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Immunogenicity and safety of monovalent and bivalent SARS-CoV-2 variant adapted RBD-based protein booster vaccines in adults previously immunized with different vaccine platforms: A phase II/III, randomized clinical trial

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ABSTRACT

A randomized, placebo-controlled, crossover, double-blind, phase II/III study was conducted to evaluate the immunogenicity, safety, and tolerability of a recombinant booster vaccine (ARVAC) containing the SARS-CoV-2 spike protein receptor binding domain in three versions: $ARVAC_{Gamma}$, $ARVAC_{Omicron}$, and $ARVAC_{Bivalent}$ in adults with ≤3 previous SARS-CoV-2 booster doses. Primary endpoint was seroconversion rate of neutralizing antibodies compared to placebo and to a > 75 % seroconversion rate to vaccine antigen homologous variants. All vaccine versions significantly increased seroconversion rates to SARS-CoV-2 variants compared to placebo. In participants aged 18–60 years, all versions met the primary endpoint; in those over 60 years old, $ARVAC_{Omicron}$ and $ARVAC_{Bivalent}$ met this endpoint. No vaccine-related serious adverse events were recorded, and most adverse events were mild. Plasma levels of anti-spike-specific IgG and anti-S1-specific IgA in saliva increased in participants receiving any vaccine. The increase in plasma neutralizing antibodies induced by the vaccine was independent of the number of previous booster doses (0, 1 or 2), the primary vaccine platform (adenovirus, single-dose adenovirus, mRNA, inactivated virus, heterologous vaccination, and virus-like particle [VLP]) and the history of previous COVID-19. The neutralizing Ab response induced by the vaccine in healthy participants was similar to that triggered in participants with underlying medical conditions associated with an increased risk of severe COVID-19. $ARVAC_{Bivalent}$ induced high seroconversion rates (>90 %) against multiple variants and was superior to other ARVAC-versions. It increased neutralizing antibodies against SARS-CoV-2 variants (Ancestral, Gamma, Omicron, XBB and JN.1) and SARS-CoV-1. (NCT05752201).

1. Background

Coronavirus disease 2019 (COVID-19) continues to be a global health threat [1,2]. Public health measures and vaccination contributed to decreasing SARS-CoV-2 virus circulation, disease severity, and associated mortality [3]. However, vaccine-induced immunity progressively wanes [4,5] and new, highly contagious SARS-CoV-2 variants that escape from vaccine-induced immunity continue to emerge [5,6]. In this scenario, primary vaccination schemes and boosters based on the ancestral SARS-CoV-2 variants fail to provide sufficient long-term protection [4,7].

To ensure long-term immune memory, the World Health Organization (WHO) recommends homologous and heterologous booster doses with variant-adapted formulations for protection against severe COVID-19 disease and death [8]. Of the most common COVID-19 vaccine platforms, including inactivated viruses, viral vectors, mRNA, and recombinant protein subunits, mRNA vaccines are the most widely used. However, they are unstable and require storage at freezing temperatures (−20 °C or −80 °C), limiting their distribution, particularly in low- and middle-income countries [5,9]. Conversely, recombinant subunit vaccines may be stored in coolers, simplifying the storage and distribution logistics. Recombinant protein large-scale production is available in several countries, enabling local manufacturing and widespread distribution with lower production costs [10]. Despite their slower development speed, recombinant subunit vaccines can be modified to induce immunity against novel SARS-CoV-2 variants [11]. Moreover, their safety profile record has been well known for over 30 years, enabling their use in children, elderly, and pregnant women [12].

Argentina has developed and manufactured a recombinant protein subunit vaccine, ARVAC, which has been approved [13] and is now available for administration in pharmacies and vaccination centers in Argentina. The first version of the vaccine contains the receptor binding domain (RBD) of the spike protein of the SARS-CoV-2 Gamma variant, with K417T, E484K, and N501Y mutations. Preclinical studies demonstrated that the Gamma RBD version is more immunogenic than the ancestral RBD at inducing broader neutralizing antibodies (nAbs) even against distant variants, such as Omicron BA.5 [14]. In a Phase I trial, the vaccine was safe and elicited a robust and broad nAb response against several SARS-CoV-2 variants [15].

In this work, we present the results of a randomized, placebo-controlled Phase II/III trial assessing the immunogenicity, safety, and tolerability of the Gamma, Omicron BA.4/5, and bivalent versions of ARVAC used as boosters in adult volunteers previously immunized with different SARS-CoV-2 vaccine platforms.

2. Methods

2.1. Study design, participants, and oversight

The ARVAC-F2–3-002 study is a multicenter, randomized, double-blind, crossover, placebo-controlled Phase II/III trial evaluating the immunogenicity, safety, and tolerability of a recombinant protein vaccine against SARS-CoV-2 in adult (≥18 years) volunteers previously vaccinated against SARS-CoV-2 with ≤3 booster doses. Inclusion and exclusion criteria are provided in the Supplementary Data. Investigators from 11 participating centers in Argentina (Supplementary Data) consecutively recruited volunteers.

The *Centro de Educación Médica e Investigaciones Clínicas – CEMIC* (Argentina) was the sponsor. An external, independent data safety monitoring board reviewed safety data. The trial adhered to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline for Good Clinical Practice and the local data protection law 25,326. All participants signed the informed consent form. The *Administración Nacional de Medicamentos, Alimentación y Tecnología Médica* (ANMAT), the local ethics committee of the Autonomous City of Buenos Aires (PRIISA, *Plataforma de Registro Informatizado de Investigaciones en Salud de Buenos Aires*), and the ethics committee of the *Centro de Estudios Infectológicos* (CEI) - Stamboulian approved the protocol, which was registered at ANMAT, PRIISA, and clinicaltrials.gov (NCT05752201). The local ethics committees that approved the study protocol are listed in the Supplementary Data. Informed consent was obtained after the nature and possible consequences of the study had been fully explained to the subjects.

2.2. Recombinant protein vaccines

The ARVAC vaccine is a liquid suspension containing 50 μg of recombinant protein adjuvanted with 0.5 mg aluminum hydroxide gel (alhydrogel). The vaccine antigen encompasses aminoacids 319R-537 K in the RBD of the SARS-CoV-2 spike protein. Recombinant proteins were produced in CHO-S cells [15].

2.3. Randomization and procedures

Participants were recruited in two stages. In stage 1 (Phase II), participants were randomized into two subgroups at a 1:1 ratio to receive

Gamma-based vaccine ($ARVAC_{Gamma}$) (50 μg) + placebo (group A) and placebo + $ARVAC_{Gamma}$ (50 μg) (group B) 28 days apart. In stage 2 (Phase III), participants were randomized into three groups to receive the $ARVAC_{Gamma}$ (50 μg), the Omicron BA.4/5-based ($ARVAC_{Omicron}$) (50 μg), and the Bivalent (Gamma/Omicron BA.4/5 25 μg/25 μg, $ARVAC_{Bivalent}$) vaccine, with two subgroups each receiving vaccine + placebo (group A) or placebo + vaccine (group B) 28 days apart (1,1,1,1,1:1 ratio) (Fig. S1). Within each group, individuals were assigned to age subgroups (18–60 years and > 60 years) and immunogenicity subsets (Supplementary Data).

Assessments were performed during five visits (V): V1 on day 1 (inclusion visit); V2, 14 ± 2 days later; V3, 28 ± 2 days after V1; V4, 56 ± 2 days after V1; and V5, 90 ± 2 days after V1. In all groups, the first treatment was administered on V1 and the second on V3. Volunteers had to be negative for SARS-CoV-2 in a polymerase chain reaction or antigen test at study inclusion and before any treatment.

2.4. Immunogenicity endpoints and variables

NAbs against different SARS-CoV-2 variants were measured on plasma samples obtained before (day 1, d1) and after (day 14, d14 and day 90, d90) the first treatment, using a SARS-CoV-2 live virus assay in VERO E6 cells (ATCC) as was described in [15]. Results are presented as geometric mean titers (GMTs); geometric mean fold rises (GMFR) and GMT ratios (GMTR). Additionally, titers against the SARS-CoV-2 Ancestral (Wuhan) variant were transformed to IU/mL using a secondary standard calibrated with a WHO international standard [16]. Based on previous studies, a > 1030 UI/mL threshold of nAbs was associated with a 90 % efficacy against symptomatic infection [17].

The primary endpoint was the seroconversion rate at d14 after receiving the vaccine compared to placebo. The vaccine immunogenicity was considered acceptable at seroconversion rates >75 % to variants homologous to the antigen contained in the vaccine (prespecified primary endpoint). The threshold for seroconversion was defined as a 4-fold or a 2-fold increase in nAb titers for individuals with "low" or "high" baseline nAb levels against Ancestral SARS-CoV-2 (<949 or ≥ 949 IU/mL), respectively.

Additional secondary and exploratory endpoints and methods are described in the Supplementary Data.

2.5. Safety endpoints and assessments

Safety endpoints were solicited local and systemic adverse events (AEs), registered daily in the participants' diary within seven days after each dose, and unsolicited local AEs occurring within 20 min after administration. Based on published guidelines, AEs were classified according to severity and their relationship to the study medication [18]. Additional details are provided in the Supplementary Data.

2.6. Statistical analysis

The sample size was calculated based on the previously obtained $ARVAC_{Gamma}$ vaccine seroconversion rates [15]. For the prespecified primary endpoint, the estimated sample size was 113 participants for each vaccine candidate, considering a 10 % dropout rate. For the exploratory endpoint of seroconversion superiority of bivalent vs. the monovalent vaccines, a sample size of 248 participants for each vaccine candidate was estimated (276, considering a 10 % dropout rate). For the safety endpoints, 2014 participants, 232 in Phase II and 1782 in Phase III, were estimated to detect AEs with a 0.1 % prevalence, considering a 20 % dropout rate. The Supplementary Data includes a detailed description of the sample size calculation.

Statistical methods are included in the Supplementary Data.

3. Results

3.1. Participants

Of 2126 participants who signed the informed consent, 2012 were included (232 in Phase II and 1780 in Phase III) and randomized. All were administered the first treatment (vaccine or placebo) and 1905, the second; 138 discontinued the study and 1874 finished the study protocol (Fig. 1).

Among the 2012 enrolled participants, the median age was 49 years (IQR: 34–63), with 48.1 % being women and 44.2 % having a prior COVID-19 diagnosis. The median age in Phase II was 34 years (IQR: 27–45.8), whereas the median age in Phase III was 51 years (IQR: 36–64), reflecting an older cohort in the latter phase (Table 1).

The baseline characteristics of the 2012 enrolled participants demonstrate the broad inclusion criteria, resulting in a highly diverse population. This diversity extended to vaccination history, including variation in vaccine platforms, number of boosters received, and time since the last dose. While all participants had completed a SARS-CoV-2 primary vaccination scheme, some had not received any previous additional booster (14,5 %), or had received one (60.1 %), two (13.3 %) or three additional booster doses (12.1 %) prior to their inclusion in the study. The median time since the last vaccine dose was 15 months (IQR: 12–17) (Table 1).

Phase III participants also exhibited considerable diversity in their underlying medical conditions, since the inclusion criteria (see supplementary material) allowed healthy volunteers or volunteers with stable and controlled chronic comorbidities not associated with a reduced immune response according to the investigators' criteria. In total, 44.8 % of participants reported at least one chronic comorbidity or medical condition linked to an increased risk of severe COVID-19, as defined by the CDC [19]. These included asthma (2.73 %), chronic lung diseases (1.94 %), diabetes (8.60 %), heart conditions (2.49 %), HIV (1.84 %), mental health conditions (4.62 %), obesity (BMI >30; 23.7 %), smoking (0.75 %), and hypertension (18.4 %), among others (Table S1).

The immunogenicity subset included all volunteers from Phase II and 1053 participants from Phase III (Fig. 1). Demographic characteristics of Phase III immunogenicity subset are described in Table S2. The median age of this subset was 52 years with 46.2 % being women. Participants were divided into two age groups: 18–60 years ($n = 628$) and > 60 years ($n = 425$). Most participants (71.6 %) had received one booster dose, while 15.4 % had received two boosters, and 13.0 % had not received a booster. Among participants aged 18–60, 82.5 % had received one booster, while in the >60 group, this proportion was lower (55.5 %), with a higher percentage receiving two boosters (37.4 %). The primary vaccine platforms included: two dose adenovirus-based primary schemes (54.9 %), adenoviral single-dose vaccines (4.0 %), mRNA vaccines (3.7 %), inactivated (20.8 %) vaccines, heterologous platforms (16.2 %, mixing Adenoviral, inactivated, mRNA), and recombinant protein vaccines (0.4 %, Virus like particles, VLP). The median time since the last vaccine administration was approximately 504 days (16.8 months).

3.2. Seroconversion rates (primary endpoint)

Seroconversion rates to homologous and non-homologous SARS-CoV-2 variants were higher after receiving any vaccine than placebo overall and in the two age groups (Table 2). All vaccine versions met the prespecified primary endpoint (i.e., seroconversion rate > 75 % for the homologous variant) in all participants and those aged 18–60 years. In participants >60 years, $ARVAC_{Omicron}$ and $ARVAC_{Bivalent}$ met the prespecified primary endpoint, whereas $ARVAC_{Gamma}$ did not. $ARVAC_{Bivalent}$-induced seroconversion rates were > 90 % against Ancestral (Wuhan), Gamma, and Omicron BA.5 SARS-CoV-2 variants in all age groups (Table 2). Analyses using normalized antibody titers (Table S3) or excluding anti-nucleoprotein IgG seroconverted participants yielded

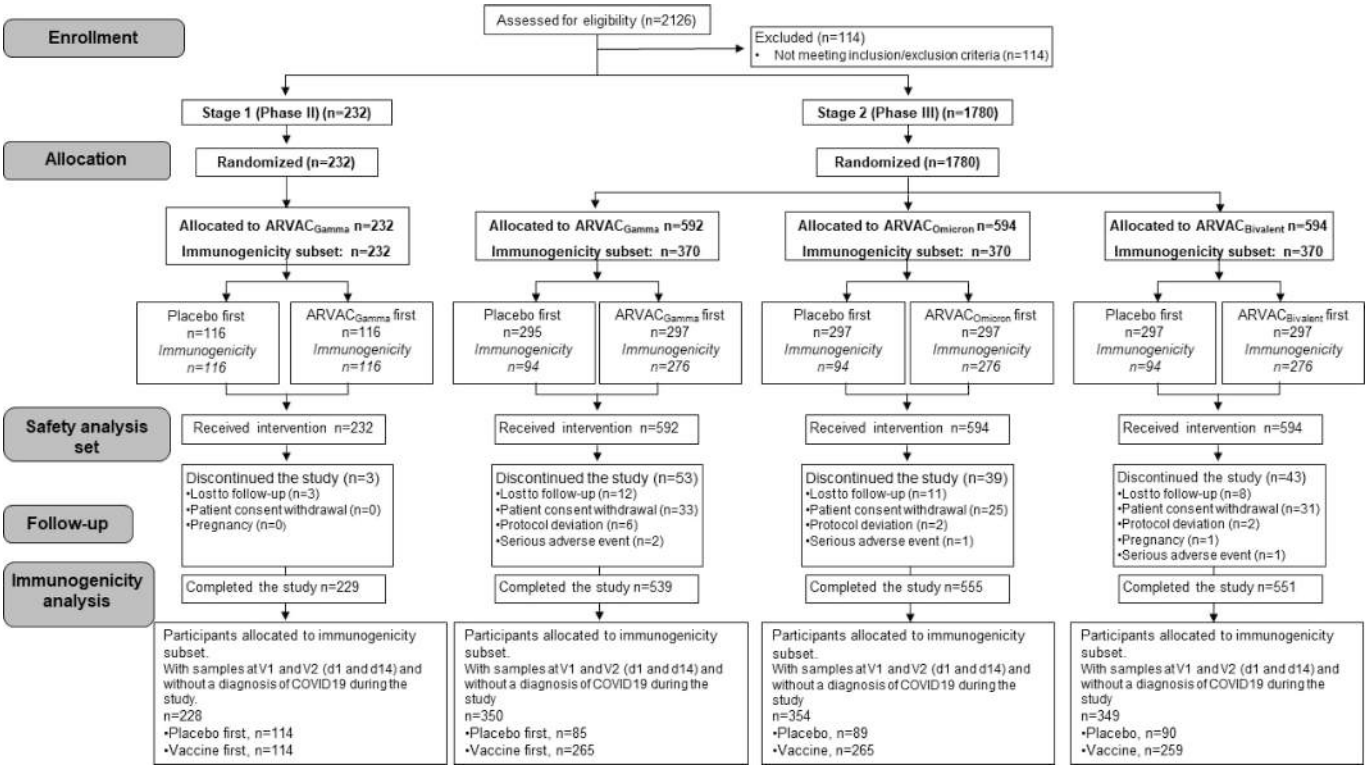

Fig. 1. Flow chart of study participants.

similar results (Table S4).

3.3. NAb Titers

GMTs to Ancestral, Gamma, and Omicron variants increased (d1-d14) in participants receiving any vaccine, but not in those receiving placebo (Fig. 2). At day 90 (d90), GMFR remained statistically significant across vaccine versions, SARS-CoV-2 variants, and age groups (Fig. S2).

The percentage of participants with nAbs to the Ancestral variant >1030 UI/mL increased at d14 for all vaccine versions, with similar results in the two age groups. For $ARVAC_{Gamma}$, $ARVAC_{Omicron}$, and $ARVAC_{Bivalent}$ vaccines, percentages increased from 30.8 %, 31.2 %, and 24.8 % to 87.2 %, 85.4 %, and 87.9 %, respectively, in participants aged 18–60 years; and from 40.4 %, 47.2 %, and 45.1 % to 84.4 %, 89.8 %, and 92.2 %, respectively, in participants >60 years (Fig. S3).

3.4. Analyses according to previous vaccination and COVID-19 infection

The nAb response was evaluated in study participants categorized by the number of previous booster doses (0, 1 or 2), by the primary vaccine platform (adenovirus, single-dose adenovirus, mRNA, inactivated virus, heterologous vaccination, and virus-like particle [VLP]) in their schemes or by their previous history of COVID-19. GMTs to all SARS-CoV-2 variants increased in participants receiving the ARVAC vaccine regardless of previous booster doses (Fig. S4) or primary vaccine platforms (Figs. S5-S8). GMTs and GMFRs were similar regardless of previous COVID-19 infection (Fig. S9).

3.5. Analyses according to the study participants baseline underlying medical conditions or chronic comorbidities

GMTs to all SARS-CoV-2 variants increased in participants with underlying medical conditions after receiving any ARVAC vaccine (Fig. S10). The response in healthy participants was similar to that observed in participants with underlying medical conditions associated with an increased risk of severe COVID-19 (higher risk, listed in Table S1) (Fig. S11). GMTs to all SARS-CoV-2 variants increased overall in subgroups of participants with any of the following conditions: hypertension, diabetes, asthma, HIV, obesity, heart conditions, or mental health conditions. Although the number of participants with chronic lung diseases in the three vaccine groups and those with HIV in the ARVAC Gamma and ARVAC Omicron cohorts was small (n = 2–5), an increase in nAb titers was still observed in these individuals (Fig. S11).

3.6. Seroconversion rates and nAb titers according to vaccine versions

$ARVAC_{Bivalent}$ was non-inferior to $ARVAC_{Omicron}$ and $ARVAC_{Gamma}$ in seroconversion rates against all three SARS-CoV-2 variants (Table S5). $ARVAC_{Bivalent}$ seroconversion rates were superior to $ARVAC_{Gamma}$'s against the Omicron variant and to $ARVAC_{Omicron}$'s against the Ancestral and Gamma variants. In participants aged 18–60 years, $ARVAC_{Bivalent}$ seroconversion rates were superior to $ARVAC_{Omicron}$'s against the Ancestral variant. In participants >60 years, $ARVAC_{Bivalent}$ seroconversion rates were superior to $ARVAC_{Gamma}$' s against the Omicron variant and to $ARVAC_{Omicron}$'s against the Ancestral and Gamma variants (Table S6).

An adjusted multivariate analysis confirmed the superiority of $ARVAC_{Bivalent}$ to $ARVAC_{Gamma}$ in seroconversion rates against all variants, and to $ARVAC_{Omicron}$ against the Ancestral and Gamma variants (Tables S7-S9).

Regarding GMTs of neutralizing antibodies, $ARVAC_{Bivalent}$ was non-inferior to the monovalent versions regardless of age (Table S10). While all vaccine versions induced similar GMTs to the Ancestral and Gamma variants, $ARVAC_{Bivalent}$ and $ARVAC_{Omicron}$ induced higher titers against the Omicron compared to $ARVAC_{Gamma}$, both in the overall population and in participants >60 years, (Tables S11-S13).

$ARVAC_{Bivalent}$ induced higher GMFRs than $ARVAC_{Omicron}$ for the Ancestral and Gamma variants and higher GMFRs than $ARVAC_{Gamma}$ for the Omicron variant (Table S14).

Table 1
Characteristics of study participants according to study phase and sex ($n = 2012$).

	Phase II			Phase III			All		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
n (%)	134 (57.8)	98 (42.2)	232	834 (46.9)	946 (53.1)	1780	968 (48.1)	1044 (51.9)	2012 (100)
Age (years), *median (IQR)*	34 (27, 46)	35 (25.8, 45)	34 (27, 45.8)	48 (33, 62)	55 (40, 65)	51 (36, 64)	46 (31, 61)	51 (37, 64)	49 (34, 63)
BMI (kg/m^2), *median (IQR)*	26.7 (23.3, 31.2)	26.7 (24.2, 29.4)	26.7 (23.4, 30.8)	26.5 (23, 30.4)	27.4 (24.3, 30.4)	27 (23.8, 30.4)	26.5 (23, 30.7)	27.3 (24.3, 30.3)	27 (23.7, 30.5)
Number of boosters after completing primary vaccination scheme, *n (%)*									
0	46 (34.3)	40 (40.8)	86 (37.1)	93 (11.2)	113 (19.9)	206 (11.6)	139 (14.4)	153 (14.7)	293 (14.5)
1	88 (65.7)	58 (59.2)	146 (62.9)	538 (64.5)	526 (55.6)	1064 (59.8)	626 (64.7)	584 (55.9)	1210 (60.1)
2				101 (12.1)	166 (17.5)	267 (15.0)	101 (10.4)	166 (15.9)	267 (13.3)
3				102 (12.2)	141 (14.9)	243 (13.6)	102 (10.5)	141 (13.5)	243 (12.1)
Time since last vaccination (months), *median (IQR)*	13 (12, 14.3)	13 (11, 15.3)	13 (12, 15)	15 (12, 17)	15 (12, 17)	15 (12, 17)	15 (12, 17)	15 (12, 17)	15 (12, 17)
Previous COVID-19, *n (%)*									
No	87 (64.9)	73 (74.5)	160 (69.0)	406 (48.7)	557 (58.9)	963 (54.1)	493 (50.9)	630 (60.3)	1123 (55.8)
Yes	47 (35.1)	25 (25.5)	72 (31.0)	428 (51.3)	389 (41.1)	817 (45.9)	475 (49.1)	414 (39.7)	889 (44.2)
Time since infection (months), *median (IQR)*	19 (14, 27)	20 (12, 30.5)	19 (14, 27.8)	18 (14, 27)	18 (15, 28)	18 (15, 27)	18 (14, 27)	19 (15, 28)	18 (14, 27)
Participants with underlying medical conditions*, *n (%)*				384 (46.0)	517 (54.7)	901 (50.6)	384 (39.7)	517 (49.5)	901 (44.8)
One condition				246 (29.5)	301 (31.8)	547 (30.7)	246 (25.4)	301 (28.8)	547 (27.2)
Two conditions				93 (11.2)	146 (15.4)	239 (13.4)	93 (9.6)	146 (14.0)	239 (11.9)
Three or more conditions				45 (5.4)	70 (7.4)	115 (6.5)	45 (4.6)	70 (6.7)	115 (5.7)
Study treatment, *n (%)*									
Bivalent				139 (16.7)	158 (16.7)	297 (16.7)	139 (14.3)	158 (15.1)	297 (14.8)
Gamma	70 (52.2)	46 (46.9)	116 (50.0)	136 (16.3)	161 (17.1)	297 (16.7)	206 (21.3)	207 (19.9)	413 (20.5)
Omicron				144 (17.2)	153 (16.2)	297 (16.7)	144 (14.9)	153 (14.6)	297 (14.8)
Placebo	64 (47.8)	52 (53.1)	116 (50.0)	415 (49.8)	474 (50.1)	889 (49.9)	479 (49.5)	526 (50.4)	1005 (50.0)

IQR, interquartile range; BMI, body mass index.

* A list of baseline underlying medical conditions or comorbidities in study participants with the number and frequency of participants with each underlying condition is presented in Table S1.

Table 2
Seroconversion rates in Phase II ($n = 228$) and Phase III ($n = 1053$) participants for the different vaccine variants and age groups compared to placebo and a > 75 % reference.

Study phase and treatment	SARS-CoV-2	Seroconversion rate (%)	95 % CI	*p*-value[a] Vaccine vs. placebo	*p*-value[b] Vaccine vs. >75 %
Phase II, $n = 228$					
	Ancestral	9.6	5.5, 16.5	NA	NA
	Gamma	18.4	12.4, 26.5	NA	NA
Placebo, $n = 114$	Omicron BA.5	10.5	6.1, 17.5	NA	NA
	Ancestral	87.7	80.4, 92.5	<0.0001	0.0004
	Gamma	90.4	83.5, 94.5	<0.0001	<0.0001
ARVAC Gamma, n = 114	Omicron BA.5	84.2	76.4, 89.8	<0.0001	0.0017
Phase III all participants, n = 1053					
	Ancestral	12.5	9.0, 17.0	NA	NA
	Gamma	12.1	8.7, 16.6	NA	NA
Placebo, $n = 264$	Omicron BA.5	15.2	11.3, 20.0	NA	NA
	Ancestral	86.8	82.2, 90.3	<0.0001	<0.0001
	Gamma	84.2	79.3, 88.1	<0.0001	0.0006
ARVAC Gamma, $n = 265$	Omicron BA.5	81.9	76.8, 86.1	<0.0001	0.0105
	Ancestral	80.0	74.8, 84.4	<0.0001	0.0694
	Gamma	82.3	77.2, 86.4	<0.0001	0.0031
ARVAC Omicron BA.5, n = 265	Omicron BA.5	87.5	83.0, 91.0	<0.0001	<0.0001
	Ancestral	92.7	88.8, 95.3	<0.0001	<0.0001
	Gamma	91.1	87.0, 94.0	<0.0001	<0.0001
ARVAC Bivalent, $n = 259$	Omicron BA.5	92.7	88.8, 95.3	<0.0001	<0.0001
Phase III, participants aged 18–60 years					
	Ancestral	7.6	4.4, 12.8	NA	NA
	Gamma	9.5	5.8, 15.1	NA	NA
Placebo, $n = 158$	Omicron BA.5	13.3	8.9, 19.5	NA	NA
	Ancestral	89.1	83.2, 93.1	<0.0001	<0.0001
ARVAC Gamma, $n = 156$	Gamma	89.1	83.2, 93.1	<0.0001	<0.0001
	Omicron BA.5	86.5	80.3, 91.0	<0.0001	0.0009
	Ancestral	81.5	74.7, 86.8	<0.0001	0.0589
	Gamma	87.3	81.1, 91.6	<0.0001	0.0004
ARVAC Omicron BA.4/5, $n = 157$	Omicron BA.5	87.3	81.1, 91.6	<0.0001	0.0004
	Ancestral	93.6	88.7, 96.5	<0.0001	<0.0001
	Gamma	91.7	86.3, 95.1	<0.0001	<0.0001
ARVAC Bivalent, n = 157	Omicron BA.5	93.0	87.9, 96.0	<0.0001	<0.0001
Phase III, participants > 60 years					
	Ancestral	19.8	13.3, 28.4	NA	NA
	Gamma	16.0	10.3, 24.2	NA	NA
Placebo, $n = 106$	Omicron BA.5	17.9	11.8, 26.3	NA	NA
	Ancestral	83.5	75.4, 89.3	<0.0001	0.0407
	Gamma	77.1	68.3, 84.0	<0.0001	0.6187
ARVAC Gamma, $n = 109$	Omicron BA.5	75.2	66.4, 82.4	<0.0001	0.9559
	Ancestral	77.8	69.1, 84.6	<0.0001	0.5050
	Gamma	75.0	66.1, 82.2	<0.0001	>0.9999
ARVAC Omicron BA.4/5, $n = 108$	Omicron BA.5	88.0	80.5, 92.8	<0.0001	0.0019
	Ancestral	91.2	84.1, 95.3	<0.0001	0.0002
	Gamma	90.2	82.9, 94.6	<0.0001	0.0004
ARVAC Bivalent, $n = 102$	Omicron BA.5	92.2	85.3, 96.0	<0.0001	<0.0001

CI, confidence interval; NA, not applicable.
[a] Chi-square test for Phase II and Fisher's exact test for Phase III.
[b] Z-test.

$ARVAC_{Bivalent}$ was superior to $ARVAC_{Gamma}$ in nAb responses (i.e., GMTR) against the Gamma and Omicron variants. $ARVAC_{Bivalent}$ was superior to $ARVAC_{Omicron}$ in nAb responses against the Ancestral and Gamma variants (Table S15).

3.7. Anti-spike-specific antibodies and mucosal response

Plasma levels of anti-spike-specific IgG increased (d1 to d14) in participants receiving any vaccine, regardless of age group (Fig. S12); changes remained significant at d90 (Fig. S13). Anti-S1-specific IgA in saliva increased in participants receiving any vaccine (Fig. S14).

3.8. NAbs against new emerging virus variants and SARS-CoV-1

GMTs to the XBB.1.18 and JN.1 subvariants increased significantly in participants aged 18–60 years and in those >60 years after $ARVAC_{Bivalent}$ administration (Fig. S15). While most participants (>89 %) had detectable nAb titers against the Ancestral, Gamma, and Omicron BA.5 variants before vaccination, nAbs to XBB.1.18 and JN.1 were detectable in 50.0 % and 18.8 % of participants aged 18–60 and in 61.5 % and 33.0 % of those >60 years, respectively. These percentages increased to 91.7 % and 83.3 % in participants aged 18–60 and to 100 % and 92.3 % in participants >60 years after $ARVAC_{Bivalent}$ administration (Fig. S15). In addition, GMTs to the SARS-CoV-1 virus increased significantly in participants >60 years after $ARVAC_{Bivalent}$ administration (Fig. S16).

3.9. Safety

Most local and systemic AEs were Grade 1 and 2 (Table 3), and no SAEs related to the vaccine were reported. The most frequent local AEs were pain and sensitivity/discomfort in the injection site and were more frequent in participants receiving the vaccine than placebo (Table 3). Pain was more frequent after administration of $ARVAC_{Omicron}$ and $ARVAC_{Bivalent}$ (Table S16).

The most frequent systemic AEs were headache and fatigue/tiredness/weakening (Table 3). A description of AEs per vaccine version is included in Table S17.

4. Discussion

This Phase II/III trial showed that booster vaccination with Gamma, Omicron BA.4/5, and Bivalent versions of a recombinant protein subunit vaccine elicited robust antibody responses to SARS-CoV-2 Ancestral, Gamma, and Omicron BA.5 variants in adults, regardless of primary vaccination platform and previous SARS-CoV-2 infection. At d14 post-vaccination, seroconversion rates to homologous and non-homologous SARS-CoV-2 variants were higher than placebo, with a favorable safety and tolerability profile. NAb GMTs against the three SARS-CoV-2 variants were significantly increased, and antibody responses persisted for at least 90 days, even in participants >60 years. NAb levels suggested that the vaccine versions achieved an estimated efficacy of ≥90 % against symptomatic infection in 84.4 %–92.2 % of participants. Additionally, all vaccine versions increased anti-spike-specific IgG antibodies in plasma and IgA in saliva.

Results from this trial are consistent with a previous Phase I study, which included younger participants (18–55 years) with various primary vaccination schemes [15]. Moreover, they align with previous research indicating that a Gamma-variant vaccine may enhance immunogenicity and breadth compared to an ancestral-variant vaccine [14,15].

To our knowledge, few trials have simultaneously assessed and compared the immunogenicity of several vaccine variant versions, as in this Phase III trial [20,21]. Bivalent Ancestral/Omicron, Alpha/Beta, and Ancestral/Beta recombinant boosters have shown robust nAb responses in individuals [20–23], but a bivalent recombinant booster vaccine lacking the Ancestral/Alpha variants remained unassessed. Despite including a lower dose of each monovalent antigen, the $ARVAC_{Bivalent}$ booster was non-inferior regarding seroconversion rates and GMTs, and, remarkably, it was superior to monovalent vaccines against heterologous SARS-CoV-2 variants. Furthermore, $ARVAC_{Bivalent}$-induced seroconversion rates were > 90 % against Ancestral, Gamma and Omicron SARS-CoV-2 in all age groups. Similar results, superiority to monovalent versions against heterologous variants and non-inferiority against homologous variants, were described for a bivalent Omicron/Ancestral recombinant spike protein vaccine as a heterologous booster dose [21,23].

Unlike most trials on COVID-19 boosters, in which enrolled participants were highly homogeneous regarding their primary vaccination scheme, (mostly based on mRNA or adenoviral vaccines [20–24]), this study reflects the diverse primary vaccination schemes used in Argentina and Latinoamerica, where at least seven different vaccines based on different platforms were applied as primary and booster doses [25]. This trial showed robust nAb responses regardless of the previous vaccination scheme and the number of previous booster doses (no booster, one, or two). Additionally, the trial included a large population with no strict selection criteria, providing valuable information for applying the ARVAC vaccine in real-world populations. In this regard, an adjusted multivariate analysis revealed the superiority of $ARVAC_{Bivalent}$ compared to $ARVAC_{Gamma}$ or $ARVAC_{Omicron}$ regardless of age, sex, previous vaccine doses and platform, time since last vaccination, and previous COVID-19 history. Moreover, these results contribute to the increasing evidence that heterologous schedules may provide superior immunogenicity to homologous booster schedules [26–28]. Furthermore, the WHO includes older adults as well as younger adults with significant comorbidities (e.g. diabetes, heart disease, HIV) as high priority [8]. Hence, the strong immune response observed in participants over 60 years and in participants with underlying medical conditions, provide data from relevant populations.

In this study, both a booster dose with a protein bivalent vaccine -combining Gamma and Omicron antigens- or a monovalent Omicron vaccine elicited comparable high levels of neutralizing antibody and seroconversion rates against Omicron. Yet the bivalent formulation demonstrated ≥90 % seroconversion rate and a broad response. Similar results were obtained with other COVID-19 vaccines such as bivalent (omicronBA.5/ancestral) mRNA-1273.222 [29]) or protein based (NVX-CoV2373/NVX-CoV2540 [21]). Conversely, some studies indicated that bivalent (Ancestral/Omicron) mRNA vaccines elicit weaker immunity against Omicron than the ancestral strain, with concerns that the ancestral antigen may cause immunological imprinting, limiting the response to newer variants [30–34]. In contrast, the RBD-based bivalent protein subunit vaccine (Gamma/ Omicron) evaluated in this work generated strong immunity against its target antigens and the ancestral strain, potentially due to differences in antigen expression and formulation. The use of the Gamma RBD antigen, rather than the ancestral strain and targeting the RBD instead of the full spike protein may mitigate imprinting issues [35]. However other variations in the study population may also contribute to the above-mentioned differences.

The Omicron BA.4/5 and Gamma antigens in the Bivalent vaccine contain key mutations associated with immune evasion and are, therefore, likely to elicit the production of antibodies neutralizing other Omicron subvariants. The vaccine's immunogenicity against the Omicron XBB.1.18 and JN.1 subvariants is relevant, as these variants contain more immune-evasive mutations than most others detected to date and are predominant in many geographical centers. Notwithstanding this, future adaptations of the vaccine may further enhance immunogenicity against emerging strains. The induced broad nAb response against multiple and phylogenetically distant variants. Including Ancestral, Gamma, Omicron, XBB, JN.1 SARS-CoV-2 and SARS-CoV-1, highlights the potential of the ARVAC platform as initial point to develop a protein subunit pan-sarbecovirus vaccine accessible in the Global South.

This vaccine demonstrated a favorable safety profile with low reactogenicity, as expected for a protein-based recombinant platform. Notably, the rate of solicited adverse events in the clinical trial was comparable to—or even lower than—that reported for other COVID-19 vaccines based on the same platform [22,36,37]. In contrast, mRNA-based booster vaccines have shown higher frequencies of solicited adverse reactions, particularly systemic events such as headache, fatigue, and myalgia, with reported rates exceeding 50 %, 60 %, and 26 %, respectively [29,38–40]. Although COVID-19 vaccine hesitancy is a complex and multifactorial issue, one of its key determinants is fear and the perceived risk of adverse events [41,42]. The availability of an alternative vaccine platform with a well-established safety record provides an additional option for public health strategies in our region and could contribute to improving vaccination coverage.

A difference between ARVAC and other available vaccines in the region is its storage conditions and stability. ARVAC has a 24-month shelf life at 2–8 °C, while widely used mRNA vaccines require lower temperatures for long-term storage [43–45].

One limitation of this study is the short follow-up for immunogenicity (3 months). However, in the Phase I study, nAb titer increases remained significant even six [15] and 12 months after vaccine administration (unpublished results). Extended follow-up studies in phase 4 will provide valuable insights into the long-term durability of immune responses and the vaccine's safety profile. Despite this limitation, this study showed that an adapted RBD-based protein subunit vaccine used as a booster in previous vaccinated individuals, elicited robust, protective, long-lasting and broad antibody responses across diverse human demographic and immunological backgrounds.

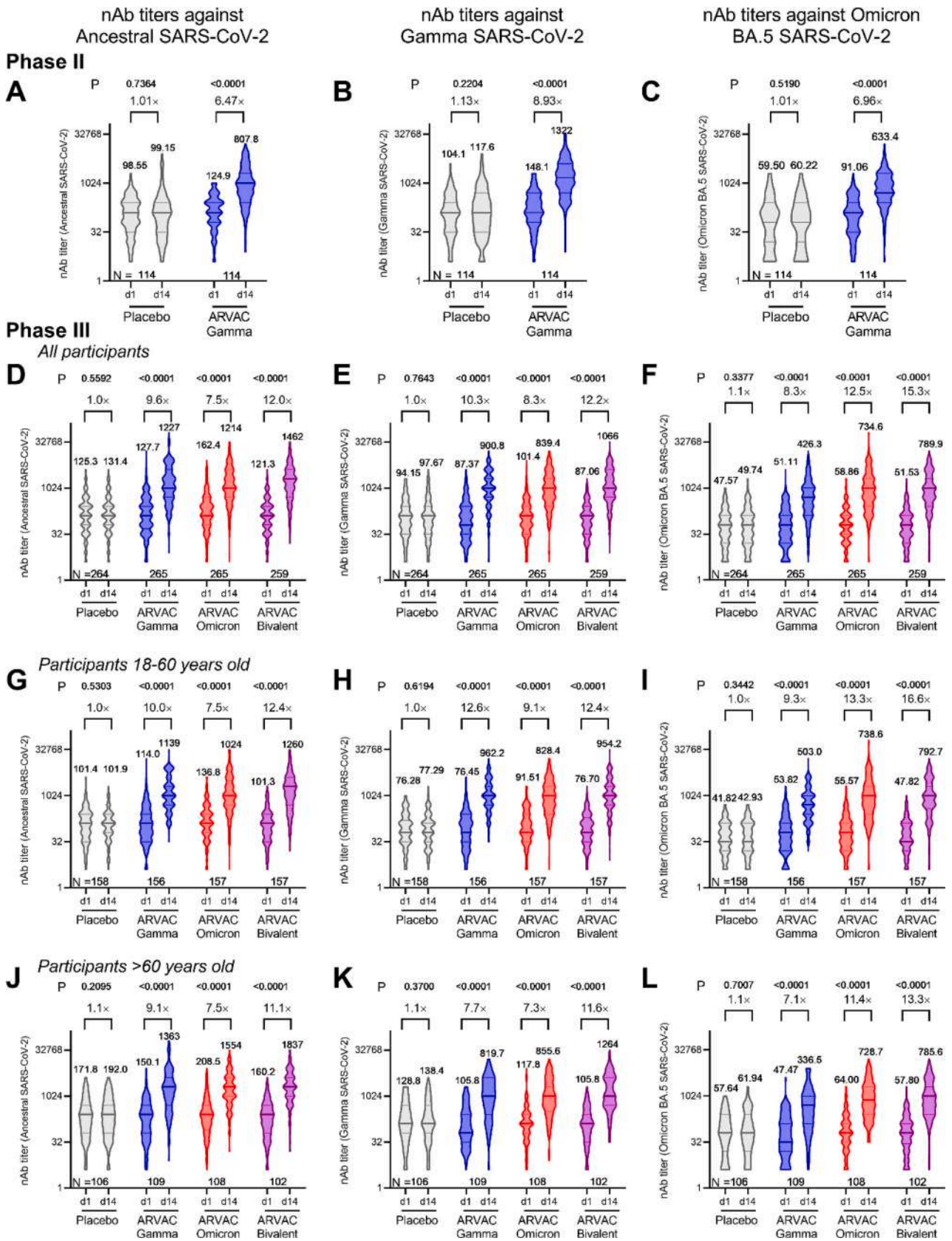

Fig. 2. Neutralizing antibody (nAb) titers to SARS-CoV-2 variants before (d1) and 14 days (d14) after vaccine/placebo administration. The plots represent antibody titers to SARS-CoV-2 ancestral (A, D, G, J), Gamma (B, E, H, K), and omicron variants (C, F, I, L) obtained from plasma samples of participants in Phase II (A-C) and Phase III (D—F). Participants in Phase III were classified according to age into 18–60 years (G-I) and > 60 years (J-L). The thick horizontal lines in the violin plots represent the medians. Geometric mean titer (GMT) values are indicated above the plots. Geometric mean fold rises (GMFRs) and *p*-values comparing titers before and after administration are indicated. *p*-values were calculated using the non-parametric paired.

Table 3
Local and systemic adverse reactions according to severity and treatment (vaccine vs. placebo), $n = 2012$.

	Vaccine ($n = 1960$)					Placebo ($n = 1957$)				*p*-value**
	Grade 1	Grade 2	Grade 3	Grade 4	Total*	Grade 1	Grade 2	Grade 3	Total*	
Local, *n (%)*#										
Pain	810 (96.3)	31 (3.7)	0	0	841 (42.9)	601 (98.0)	11 (1.8)	1 (0.2)	613 (31.3)	<0.001
Sensitivity/discomfort	565 (88.6)	69 (10.8)	4 (0.6)	0	638 (32.5)	385 (92.1)	30 (7.2)	2 (0.5)	417 (21.3)	<0.001
Swelling/induration	155 (98.1)	3 (1.9)	0	0	158 (8.1)	73 (100)	0	0	73 (3.8)	<0.001
Erythema/redness	74 (96.1)	3 (3.9)	0	0	77 (3.9)	44 (97.8)	1 (2.2)	0	45 (2.3)	0.003
Itching	54 (100)	0	0	0	54 (2.8)	28 (96.6)	1 (3.4)	0	29 (1.5)	0.006
Systemic, *n (%)*#										
Diarrhea	43 (91.5)	3 (6.4)	1 (2.1)	0	47 (2.4)	38 (97.4)	1 (2.6)	0	39 (2.0)	0.388
Headache	203 (83.9)	37 (15.3)	2 (0.8)	0	242 (12.3)	170 (90.4)	17 (9.0)	1 (0.5)	188 (9.6)	0.006
Joint pain	48 (85.7)	7 (12.5)	1 (1.8)	0	56 (2.9)	39 (84.8)	7 (15.2)	0	46 (2.4)	0.321
Muscle pain/myalgia	103 (86.6)	15 (12.6)	1 (0.8)	0	119 (6.1)	93 (86.9)	14 (13.1)	0	107 (5.5)	0.420
Chills	34 (81.0)	7 (16.7)	1 (2.4)	0	42 (2.1)	26 (89.7)	3 (10.3)	0	29 (1.5)	0.122
Fatigue/tiredness/weakening	206 (88.8)	23 (9.9)	2 (0.9)	1 (0.4)	232 (11.8)	169 (89.9)	19 (10.1)	0	188 (9.6)	0.024
Fever	22 (75.9)	6 (20.7)	1 (3.4)	0	29 (1.5)	14 (93.3)	1 (6.7)	0	15 (0.8)	0.034
Nausea	32 (94.1)	2 (5.9)	0	0	34 (1.7)	31 (91.2)	3 (8.8)	0	34 (1.7)	0.993
Palpitations	15 (83.3)	3 (16.7)	0	0	18 (0.9)	12 (92.3)	1 (7.7)	0	13 (0.7)	0.370
Drowsiness	196 (88.3)	24 (10.8)	2 (0.9)	0	222 (11.3)	183 (92.0)	16 (8.0)	0	199 (10.2)	0.243
Vomiting	6 (100)	0	0	0	6 (0.3)	3 (50.0)	3 (50.0)	0	6 (0.3)	0.997

Adverse reactions to all vaccine and placebo administrations are included.
The percentage next to each grade was calculated over the total number of cases for each adverse reaction.
* Percentage of each adverse reaction calculated over the total number of administrations.
** Chi-square test.

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5. Conclusions

Booster vaccination with the Gamma, Omicron BA.4/5, and Bivalent versions of the recombinant protein subunit ARVAC vaccine elicited protective neutralizing antibody responses to several SARS-CoV-2 variants and SARS-CoV-1. Additionally, all vaccine versions increased anti-spike-specific IgG antibodies in plasma and IgA in saliva. The increase in plasma neutralizing antibodies induced by the vaccine was independent of the number of previous booster doses, the primary vaccine platform and the history of previous COVID-19. The neutralizing Ab response induced by the vaccine in healthy participants was similar to that triggered in participants with underlying medical conditions associated with an increased risk of severe COVID-19. ARVAC showed very low reactogenicity and a favorable safety profile, as expected for a recombinant protein alhydrogel-adjuvanted vaccine. The ARVAC vaccine is a valuable booster option since it induced a strong and broad nAb response in high-priority populations previously vaccinated with a variety of approved primary vaccination schemes, its feasibility and low cost of large-scale recombinant vaccine production, its potential for adaptation, its safety profile, and its viable widespread distribution.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.127045.

Data availability

Data will be made available on request.

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