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Safety and immunogenicity of PIKA-adjuvanted recombinant SARS-CoV-2 spike protein subunit vaccine as a booster against SARS-CoV-2: a phase II, open-label, randomized, double-blinded study

Renan James Lim[1], Xiangyan Qiu[2], Edison Alberto[3], Maria Rosario Capeding[4], Josefina Carlos[5], Robert Neil Leong[1], Jose Limuel Gutierrez[1], Maricris Trillana[1], Yuan Liu[2], Zenaida Mojares[6]

[1]YS Biopharma Co. Ltd., Bonifacio Global City, Taguig, Philippines; [2]YS Biopharma (China) Co. Ltd., Beijing, China; [3]Clinical Research Center, Health Index Multispecialty Clinic, Imus; [4]Clinical Research Unit, Tropical Disease Foundation, Muntinlupa; [5]Research Center, University of the East Ramon Magsaysay Memorial Medical Center Inc., Quezon City, Philippines; [6]YS Biopharma (Singapore) Co. Ltd., Singapore, Singapore

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Corresponding author: Renan James Lim, MD
YS Biopharma (Philippines) Co. Ltd., 19th floor High Street South Corporate Plaza, 26th St., Bonifacio Global City, Taguig, Philippines
Tel: +63-9178972958
E-mail: renan.lim@lakeshorebio.com; renan.lim@yishengbio.com

Yuan Liu is listed as an inventor on patent applications for PIKA COVID-19 vaccine (PCT/CN2021/096048). Zenaida Mojares, Maricris Trillana, Renan James Lim, Xiangyan Qiu, Robert Neil Leong, and Jose Limuel Gutierrez are employees of YS Biopharma Co. Ltd. and have no stock options. Except for that, no potential conflict of interest relevant to this article was reported.

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Purpose: This study evaluated the safety and immunogenicity of the PIKA-adjuvanted recombinant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein subunit vaccine as a booster dose for healthy adults who had previously received two or more doses of an inactivated coronavirus disease 2019 (COVID-19) vaccine.

Materials and Methods: The study was a phase II multicenter, double-blinded, comparator-controlled, randomized trial. Participants were randomly assigned to receive either the PIKA COVID-19 vaccine booster dose or an inactivated COVID-19 vaccine (Sinovac, China). Safety was assessed based on adverse events, while immunogenicity was measured by neutralizing antibodies against SARS-CoV-2 and serum immunoglobulin G (IgG) levels. Data on safety and immunogenicity were collected in the short-term (within 14 days after the booster dose) and long-term (from 90 to 365 days after the booster dose).

Results: The PIKA-adjuvanted vaccine demonstrated a significant increase in neutralizing antibodies against the Omicron variant (geometric mean ratio [GMR]=2.0 on day 7, p-value <0.001; GMR=2.7 on day 14, p-value <0.001) and the wild type SARS-CoV-2 virus (GMR=2.3 on day 7, p-value <0.001; GMR=2.8 on day 14, p-value<0.001) in the early post-vaccination period when compared to the inactivated vaccine. Additionally, the PIKA COVID-19 vaccine showed higher seroconversion rates for neutralizing antibodies against both variants during the first 14 days post-vaccination. However, there were no significant differences in neutralizing antibody levels between the two vaccines from day 90 to day 360 post-vaccination. Serum IgG antibody levels for the PIKA COVID-19 vaccine were also higher throughout the study period. The incidence of adverse events was slightly higher in the PIKA COVID-19 group, with the most common events being pain at the injection site and headache. All adverse events were mild or moderate, with no reports of severe or life-threatening adverse events in either group.

Conclusion: The PIKA COVID-19 vaccine, when administered as a booster dose, showed promising short- and long-term immunogenicity with no emergent safety issues identified. The booster dose of the PIKA COVID-19 vaccine elicited a robust immune response against various SARS-CoV-2 variants and provided some seroprotection for up to 360 days (ClinicalTrials.gov registration number: NCT05463419).

Keywords: Vaccine, COVID-19, PIKA adjuvant, Recombinant subunit COVID-19 vaccine, Phase II clinical trial

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for causing coronavirus disease 2019 (COVID-19), has sparked the third major coronavirus outbreak in the last two decades [1]. Even with the administration of nearly 13.58 billion vaccine doses as of July 2024, SARS-CoV-2 continues to spread globally due to ongoing transmission and waning immune responses over time [2,3]. Consequently, there is an urgent need to develop safe and effective vaccines capable of eliciting rapid, potent, and enduring immune responses [4].

Recently, a new vaccine, the refined polyinosinic-polycytidylic acid (Poly:C) stabilized with kanamycin and calcium-(PIKA) adjuvanted recombinant SARS-CoV-2 spike (S) protein subunit vaccine, has been developed to combat COVID-19 [5-7]. It contains a recombinant S protein antigen (5 μg/mL) as a trimer based on the coronavirus S protein and a novel double-stranded RNA (dsRNA) PIKA adjuvant (1 mg). He PIKA adjuvant is a chemically synthesized double-stranded RNA analogue comprising double-stranded polyinosinic acid-polycytidylic acid, kanamycin monosulfate, and calcium chloride. It functions as an agonist of toll-like receptor 3, a crucial receptor for recognizing dsRNA viral infections and activating the innate immune system [6,7]. The S protein plays a crucial role in the SARS-CoV-2 viral cycle by binding to human angiotensin-converting enzyme 2 (ACE2) to enter the body [8-10]. The PIKA COVID-19 vaccine enhances the production of neutralizing antibodies against the SARS-CoV-2 S protein, thereby preventing viral entry and subsequent infection. Preclinical and phase I clinical studies have demonstrated its potential to induce both humoral and cellular rapid, robust, and long-lasting immune responses [5,11,12].

This phase II study evaluated the safety and immunogenicity of a booster dose of the PIKA COVID-19 vaccine in adults aged 18 years and over who have received two or more doses of inactivated COVID-19 vaccine as their primary series. The objectives included assessing the vaccine's safety and immune response in the short term (primary) for the first 28 days after vaccination and in the long term (secondary) from 90 up to 360 days.

Materials and Methods

Study design

This phase II study was conducted at three sites in the Philippines: the Health Index Multispecialty Clinic, Clinical Research Center; the Tropical Disease Foundation, Clinical Research Unit; and the University of the East Ramon Magsaysay Research Center. Participants were randomly assigned in a 1:1 ratio, with stratification based on age groups (≥18–<60 years old versus ≥60 years old) to receive either a booster dose of the PIKA COVID-19 vaccine (group 1) or the inactivated COVID-19 vaccine, Sinovac (Beijing, China) (group 2).

Subject enrollment and randomization

A total of 300 participants were enrolled in the study. Eligible participants were healthy adults aged 18 years or older who had previously received two or more doses of an inactivated COVID-19 vaccine. They tested negative for SARS-CoV-2 through nasopharyngeal swabs using reverse transcription-polymerase chain reaction at the time of enrollment, had a body temperature of ≤37.5°C, had no known allergy to any component of the two vaccines, and had not been on any systemic immunosuppressants or other immunomodulators in the 30 days prior to enrollment. Vaccine group assignment was based on a randomization schedule centrally managed by a validated Interactive Web Response System and prepared by an independent statistician for the study team.

Vaccine formulation

The investigational PIKA COVID-19 vaccine contains 5 μg of the SARS-CoV-2 S subunit protein, 1.0 mg/mL of the PIKA adjuvant, and other components. This vaccine is provided in a freeze-dried powder form and must be mixed with a clear, colorless solution before administration. The standard vial volume is 1 mL.

The antigen used in this candidate vaccine is a recombinant SARS-CoV-2 S protein, produced in Chinese hamster ovary cells. The S protein plays a crucial role in mediating the virus's entry into human cells by binding to the ACE2 receptor via its receptor binding domain (RBD) and undergoing proteolytic activation by human proteases. This RBD exhibits high affinity for the human ACE2 receptor, facilitating efficient viral entry. Additionally, the S protein's pre-activation by the proprotein convertase furin may reduce its reliance on target cell proteases for entry, potentially aiding immune evasion by the virus. The exact S sequence used in this study is based on the original Wuhan strain. Although some variants carry mutations in the S protein, previous studies suggest that vaccines based on the ancestral strain can still generate cross-reactive antibodies, potentially due to shared epitopes be-

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tween variants [13,14].

Vaccine schedule

Following randomization and confirmation of subject eligibility, a singular dose of either the PIKA COVID-19 vaccine or the inactivated COVID-19 vaccine, according to the assigned vaccine group, was administered in the deltoid muscle on study day 0.

Study endpoints

The primary immunogenicity endpoint of the study was the geometric mean titer (GMT) of neutralizing antibodies against the Omicron virus 14 days after the booster dose administration. Secondary endpoints included evaluating the seroconversion rate on day 14 and early-term and long-term immunogenicity endpoints. Early-term immunogenicity was measured using GMT of neutralizing antibodies, seroconversion rates, and geometric mean titer fold growth ratio (GMFR) of neutralizing antibodies against both the wild-type SARS-CoV-2 virus and the Omicron variant, as well as GMT and seroconversion rate of serum immunoglobulin G (IgG) antibodies on day 7. Long-term immunogenicity was assessed on days 90, 180, and 360 using the same endpoints. Seroconversion was defined as at least a four-fold increase in antibody titer from baseline.

Safety endpoints examined were the incidence of expected local and systemic adverse events (AEs) for 7 days following the booster dose, unsolicited AEs and medically attended AEs (MAAEs) for 28 days after the booster dose, and serious adverse events (SAEs), suspected unexpected serious adverse reactions (SUSARs), and adverse events of special interest (AESIs) for up to 360 days after the booster dose.

Study procedures for safety monitoring and follow-up

Following the vaccine's administration, all participants were monitored for a minimum of 30 minutes. Any solicited AEs were recorded over the next 7 days, while unsolicited AEs and MAAEs were collected for 28 days. SAEs, SUSARs, and AESIs were documented throughout the entire duration of the study.

Furthermore, blood samples for assessing immunogenicity were collected on days 0, 14, 90, 180, and 360 for all participants. To facilitate early immunogenicity assessment, an additional subset of the first 100 randomized subjects underwent blood sampling on day 7.

Statistical analysis

Immunogenicity analysis set consisted of all randomized subjects who received a booster dose of the study vaccines and contributed valid results for both pre-and at least one post-vaccination blood samples. Log-transformed neutralizing antibody titers were analyzed using the analysis of covariance (ANCOVA) method. The ANCOVA model controlled for the age group and log-transformed baseline titer. Geometric mean ratios (GMRs) were reported based on the exponentially back-transformed least squares mean differences between the log-transformed neutralizing antibody titers. Seroconversion rates were presented as percentages and were compared between the two vaccine groups using the Cochran-Mantel-Haenszel method to allow stratified analysis by age group.

Safety analysis was performed on all subjects who received booster dose of the study vaccines. Safety parameters were summarized per vaccine group using descriptive statistics. All statistical analyses were performed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA). Whenever applicable, 95% confidence intervals (CIs) were calculated.

Ethical approval

The study protocol and study-related documents were approved by the Single Joint Ethics Review Board of the Philippines with document approval identifier: SJREB 2022-62. 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 (GCP) and applicable national and institutional regulations and guidelines which govern GCP operations. All subjects provided written informed consent to be part of the study (ClinicalTrials.gov registration number: NCT05463419).

Results

Subject disposition and baseline demographic characteristics

Overall, 426 subjects were screened before reaching the enrollment target of 300, with 150 randomized into each vaccine group. Of these 300 subjects, only 294 were able to complete the study; the four subjects who did not finish the study were due to consent withdrawal (n=1), reason not disclosed (n=1), or lost to follow-up (n=2) (Fig. 1).

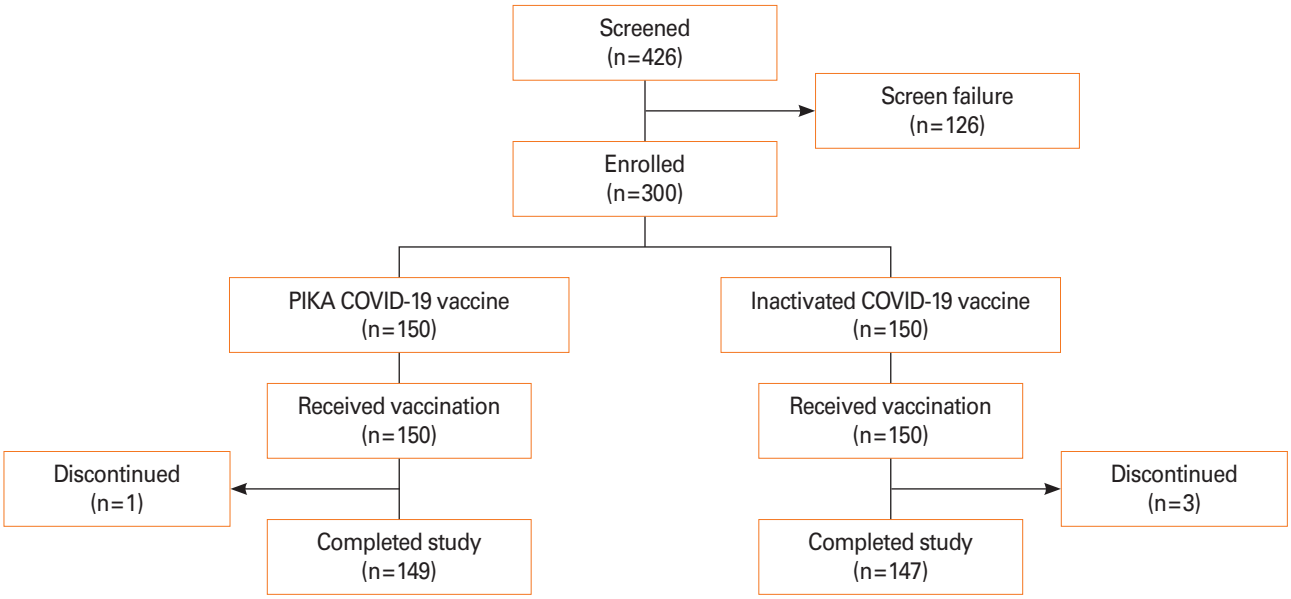

Fig. 1. Subject enrollment flow diagram. COVID-19, coronavirus disease 2019.

The baseline demographic characteristics of the subjects were consistent between the two vaccine groups. The majority (98.7%) of subjects were under the age of 60 years, with a higher proportion being female (60.3%). Of these females, 93.4% of were of childbearing potential. All participants were of Asian ethnicity, and the average body mass index was 23.615 kg/m^2 (Table 1).

Table 1. Subject demographic characteristics

Characteristic	PIKA COVID-19 vaccine (n=150)	Inactivated COVID-19 vaccine (n=150)	Overall (n=300)
Age at enrollment (yr)	30.8±10.7	30.4±9.2	30.6±10.0
<60	147 (98.0)	149 (99.3)	296 (98.7)
≥60	3 (2.0)	1 (0.7)	4 (1.3)
Gender			
Male	63 (42.0)	56 (37.3)	119 (39.7)
Female	87 (58.0)	94 (62.7)	181 (60.3)
Childbearing potential[a]			
Of childbearing potential	81 (93.1)	88 (93.6)	169 (93.4)
Postmenopausal	6 (6.9)	5 (5.3)	11 (6.1)
Permanently sterilized	0	1 (1.1)	1 (0.6)
Race			
Asian	150 (100.0)	150 (100.0)	300 (100.0)
Weight (kg)	58.56±12.22	60.22±12.78	59.39±12.51
Height (cm)	158.82±7.70	158.28±7.89	158.55±7.79
Body mass index (kg/m^2)	23.23±4.81	24.01±4.56	23.62±4.69

Values are presented as mean±standard deviation or number (%).
COVID-19, coronavirus disease 2019.
[a]For childbearing potential, percentages are based on the number of female subjects.

Immunogenicity

SARS-CoV-2 neutralizing antibody against omicron variant

Baseline GMTs on day 0 (pre-vaccination) of neutralizing antibodies against the Omicron variant were similar for both the PIKA and inactivated vaccine groups, with levels of 252.4 IU/mL (95% CI, 205.4–310.2 IU/mL) and 282.6 IU/mL (95% CI, 230.1–346.9 IU/mL), respectively.

Following the booster dose, the PIKA vaccine group demonstrated notably higher levels of neutralizing antibodies against the Omicron variant early on, particularly on days 7 and 14 post-vaccination. On day 14, the PIKA group had a GMT of 749.5 IU/mL (95% CI, 458.0–1,226.6 IU/mL), compared to 280.2 IU/mL (95% CI, 166.8–470.6 IU/mL) in the inactivated group, with a GMR of 2.7 ($p<0.001$). Similarly, on day 7, the GMT for the PIKA group was 371.5 IU/mL (95% CI, 219.6–628.5 IU/mL), while for the inactivated group, it was 185.1 IU/mL (95% CI, 105.1–326.1 IU/mL), with GMR=2.0 ($p<0.001$) (Fig. 2). GMT fold growths of neutralizing antibodies against the Omicron variant from day 0 for the PIKA vaccine group compared to the inactivated vaccine group were 1.5 versus 0.7 (GMFR=2.0, $p<0.001$) on day 7 and 2.7 versus 1.0 (GMFR=2.7, $p<0.001$) on day 14. Furthermore, seroconversion rates for neutralizing antibodies against the Omicron virus from day 0 to days 7 and 14 were also significantly higher in the PIKA vaccine group compared to the inactivated

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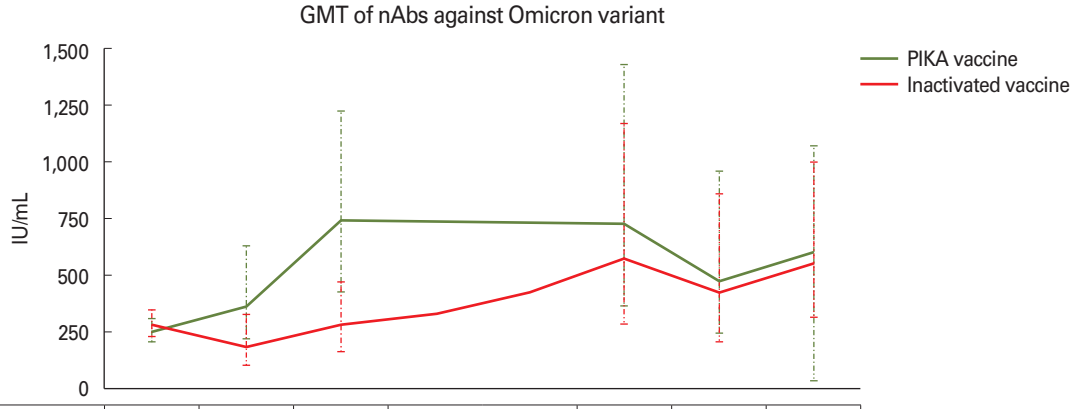


GMFR (From baseline)	Day 0	Day 7	Day 14	...	...	Day 90	Day 180	Day 360
PIKA		1.5	2.7			2.7	1.8	2.3
Inactivated		0.7	1.0			2.1	1.6	2.1
GMR (PIKA vs. inactivated)		2.0[a)]	2.7[a)]			1.3	1.1	1.1

Fig. 2. Geometric mean titers of neutralizing antibodies against Omicron variant at different timepoints. GMT, geometric mean titer; GMFR, geometric mean titer fold growth ratio; GMR, geometric mean ratio. [a)]Significantly higher GMT for PIKA vaccine at $\alpha=5\%$.

vaccine group: 37.5% versus 4.2% on day 7 ($p<0.001$) and 45.1% versus 12.3% on day 14 ($p<0.001$). Altogether, these support the claim of early onset of significantly higher seroprotection by the PIKA vaccine as a booster dose against the COVID-19 Omicron variant.

Conversely, there were no significant differences in neutralizing antibody levels between the two groups on days 90, 180, and 360 post-vaccination, with GMRs between 1.1 and 1.3 ($p=0.114$, $p=0.349$, and $p=0.447$, respectively). However, the GMT levels among PIKA vaccine recipients remained at least 485 IU/mL which was higher than at baseline pre-vaccination, potentially indicating sustained seroprotection as a booster dose for at least a year post-vaccination (Fig. 2).

SARS-CoV-2 neutralizing antibody against wild type SARS-CoV-2 variant

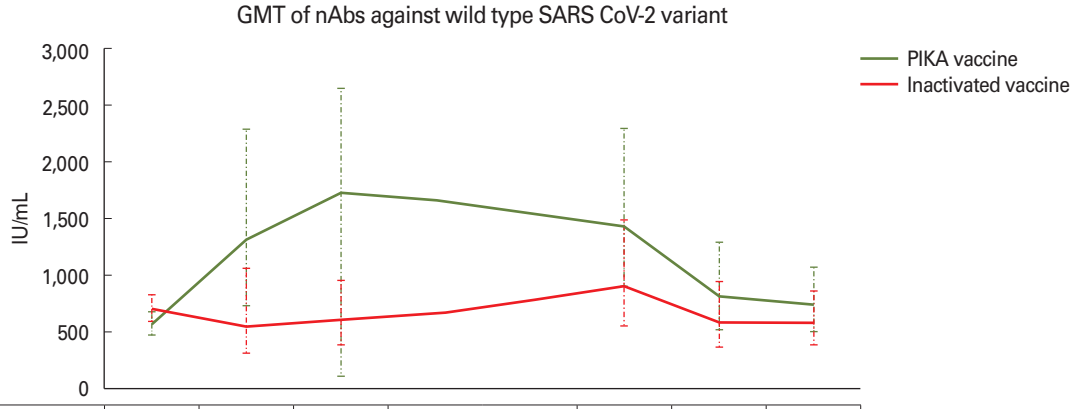
Baseline GMTs on day 0 (pre-vaccination) of neutralizing antibodies against the wild type SARS-CoV-2 variant were similar for both the PIKA and inactivated vaccine groups, with levels of 564.9 IU/mL (95% CI, 467.7–682.2 IU/mL) and 703.6 IU/mL (95% CI, 594.7–832.6 IU/mL), respectively. Following the booster dose, the PIKA vaccine group not only again demonstrated notably higher levels of neutralizing antibodies against the wild type SARS-CoV-2 variant early on days 7 and 14 post-vaccination but also showed significantly higher GMTs on days 90 up to 360 post-vaccination. Moreover, the GMT levels among PIKA vaccine recipients remained at least 734.5 IU/mL, which was higher than at baseline pre-vaccination (at least GMFR=1.1 compared to baseline) (Fig. 3). When administered as a booster dose, the PIKA vaccine demonstrated early onset and durable and sustained seroprotection up to 360 days post-vaccination against the COVID-19 wild type SARS-CoV-2 variant.

Serum immunoglobulin antibody

The GMT of serum IgG antibody in the PIKA vaccine group was significantly higher than in the inactivated vaccine group on days 7, 14, 90, and 180 post-vaccination. GMRs were 3.1 ($p<0.001$), 3.4 ($p<0.001$), 1.8 ($p<0.001$), and 1.7 ($p<0.001$), respectively, on these timepoints. Similarly, the seroconversion rates of serum IgG antibody in the PIKA vaccine group were significantly higher than in the inactivated vaccine group on day 7 (44.9% versus 2.0%, $p<0.001$) and day 14 (45.3% versus 4.3%, $p<0.001$) post-vaccination (Table 2).

Safety

A total of 106 subjects (35.3%) experienced 394 AEs, with 55 subjects (36.7%) in the PIKA vaccine group and 51 subjects (34.0%) in the inactivated vaccine group reporting a total of 192 and 202 events, respectively. Among these, 96 subjects (32.0%) reported 361 instances of solicited AEs, with 51 subjects (34.0%) in the PIKA vaccine group and 45 subjects



GMFR (From baseline)	Day 0	Day 7	Day 14	...	...	Day 90	Day 180	Day 360
PIKA		2.2	2.7			2.2	1.3	1.1
Inactivated		1.0	1.0			1.4	0.9	0.9
GMR (PIKA vs. inactivated)		2.3[a)]	2.8[a)]			1.6[a)]	1.4[a)]	1.3[a)]

Days since vaccination

Fig. 3. Geometric mean titers of neutralizing antibodies against wild type severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant at different timepoints. GMFR, geometric mean titer fold growth ratio; GMR, geometric mean ratio; GMT, geometric mean titer. [a)]Significantly higher GMT for PIKA vaccine at α=5%.

(30.0%) in the inactivated vaccine group. Most frequently reported solicited AE was pain at injection site which was also higher in the PIKA vaccine group compared to the inactivated vaccine group (30.0% versus 20.7%). Incidence of solicited systemic reactions were consistent between the two groups, and the most frequently reported of which were headache (14.7% versus 13.3%) and fatigue (7.3% versus 10.7%). All solicited AEs were only mild or moderate.

Twenty-six (8.7%) reported a total of 33 unsolicited AEs, with 12 subjects (8.0%) in the PIKA vaccine group and 14 subjects (9.3%) in the inactivated vaccine group. The most frequently reported unsolicited AEs were related to infections and infestations. All unsolicited AEs were either mild or moderate. Two subjects, however, experienced unsolicited AEs which were assessed as vaccine-related: hyperfibrinogenemia for the one in the PIKA vaccine group, hypersensitivity for the one in the inactivated vaccine group.

Notably, no subjects experienced MAAEs in the PIKA vaccine group, while three subjects experienced four MAAEs in the inactivated group, one of which assessed as inactivated vaccine-related (hypersensitivity). There were no SAEs. SUSARs, nor AESIs reported in the study. No clinically significant abnormalities were also found in clinical laboratory examinations, vital signs, nor physical examinations. Lastly, no AEs leading to death nor study discontinuation were also reported.

Discussion

The PIKA COVID-19 vaccine, comprising a recombinant S-trimer protein antigen and the innovative double-stranded RNA PIKA adjuvant, has shown promising results in preclinical and our phase I studies [5-7,11,12]. The S-trimer protein has demonstrated a strong binding affinity to human ACE2 [9,10], while the PIKA adjuvant has elicited a more potent immune response compared to other adjuvants [7]. This phase II study further confirmed the main findings of our phase I study [12]. When administered as a booster vaccine, the PIKA COVID-19 vaccine exhibited a favorable safety and immunogenicity profile against various SARS-CoV-2 variants such as Omicron and the wild type.

Our phase II study demonstrated that a booster of this PIKA COVID-19 vaccine generated a stronger humoral immune response to the Omicron virus and wild type SARS-CoV-2 virus compared to the inactivated COVID-19 vaccine, with rapid and enduring immune responses observed. The faster and more robust neutralizing antibody titers induced by PIKA COVID-19 vaccine suggest that this may serve as a promising booster to effectively combat diverse SARS-CoV-2 variants, including the immune-evading Omicron variant and its sublineages [13,14].

In response to the emergence of immune-evading variants

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Table 2. Geometric mean titer levels of serum immunoglobulin antibody and seroconversion from baseline, when applicable, at different timepoints

Timepoint and statistic	PIKA COVID-19 vaccine (n=150)	Inactivated COVID-19 vaccine (n=150)
Day 0 (baseline, pre-vaccination)		
GMT (IU/mL)	240.2	241.3
95% CI	(203.5–283.5)	(202.3–287.8)
Day 7		
GMT (IU/mL)	850.1	273.3
95% CI	(558.3–1,294.4)	(176.9–422.0)
GMR (PIKA vs. inactivated)	3.1	
p-value, GMR	<0.001	
SCR (%)	44.9	2.0
95% CI	(30.7–59.8)	(0.0–10.4)
p-value, SCR difference	<0.001	
Day 14		
GMT (IU/mL)	1,242.9	365.3
95% CI	(874.3–1,766.7)	(254.2–525.1)
GMR (PIKA vs. inactivated)	3.4	
p-value	<0.001	
SCR (%)	45.3	4.3
95% CI	(36.1–54.8)	(1.4–9.7)
p-value, SCR difference	<0.001	
Day 90		
GMT (IU/mL)	1,184.2	656.9
95% CI	(705.4–1,987.7)	(386.2–1,117.1)
GMR (PIKA vs. inactivated)	1.8	
p-value	<0.001	
Day 180		
GMT (IU/mL)	1,347.3	810.7
95% CI	(914.1–1,985.9)	(544.5–1,206.9)
GMR (PIKA vs. inactivated)	1.7	
p-value	<0.001	

Values are presented as mean±standard deviation or number (%).
COVID-19, coronavirus disease 2019; GMT, geometric mean titer; CI, confidence interval; GMR, geometric mean ratio; SCR, seroconversion rate.

of SARS-CoV-2, specific vaccines have been developed utilizing various technologies, including inactivated virus-based, messenger RNA-based, and recombinant protein-based approaches [4,15]. These vaccines target updated variants such as Beta, Delta, Omicron BA.1, or Omicron BA.2. Additionally, research has explored the combination of variant-specific and prototype vaccines to evoke wide-ranging responses against diverse SARS-CoV-2 strains [16-18]. Nonetheless, ensuring the safety and tolerability of these vaccines remains a significant concern. The unpredictable evolution of SARS-CoV-2 can lead to increased transmissibility, immune evasion, and virulence in the future. Developing new variant-specific vaccines for each occurrence may not be sufficiently expedient [13,15,18]. Consequently, it is imperative to develop technologies that can effectively address the majority of variants while ensuring the safety and tolerability of these vaccines, including as boosters, for a wide demographic. From this study, our PIKA COVID-19 vaccine holds promise in meeting these crucial criteria.

SARS-CoV-2 infection has been associated with specific patterns of anti-S IgG1 Fc glycosylation [1,9,10,19]. In severe COVID-19 cases, there has been an observed increase in afucosylated anti-S IgG, particularly during seroconversion [20,21]. IgG is believed to play a key role in helping to protect against infection by various SARS-CoV-2 variants and in mitigating the progression of COVID-19. Given that our PIKA COVID-19 vaccine utilizes a recombinant antigen and the PIKA adjuvant, it is reasonable to hypothesize that the vaccine may contribute to generating IgG responses against the S protein, similar to those seen in natural infection. While this hypothesis requires further validation in future clinical studies, it provides a basis for early indications that our vaccine could offer protection against infection from different SARS-CoV-2 variants and potentially reduce the severity of COVID-19. Furthermore, the safety of our vaccine is attributed to the use of PIKA as the adjuvant, which has been found to have a tolerable and comparable, if not more favorable, safety profile to other COVID-19 vaccines [22-24]. Thus, our vaccine development approach of combining the PIKA adjuvant with a well-designed antigen represents a safe and immunogenic option to support large-scale booster vaccination efforts.

Our study comes with certain limitations. Firstly, only a small percentage of subjects underwent early immunogenicity testing, which may not fully reveal the extent of the rapid immune response. Secondly, the trials primarily involved an Asian population, with a minimal number of elderly individuals, making it challenging to generalize the findings to other racial groups and older age categories. Thirdly, the PIKA COVID-19 vaccine, while relatively broad-reactive, may require periodic assessments and updates to maintain high neutralizing antibody titers in response to new SARS-CoV-2 variants with significant antigenic changes [14,25]. However, the clinical trial design, randomization, and statistical methods may have helped address some of these limitations, particularly reducing the impact of biases and potentially confounding factors. Furthermore, our ongoing phase III study, planned to be conducted in several countries, aims to analyze more end-

points, including vaccine effectiveness against actual COVID-19 infection, which should help address some of these limitations.

In conclusion, the PIKA COVID-19 vaccine, when administered as a booster dose, showed promising short- and long-term immunogenicity with no emergent safety issues identified. The booster dose of the PIKA COVID-19 vaccine elicited a robust immune response against various SARS-CoV-2 variants and provided some seroprotection for up to 360 days. The ongoing phase III study of this vaccine is expected to provide more data to further establish the early and long-lasting vaccine-induced protection and safety profile of this vaccine, supporting its potential as a booster dose.

ORCID

Renan James Lim *https://orcid.org/0009-0007-4521-3009*
Xiangyan Qiu *https://orcid.org/0009-0005-5914-4361*
Edison Alberto *https://orcid.org/0000-0002-0416-2221*
Maria Rosario Capeding *https://orcid.org/0000-0002-1646-6521*
Josefina Carlos *https://orcid.org/0000-0002-2680-4918*
Robert Neil Leong *https://orcid.org/0000-0003-2230-9247*
Jose Limuel Gutierrez *https://orcid.org/0009-0003-3509-2363*
Maricris Trillana *https://orcid.org/0009-0002-8354-5972*
Yuan Liu *https://orcid.org/0000-0003-1204-9648*
Zenaida Mojares *https://orcid.org/0000-0003-1632-9268*

References

1. Ganesh B, Rajakumar T, Malathi M, et al. Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: an updated overview of current knowledge and future perspectives. Clin Epidemiol Glob Health 2021;10:100694.
2. World Health Organization. WHO COVID-19 Dashboard. Geneva: World Health Organization; 2020.
3. World Health Organization. COVID-19 advice for the public: getting vaccinated. Geneva: World Health Organization; 2023.
4. Nagy A, Alhatlani B. An overview of current COVID-19 vaccine platforms. Comput Struct Biotechnol J 2021;19:2508-17.
5. Lau YF, Tang LH, McCall AW, Ooi EE, Subbarao K. An adjuvant for the induction of potent, protective humoral responses to an H5N1 influenza virus vaccine with antigen-sparing effect in mice. J Virol 2010;84:8639-49.
6. Lau YF, Tang LH, Ooi EE, Subbarao K. Activation of the innate immune system provides broad-spectrum protection against influenza A viruses with pandemic potential in mice. Virology 2010;406:80-7.
7. Shen E, Li L, Li L, et al. PIKA as an adjuvant enhances specific humoral and cellular immune responses following the vaccination of mice with HBsAg plus PIKA. Cell Mol Immunol 2007;4:113-20.
8. Bezbaruah R, Borah P, Kakoti BB, et al. Developmental landscape of potential vaccine candidates based on viral vector for prophylaxis of COVID-19. Front Mol Biosci 2021;8:635337.
9. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 2020;41:1141-9.
10. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281-92.
11. Gai WW, Zhang Y, Zhou DH, Chen YQ, Yang JY, Yan HM. PIKA provides an adjuvant effect to induce strong mucosal and systemic humoral immunity against SARS-CoV. Virol Sin 2011;26:81-94.
12. Liu Y, Tan LH, Zhang N, Zhang Y, Mojares ZR. Safety, tolerability, and immunogenicity of PIKA-adjuvanted recombinant SARS-CoV-2 spike (S) protein subunit vaccine in healthy adults: interim results of an open-label and randomised phase 1 clinical trial. medRxiv [Preprint] 2022 Nov 22. https://doi.org/10.1101/2022.11.20.22282565
13. Chakraborty C, Sharma AR, Bhattacharya M, Lee SS. A detailed overview of immune escape, antibody escape, partial vaccine escape of SARS-CoV-2 and their emerging variants with escape mutations. Front Immunol 2022;13:801522.
14. Chatterjee S, Bhattacharya M, Nag S, Dhama K, Chakraborty C. A detailed overview of SARS-CoV-2 Omicron: its sub-variants, mutations and pathophysiology, clinical characteristics, immunological landscape, immune escape, and therapies. Viruses 2023;15:167.
15. Chi WY, Li YD, Huang HC, et al. COVID-19 vaccine update: vaccine effectiveness, SARS-CoV-2 variants, boosters, adverse effects, and immune correlates of protection. J Biomed Sci 2022;29:82.
16. Ghildiyal T, Rai N, Mishra Rawat J, et al. Challenges in emerging vaccines and future promising candidates

against SARS-CoV-2 variants. J Immunol Res 2024;2024:9125398.
17. Wang Z, An J, Liu K, et al. A potent, broadly protective vaccine against SARS-CoV-2 variants of concern. NPJ Vaccines 2022;7:144.
18. Zhao F, Zai X, Zhang Z, Xu J, Chen W. Challenges and developments in universal vaccine design against SARS-CoV-2 variants. NPJ Vaccines 2022;7:167.
19. Larsen MD, de Graaf EL, Sonneveld ME, et al. Afucosylated IgG characterizes enveloped viral responses and correlates with COVID-19 severity. Science 2021;371:eabc8378.
20. Pongracz T, Nouta J, Wang W, et al. Immunoglobulin G1 Fc glycosylation as an early hallmark of severe COVID-19. EBioMedicine 2022;78:103957.
21. Siekman SL, Pongracz T, Wang W, et al. The IgG glycome of SARS-CoV-2 infected individuals reflects disease course and severity. Front Immunol 2022;13:993354.
22. Asyura MM, Chandra AP, Agussalim A, Soloan G. Immunogenicity and safety analysis of inactivated virus vaccine against SARS-CoV-2: a systematic review of phase 1/2 clinical trials. J Asian Med Stud Assoc 2021;9:169-84.
23. Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:803-12.
24. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:181-92.
25. Hattab D, Amer MF, Al-Alami ZM, Bakhtiar A. SARS-CoV-2 journey: from Alpha variant to Omicron and its sub-variants. Infection 2024;52:767-86.