baseline at Day 14, reaching a GMT of 105,820.40 (95% CI: 79,582.50, 140,708.80), which declined to a still-robust 324.00-fold increase (GMT: 64,800.66; 95% CI: 49,005.46, 85,686.88) by Day 28. In comparison, CoronaVac elicited more modest fold-rises of 32.12-fold at Day 14 (GMT: 5,992.96; 95% CI: 4,004.66, 8,968.43) and 28.24-fold at Day 28 (GMT: 5,271.97; 95% CI: 3,550.72, 7,827.61) from baseline. These within-group responses translated to substantial between-group differences, with RBMRNA-405 demonstrating 17.66-fold higher GMTs than CoronaVac at Day 14 (p<0.0001) and 12.29-fold higher GMTs at Day 28 (p<0.0001) (Fig. 6; Supplementary Table 4).

### Age-Specific Anti-Spike IgG Trends

In both adult (18–59 years) and older adult (≥60 years) cohorts, the RBMRNA-405 booster elicited significantly higher anti-spike IgG GMTs compared to the CoronaVac group at Days 14 and 28, demonstrating robust immunogenicity across age groups (Supplementary Table 4). For adults, the RBMRNA-405 group achieved a peak GMT of 154,719.74 (95% CI: 101,995.57, 234,698.43) at Day 14, a 20.91-fold increase over the CoronaVac group's 7,398.27 (95% CI: 4,100.96, 13,346.76) (p<0.0001; Fig. 7A). This trend persisted at Day 28, with RBRMNA-405 GMTs at 94,367.50 (95% CI: 63,174.78, 140,961.70), a 12.52-fold increase compared to 7,535.90 (95% CI: 4,269.30, 13,301.89) in the CoronaVac group (p<0.0001; Fig. 7A).

Similarly, in older adults, RBMRNA-405 induced a peak GMT of 72,407.73 (95% CI: 47,543.50, 110,275.42) at Day 14, a 14.93-fold increase over the CoronaVac group's 4,850.29 (95% CI: 2,675.47, 8,792.98) (p<0.0001; Fig. 7B). By Day 28, RBMRNA-405 maintained significantly higher GMTs at 44,572.19 (95% CI: 29,217.04, 67,997.32), a 12.13-fold increase compared to 3,675.83 (95% CI: 2,022.79, 6,679.78) in the CoronaVac group (p<0.0001; Fig. 7B).

To assess the concordance between the binding antibody and neutralizing antibody assays, we performed a Pearson correlation analysis between anti-S IgG levels and neutralizing antibody titers against the Omicron BA.1 variant. The analysis included data from both pre-vaccination and day 28 post-vaccination time points. A strong, statistically significant positive correlation was observed between the two measures (r = 0.73657, p < 0.0001).

### Long-Term Anti-Spike IgG Trends

The CoronaVac group exhibited a significant rise in anti-spike IgG geometric mean titers (GMTs) at Day 360, reaching 8,560.68 (95% CI: 6,128.61, 11,957.89), compared to 2,466.87 (95% CI: 1,688.37, 3,604.33) at Day 180, representing a 3.5-fold increase (p<0.0001; Fig. 8). Conversely, the RBMRNA-405 group showed a modest decline in IgG GMTs at Day 360 [21,758.46 (95% CI: 17,126.47, 27,643.20)] relative to Day 180 [22,806.21 (95% CI: 17,382.15, 29,922.85)], as detailed in Supplementary Table 5.

In the adult cohort (18–59 years), a significantly higher proportion of CoronaVac recipients (40.0%) exhibited an unexpected increase in anti-spike IgG levels at Day 360 compared to none (0.0%) in the RBMRNA-405 group ($p=0.0077$). This pattern was consistent in older adults (≥60 years), where all CoronaVac participants (100%) displayed late-stage IgG elevation at Day 360, compared to 35.0% in the RBMRNA-405 group ($p=0.0011$), as shown in Table 2.

## Discussion

Our study evaluated the safety and immunogenicity of a heterologous prime-boost regimen using RBMRNA-405 following a primary series of inactivated CoronaVac, compared to a homologous CoronaVac prime-boost regimen. The results indicate that the heterologous regimen induced a superior immune response while maintaining a favorable safety profile. Notably, the frequency and severity of adverse events were comparable between the RBMRNA-405 and CoronaVac groups, with the exception of increased injection-site pain in the RBMRNA-405 cohort. Additionally, longitudinal analysis of IgG antibody levels through Day 360 revealed a robust and sustained immune response against SARS-CoV-2 in participants receiving the heterologous regimen.

The observed superiority of heterologous prime-boost regimens over homologous inactivated vaccine schedules can be attributed to fundamental differences in immune activation pathways. mRNA vaccines have been shown to elicit robust CD4+ T-follicular helper (Tfh) cell responses and promote vigorous germinal center (GC) reactions, which are critical for the generation of high-affinity antibodies and durable memory B cell responses[22,23]. In contrast, inactivated vaccines typically induce weaker Tfh responses and less efficient GC formation, resulting in antibodies with lower affinity maturation and reduced breadth of neutralization[24,25]. The heterologous approach leverages these complementary mechanisms: the inactivated vaccine priming establishes initial immune recognition, while the mRNA boost drives affinity maturation through enhanced GC activity and Tfh-mediated B cell selection. This sequential engagement of distinct immunological pathways likely accounts for the enhanced neutralizing antibody titers and broader cross-variant protection observed in our heterologous regimen compared to homologous inactivated vaccine schedules.

In both the RBMRNA-405 and CoronaVac booster groups, solicited local and systemic adverse events were more frequent among adults aged 18–59 years compared to older adults above 60 years, consistent with prior findings from homologous and heterologous third-dose booster studies[26,27]. This observation underscores an age-dependent variation in immune responses to booster vaccinations. The safety profile of RBMRNA-405 was favorable, with no vaccine-related SAEs, SUSARs, AESIs, deaths, or AEs leading to withdrawal reported in either age group, indicating that RBMRNA-405 was well-tolerated across the study population.

The most common solicited adverse event (≥10.0%) associated with RBMRNA-405 was injection-site pain, observed in both age groups, with all occurrences classified as grade 1 or 2 in severity. This underscores the favorable safety profile of RBMRNA-405, particularly in adults aged 60 years and older. These findings are consistent with preclinical studies in animal models and RBMRNA vaccine series, which similarly demonstrated acceptable safety and tolerability profiles for RBMRNA-405[20,21]. The reduced adverse event frequency in older adults may reflect not only lower reactogenicity but also age-related immunological hyporesponsiveness or potential underreporting bias. Older participants may have attenuated inflammatory responses to vaccination or different thresholds for symptom reporting compared to younger age groups, which should be considered when interpreting these safety profiles.

In alignment with the current study, a prior randomized, double-blind, placebo-controlled phase I/II clinical trial (NCT05903118) evaluating a related vaccine platform (RBMRNA-176) against the Wuhan-Hu-1 SARS-CoV-2 strain reported a comparable safety profile. That trial, involving 390 healthy adults who received a two-dose primary series of RBMRNA-176, identified injection-site pain as the most frequent solicited local adverse event (65.9%), consistent with the observations in this study. No vaccine-related SAEs, SUSARs, AESIs, deaths, or adverse events leading to withdrawal were reported (Supplementary Table 6). These results align with established safety profiles of mRNA vaccines, where injection-site pain is the predominant reactogenicity feature, distinguishing the heterologous and homologous booster groups in this study. The frequencies of solicited systemic adverse events, such as fever (12.5%) and fatigue (7.5%), were consistent with prior reports and within expected ranges[28,29]. Over a 12-month follow-up period, the heterologous booster vaccination with RBMRNA-405 maintained a favorable safety profile in both adults and older adults, with no vaccine-related adverse events reported after Day 14.

The humoral responses elicited by RBMRNA-405 in heterologous boosting align with the established pattern for mRNA vaccines following inactivated vaccine priming, characterized by a strong boost in anti-spike IgG and, most importantly, the consistent elicitation of neutralizing activity against variants like Omicron BA.1 in most recipients[30]. While these functional benchmarks affirm RBMRNA-405's comparable humoral responses, our study's distinctive value emerges from its year-long (Day 360) monitoring of IgG persistence in older adults (≥60 years)—a vulnerable group underrepresented in prior trials. This extended follow-up illuminates the durability of mRNA-boosted immunity in elderly cohorts from low- and middle-income countries reliant on inactivated platforms, bridging a critical gap in evidence for optimizing booster strategies amid resource constraints.

Our immunogenicity findings corroborate previous studies demonstrating enhanced immune responses with a heterologous prime-boost regimen following a primary series of inactivated vaccine[33,30]. Recent real-world evidence from large-scale cohort studies further supports the superiority of heterologous boosting strategies. Notably, a prospective study in Chile involving over 11 million individuals primed with CoronaVac demonstrated that heterologous boosters with BNT162b2 or AZD1222 provided substantially higher vaccine effectiveness against symptomatic COVID-19 (96.5% and 93.2%, respectively) compared to homologous CoronaVac boosting (78.8%), with even more pronounced benefits against severe outcomes and mortality[34]. Similarly, comparative analyses across different vaccine platforms have shown that heterologous prime-boost combinations, particularly those involving viral vector and mRNA vaccines, elicit comparable or superior antibody titers and enhanced T-cell responses relative to homologous regimens[35]. Moreover, immunological assessments of triple vaccination regimens have revealed that while homologous and heterologous approaches both generate neutralizing antibodies against ancestral strains, heterologous strategies demonstrate more robust and sustained immune activation, particularly against emerging variants with significant immune evasion capabilities[35]. These converging lines of evidence underscore the immunological advantage of platform-switching boosters in populations initially primed with inactivated vaccines, reinforcing our findings of enhanced antibody responses with RBMRNA-405. At baseline (Day 0), at least six months post-administration of two doses of inactivated vaccine, NAb concentrations against Omicron BA.1 were low to undetectable across all groups. Following a single booster dose, NAb concentrations increased in both groups by Day 14. However, the RBMRNA-405 group exhibited a substantial but rapid decline in neutralizing antibody titers, dropping approximately 15-fold from Day 14 to Day 28. This rapid waning is characteristic of mRNA vaccine-induced neutralizing responses and contrasts with the more stable anti-spike IgG levels, which declined only 1.63-fold over the same period. Despite this decline, RBMRNA-405 maintained significantly higher NAb titers compared to the CoronaVac group, which showed an even more pronounced reduction from already lower peak levels. Notably, RBMRNA-405 induced a 2-fold increase in live NAb titers in adults and a 12-fold increase in older adults compared to the homologous booster by Day 28. Moreover, the observed age-independent neutralizing antibody response following revaccination with CoronaVac, yielding similar GMTs with less than a 1.05-fold difference between adults (18-59 years) and older adults (≥60 years) at Day 14, stands in contrast to the age-stratified response elicited by the RBMRNA-405 mRNA booster, where younger participants demonstrated more robust immunogenicity. This disparity may stem from fundamental differences in vaccine platforms and their interactions with age-related immunosenescence. In such cases, the immune response relies heavily on recalling existing memory B and T cells established during primary vaccination, which may be comparably constrained in older adults due to reduced naïve lymphocyte pools and chronic low-grade inflammation, resulting in minimal age-based variation. mRNA vaccines like RBMRNA-405, which encode the spike protein for endogenous expression, provoke stronger de novo adaptive responses that are more susceptible to immunosenescence; older individuals often exhibit pronounced declines in antibody titers, neutralizing capacity, and cellular immunity. Additionally, the primary vaccination schedule—here, homologous CoronaVac priming—may cap the booster potential in older cohorts, as preexisting immunity could saturate the response ceiling, whereas the heterologous mRNA boost exploits a novel mechanism that amplifies disparities in immune competence. These platform-specific dynamics underscore the need for tailored booster strategies in elderly populations to optimize protection against variants like Omicron BA.1. These results align with prior reports highlighting the greater magnitude of immune responses elicited by heterologous boosting, indicating a more effective strategy for addressing emerging VOC[15].

Anti-spike IgG titers followed a consistent pattern across all groups, peaking at Day 14 and declining by Day 28. However, notable differences were observed between the heterologous and homologous boosting strategies. The heterologous booster, RBMRNA-405, consistently induced higher anti-spike IgG titers at all time points and in both age groups compared to the homologous CoronaVac booster. Among adult participants, RBMRNA-405 elicited a 20.91-fold increase in anti-spike IgG titers on Day 14 and a 12.52-fold increase on Day 28 relative to CoronaVac. Similarly, in older adults, RBMRNA-405 resulted in a 14.93-fold increase on Day 14 and a 12.13-fold increase on Day 28 compared to the homologous booster. These sustained and significantly higher anti-spike IgG titers, particularly in older adults, suggest that heterologous boosting with RBMRNA-405 generates a more robust immune response than homologous boosting. These findings provide valuable insights for optimizing vaccination strategies, especially for vulnerable populations.

The evaluation of long-term anti-spike IgG trends was incorporated into this study to assess the durability of the humoral immune response induced by the vaccines under investigation. Extended follow-up, extending to Day 360 post-vaccination, enables a comprehensive examination of antibody persistence. This approach is essential for elucidating potential differences in vaccine-induced immunity over time, informing strategies for booster regimens. Consistent with prior studies[36-38], anti-spike IgG antibody levels generally declined over time across all groups. However, in the CoronaVac group, a notable increase in anti-spike IgG titers was observed from Day 180 to Day 360, coinciding with the relaxation of China's Zero-COVID policy in December 2022 and the subsequent spread of BA.5 and BF.7 variants approximately one to two months post-Day 180. This suggests potential breakthrough infections during this period, supported by elevated IgG titers in both adult and older adult CoronaVac participants at Day 360. In contrast, this trend was not observed in the RBMRNA-405 group, indicating that heterologous boosting with an mRNA vaccine may enhance the durability of the humoral response. While the potent peak NAbs elicited by heterologous boosting are known to wane over time[39], our observation of sustained anti spike IgG levels over 360 days points to the establishment of a durable humoral response. This kinetic profile, characterized by a strong initial peak followed by a stable baseline, aligns with the established model of immune memory development. The initial high titers reflect robust activation, while the sustained IgG levels are consistent with the persistence of long lived plasma cells. Furthermore, the absence of a late serological boost suggestive of widespread infection implies that this sustained response may have contributed to reducing antigen exposure. Ultimately, long term defense against severe disease is mediated by rapid anamnestic responses from memory B and T cells. Our data on IgG durability provide a positive immunological indicator that this foundational memory was effectively generated in this vulnerable, older adult population. These findings underscore the value of heterologous boosting strategies in strengthening vaccine-induced antibody responses, aligning with the need for effective immunization strategies to address infectious disease challenges, such as those posed by the COVID-19 pandemic and rising measles cases globally.

Collectively, our results demonstrate that a heterologous RBMRNA-405 mRNA booster elicits a robust humoral immune response and a favorable safety profile compared to a homologous CoronaVac booster in individuals previously vaccinated with two doses of inactivated vaccine. This study contributes to the evidence supporting heterologous boosting with mRNA vaccines, particularly for older adults and populations in LMICs reliant on inactivated vaccines[40]. The observed age-dependent decline in antibody responses with homologous boosting further highlights the potential of RBMRNA-405 or other mRNA vaccines to optimize long-term immunity[32]. These insights are critical for developing vaccination strategies that ensure safe and effective protection against infectious diseases, supporting further evaluation of RBMRNA-405 in larger Phase II and III clinical trials to assess efficacy and safety across diverse populations.

This study has several limitations. The sample size was relatively small, potentially limiting generalizability. The protocol's exclusion of individuals with severe or immunocompromising conditions means the remaining stable comorbidities are unlikely to confound the results in this randomized population. However, their absence limits the detailed characterization of the cohort. This study was conducted exclusively in a Han Chinese population. While this homogeneity ensures a well-controlled genetic and environmental background for the initial assessment, it limits the immediate generalizability of the findings to other ethnic groups. Future studies in more diverse populations are warranted to confirm the broader applicability of the results. The study design was open label. Due to the study design, frequent PCR testing was not readily available for all participants, which prevented detection of breakthrough infections following the lift of the Zero COVID Policy. This study is limited by its focus on humoral immunity, without corresponding data on T-cell responses. Given the established role of cellular immunity in durable protection and defense against severe outcomes, this gap prevents a full assessment of the regimen's immunologic profile. Correlating humoral kinetics with T-cell measures remains an essential objective for future research.

## Methods

### Study Design

This is a single-center, randomized, positive-controlled phase I study carried out in Chengdu, Sichuan, China, to evaluate the safety and immunogenicity of the SARS-CoV-2 mRNA vaccine RBMRNA-405 as a heterologous third-dose booster in adults and older adults, who received 2 doses of inactivated vaccine primary series at least 6 to 12 months prior.

This trial was registered on ClinicalTrials.gov on May 28, 2023 (registration ID: NCT05897190). As an Investigator-Initiated Trial (IIT), the public registration was completed following the submission and review of the RBMRNA-405 Investigational New Drug (IND) application, in accordance with regulatory procedures. The study protocol was finalized prior to participant enrollment in

May 2022 and remained strictly unchanged throughout the trial, with no post-hoc modifications to the endpoints, procedures, or analysis plan.

The trial was conducted in accordance with the Declaration of Helsinki, Consolidated Standards of Reporting Trials (CONSORT) guidelines and local guidelines. The study protocol was reviewed and approved by the Ethics Committee of Chengdu Xinhua Hospital Affiliated to North Sichuan Medical College, China on April 27, 2022 (Ethics Approval ID: 2022lunkeyan004). Written informed consent was obtained from all study participants before enrollment.

### Participants

Adults aged 18 years and above inclusive, who received 2 doses of inactivated vaccine primary series at least 6 to 12 months prior, were eligible. ‘High-risk’ individuals, such as long-term use of immunopotentiator, immunosuppressant therapy 6 months prior to vaccination, serious chronic illness were excluded (See clinicaltrials.gov: NCT05897190 for eligibility criteria).

### Randomization

Eligible participants were randomly assigned in a 2:1 ratio to receive a booster immunization (third dose of vaccine) either with the heterologous booster RBMRNA-405 or homologous booster CoronaVac based on a computer-generated randomization schedule prepared by the randomization statistician.

Subjects were recruited on May 16, 2022 with follow-up visits to May 2023. Due to the COVID-19 Prevention and Control Health Code System in China in 2022, all commercial COVID-19 vaccinations including boosters must be collected and recorded in the Health Code System. Therefore, the homologous booster group was open label.

### Procedures

Participants received intramuscular injections in the deltoid region. RBMRNA-405 was administered in the dose of 50 μg in 0.25mL per dose. CoronaVac was administered the recommended dose of 0.5mL. After booster dose vaccination, participants were closely monitored for safety evaluation on days 0, 7, 14, 28, 90, 180 and 360. Post-boost adverse events (AEs) were recorded by diary cards and recorded during follow-up visits. Serum samples were collected on days 0, 7, 14, 28, 90, 180 and 360 for immunogenicity evaluation.

### Outcomes

The primary outcome was the occurrence of solicited local and systemic AEs within 14 days following the booster immunization. Secondary safety outcomes included severe adverse events (SAEs) within 360 days as well as the live neutralization of SARS-CoV-2 Omicron BA.1 (Guangzhou City Eighth People’s Hospital) at 14 and 28 days post-boost [State Key Laboratory of Respiratory

Disease, National Clinical Research Center for Respiratory Disease (Guangzhou, China)], geometric mean concentrations of IgG responses against spike (s)-protein of SARS-CoV-2 at 7, 14, 28, 90, 180 and 360 days following booster vaccination (Supplemental Appendix, Supplementary Immunologic Assay Methods).

## Statistical analysis

A total of 60 randomized participants were included in the statistical analysis. The occurrences of safety outcomes are presented as percentages (%). Demographic information and AE data are summarized using descriptive statistics, including mean, standard deviation (SD), median, and range. Missing data were not imputed and participants with missing data were excluded from the respective analyses.

For immunogenicity data, neutralizing antibody titers against SARS-CoV-2 Omicron BA.1 measured by $TCID_{50}$ were log-transformed for analysis using a linear regression fixed effects model, including analysis of covariance (ANCOVA) with adjustments for baseline titer as covariate. For each time point, an ANCOVA model was fitted with the log-transformed pre-vaccination titer as covariate and including treatment group (RBMRNA-405 vs. CoronaVac) and age stratum (18-59 vs. ≥60 years) as fixed effects, to estimate least-squares means for the log-transformed antibody levels by group and their differences, which were then back-transformed to report adjusted geometric means, the ratio of geometric means (RBMRNA-405/ CoronaVac), and its 95% confidence intervals (CI). All immunogenicity data, including neutralizing antibody titers and IgG titers, were analyzed using the same analytical method and expressed as geometric mean titers (GMTs) with corresponding 95% CIs. Differences between groups were assessed by calculating the ratio of GMTs with corresponding 95% CIs. Subgroup analyses were performed for safety and immunogenicity outcomes by age (18-59 and ≥60 years). Given the exploratory nature of the analyses, no formal adjustment for multiple comparisons was applied; p-values are presented as unadjusted to reflect this approach. Statistical analyses were performed with SAS software (Version 9.4).

## Anti-spike IgG ELISA

Serum anti-spike IgG antibody levels were determined by enzyme-linked immunosorbent assay (ELISA) using recombinant full-length SARS-CoV-2 Omicron BA.1 Spike Trimer protein (ACROBiosystems, SPN-C52Hz) as the coating antigen at 200 ng/well. Ninety-six-well plates were coated overnight at 4°C, blocked with 1% BSA, and incubated with serially diluted heat-inactivated serum samples for 1 hour at 37°C. After washing, HRP-conjugated goat anti-human IgG (Proteintech, SA00001-17, 1:1,000) was added for 1 hour at 37°C, followed by TMB substrate development. Absorbance was measured at 450 nm, and endpoint titers were calculated as the highest serum dilution exceeding the cutoff value (mean ± SD of negative controls at the lowest dilution). Complete protocol details, including detailed reagent specifications, incubation conditions, and washing procedures, are provided in Supplementary Immunologic Assay Methods.

### Role of the funding source

This study was an investigator-initiated trial (IIT). The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Author Contributions

X.Y. was the principal investigator and J.H. was the co-principal investigator. X.Y. and J.H. conceived the trial and contributed to the protocol and design of the study. X.Y. led the implementation of the study. Y.W. performed the statistical analysis and has verified the underlying data. Y.O. and C.Z. drafted the original manuscript. X.Y., J.H., and B.Z. reviewed and revised the original draft. All authors contributed to the article and approved the submitted version.

## Data Availability

Anonymized individual participant data will be made available when the study is complete, on reasonable requests made to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform. The trial protocol and statistical analysis plan will also be made available upon reasonable request to the corresponding author.

## Additional Information

### Competing Interest Statement

This trial was sponsored by Argorna Pharmaceuticals, which provided the study vaccine RBMRNA-405. RBMRNA-405 was co-developed by Argorna and RiboBio. YO and BZ are employees of Argorna. CZ is an employee of RiboBio. The other authors declare no competing interests.

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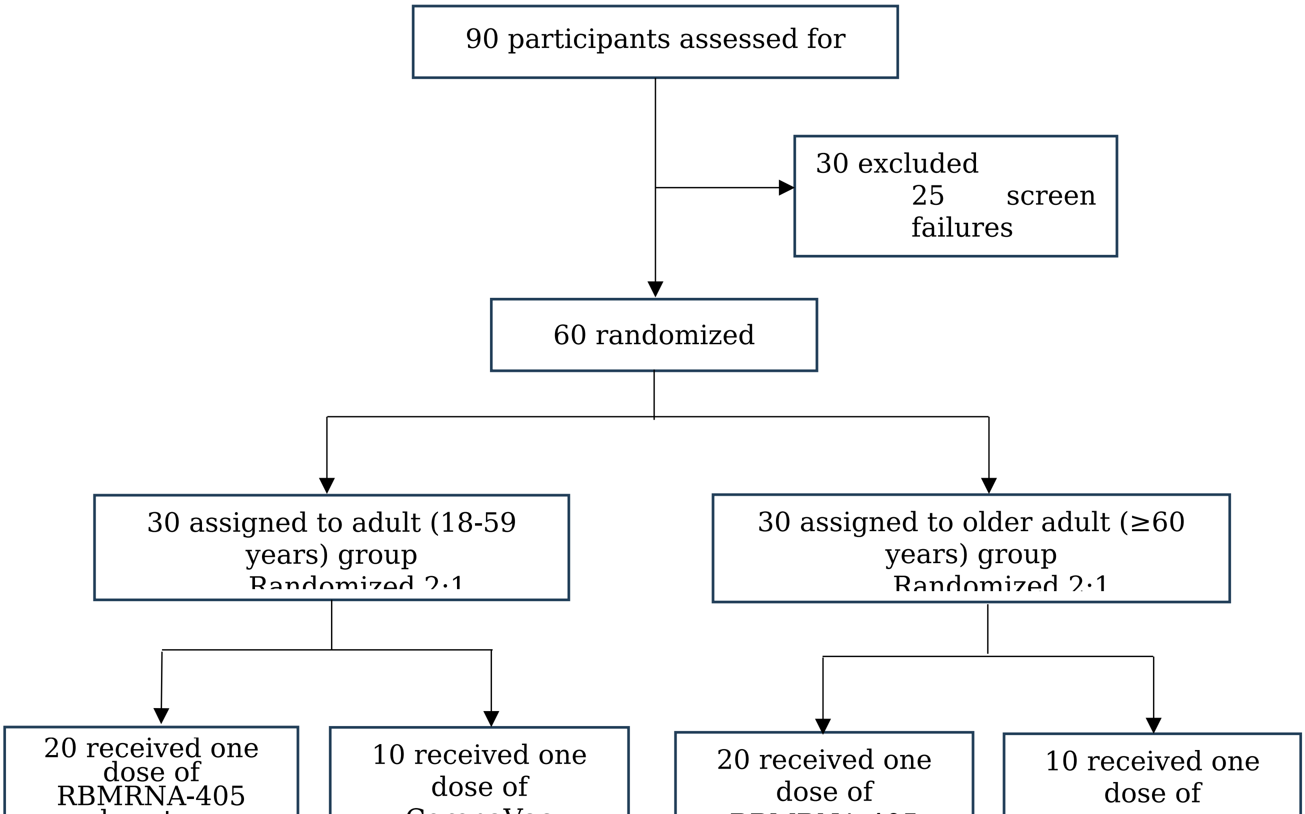

**Fig.1. Trial profile**

*One subject dropout: lost to follow-up after 28 days due to other personal reasons.

** Immunogenicity analysis within 28 days: n=20; after 28 days: n=19.

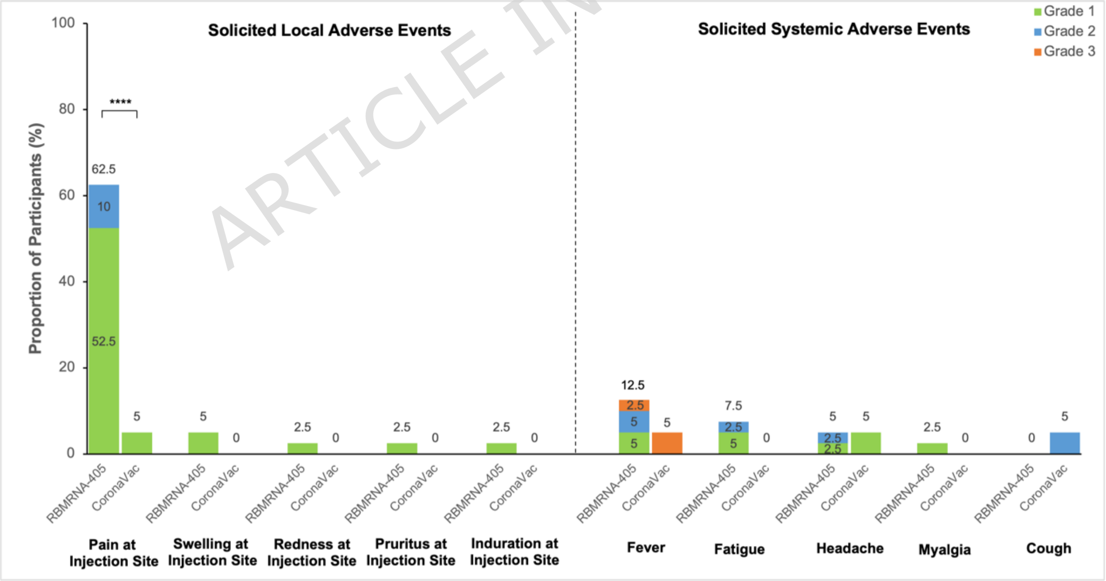

**Fig.2 Solicited local and systemic adverse events in participants aged ≥18 years-old**

The percentage of participants aged 18 years old and above who reported solicited local and systemic adverse events within 14 days following booster vaccination. (RBMRNA-405 group: n=40, CoronaVac group: n=20). The severity of solicited adverse events were graded as Grade 1, Grade 2 and Grade 3 (no Grade 4 or 5 reported). See classification definitions detailed in Supplementary Materials. Asterisks denote statistically significant differences between groups.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

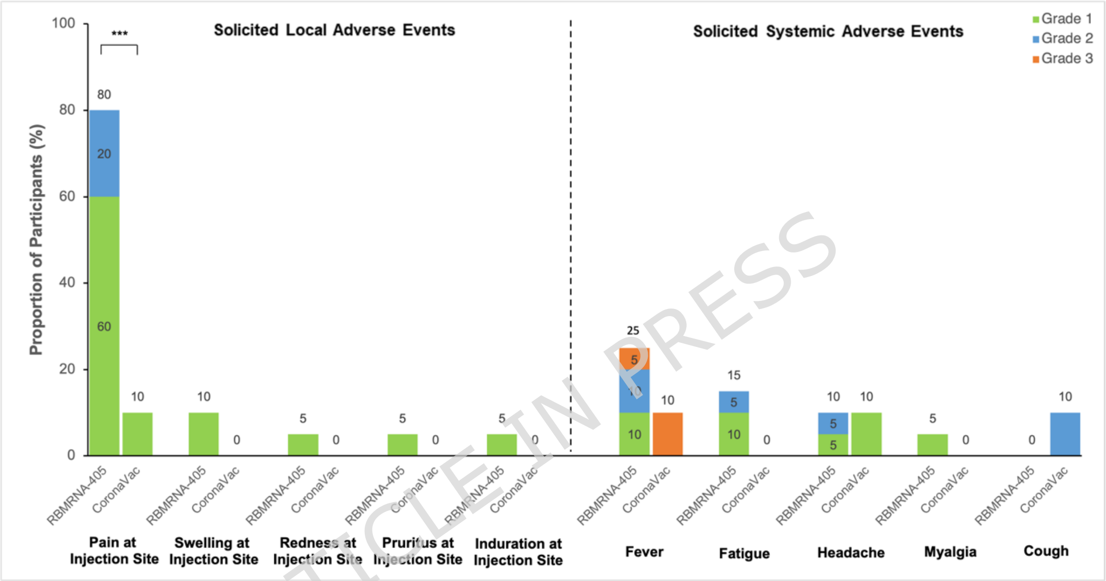

**Fig.3A Solicited local and systemic adverse events in participants aged 18-59 years-old**

Percentages of participants aged 18-59 years old who reported solicited local and systemic adverse events within 14 days following booster vaccination (RBMRNA-405 group: n=20, CoronaVac group: n=10). The severity of solicited adverse events were graded as Grade 1, Grade 2 and Grade 3 (no Grade 4 or 5 reported). See classification definitions detailed in Supplementary Materials. Asterisks denote statistically significant differences between the groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

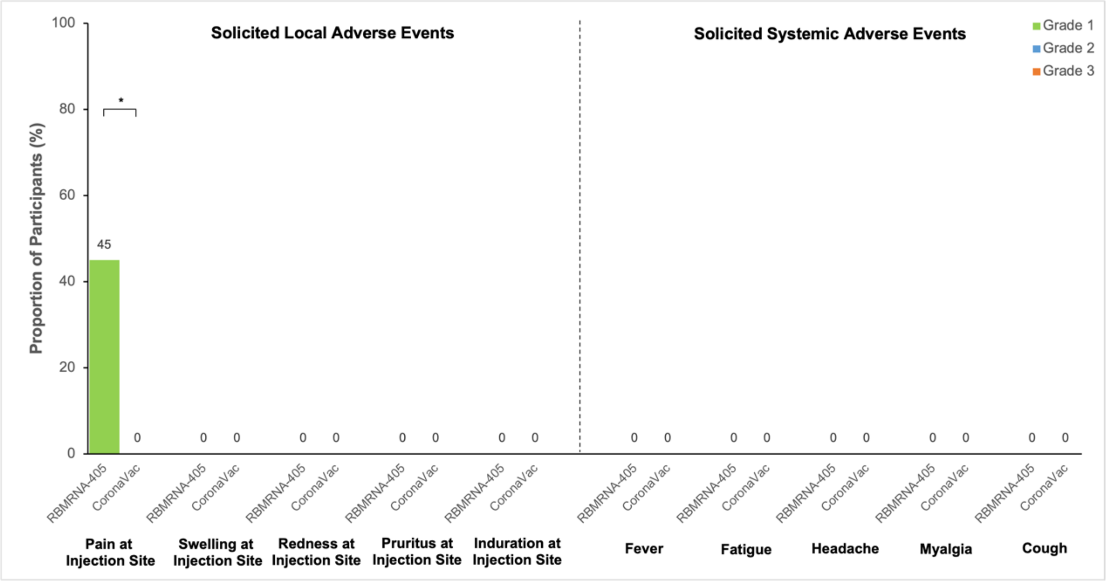

**Fig.3B Solicited local and systemic adverse events in participants aged ≥60 years-old**

Percentages of participants aged 60 years old and above who reported solicited local and systemic adverse events within 14 days following booster vaccination (RBMRNA-405: n=20, CoronaVac group: n=10). The severity of solicited adverse events were graded as Grade 1, Grade 2 and Grade 3 (no Grade 4 or 5 reported). See classification definitions detailed in Supplementary Materials. Asterisks denote statistically significant differences between groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

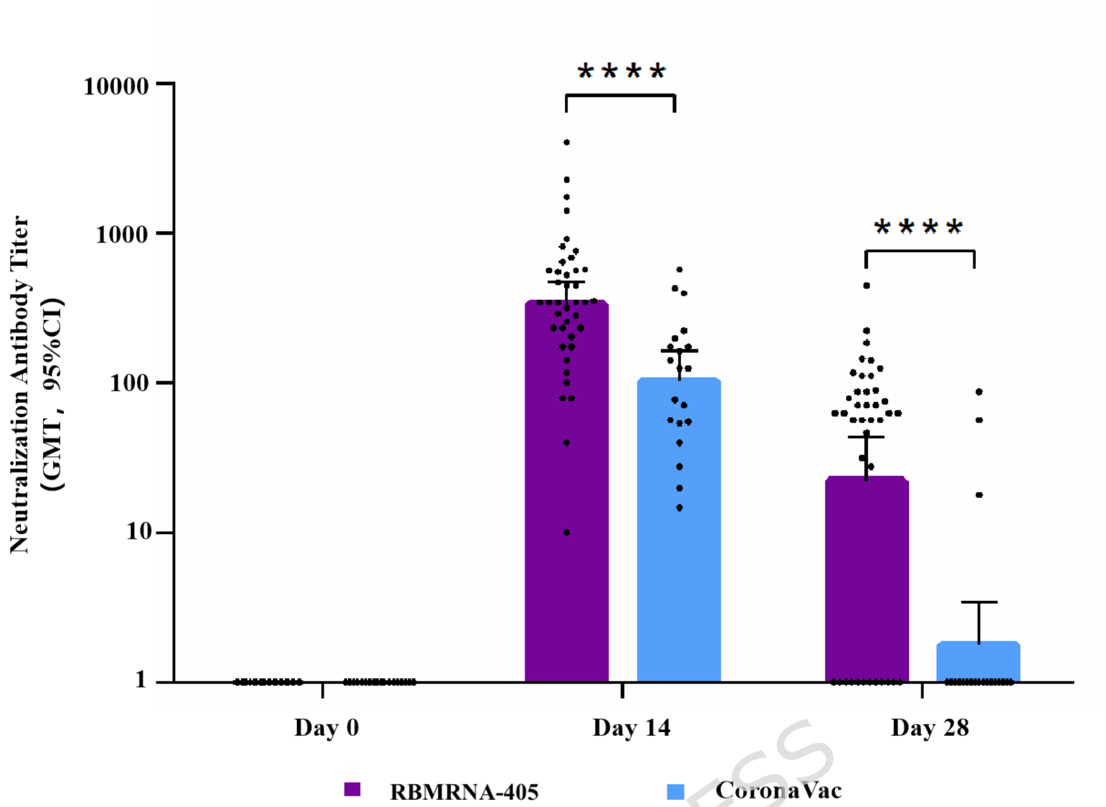

**Fig.4 Neutralizing antibody titers against SARS-CoV-2 Omicron BA.1 following booster vaccination**

GMTs of neutralizing antibodies against Omicron BA.1 in all 60 participants aged 18 years and older, measured before and 14 days, 28 days after receiving a booster dose (RBMRNA-405: n=39, CoronaVac: n=20; note: one participant in the RBMRNA-405 18-59 subgroup was excluded from immunogenicity analysis as they were lost to follow-up after 28 days due to other personal reasons). Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences between groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

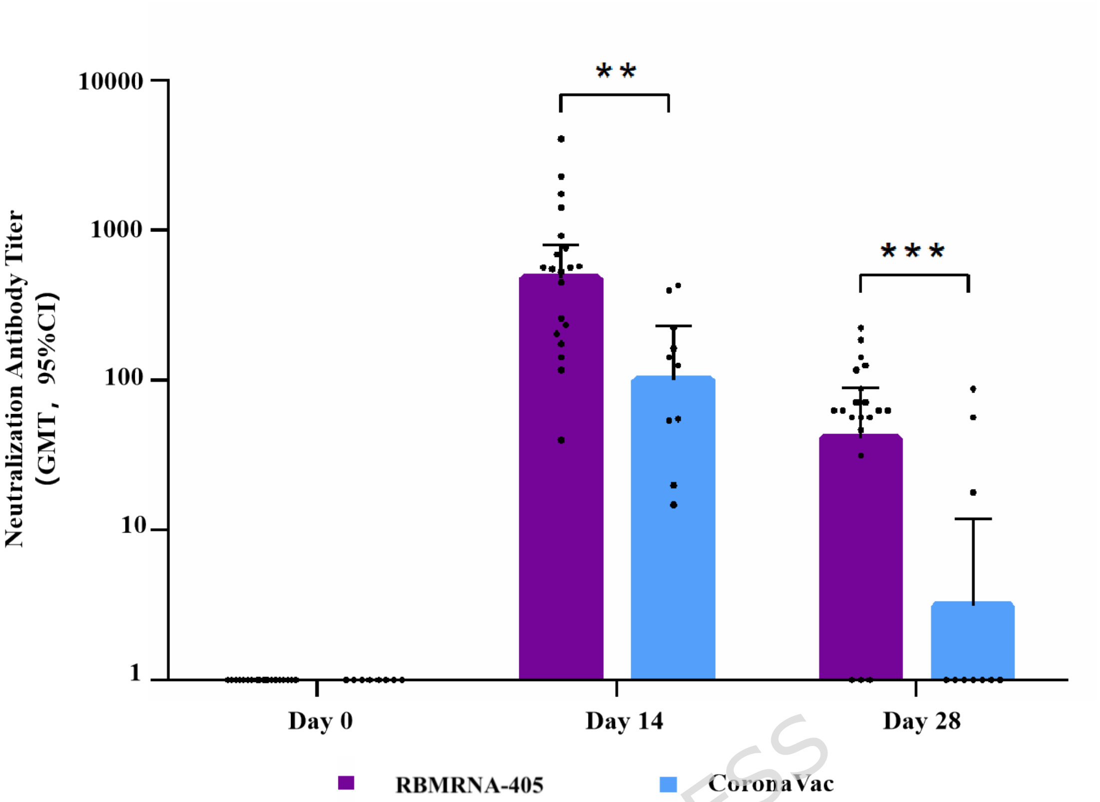

**Fig.5A Neutralizing antibody titers against SARS-CoV-2 Omicron BA.1 in adult participants aged 18-59 years-old**

GMTs of neutralizing antibodies against Omicron BA.1 in 30 participants aged 18-59 years, measured before and 14 days, 28 days after receiving a booster dose (RBMRNA-405: n=19, CoronaVac: n=10; note: one participant in the RBMRNA-405 subgroup was excluded from immunogenicity analysis as they were lost to follow-up after 28 days due to other personal reasons). Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences between groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

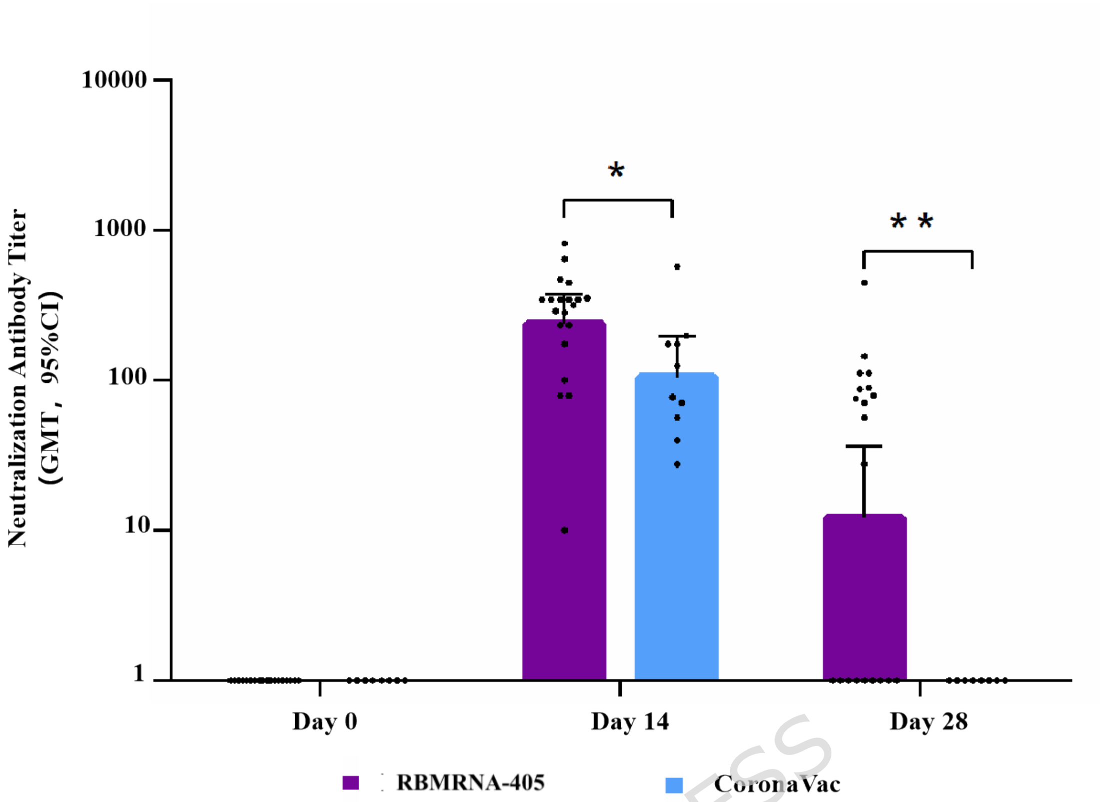

**Fig.5B Neutralizing antibody titers against SARS-CoV-2 Omicron BA.1 in older adult participants aged ≥60 years-old**

GMTs of neutralizing antibodies against Omicron BA.1 in 30 participants aged 60 and above, measured before and 14 days, 28 days after receiving a booster dose (RBMRNA-405: n=20, CoronaVac: n=10). Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences between groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

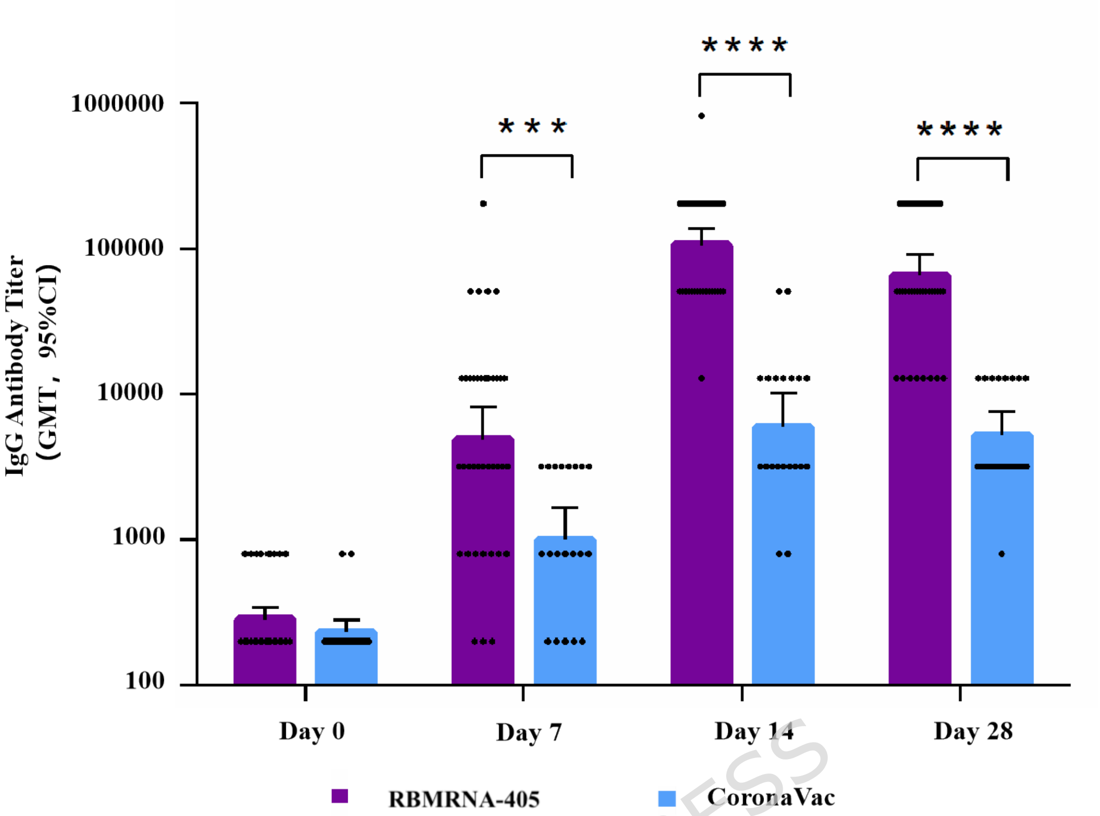

**Fig.6 Anti-spike IgG antibody titers against SARS-CoV-2 Omicron BA.1 following booster vaccination**

GMTs of anti-spike IgG antibody against Omicron BA.1 in all 60 participants aged 18 years and older, measured before and 7days,14 days, 28 days after receiving a booster dose. (RBMRNA-405: n=39, CoronaVac: n=20; note: one participant in the RBMRNA-405 18-59 subgroup was excluded from immunogenicity analysis as they were lost to follow-up after 28 days due to other personal reasons). Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences between groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

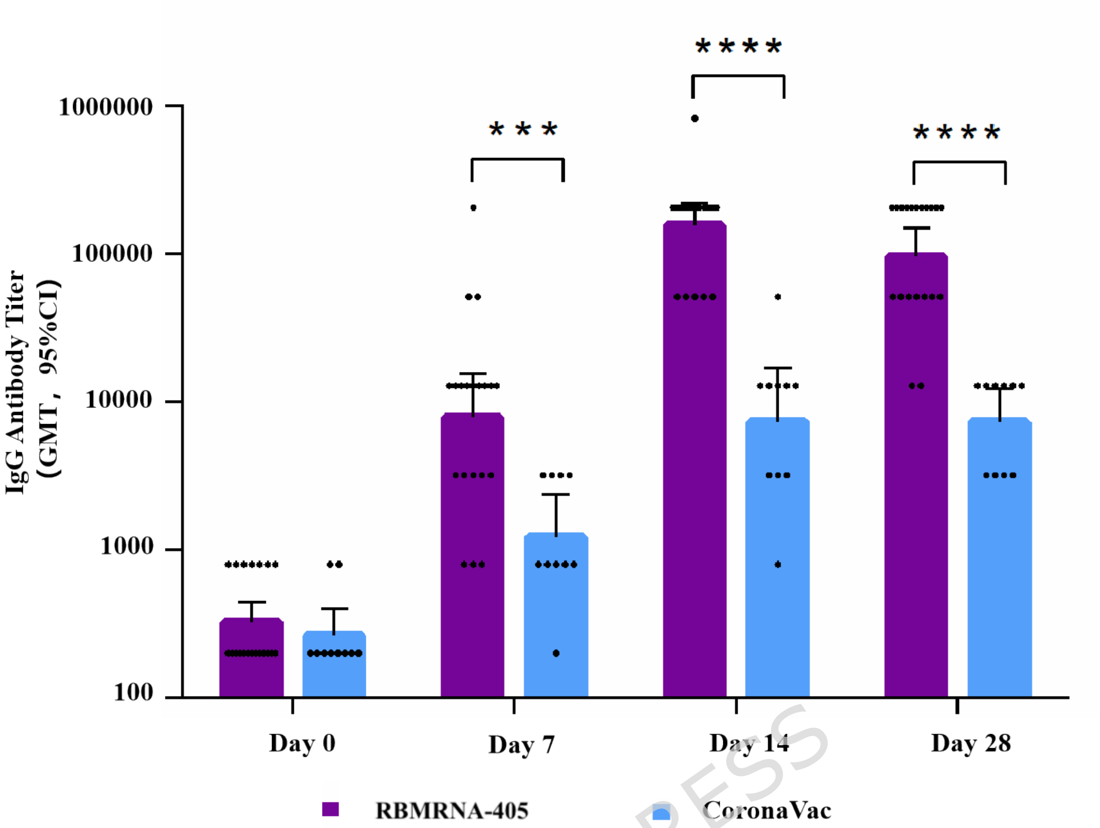

**Fig.7A Anti-spike IgG antibody titers against SARS-CoV-2 Omicron BA.1 in adult participants aged 18-59 years-old**

GMTs of anti-spike IgG antibody against Omicron BA.1 in 30 participants aged 18-59 years, measured before and 7 days,14 days, 28 days after receiving a booster dose (RBMRNA-405: n=19, CoronaVac: n=10; note: one participant in the RBMRNA-405 subgroup was excluded from immunogenicity analysis as they were lost to follow-up after 28 days due to other personal reasons). Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences between groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

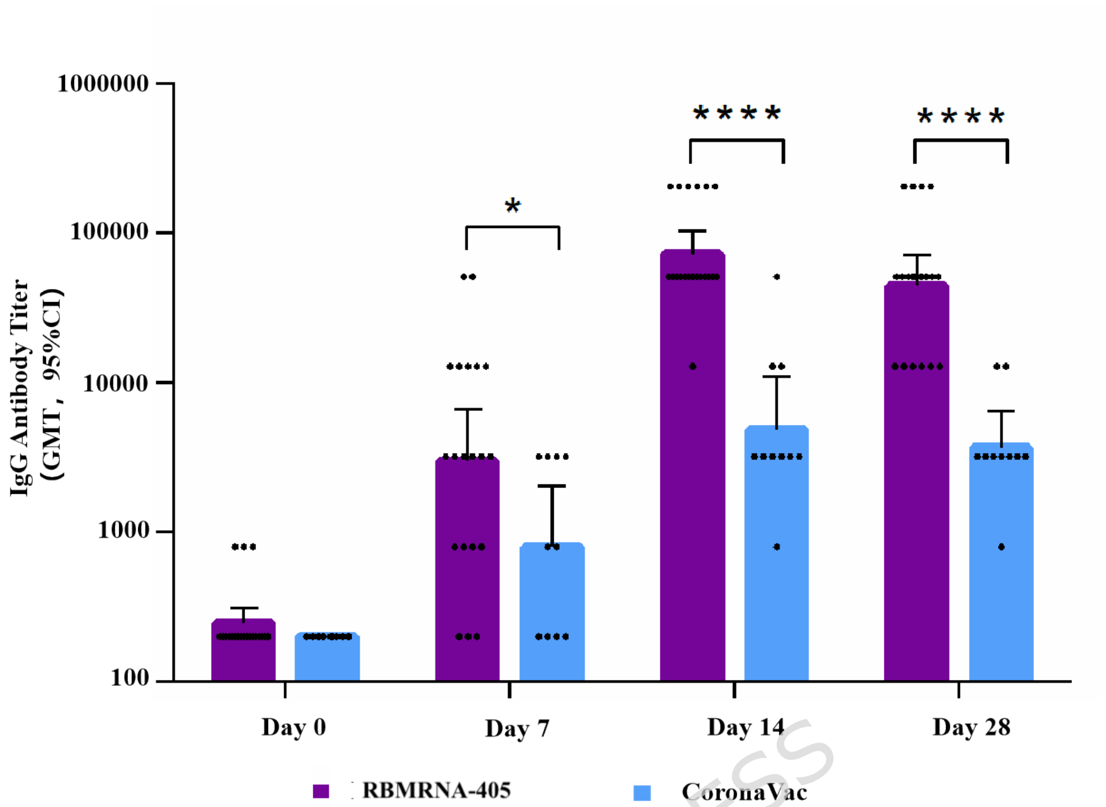

**Fig.7B Anti-spike IgG antibody titers against SARS-CoV-2 S Omicron BA.1 in older adult participants aged ≥60 years-old**

GMTs of anti-spike IgG antibody against Omicron BA.1 in 30 participants aged 60 and above, measured before and 7 days,14 days, 28 days after receiving a booster dose (RBMRNA-405: n=20, CoronaVac: n=10). Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences between groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

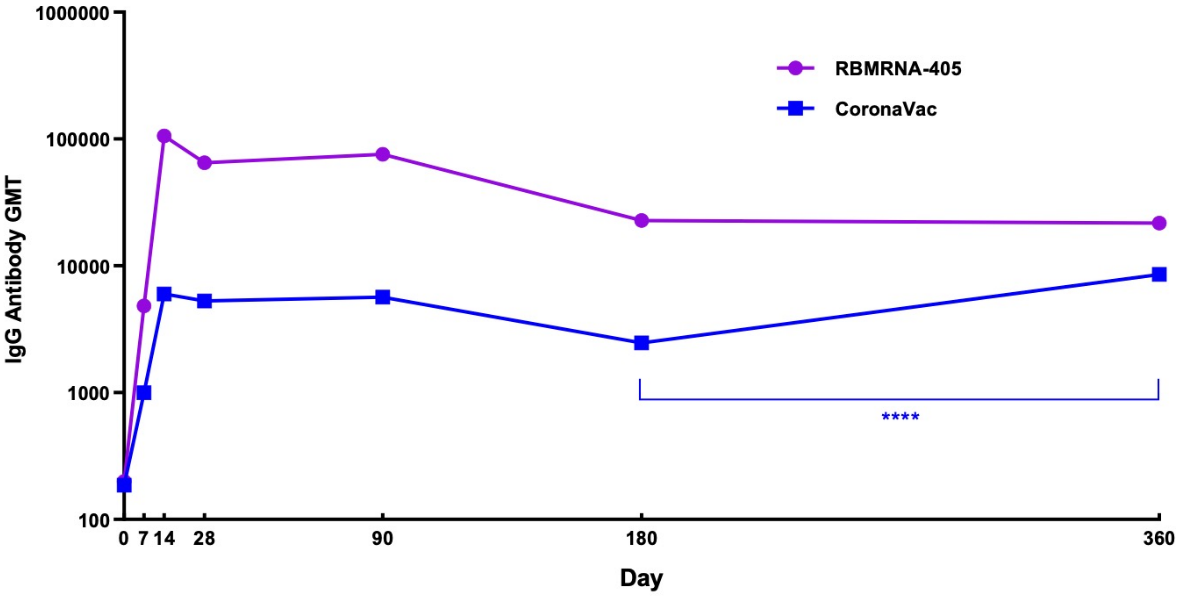

**Fig.8 Anti-spike IgG antibody titers against SARS-CoV-2 S Omicron BA.1 in participants aged ≥18 years over time (Day 0 to Day 360)**

Asterisks denote a significant increase in GMT of IgG on Day 360 compared to on Day 180 for the CoronaVac group. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.

**Table 1. Characteristics of all participants at baseline. (Full Analysis Set for Booster, bFAS）**

| **Characteristic** | Adult group (18-59 years) | | Older Adult group (≥60 years) | |
|---|---|---|---|---|
| | **RBMRNA-405** (N=20) | **CoronaVac** (N=10) | **RBMRNA-405** (N=20) | **CoronaVac** (N=10) |
| Sex | | | | |
| Male n (%) | 12 (60.00) | 6 (60.00) | 7 (35.00) | 6 (60.00) |
| Female n (%) | 8 (40.00) | 4 (40.00) | 13 (65.00) | 4 (40.00) |
| Total (Missing) | 20 (0) | 10 (0) | 20 (0) | 10 (0) |
| Age (years) | | | | |
| n (Missing) | 20 (0) | 10 (0) | 20 (0) | 10 (0) |
| Mean (SD) | 30.4 (10.3) | 27.9 (4.6) | 64.7 (3.7) | 68.1 (5.0) |
| Median | 27.0 | 27.5 | 64.0 | 67.5 |
| Min, Max | 23, 59 | 21, 37 | 60, 75 | 62, 77 |
| Ethnicity | | | | |
| Han Chinese n (%) | 20 (100.00) | 10 (100.00) | 20 (100.00) | 10 (100.00) |
| Others n (%) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| Total (Missing) | 20 (0) | 10 (0) | 20 (0) | 10 (0) |

| **Characteristic** | Adult group (18-59 years) | | Older Adult group (≥60 years) | |
|---|---|---|---|---|
| | **RBMRNA-405** (N=20) | **CoronaVac** (N=10) | **RBMRNA-405** (N=20) | **CoronaVac** (N=10) |
| BMI (kg/m$^2$) | | | | |
| n (Missing) | 20 (0) | 10 (0) | 20 (0) | 10 (0) |
| Mean (SD) | 21.89 (2.92) | 22.25 (2.02) | 23.61 (2.58) | 24.88 (3.38) |
| Median | 21.35 | 21.50 | 23.40 | 24.20 |
| Min, Max | 18.1, 27.5 | 20.0, 25.1 | 19.6, 29.1 | 20.7, 30.0 |
| Time interval since the last COVID-19 vaccination (days) | | | | |
| n (Missing) | 20 (0) | 10 (0) | 20 (0) | 10 (0) |
| Mean (SD) | 288.3 (40.9) | 284.0 (36.3) | 269.6 (33.0) | 253.4 (46.7) |
| Median | 282.5 | 268.5 | 272.5 | 233.0 |
| Min, Max | 228, 347 | 234, 339 | 213, 329 | 202, 327 |

SD: standard deviation; BMI: Body Mass Index.

**Table 2. Prevalence of Anti-spike IgG Elevation on Day 360 by Group**

| **Group**<br>**Age (yrs)** | **RBMRNA-405** (AD: n=19, OD: n=20) | **CoronaVac** (AD: n=10, OD: n=10) | **P Value** |
|---|---|---|---|
| Adult (18-59) | 0 (0.0%) | 4 (40.0%) | 0.0077** |
| Older Adult (≥60) | 7 (35.0%) | 10 (100%) | 0.0011** |

AD= Adult Group, OD= Older Adult Group, yrs= years-old

Prevalence of Anti-spike IgG elevation on Day 360 compared to Day 180.

Asterisks denote significant differences between groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Non-significant comparisons are omitted, unless explicitly indicated as “ns”.