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Safety, tolerability, and immunogenicity of PIKA-adjuvanted recombinant SARS-CoV-2 spike protein subunit vaccine in healthy adults: an open-label randomized phase I clinical trial

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Purpose: This phase I study aimed to assess the safety, tolerability, and immunogenicity of the PIKA-adjuvanted recombinant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein subunit vaccine in healthy adults aged 18 years and older.

Materials and Methods: This is a phase I, open-label, dose-escalation study at three dose levels (5 μg, 10 μg, and 20 μg) of the PIKA coronavirus disease 2019 (COVID-19) vaccine administered intramuscularly. The three vaccine arms are (A) subjects who have never received any COVID-19 vaccination or have had COVID-19 infection for >6 months prior to enrolment; (B1) subjects whose COVID-19 primary vaccination series was completed with an inactivated COVID-19 vaccine; and (B2) subjects whose primary series was completed with messenger RNA COVID-19 vaccine.

Results: Subjects who reported solicited adverse events (AEs) within seven days post-vaccination ranged from 35% to 60% within each vaccine arm. Most solicited AEs were mild local pain and tenderness. Systemic solicited AEs were only reported in Arm A. In all three vaccine arms, neutralizing antibody geometric mean titers were highest at day 28 (Arms B1 and B2) or day 35 (Arm A) than at baseline for all dose levels against the Wuhan (wild original SARS-CoV-2 virus, Wuhan-Hu-1), Delta (B.1.617.2), and Omicron (B.1.1.529) variants. These were sustained at day 183. Seroconversion rates at day 35 (Arm A, 85.7%–92.9%) or day 183 (Arms B1, 90.9%–100.0%, and B2, 18.2%–36.4%) and geometric mean fold rises were highest in the 5-μg dose level against all three variants.

Conclusion: The PIKA-adjuvanted recombinant SARS-CoV-2 S protein subunit vaccine showed promising immunogenicity profile with no safety concerns. A dose-dependent immune response was observed, with slight advantages seen in low-dose (5 μg and 10 μg) groups (ClinicalTrials.gov registration number: NCT05305300).

Keywords: Vaccine, COVID-19, PIKA adjuvant, Phase I clinical trial

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), is a Beta-coronavirus belonging to the *Coronaviridae* family. It has caused the third human coronavirus outbreak in the last 20 years,

following SARS (SARS-CoV-1) in 2003 and MERS (Middle East respiratory syndrome) in 2012 [1]. The COVID-19 pandemic has had a significant impact, leading to widespread illness, death, and global economic instability. As of September 2023, there have been over 770 million reported cases of COVID-19 and 7 million related deaths worldwide [2].

SARS-CoV-2 is highly pathogenic and spreads mainly via respiratory droplets and aerosols. It is comprised of an approximately 30 kilobase single-stranded positive-strand RNA genome that encodes four major structural proteins [3]. The spike (S) protein facilitates viral attachment and entry into host cells via human angiotensin-converting enzyme 2 receptors [4]. This protein is a significant target of new COVID-19 vaccines [5].

Vaccine platforms include subunit or virus-like particle vaccines, virus-vectored vaccines, nucleic acid vaccines, and inactivated vaccines [6]. Several vaccines that use different platforms have been found to be effective against COVID-19 [7-10]. Preliminary and published data from phase I–III trials showed that S antigen-based vaccines provided high-level protection against multiple SARS-CoV-2 variants, particularly against severe COVID-19 [11-16].

Building on this promising evidence, we explored the potential of the recombinant SARS-CoV-2 protein as a COVID-19 vaccine candidate that uses PIKA as adjuvant. YS Biopharma Co. Ltd. has developed this chemical as its proprietary vaccine platform. It interacts specifically with toll-like receptor 3 (TLR3) in a dose-dependent manner, promoting the activation and maturation of dendritic cells, inducing the activation and proliferation of B and NK cells, and eliciting both type 1 T helper (Th1) and type 2 T helper (Th2) immune responses [17,18]. In pre-clinical and early clinical studies, PIKA has shown excellent tolerability and the ability to elicit potent local and systemic immune responses [19,20].

This phase I study aimed to assess the safety, tolerability, and immunogenicity of the PIKA-adjuvanted recombinant SARS-CoV-2 S protein subunit vaccine in healthy adults aged 18 years and older. An exploration of the vaccine's efficacy against the Delta and Omicron variants of SARS-CoV-2 was also done.

Materials and Methods

Study design

This was a single-center, open-label, randomized phase I dose-escalation trial conducted at Al Kuwait (Al Bahara) Hospital in the United Arab Emirates (UAE). The trial evaluated three dose levels (5 μg, 10 μg, and 20 μg) of the SARS-CoV-2 S antigen vaccine administered intramuscularly in combination with 1 mg of PIKA adjuvant. The study consisted of three vaccine arms: Arm A, which included subjects who had not received a COVID-19 vaccine or had a history of COVID-19 infection not less than six months prior to study participation; and Arms B1/2, which included subjects who received either an inactivated (B1) or messenger RNA (mRNA) (B2) COVID-19 vaccine as primary series.

Subject enrolment

In Arm A, 45 subjects were divided into three dose groups. The vaccines were given on days 0 and 7. Enrolment of each dose group started with three sentinel subjects who were followed up by phone call every 24 hours during the first 48 hours after vaccine administration. After 48 hours with no safety concerns on the sentinel subjects, 12 more subjects were subsequently enrolled. Within Arm A, subjects were enrolled in different dose level groups sequentially, starting with the lowest dose group 1 (5 μg), followed by group 2 (10 μg), and lastly group 3 (20 μg). Sequential enrolment to a higher dose group was only performed when no safety concerns leading to study discontinuation were observed. Arm B enrolled 90 subjects, with 45 participants randomly assigned either Arms B1 and B2. A sequential enrolment process similar to Arm A was followed.

Vaccine formulation

The investigational PIKA COVID-19 vaccine combines the SARS-CoV-2 S subunit protein and the PIKA adjuvant. The vaccine antigen used is the S1 subunit of the S protein, which contains the receptor-binding domain, derived from the wild original SARS-CoV-2 virus, Wuhan-Hu-1 strain. The antigen sequence includes specific modifications: the 2P mutation, which stabilizes the prefusion conformation of the S protein, and the deletion of the furin cleavage site, which can influence the protein's processing and immunogenicity. The antigen is expressed using a mammalian cell expression system, specifically CHO cells, ensuring proper protein folding and post-translational modifications.

PIKA COVID-19 vaccine is a sterile liquid packaged in a single-use vial for intramuscular injection. The vial is a 1-mL neutral borosilicate glass vial, sealed with a halogenated butyl rubber stopper (brominated). Each vial contains 5 μg/mL, 10 μg/mL, or 20 μg/mL of S protein trimer antigen, 1 mg of

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PIKA adjuvant, and excipients (e.g., polysorbate 80, arginine hydrochloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride) in water-for-injection. It comes as a freeze-dried powder and is reconstituted into a clear, colorless solution. For storage and handling, the investigational vaccine was kept at a temperature of 5±3°C and protected from light. Vaccine storage in syringes was also avoided to maintain its integrity.

The PIKA adjuvant is a chemically synthesized double-stranded RNA analogue consisting of double-stranded polyinosinic acid-polycytidylic acid, kanamycin monosulfate, and calcium chloride. It is an agonist of TLR3, a key pathogen-associated molecular pattern receptor for recognizing double-stranded RNA viral infections and activating the innate immune system [17,21].

The PIKA COVID-19 vaccine has several key physical and chemical properties. Its isoelectric point, which is the pH at which the vaccine carries no net electrical charge, is 6.10 (range, 4.71–6.72). The theoretical extinction coefficient is 0.968 at 280±3 mm. This indicates how much light the vaccine absorbs at this wavelength, aiding in its quantification. The molecular mass of the monomer form is 176 kDa, while the deglycosylated molecular weight is 142.4 kDa for the monomer and 428.1 kDa for the trimer. The vaccine's binding affinity to the anti-SARS-CoV-2 S protein antibody, as measured by surface plasmon resonance, is characterized by an association rate constant (Ka) of 3.83×10^{4} 1/M·s, a dissociation rate constant (Kd) of 9.12×10^{-4} 1/sec, and an equilibrium dissociation constant (KD) of 2.38×10^{-8} M, indicating high affinity.

Vaccine schedule

In Arm A, since subjects were naïve against COVID-19, two doses of the PIKA COVID-19 vaccine were administered on study days 0 and 7 in the deltoid muscle. In Arms B1 and B2, only one PIKA COVID-19 vaccine dose was administered as booster on study day 0 in the deltoid muscle. Study day 0 refers to the day when the subject was enrolled.

Study endpoints

The safety endpoints of this study included the following: (1) frequency of subjects with solicited local and/or systemic adverse events (AEs) within 7 days after each vaccination; (2) frequency of subjects with unsolicited AEs within 28 days after each vaccination; (3) frequency of serious adverse events (SAEs), including suspected unexpected serious adverse reactions (SUSARs), medically attended adverse events (MAAEs), and/or adverse events of special interest (AESIs) throughout the study; and (4) changes in safety laboratory parameters from baseline.

The immunogenicity endpoints of this study were: (1) geometric mean titers (GMTs) of neutralizing antibody against various SARS-CoV-2 virus variants (e.g., wild original SARS-CoV-2 virus, Delta B.1.617.2, and Omicron B.1.1.529) at baseline and at pre-defined post-vaccination time points; (2) seroconversion rates of neutralizing antibody at pre-defined post-vaccination time points; (3) geometric mean fold rises (GMFRs) of neutralizing antibody titer from baseline; (4) GMT of serum immunoglobulin G (IgG) against SARS-CoV-2 as measured by the enzyme-linked immunosorbent assay (ELISA); (5) seroconversion rates of serum IgG; (6) frequency of CD4+ and CD8+ T cells cytokine expression by intracellular and extracellular staining using S protein overlapping peptide pool; and (7) GMT of neutralizing antibody against Delta (B.1.617.2) and Omicron (B.1.1.529) variants of SARS-CoV-2 at baseline and at pre-defined post-vaccination time points. Seroconversion was defined as at least 4-fold increase from baseline.

Immunogenicity was measured by examining both humoral and cellular immune responses. The functional assay of humoral immunity involves the collection of serum samples to measure the immune response against different SARS-CoV-2 variants. The primary assays used are the serum neutralizing antibody test and the measurement of serum IgG levels. In particular, humoral responses were assessed by measuring SARS-CoV-2 neutralizing antibody levels in the blood using a virus neutralization assay with live virus via Plaque Reduction Neutralization Test (PRNT), as well as SARS-CoV-2 binding antibodies to S protein using ELISA. For the PRNT, heat-inactivated 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2% L-glutamine comprised the minimum essential media which was used as the culture medium with Caco-2 cells used as the cell line. Cell cultures were maintained in 175 cm^2 cell culture flasks in a humidified incubator at 37°C and 5% CO_2. All humoral immunogenicity tests were performed under sterile conditions.

Cellular immune responses were evaluated by measuring cytokine-secreting peripheral blood mononuclear cells (PBMCs) using T-spot, and by assessing Th1 and Th2 immune responses through flow cytometry after stimulating PBMCs with SARS-CoV-2 S protein peptides and conducting intracellular staining to identify CD4+ and CD8+ T cells ex-

pressing interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α), and other Th1/Th2 markers. Preparatory steps included PBMC isolation from human blood samples using density gradient centrifugation, then preparing the ELISpot plate by pre-wetting with 70% ethanol, washing with PBS, and blocking with culture medium. The PBMCs were cultured in RPMI-1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin, and 1% L-glutamine. PBMCs were then added to each well at a concentration of 2×10^5 cells/well. Positive and negative controls were included, and the plate was incubated for 18–24 hours at 37°C in a 5% CO_2 incubator. Spots, representing individual cytokine-secreting cells, were counted using an automated ELISpot reader. Data analysis involved calculating the mean number of spots for triplicate wells, subtracting the mean spot count of negative control wells from antigen-stimulated wells to account for background, and expressing results as spot-forming units per 10^6 PBMCs. All immunogenicity tests were performed by Biogenix Labs located in Masdar City, Abu Dhabi.

These immune responses were measured at baseline and at various time points after the study injections. Pre-defined post-vaccination time points were days 0, 14, 21, 35, and 183 from first study injection. A total of five blood draws were performed during six visits.

Subject safety monitoring and follow-up

All subjects were monitored for at least 60 minutes after each study injection. Both solicited and unsolicited AEs were recorded for 7 days and 28 days, respectively, following each study injection. SAEs, including SUSARs, AESIs, and MAAEs were recorded for the entire duration of the study. All subjects were followed up for 180 days from the first study injection. Clinical safety laboratory evaluations were performed at screening and 7 days after each vaccination.

Statistical analysis

The sample sizes for each study group and the overall study were determined based on planned descriptive analyses of safety and immunogenicity data. Safety analysis was performed on all enrolled subjects who received at least one dose of the PIKA COVID-19 vaccine. Safety parameters were summarized using descriptive statistics and presented by vaccine arm and dose group. Immunogenicity analysis was conducted on the per protocol set, consisting of all enrolled subjects who received all scheduled doses of the PIKA COVID-19 vaccine and had no major protocol deviation. Assessments were performed for each vaccine arm separately. GMTs at each visit day of neutralizing antibodies against each variant, ELISA, and serum IgG were calculated and estimated by exponentiating the corresponding log10-transformed values. Seroconversion rates were defined as the percentage of subjects with at least a 4-fold increase in titers from day 0 pre-vaccination. GMFRs were defined as the ratio between GMTs at the day of visit to the GMT at day 0 as reference. CD4+ and CD8+ cytokine expressions were summarized as percentages. All statistical analyses were performed using IBM SPSS ver. 28.0 (IBM Corp., Armonk, NY, USA). Whenever applicable, 95% confidence intervals were calculated.

Ethical approval

The study protocol and related documents were approved by an independent ethics committee, with the approval identifier MOHAP/DXB-REC/JJJ/no.70/2021, dated July 13, 2021. The study was carried out in compliance with the protocol, in accordance with the International Council for Harmonization Technical Requirements for Registration of Pharmaceuticals for Human Use harmonized tripartite guideline for Good Clinical Practice and under the Ministry of Health and Prevention (MOHAP) of UAE Research Ethics Committee with MOHAP permission identifier No.FK:RCMOHP/CT1/0112/2021, dated July 18, 2021. All subjects provided written informed consent to be part of the study (ClinicalTrials.gov registration number: NCT05305300).

Results

Subject disposition

The safety analysis set included 45 subjects in each vaccine arm, equally randomized to three dose groups (5 μg, 10 μg, and 20 μg). In the immunogenicity analysis per-protocol set, seven subjects were excluded due to either delayed positive results obtained from SARS-CoV-2 reverse transcription-polymerase chain reaction tests following false negative results from rapid antigen kits prior to randomization (n=5) or due to existing medical histories of uncontrolled diabetes mellitus (n=1) or hepatitis C infection (n=1) (Fig. 1).

Demographic characteristics

The average age of subjects across the three Arms A, B1, and B2 were 33.6 years, 31.1 years, and 32.2 years, respectively. Most subjects were males. Asians comprised one-third, one-half, and almost all subjects in Arms A, B1, and B2, respec-

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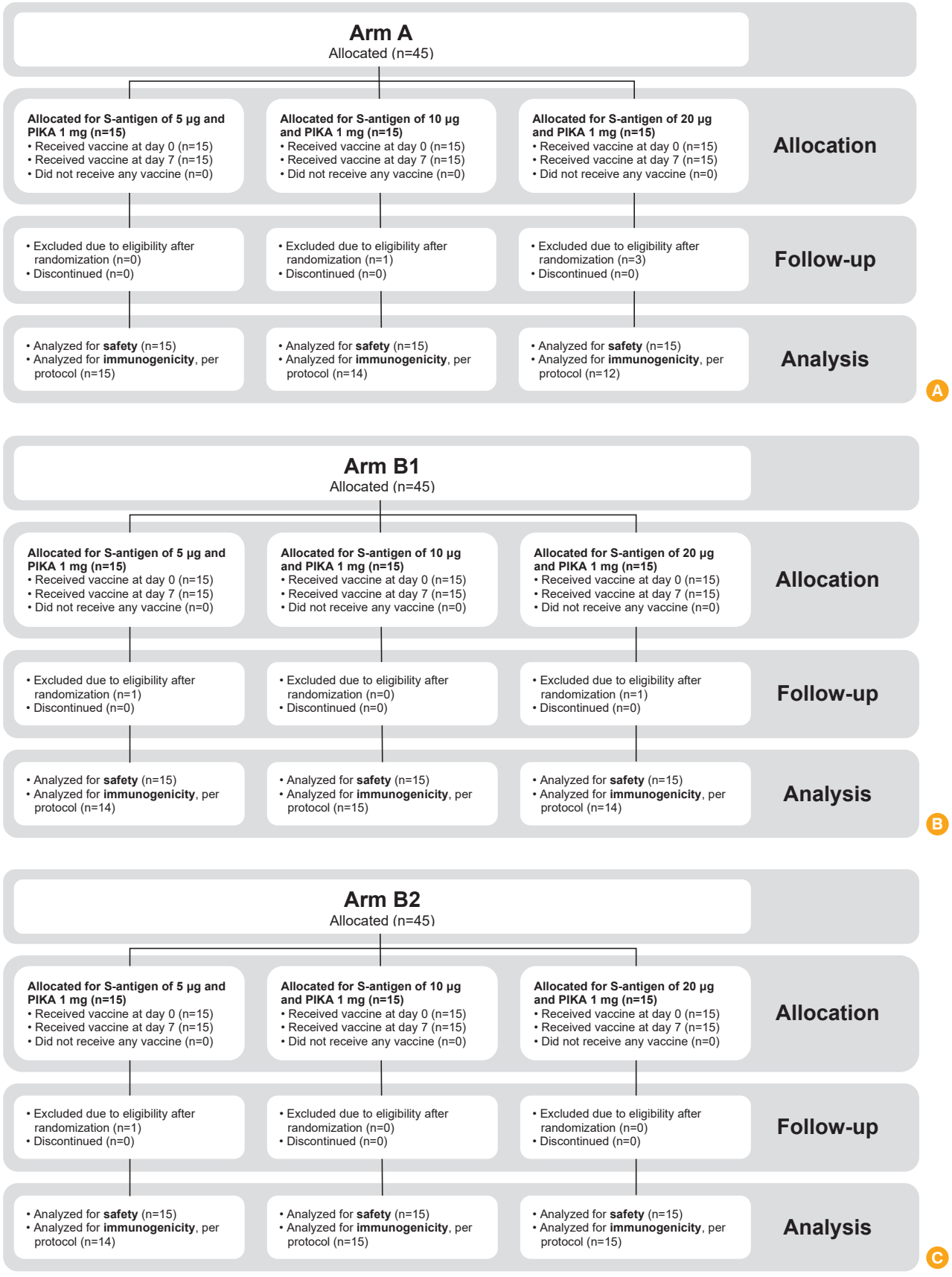

Fig. 1. (A) Subject disposition, in Arm A. **(B)** Subject disposition, in Arm B1. **(C)** Subject disposition, in Arm B2.

Table 1. Subject demographic characteristics

Vaccine arm	Dose group	Age (yr)		Gender (no., %)		Race (no., %)			
		Mean±SD (range)	Median (Q1–Q3)	Male	Female	Asian	African or Black	Arab or White or Caucasian	Others
Arm A	5 μg	31.7±9.5 (21–48)	31 (23–39)	9 (60)	6 (40)	8 (54)	3 (20)	2 (13)	2 (13)
	10 μg	33.0±6.5 (23–46)	32 (28–36)	13 (93)	1 (7)	3 (21)	7 (50)	3 (21)	1 (8)
	20 μg	36.7±5.5 (26–43)	37 (34–42)	10 (83)	2 (17)	3 (25)	4 (33)	5 (42)	0
	Overall	33.6±7.6 (21–48)	33 (27–40)	32 (78)	9 (22)	14 (34)	14 (34)	10 (24)	3 (8)
Arm B1	5 μg	28.3±6.5 (22–47)	34 (28–35)	12 (86)	2 (14)	7 (50)	2 (14)	5 (36)	0
	10 μg	33.0±7.3 (21–42)	26 (22–33)	13 (87)	2 (13)	8 (53)	4 (27)	3 (20)	0
	20 μg	31.4±8.6 (21–50)	31 (24–36)	13 (93)	1 (7)	6 (43)	3 (21)	4 (29)	1 (7)
	Overall	31.1±7.5 (21–50)	31 (25–35)	38 (88)	5 (12)	21 (49)	9 (21)	12 (28)	1 (2)
Arm B2	5 μg	33.3±10.8 (20–58)	31 (25–40)	14 (100)	0	10 (71)	1 (7)	3 (22)	0
	10 μg	32.7±9.2 (20–55)	33 (25–39)	15 (100)	0	14 (93)	0	1 (7)	0
	20 μg	30.5±6.8 (21–44)	30 (25–37)	15 (100)	0	14 (93)	0	1 (7)	0
	Overall	32.2±9.0 (20–58)	31 (25–38)	44 (100)	0	38 (86)	1 (2)	5 (12)	0

Percentages are computed using dose group in each arm as reference (e.g., per row).
SD, standard deviation.

tively. Randomization of the 45 participants to the three doses in each study arm did not produce well-balanced groups according to subjects' demographics due to the small sample size and lack of stratification (Table 1).

Safety events

Across all three vaccine arms, the most common AEs reported were mild pain/tenderness at the local injection site and mild fever or mild headaches among systemic AEs. Unsolicited AEs were also common, but most were mild and resolved spontaneously, and none were deemed related to the study vaccine. No SAE or MAAE was deemed related to the study vaccine (Table 2).

In Arm A, 60% of subjects reported solicited AEs after the first vaccination, decreasing to 27% after the second. At least 80% of the reported events in each dose group were considered mild. Unsolicited adverse events were reported by 67% of subjects, with a decreasing trend after the second vaccination. Four patients were diagnosed with COVID-19 infection, an SAE, and two of which resulted to being MAAEs (one for each of the 5 μg and 10 μg dose groups). All cases, including the two which were deemed MAAEs, were mild, resolved quickly, and were determined to be unrelated to the study vaccine. In Arm B1, most reported AEs (at least 60%) were considered mild. Unsolicited AEs were reported among 67%, 53%, and 47% of the subjects for the 5 μg, 10 μg, and 20 μg dose groups, respectively. No SAE or MAAE was reported in this arm. Fairly similar patterns with Arm B1 were observed in Arm B2.

Laboratory safety parameters

In all three arms, common laboratory test abnormalities included glucose or white blood cells in the urine. Other abnormalities were rare, and no severe abnormalities were reported. In Arm A, clinically significant hematuria was reported in the 10 μg dose group on day 14. Glycosuria was found in three subjects, and one subject had a significant abnormality in the coagulation test on day 14 in the 20 μg dose group. In Arm B1, clinically significant pyuria was noted in the 10 μg dose group on day 14. Glycosuria was recorded in four instances, and four significantly elevated aspartate aminotransferase and alanine transaminase were reported in the 10 μg dose group. In Arm B2, clinically significant white blood cell abnormalities were reported on day 7 in the 10 μg dose group. Additionally, two instances of significant pyuria were recorded in the 10 μg dose group on days 7 and 14. Glycosuria was also reported in three instances. Nevertheless, none of the clinically significant laboratory abnormalities were deemed related to the study vaccine.

Immunogenicity

The study found that after vaccination, levels of neutralizing antibodies increased significantly against all three variants of SARS-CoV-2 (Table 3). Although these levels decreased over time, they remained higher than baseline, even at day 183. Seroconversion rates were high for all three variants, with the lowest rates observed in the 20 μg dose group (Fig. 2). The GMFRs were highest on days 14 and 28 but decreased over

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Table 2. Safety summary

Variable	Arm A			Arm B1			Arm B2		
	5 μg	10 μg	20 μg	5 μg	10 μg	20 μg	5 μg	10 μg	20 μg
At least one solicited AE, days 0 to 7	9 (60.0)	9 (60.0)	9 (60.0)	5 (33.3)	9 (60.0)	7 (46.7)	5 (33.3)	2 (13.3)	4 (26.7)
At least one solicited local AE, days 0 to 7	6 (40.0)	7 (46.7)	8 (53.3)	3 (20.0)	8 (53.3)	5 (33.3)	4 (26.7)	2 (13.3)	2 (13.3)
Pain/tenderness	6 (40.0)	7 (46.7)	8 (53.3)	3 (20.0)	8 (53.3)	5 (33.3)	4 (26.7)	2 (13.3)	2 (13.3)
Erythema/redness	-	1 (6.7)	1 (6.7)	-	-	-	-	-	-
Swelling/induration	-	-	-	-	-	-	-	-	-
At least one solicited systemic AE, days 0 to 7	3 (20.0)	2 (13.3)	5 (33.3)	2 (13.3)	3 (20.0)	4 (26.7)	2 (13.3)	2 (13.3)	2 (13.3)
Fever	1 (6.7)	1 (6.7)	2 (13.3)	1 (6.7)	2 (13.3)	-	2 (13.3)	2 (13.3)	1 (6.7)
Headache	2 (13.3)	1 (6.7)	2 (13.3)	-	1 (6.7)	4 (26.7)	1 (6.7)	2 (13.3)	1 (6.7)
Others[a]	-	-	1 (6.7)	1 (6.7)	1 (6.7)	-	1 (6.7)	1 (6.7)	-
At least one solicited AE, days 8 to 14	4 (26.7)	5 (33.3)	5 (33.3)						
At least one solicited local AE, days 8 to 14	2 (13.3)	4 (26.7)	5 (33.3)						
Pain/tenderness	1 (6.7)	2 (13.3)	5 (33.3)						
Eryhtema/redness	-	1 (6.7)	-						
Swelling/induration	1 (6.7)	1 (6.7)	-						
At least one solicited systemic AE, days 8 to 14	2 (13.3)	3 (20.0)	-						
Fever	2 (13.3)	2 (13.3)	-						
Headache	-	1 (6.7)	-						
Others[a]	-	2 (13.3)	-						
At least one unsolicited AE, days 0 to 28	1 (6.7)	8 (53.3)	7 (46.7)	1 (6.7)	8 (53.3)	7 (46.7)	7 (46.7)	1 (6.7)	1 (6.7)
At least one unsolicited AE, day 29 until end of study	2 (13.3)	2 (13.3)	1 (6.7)	2 (13.3)	2 (13.3)	1 (6.7)	1 (6.7)	1 (6.7)	-
At least one SAE	2 (13.3)	-	2 (13.3)	-	-	-	-	-	-
Not related	2 (13.3)	-	2 (13.3)	-	-	-	-	-	-
At least one SUSAR	-	-	-	-	-	-	-	-	-
At least one MAAE	1 (6.7)	1 (6.7)	-	-	-	-	-	-	-
Severity (all AEs)									
Mild	12 (80.0)	13 (86.7)	13 (86.7)	10 (66.7)	12 (80.0)	9 (60.0)	10 (66.7)	10 (66.7)	12 (80.0)
Moderate	2 (13.3)	1 (6.7)	1 (6.7)	2 (13.3)	2 (13.3)	1 (6.7)	1 (6.7)	1 (6.7)	2 (13.3)

Values are presented as number (%), unless otherwise stated. Day 0 is the first day of receiving (first, for Arm A) vaccination. Percentages are computed using dose group in each arm as reference (e.g., per column).
AE, adverse event; SAE, serious adverse event; SUSAR, suspected unexpected serious adverse event; MAAE, medically attended adverse event.
[a]Others include chills, myalgia, arthralgia, nausea/vomiting, diarrhea, fatigue/malaise, pain, and axillary lymphadenopathy.

time, remaining above 1.0 on day 183. Almost all subjects in all dose groups showed increased levels of serum IgG by day 21, which was sustained on days 35 and 183. Supplement 1 summarizes the GMTs and GMFRs of serum IgG at different time points across all vaccine arms against all variants.

In Arm A, neutralizing antibody levels increased significantly from pre-vaccination to day 14 against all three variants. While levels against the Wuhan variant continued to increase on day 21 and then decreased by day 35, levels for the Delta and Omicron variants decreased from day 14 to days 21 and 35. However, all levels remained above baseline at day 183. Seroconversion rates were high in the 5 μg and 10 μg dose groups but slightly lower in the 20 μg group. GMFRs for all three variants were highest on days 14 and 21.

In Arm B1, neutralizing antibody levels increased significantly from pre-vaccination to day 28 against all three variants, remaining above baseline levels at day 183. The highest seroconversion rates were recorded in the 5 μg dose group, followed by the 10 μg dose group, and lowest in the 20 μg group. GMFRs for all three variants were highest at days 14 and 28.

In Arm B2, neutralizing antibody levels increased significantly from pre-vaccination to day 14 against all three variants. While levels started declining on day 28, they slightly increased by day 183. Seroconversion rates were modest across all dose groups, with the 20 μg group showing the highest GMFRs at day 183 for all three variants. Overall, the GMFRs for all three variants were above 1.0 but relatively lower than those observed in Arms A and B1.

Table 3. GMTs of neutralizing antibodies against each variant and corresponding GMFRs

Vaccine arm	Day	Dose group					
		5 μg		10 μg		20 μg	
		GMT	GMFR	GMT	GMFR	GMT	GMFR
Wuhan (wild original SARS-CoV-2 virus, Wuhan-Hu-1) variant							
Arm A	0	52.8 (23.9–116.5)		48.8 (20.2–117.6)		65.0 (11.5–368.1)	
	14	3,225.4 (1,197.2–8,689.9)	61.1 (24.3–153.8)	1,762.6 (406.4–7,644.2)	33.8 (9.6–119.2)	1,280.0 (159.8–10,252.4)	32.0 (4.1–247.6)
	21	4,255.9 (2,046.6–8,850.4)	80.6 (31.6–205.7)	2,969.9 (1,253.8–7,034.9)	60.9 (23.9–155.1)	2,743.7 (539.2–13,961.8)	42.2 (7.1–251.2)
	35	4,413.3 (1,976.8–9,853.1)	90.5 (34.9–234.4)	2,206.7 (931.6–5,226.9)	45.3 (19.0–108.1)	2,388.6 (502.7–11,348.5)	36.8 (5.3–255.5)
	183	1,150.5 (655.1–2,020.6)	20.9 (9.1–47.9)	508.0 (130.6–1,975.9)	8.0 (2.4–27.1)	640.0 (145.6–2,812.8)	16.0 (4.0–63.9)
Arm B1	0	76.1 (27.4–211.8)		133.0 (40.8–434.0)		304.5 (99.9–928.8)	
	14	1,413.2 (566.9–3,522.9)	18.6 (6.2–55.8)	3,079.7 (1,808.4–5,244.9)	23.2 (6.1–87.4)	1,810.2 (1,011.4–3,239.8)	5.9 (1.5–22.9)
	21	3,620.4 (2,484.7–5,275.1)	47.6 (15.4–147.0)	5,652.9 (3,999.7–7,989.5)	47.6 (11.0–205.1)	3,620.4 (1,868.7–7,014.2)	11.9 (2.7–52.1)
	28	4,413.3 (2,987.5–6,519.6)	58.0 (17.9–187.4)	5,881.3 (4,368.6–7,917.8)	44.2 (11.8–165.7)	3,120.7 (1,620.5–6,009.8)	10.2 (2.4–43.4)
	183	2,119.0 (1,568.1–2,863.7)	24.9 (7.3–84.2)	1,859.1 (966.9–3,574.5)	22.0 (5.0–96.5)	1,859.1 (966.9–3,574.5)	4.7 (1.1–19.7)
Arm B2	0	1,050.0 (617.6–1,785.1)		1,167.0 (514.5–2,647.0)		1,159.3 (675.2–1,990.5)	
	14	1,103.3 (834–1,459.6)	1.1 (0.8–1.5)	2,301.1 (1,645.4–3,217.9)	1.5 (0.7–3.1)	1,902.1 (1,166.1–3,102.5)	1.6 (1.1–2.4)
	21	1,722.8 (1,006.7–2,948.2)	1.6 (1.1–2.5)	2,560.0 (1,628.4–4,024.6)	1.6 (0.8–3.4)	2,436.3 (1,399.8–4,240.6)	2.1 (1.5–2.9)
	28	1,485.0 (922.9–2,389.2)	1.4 (1.0–2.1)	1,859.1 (1,343.1–2,573.4)	1.2 (0.6–2.3)	1,998.6 (1,144.6–3,489.7)	1.7 (1.1–2.8)
	183	1,280.0 (641.6–2,553.7)	1.4 (0.8–2.2)	1,960.9 (1,156.4–3,325.2)	1.4 (0.7–2.6)	2,743.7 (1,589.8–4,735.1)	2.3 (1.3–4.0)
Delta (B.1.617.2) variant							
Arm A	0	38.2 (16.7–87.3)		40.0 (16.5–97.2)		37.3 (9.5–147.2)	
	14	2,560.0 (999.8–6,555.1)	67.0 (23.2–193.6)	1,150.5 (264.7–5,001.3)	25.9 (7.1–94.4)	592.6 (61.4–5,721)	23.5 (2.7–208.5)
	21	2,334.0 (1,016.0–5,362.0)	61.1 (23.7–157.3)	1,413.2 (574.7–3,475.3)	35.3 (13.1–95.2)	1,280.0 (257.8–6,355.6)	34.3 (6.1–192.5)
	35	1,722.8 (777.2–3,818.8)	47.6 (17.5–129.5)	1,159.3 (430.3–3,123.2)	29.0 (9.8–85.9)	1,280.0 (249.2–6,573.7)	34.3 (6.3–186.4)
	183	1,424.0 (832.7–2,435.3)	33.8 (14.0–81.3)	435.5 (131.9–1,438.1)	8.0 (3.0–21.7)	430.7 (63.3–2,932.0)	16.0 (3.3–76.9)
Arm B1	0	40.0 (17.7–90.4)		87.7 (32.8–234.4)		152.3 (54.1–428.6)	
	14	861.4 (429.0–1,729.4)	21.5 (7.8–59.4)	1,470.3 (996.2–2,170.1)	16.8 (5.7–49.4)	1,998.6 (1,054.2–3,789.2)	13.1 (3.9–44.5)
	21	1,902.1 (1,137.8–3,179.6)	47.6 (17.0–132.7)	3,620.4 (2,484.7–5,275.1)	45.3 (13.2–155.4)	2,181.6 (909.9–5,230.8)	16.0 (3.5–73.1)
	28	2,206.7 (1,371.5–3,550.4)	55.2 (21.2–143.7)	2,807.9 (1,919.9–4,106.6)	32.0 (9.8–104.9)	1,810.2 (856.2–3,827.3)	11.9 (2.8–50.8)
	183	1,280.0 (803.5–2,039.1)	28.2 (8.7–91.3)	1,424.0 (790.5–2,565.2)	23.2 (6.7–80.3)	1,350.1 (689.2–2,644.7)	7.2 (2.0–26.4)
Arm B2	0	551.7 (362.3–840.1)		769.9 (324.5–1,826.8)		819.8 (503.9–1,333.5)	
	14	861.4 (557.0–1,332.1)	1.6 (1.2–2.0)	1,992.0 (1,151.2–3,446.7)	1.8 (0.7–4.7)	1,485.0 (922.9–2,389.2)	1.8 (1.3–2.5)
	21	951.0 (531.8–1,700.8)	1.7 (1.2–2.5)	1,859.1 (1,197.5–2,886.1)	1.7 (0.7–3.9)	1,639.5 (1,063.4–2,527.8)	2.0 (1.3–3.0)
	28	819.8 (459.4–1,462.7)	1.5 (1.1–2.1)	1,424.0 (977.3–2,075)	1.3 (0.6–2.9)	1,413.2 (910.2–2,194.4)	1.7 (1.2–2.5)
	183	934.1 (552.4–1,579.5)	1.9 (1.3–2.8)	1,671.1 (1,117.4–2,499)	1.7 (0.8–3.8)	1,810.2 (1,059.6–3,092.6)	2.1 (1.2–3.9)

(Continued on next page)

Table 3. Continued

Vaccine arm	Day	Dose group					
		5 μg		10 μg		20 μg	
		GMT	GMFR	GMT	GMFR	GMT	GMFR
Omicron (B.1.1.529) variant							
Arm A	0	12.6 (9.2–17.2)		14.1 (9.7–20.6)		21.4 (8.6–53.7)	
	14	422.2 (193.8–919.8)	33.5 (12.7–88.2)	245.1 (73.6–816.7)	16.9 (5.5–51.4)	160.0 (31.6–808.9)	10.1 (2.2–45.5)
	21	351.0 (150.9–816.5)	27.9 (10.2–76.3)	185.6 (83.1–414.4)	13.1 (6.2–27.6)	211.1 (43.9–1,016.3)	9.8 (2.2–44.0)
	35	320.0 (153.2–668.2)	29.0 (13.5–62.4)	275.8 (129.5–587.3)	19.5 (8.6–44.4)	226.3 (65.3–783.8)	10.6 (3.0–37.3)
	183	337.5 (168.7–675.5)	25.9 (11.3–59.1)	108.9 (31.1–381.0)	8.0 (2.2–28.7)	107.7 (30.1–385.1)	5.9 (3.2–11.1)
Arm B1	0	12.2 (9.5–15.6)		15.9 (9.1–27.7)		62.5 (25.8–151.0)	
	14	97.5 (53.7–177)	8.0 (4.2–15.3)	291.8 (132.0–644.7)	18.4 (6.9–48.7)	353.3 (154.3–809.0)	5.7 (1.9–17.1)
	21	275.8 (159.5–477.0)	22.6 (12.6–40.5)	499.7 (292.7–853.0)	30.5 (13.6–68.3)	499.7 (249.3–1,001.2)	8.0 (2.9–22.4)
	28	320.0 (181.7–563.6)	26.3 (13.9–49.6)	583.5 (369.9–920.4)	36.8 (18.8–71.7)	371.2 (183.4–751.4)	5.9 (2.0–17.6)
	183	205.9 (117.4–361.0)	16.0 (8.9–28.8)	220.3 (120.0–404.4)	17.8 (8.0–39.6)	287.6 (163.8–505.1)	4.0 (1.7–9.2)
Arm B2	0	102.5 (54.0–194.3)		145.9 (60.5–352.0)		185.6 (109.8–313.7)	
	14	185.6 (100.7–342.2)	1.8 (1.3–2.6)	375.5 (245.7–573.9)	1.8 (1.0–3.4)	371.2 (219.6–627.5)	2.0 (1.5–2.6)
	21	176.7 (89.3–349.3)	1.7 (1.2–2.5)	337.5 (211.6–538.5)	1.6 (0.8–3.2)	336.2 (197.6–572.1)	1.8 (1.2–2.7)
	28	131.3 (65.7–262.2)	1.3 (1.0–1.7)	356.0 (214.1–592.0)	1.7 (0.9–3.2)	320.0 (185.8–551.2)	1.7 (1.0–3.0)
	183	181.5 (71.8–458.6)	1.9 (1.0–3.7)	356.0 (220.5–574.8)	2.0 (0.9–4.2)	557.2 (241.4–1,285.8)	2.6 (1.2–6.0)

Values are presented as mean (95% confidence interval). GMTs of neutralizing antibodies at day 0 is measured at pre-vaccination.
GMT, geometric mean titer; GMFR, geometric mean folding ratio.

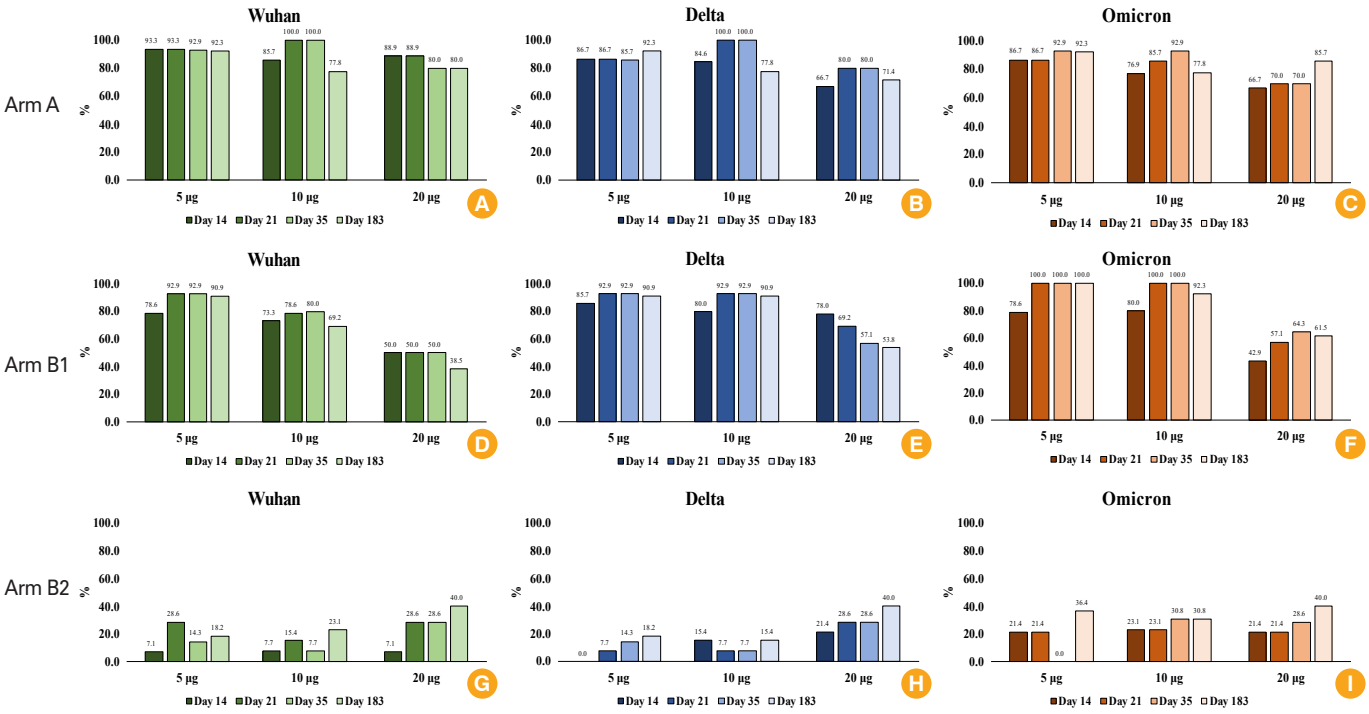

Fig. 2. (A–I) Seroconversion rates.

Cellular immunity

The analysis of cellular immunity was limited to days 0 (baseline) and day 21 for Arm A, and day 14 for Arms B1 and B2. The study measured mean levels of intracellular and extracellular cytokines (Fig. 3). A dose-dependent response was observed, with higher dosages leading to a more robust cellular immune response. In Arm A, most CD4+ T cell immunity variables (INF-γ, TNF-α, IL-4, IL-17a) increased on day 21 compared to baseline. However, for CD8+ T cell variables (INF-γ, TNF-α, IL-4, IL-17a), there was a decrease in the mean of all cellular immunity variables on day 21 compared to baseline in the 5 μg dose arm. A dose-dependent increase in mean levels of cytokines was observed from the 10 to 20 μg dose groups. In Arm B1 and B2, similar patterns of increasing cellular immune response corresponding to increasing vaccine dosage were observed. Cellular immunity results were only restricted to days 0 and 21 as very few of such tests were done at day 183.

Discussion

This first-in-human study of YS Biopharma Co. Ltd.'s PIKA COVID-19 vaccine did not find any safety concerns in all arms and dose level groups of the study. The vaccine-induced neutralizing antibody responses to all three variants of SARS-CoV-2 in subjects who had not previously been infected (Arm A) and boosted neutralizing antibody responses in subjects who had previously received a primary series of COVID-19 vaccination with either an inactivated (Arm B1) or mRNA (Arm B2) COVID-19 vaccine were promising. This study's safety and immunogenicity results are consistent with previous assessments on PIKA adjuvanted vaccines for other infections [19,20,22].

The PIKA COVID-19 vaccine showed no safety concerns, with no reported deaths in the study. Four participants experienced SAEs of mild COVID-19, all deemed unrelated to the vaccine. The most common reactions were injection site pain/tenderness and fever, which resolved within a few days. No significant SUSARs or AESIs were reported. The safety profile of the PIKA COVID-19 vaccine is comparable to that of other inactivated virus vaccines against SARS-CoV-2 [16, 23-26].

The PIKA COVID-19 vaccine induced a strong and lasting neutralizing antibody response against all three SARS-CoV-2 variants across all vaccine arms. Antibody levels increased significantly by day 14 and remained higher than baseline by day 183, with the 5 μg dose group showing the highest levels. Seroconversion rates for the PIKA COVID-19 vaccine were

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Fig. 3. (A) Cellular immunity, Arm A. **(B)** Cellular immunity, Arm B1. **(C)** Cellular immunity, Arm B2. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ag, antigen; IL, interleukin; INF-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha.

highest in dose groups 5 μg and 10 μg, especially in Arms A and B1 against all three variants (from 86% to 100%). The seroconversion rates were slightly lower for the 20 μg group, ranging from 66.7% to 88.9%. The seroconversion rates were also slightly lower in Arm B2 against all three variants. Higher baseline GMTs may explain the lowered seroconversion rates for the subjects in Arm B2. Nevertheless, the increase in antibody titers suggests that the test vaccine may still offer additional protection even in those previously inoculated with COVID-19 mRNA vaccines [27].

The PIKA COVID-19 vaccine's immunogenic properties in this phase I study were similar to other inactivated virus vaccines against COVID-19 [23,28]. The second dose yielded a 100% seroconversion rate for multiple COVID-19 variants. There was a decrease in seroconversion with the highest dose administered in this trial similar to what was observed in other trials [25,29]. This may be due to higher baseline values of neutralizing antibodies and the higher dose given for the maximum dose arm compared to other phase I studies. The PIKA COVID-19 vaccine's ability to elicit a dose-dependent cellular immune response is similar to other non-mRNA virus vaccines for COVID-19 [30,31].

This phase I clinical study showed promising immunogenicity findings for the PIKA COVID-19 vaccine, even without a control group. Pre- and post-vaccination GMTs were sufficient to suggest immunogenicity. The rise in antibody response preceding immunization was used to assess immunogenicity across different subject groups based on their COVID-19 vaccine history. These findings support the potential of the candidate vaccine to be used as a booster dose. The World Health Organization recommends any commercially available booster, and the PIKA COVID-19 vaccine can potentially alleviate vaccine supply issues, especially since there has been a recent surge in COVID-19 cases in some regions [32,33].

In many COVID-19 vaccine trials done, aluminum hydroxide was used as the adjuvant, and the agent was administered intramuscularly [34,35]. It was found that this adjuvant may not be the best choice when a cellular response is desired; furthermore, an adjuvant like PIKA that triggers dendritic cell maturation during antigen uptake to assist in the cell's cross-presentation capability may better assist in this process [36,37]. The ongoing phases II and III studies on this vaccine are expected to provide more data to substantiate this.

In conclusion, the PIKA-adjuvanted recombinant SARS-CoV-2 S protein subunit vaccine showed promising immunogenicity profile with no safety concerns. A dose-dependent immune response was observed, with slight advantages seen in low-dose (5 μg and 10 μg) groups. The ongoing phases II and III studies on this vaccine are expected to provide more data to support the potential of this candidate vaccine to be used as a booster dose.

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Supplementary Materials

Supplementary material is available at Clinical and Experimental Vaccine Research website (http://www.ecevr.org).

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