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Safety, Tolerability, and Immunogenicity of an mRNA COVID-19 Vaccine Incorporating the Novel 5′ Cap Analogue SmartCap® SC101: Results from a First-in-Human Phase I Dose-Escalation Study

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Abstract

Background/Objectives: Messenger RNA (mRNA) vaccines have enabled rapid vaccine development and have become an important platform for pandemic preparedness; however, clinical evaluation of alternative co-transcriptional 5′ cap technologies remains limited. Here, we report the first-in-human safety, tolerability, and immunogenicity of STP2104, a SARS-CoV-2 mRNA vaccine incorporating the novel co-transcriptional 5′ cap analogue SmartCap® SC101. **Methods:** In this Phase I, multicenter, open-label, dose-escalation study, 30 healthy adults with no known history of SARS-CoV-2 infection received two intramuscular doses of STP2104 at either 25 μg or 50 μg on Days 0 and 28. Safety and tolerability were evaluated, along with humoral and cellular immune responses, including neutralizing antibodies, binding antibodies, and antigen-specific IFN-γ ELISpot responses. All participants completed the planned two-dose vaccination schedule before early study termination. **Results:** STP2104 demonstrated an acceptable short-term safety and tolerability profile across both dose cohorts, with no treatment-emergent serious adverse events, Grade ≥ 3 treatment-emergent adverse events under the protocol-defined clinical severity criteria, or events leading to study discontinuation. However, one participant experienced a transient Grade 3 platelet-count laboratory abnormality, which was recorded in the Clinical Study Report (CSR) as a Grade 1 thrombocytopenia treatment-emergent adverse event. Neutralizing antibody titers increased following vaccination in both dose cohorts, reaching their post-boost maximum at Day 35 and remaining above baseline at Day 56. Binding-antibody concentrations also increased following vaccination, and antigen-specific IFN-γ ELISpot responses were detected in both dose cohorts. **Conclusions:** This first-in-human Phase I study shows that SmartCap® SC101 can be incorporated into an mRNA–LNP vaccine construct for clinical evaluation. STP2104 had an acceptable short-term safety and tolerability profile and induced measurable humoral and

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cellular immune responses. These results support further clinical evaluation of SmartCap® SC101-containing mRNA constructs with updated or alternative antigens.

Keywords: mRNA vaccine; COVID-19; SARS-CoV-2; First-in-human; Phase I clinical trial; 5′ cap analogue; co-transcriptional capping; SmartCap® SC101

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in late 2019, rapidly spread worldwide and led to the coronavirus disease 2019 (COVID-19) pandemic [1]. The need for vaccines that could be designed and manufactured rapidly accelerated the clinical development of messenger RNA (mRNA) vaccine technologies. The authorization and widespread use of the mRNA–lipid nanoparticle (LNP) vaccines BNT162b2 and mRNA-1273 subsequently established mRNA–LNP vaccination as a clinically validated modality [2,3]. Clinical trials and real-world studies demonstrated substantial protection against symptomatic and severe COVID-19, particularly during the earlier phases of the pandemic [2–4].

COVID-19 RNA vaccine development continued beyond the first ancestral strain-based vaccines. Variant-adapted formulations were introduced as SARS-CoV-2 evolved, and self-amplifying RNA (saRNA) vaccines subsequently advanced into clinical and regulatory use [5–7]. Selected examples illustrating this progression are summarized in Appendix A Table A1. This evolution highlights an important feature of RNA vaccines: the encoded antigen can be modified while retaining much of the underlying RNA production and delivery framework. The performance of an RNA vaccine, however, depends on more than the encoded antigen sequence. RNA design, 5′ capping, untranslated regions, nucleotide composition, purification, and delivery collectively influence mRNA stability, translation, innate immune recognition, and ultimately vaccine performance [8].

Among these components, the 5′ cap is an essential structural feature of eukaryotic mRNA that contributes to mRNA stability and efficient translation and participates in the discrimination of self from non-self RNA [9]. For synthetic mRNA, capping can be performed enzymatically after in vitro transcription or through incorporation of a cap analogue during transcription. Co-transcriptional capping reduces the number of processing steps, and CleanCap® reagents represent an established approach for generating Cap 1 mRNA [10]. Co-transcriptional Cap 1 capping has also been employed in clinically validated mRNA vaccines, including BNT162b2 [11]. Despite the central role of the 5′ cap in mRNA design, clinical experience with alternative co-transcriptional cap analogues remains limited.

We previously developed a Capping Library Screening (CLS) system to evaluate more than 30 cap analogues and selected SmartCap® SC101 for further development [12]. Candidate caps were evaluated for mRNA synthesis, capping efficiency, and protein-expression assays. SC101 showed capping efficiency and protein-expression performance comparable to those obtained with the established co-transcriptional capping reagent CleanCap®, although relative expression varied with the encoded sequence and experimental system [12]. SC101 was subsequently incorporated into STP2104, an LNP-formulated mRNA vaccine encoding the full-length spike antigen of ancestral SARS-CoV-2. Preclinical studies of STP2104 demonstrated spike expression, humoral and cellular immune responses, neutralizing activity, and protection in a SARS-CoV-2 challenge model using hACE2-transgenic mice [12].

These findings supported clinical evaluation of STP2104. A first-in-human Phase I study was conducted in healthy adults using two intramuscular doses of 25 μg or 50 μg

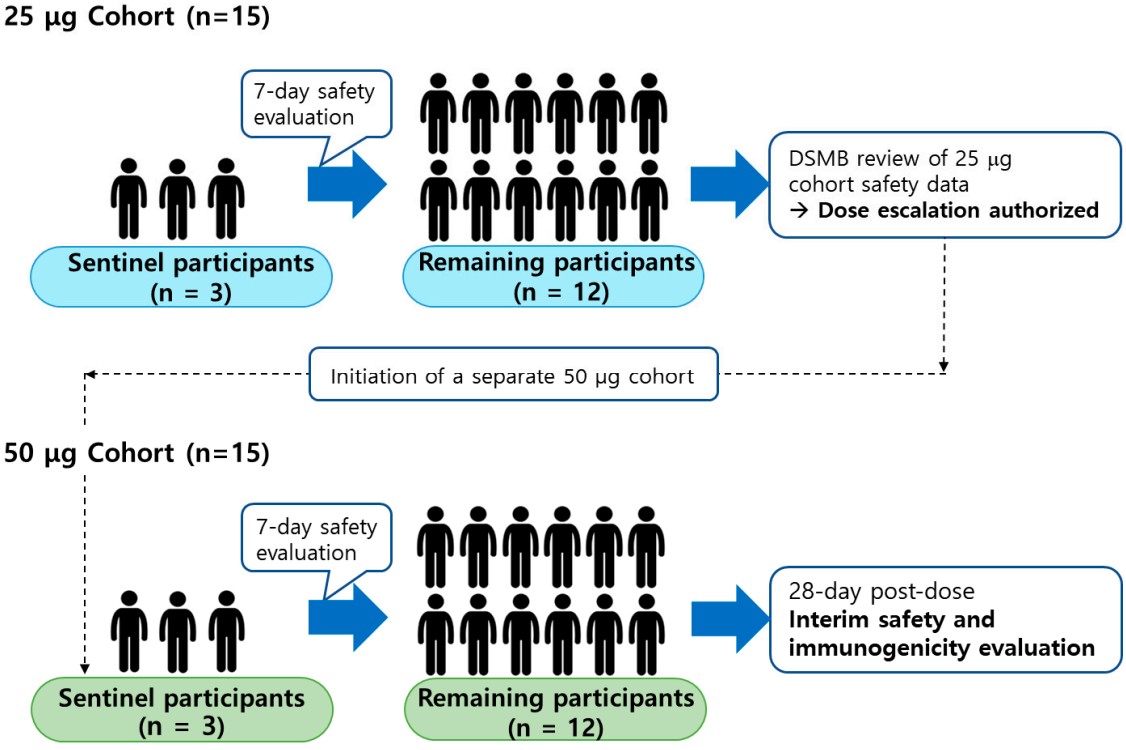
administered 28 days apart. Initial findings from this trial have been reported previously and provided the first clinical experience with an mRNA vaccine incorporating SmartCap® SC101 [13]. The present report focuses more specifically on the clinical safety and tolerability data from this Phase I study, while also providing the associated humoral and cellular immunogenicity findings. Safety assessments include solicited reactogenicity, treatment-emergent adverse events, adverse events of special interest, clinical laboratory findings, and other prespecified safety evaluations.

Accordingly, the objective of the present study was to characterize the safety, tolerability, and immunogenicity of STP2104 in this Phase I trial. Because the study did not include an active mRNA vaccine comparator, the clinical findings are not intended to establish superiority or equivalence of SmartCap® SC101 relative to other capping technologies. Rather, this study provides clinical safety and immunogenicity data for an mRNA–LNP vaccine incorporating SC101 and extends the available clinical evidence for this alternative co-transcriptional 5′ cap analogue.

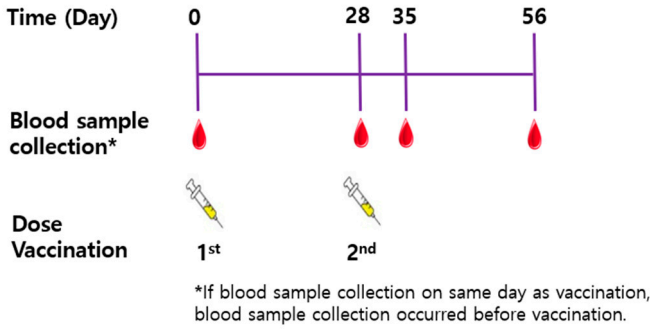
2. Materials and Methods

2.1. Study Design & Participants

This first-in-human clinical trial was designed as a multicenter, open-label, dose-escalation, Phase I study to evaluate the safety, tolerability, and immunogenicity of STP2104, a prophylactic mRNA COVID-19 vaccine incorporating the novel co-transcriptional 5′ cap analogue SmartCap® SC101, in healthy adults with no known history of SARS-CoV-2 infection. The study was conducted at clinical sites in South Africa and South Korea. Healthy volunteers were enrolled competitively according to country-specific eligibility criteria (18–55 years in South Africa and 19–55 years in South Korea), with a planned total enrollment of 30 participants (Figure 1a,b). Participants were assigned to a dose cohort and received the same dose for both vaccinations: participants in the 25 µg cohort received 25 µg on both Day 0 (prime) and Day 28 (boost), whereas participants in the 50 µg cohort received 50 µg at both time points.



(**a**)



(**b**)

Figure 1. Study design and dosing schedule of the first-in-human Phase I clinical trial of STP2104. (**a**) Overview of the multicenter, open-label, dose-escalation study evaluating the safety, tolerability, and immunogenicity of STP2104, an mRNA SARS-CoV-2 vaccine incorporating the novel 5′ cap analogue SmartCap® SC101. The 25 μg and 50 μg dose groups comprised two separate cohorts of 15 participants each; individual participants did not undergo dose escalation from 25 μg to 50 μg. In the 25 μg cohort, three sentinel participants were vaccinated first, and safety data collected during the first 7 days after vaccination were reviewed before vaccination of the remaining 12 participants. Following DSMB review of the interim safety data from the 25 μg cohort and confirmation that no safety concerns precluded further dose escalation, dose escalation was permitted and a separate 50 μg cohort was initiated using the same sentinel strategy (three sentinel participants followed by 12 remaining participants). (**b**) Dosing schedule and sampling timeline. Participants received two intramuscular doses of STP2104 on Day 0 (prime) and Day 28 (boost). Blood samples for safety laboratory testing and immunogenicity analyses were collected at baseline (Day 0), Day 28 ± 2, Day 35 ± 2, and Day 56 ± 2. When blood collection and vaccination occurred on the same day, blood samples were obtained before vaccine administration.

Written informed consent was obtained from all participants before the initiation of any study-specific procedures. The study protocol and informed consent documents were reviewed and approved by the Institutional Review Boards (or Ethics Committees) of Korea University Guro Hospital, Hallym University Kangnam Sacred Heart Hospital, Inha University Hospital, and Pusan National University Hospital in South Korea, and by Pharma-Ethics in South Africa. The corresponding approval codes and approval dates are provided in the Institutional Review Board Statement. The study protocol, informed consent forms, and all relevant study documents were reviewed and approved by the Institutional Review Boards (IRBs) and the national regulatory authorities in each participating country. The trial was conducted in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice (ICH E6(R2) GCP) guidelines, and all applicable national regulatory requirements.

2.2. Eligibility Criteria

Eligible participants were healthy adults (18–55 years of age in South Africa and 19–55 years in South Korea) who had no clinically significant medical history or abnormalities identified during screening, including physical examination and routine clinical laboratory assessments. Key inclusion criteria included a body mass index (BMI) between 18.0 and 30.0 kg/m^2, willingness to comply with all study procedures throughout the trial, and agreement to use highly effective contraception, when applicable.

The principal exclusion criteria included:

- Previous SARS-CoV-2 infection or high-risk exposure, defined as a prior diagnosis of COVID-19, positive anti-SARS-CoV-2 IgM and/or IgG serology at screening, close contact with a confirmed COVID-19 case within 14 days before the first vaccination,

or occupational exposure associated with a high risk of SARS-CoV-2 infection (e.g., healthcare personnel directly caring for patients with COVID-19).

- Previous coronavirus infection, vaccination or investigational intervention, including prior receipt of any COVID-19 vaccine, previous participation in clinical studies involving lipid nanoparticle (LNP)-based therapeutics, or a history of SARS-CoV or Middle East respiratory syndrome coronavirus (MERS-CoV) infection or vaccination.
- Medical conditions affecting vaccine safety or immune responses, including a history of severe allergic reactions or hypersensitivity to vaccine components, vaccine-related serious adverse events, chronic systemic corticosteroid therapy, or treatment with immunosuppressive or immunomodulatory agents within 6 months before enrollment.
- Recent medical interventions, including receipt of another vaccine from 4 weeks before the first vaccination until 4 weeks after the second vaccination, participation in another investigational drug or medical device study within the previous 6 months, administration of immunoglobulins or blood-derived products within 3 months before enrollment, or a history of substance abuse that could interfere with study participation.

Women of childbearing potential were required to have a negative pregnancy test before enrollment and to use highly effective contraception throughout the study. Male participants with partners of childbearing potential were also required to comply with the protocol-specified contraceptive requirements.

2.3. Vaccination Procedures, Dosing Escalation, and Study Endpoints

Participants were sequentially enrolled into two dose cohorts according to the order of enrollment. The first 15 participants received STP2104 at a dose of 25 μg, followed by enrollment of 15 participants in the 50 μg cohort. Participants remained within their assigned dose cohort throughout the study; no participant received both the 25 μg and 50 μg dose levels. Participants in both cohorts received two intramuscular (IM) injections administered 28 days apart.

Each dose cohort was initiated using a sentinel dosing strategy to enhance participant safety. Three participants were enrolled in the sentinel group, with vaccinations staggered by at least 24 h between consecutive participants. Escalation within each sentinel group proceeded only after confirmation that no protocol-defined temporary halting criteria had been met during the immediate post-vaccination safety observation period.

Following completion of the sentinel vaccinations, safety data collected during the first 7 days after vaccination were reviewed before enrollment of the remaining participants within the corresponding dose cohort. Dose escalation from the 25 μg cohort to the 50 μg cohort was permitted only after an independent Data and Safety Monitoring Board (DSMB) reviewed the interim safety data and determined that no safety concerns precluded further dose escalation. The same sentinel-based safety review process was subsequently applied to the 50 μg cohort.

Blood samples for safety laboratory testing and immunogenicity assessments were collected at baseline (Day 0, prior to vaccination), Day 28 (before the second vaccination), Day 35 (7 days after the second vaccination), and Day 56 (28 days after the second vaccination). When blood sampling and vaccination occurred on the same day, blood samples were obtained before vaccine administration.

The primary study objectives were to evaluate the safety, tolerability, and preliminary immunogenicity of STP2104 (Figure 1a,b).

2.4. Safety Oversight

An independent DSMB was established before participant enrollment and operated according to a predefined DSMB charter. The DSMB was responsible for independent oversight of participant safety throughout the study, including review of emerging safety data and recommendations regarding dose escalation, study continuation, protocol modification, or study suspension.

Dose escalation from the 25 μg cohort to the 50 μg cohort would be permitted only after DSMB review of solicited and unsolicited adverse events (AEs) collected during the first 7 days following the initial vaccination of the low-dose cohort.

An ad hoc DSMB meeting would be convened if any participant experienced an adverse event meeting the predefined temporary halting criteria within 7 days after vaccination. These criteria included:

- Fever exceeding 40 °C following vaccination;
- Two or more participants experiencing the same treatment-related Grade 3 or higher adverse event;
- Any serious adverse drug reaction (SADR) considered related to the investigational vaccine;
- Anaphylaxis or other acute systemic hypersensitivity reactions following vaccination; or
- Any other clinically significant medical condition that, in the opinion of the investigator or DSMB, warranted interruption of the study for participant safety.

If none of these criteria were met, enrollment and dose escalation proceeded according to the study protocol without additional DSMB review.

2.5. Safety Assessments

Safety was assessed by monitoring solicited local and systemic adverse events, unsolicited treatment-emergent adverse events, serious adverse events, and adverse events of special interest, together with protocol-defined safety assessments. The severity of unsolicited adverse events was graded according to the protocol-defined criteria (Grades 1–4), considering the nature and duration of clinical symptoms, their impact on daily activities, and the need for medical intervention or treatment. Clinical assessments included physical examinations, vital signs (blood pressure, pulse rate, respiratory rate, and tympanic temperature), 12-lead electrocardiography (heart rate, PR, QRS, QT, and QTc intervals), and chest radiography. Laboratory assessments included hematology (local), serum chemistry, urinalysis, and additional safety biomarkers (troponin I and D-dimer). Screening assessments included viral testing for hepatitis B surface antigen, antibodies to hepatitis C virus and HIV, and SARS-CoV-2 antigen. Pregnancy testing by serum or urine β-human chorionic gonadotropin was performed, as appropriate, in women of childbearing potential. Urinalysis included protein (albumin), ketones, blood (red blood cells), glucose, and bilirubin, with abnormal findings further evaluated by microscopy and culture when clinically indicated. A posteroanterior chest radiograph was obtained at screening, with repeat examination permitted once during screening at the investigator's discretion. All assessments were performed according to the schedule specified in the study protocol. Protocol-defined safety assessments are summarized in Table 1.

Table 1. Predefined safety assessments.

Assessment	Description
Pregnancy testing	Serum or urine β-human chorionic gonadotropin testing was performed, as appropriate, in women of childbearing potential.
Physical examination	Complete or symptom-directed examinations included general appearance, skin, head and neck, cardiopulmonary, abdominal, genitourinary, musculoskeletal, neurologic, extremity, and lymph node assessments.
Vital signs	Blood pressure, pulse rate, respiratory rate, and tympanic temperature were measured after participants had rested in a seated position for at least 5 min.
Viral testing (local)	Screening tests assessed HBsAg, antibodies to HCV and HIV, and evidence of SARS-CoV-2 antigen.
Hematology (local)	• Hemoglobin • Hematocrit • Leukocyte count • Red blood cell (RBC) count • Platelet count
	Differential (absolute) counts: Neutrophils, Lymphocytes, Basophils, Monocytes, Eosinophils
Serum chemistry	Blood urea nitrogen, Creatinine, Total bilirubin, Alkaline phosphatase, Alanine aminotransferase, Aspartate aminotransferase
Additional safety biomarkers	Cardiac/coagulation biomarkers: Troponin I and D-dimer.
Urinalysis (local)	Protein (albumin), Ketones, Blood (RBC), Glucose, and Bilirubin; abnormal findings were further evaluated by microscopy and culture, as clinically indicated.
12-lead electrocardiography	Heart rate, PR, QRS, QT, QTc intervals, with an overall clinical interpretation.
Chest radiography	A posteroanterior chest radiograph was obtained at screening; repeat examination was permitted once during screening at the investigator's discretion.

The table summarizes protocol-defined physical examinations, vital sign measurements, clinical laboratory tests, urinalysis, pregnancy testing, electrocardiography, and chest radiography performed during the study. Adverse-event monitoring is described separately in Section 2.5. Abbreviations: HBsAg, Hepatitis B Surface Antigen; HCV, Hepatitis C Virus; HIV, Human Immunodeficiency Virus; Local, assessment to be completed at local laboratory.

2.6. Study Endpoints

The primary study endpoints included assessments of the safety, tolerability, and immunogenicity of STP2104 following two intramuscular administrations.

Safety and Tolerability Endpoints:

Safety and tolerability were assessed through the incidence, severity, duration, and relationship to vaccination of adverse events (AEs). Safety evaluations included solicited local and systemic adverse reactions occurring within 7 days after each vaccination; unsolicited adverse events (AEs) occurring within 28 days after each vaccination; and serious adverse events (SAEs), medically attended adverse events (MAAEs), and adverse events of special interest (AESIs) throughout the study. MAAEs were defined as AEs resulting in hospitalization, an emergency department visit, or an unscheduled medical visit following investigational drug vaccination. AESIs or clinically important unexpected reactions that were considered to occur or to be a possible risk following investigational drug vaccination were also evaluated. Clinically significant changes in hematology, serum biochemistry, urinalysis, vital signs, and physical examination findings were also evaluated.

Immunogenicity Endpoints:

Humoral and cellular immune responses were evaluated using predefined immunological endpoints. Humoral immunity was assessed by measuring the seroresponse rate (SRR), geometric mean titer (GMT), and geometric mean fold rise (GMFR) of SARS-CoV-2 neutralizing antibodies, together with the SRR, geometric mean concentration (GMC), and GMFR of spike-specific binding antibodies.

Cell-mediated immunity was evaluated by quantifying SARS-CoV-2 spike-specific T-cell responses using interferon-γ (IFN-γ) enzyme-linked immunospot (ELISpot) assays and by characterizing T-helper (Th1/Th2) cytokine polarization using intracellular cytokine staining (ICS) followed by flow cytometric analysis.

2.7. STP2104 mRNA Vaccine Formulation and Manufacturing

STP2104 is an investigational lipid nanoparticle (LNP)-formulated mRNA vaccine encoding a codon-optimized full-length SARS-CoV-2 spike glycoprotein and incorporating the co-transcriptional 5′ cap analogue SmartCap® SC101. The formulation characteristics, manufacturing process, and preclinical evaluation of STP2104 have been described previously [12,13]. Bulk LNP-encapsulated mRNA drug product was manufactured under Good Manufacturing Practice (GMP) conditions at the ST Pharm Banwol Campus (Ansan, Republic of Korea). Manufacturing comprised mRNA synthesis, LNP formulation, and in-process and release quality control (QC) testing according to validated manufacturing procedures and predefined product specifications. The clinical trial material (CTM) was aseptically filled and finished into sterile vials at the Dong-A ST Daegu Campus (Daegu, Dalseong-gun, Republic of Korea). The Banwol manufacturing facility of the ST Pharm was licensed by the Ministry of Food and Drug Safety (MFDS) of the Republic of Korea for the manufacture and quality control of investigational medicinal products (Approval No. 100743; 24 March 2022). The Dong-A ST Daegu facility is certified in accordance with Korea Good Manufacturing Practice (KGMP) for biopharmaceutical manufacturing.

2.8. Analysis Populations

A summary of the statistical analysis populations is in Table 2.

Table 2. Definitions of the statistical analysis populations.

Subject Population	Description
Safety Analysis Set (SS)	The SS consisted of all participants who received at least 1 dose of STP2104. The primary safety analyses were performed using the SS. The SS consisted of total SS, SS for the first dose (referred to as SS1), and SS for the second dose (referred to as SS2). Safety analysis for each dose was based on the vaccinated participants of each dose, respectively. SS1 was defined as all participants who received the first dose. SS2 was defined as all participants who received the second dose.
Full Analysis Set (FAS)	The FAS consisted of participants who were vaccinated with a study vaccine at least once and had immunogenicity results before and after STP2104 vaccination.
Per Protocol Set 1 (PPS1)	The PPS1 consisted of participants who completed 2 vaccinations without major protocol deviations among FAS who completed all visits up to and including Visit 6. The main analysis of immunogenicity data was performed in PPS1 and additional analysis was performed in FAS.
Per Protocol Set 2 (PPS2)	The PPS2 consisted of participants who completed 2 vaccinations without major protocol deviations among FAS who completed all visits up to and including Visit 8 (EOS). Note: PPS2 was prespecified in the protocol but was not included in the present analysis because the interim analysis was conducted after completion of Visit 6, before the end-of-study (Visit 8). For individual immunogenicity assays, the number of evaluable participants could be smaller than the corresponding analysis population because of assay-specific sample availability or evaluability.

2.9. Immunogenicity Assessment Methods

Immunogenicity was evaluated using clinical samples collected at baseline (Day 0), 28 days post-first vaccination (Day 28), 7 days post-second vaccination (Day 35), and 28 days post-second vaccination (Day 56). Whole-blood samples collected at the study sites were transported to designated local processing facilities, where PBMCs were isolated

and serum was separated. The processed samples were stored under appropriate conditions and subsequently shipped to the Global Clinical Central Laboratory (GCCL, Yongin, Republic of Korea) for immunogenicity assessments according to the study-specific Laboratory Instruction Manual (Protocol No. STP2104-101, LIM version 1.0).

PBMCs were used to evaluate cell-mediated immune responses by interferon-γ (IFN-γ) enzyme-linked immunospot (ELISpot) and intracellular cytokine staining (ICS) assays at GCCL. Serum samples were used for the determination of SARS-CoV-2 spike-specific binding antibodies by electrochemiluminescence immunoassay (ECLIA) at GCCL. Serum samples designated for neutralizing-antibody analysis were transferred for plaque reduction neutralization testing as described below [13,14].

Seroresponse was defined as a ≥4-fold increase in antibody titer or concentration from baseline for neutralizing and binding antibodies, respectively. For baseline values below the lower limit of quantitation (LLOQ), half the corresponding LLOQ was used (2.00 IU/mL for neutralizing antibodies and 0.20 U/mL for binding antibodies). The seroresponse rate (SRR) was calculated as the proportion of evaluable participants meeting this criterion at each post-vaccination time point.

2.9.1. Quantification of Binding Antibodies by Electrochemiluminescence Immunoassay (ECLIA)

Serum antibodies against the SARS-CoV-2 spike (S) protein were quantified using the Elecsys® Anti-SARS-CoV-2 S assay (Roche Diagnostics, Basel, Switzerland), an electrochemiluminescence immunoassay (ECLIA), according to the manufacturer's instructions. Antibody concentrations were reported in U/mL, with an upper limit of quantification (ULOQ) of 250 U/mL. Individual measurements above the quantitative range were reported as >250 U/mL in the clinical study dataset.

2.9.2. Quantification of Neutralizing Antibodies by Plaque Reduction Neutralization Test (PRNT)

The neutralizing antibody (NAb) titration was conducted at the Korea National Institute of Infectious Diseases under Good Clinical Laboratory Practice (KNIID GCLP-190) guidelines, according to the institutional protocol (SOP-AS-005). The procedure was based on previously described methods [13,14] with minor modifications.

Briefly, Vero E6 cells (ATCC, catalog #CCL-81, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Upon reaching 80–90% confluency, the cells were washed with Dulbecco's Phosphate-Buffered Saline (DPBS), detached using Trypsin-EDTA, and centrifuged at 1500 rpm for 3 min. Cell viability and concentration were determined using 0.4% Trypan blue exclusion. The suspended cells were then seeded into 12-well plates at a density of 2×10^5 cells/mL (1 mL/well) and incubated to allow for attachment.

Clinical serum samples were heat-inactivated at 56 °C for 30 min and serially diluted two-fold from the stock solution using an infection medium (DMEM containing 2% FBS and 1% penicillin-streptomycin). The SARS-CoV-2 challenge virus (National Culture Collection for Pathogens, BetaCoV/Korea/KCDC03/2020, NCCP #43326, Cheongju, Republic of Korea) was diluted to 6×10^3 PFU/mL. Equal volumes of the diluted serum and virus were mixed and incubated for 1 h at 37 °C in a 5% CO_2 incubator.

The culture medium was removed from the 12-well plates, and 200 μL of the virus-serum mixture was inoculated into each well. After a 1 h incubation at 37 °C to facilitate viral adsorption, the inoculum was removed. The cells were overlaid with 1 mL of a 1:1 mixture containing 2× Minimum Essential Medium (MEM) with 4% FBS and 1.5% agar, followed by a 3-day incubation in a 5% CO_2 incubator.

After incubation, the overlay media was removed, and the cells were fixed and stained with a crystal violet solution (1 mL/well) for at least 1 h at room temperature. The plaques were enumerated after washing and drying the plates at room temperature. The NAb titer was defined as the reciprocal of the highest serum dilution that resulted in a 50% reduction in the number of plaques compared to the virus-only positive control. The 50% neutralizing dose (ND_{50}) was calculated using the Spearman-Kärber formula [15]:

$$log_{10}\, ND_{50} = m - \Delta(\Sigma\, p - 0.5)$$

where m is the log of the highest dilution factor, Δ is the log of the dilution step, and p is the sum of the proportion of plaque reduction relative to the positive control at each dilution. Finally, the ND_{50} titers were converted to International Units per milliliter (IU/mL) using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136, 1000 IU/mL) as a reference.

2.9.3. IFN-γ Enzyme-Linked Immunospot (ELISpot) Assay

Cryopreserved PBMCs isolated from study participants were analyzed using the Human IFN-γ ELISpot Pro kit (Mabtech, Nacka Strand, Sweden) according to the manufacturer's instructions. PBMCs were stimulated with overlapping 15-mer peptides (9-amino acid overlap) spanning the full-length ancestral SARS-CoV-2 spike protein (1273 amino acids), divided into five peptide pools. Cells (2×10^6 cells/mL) were incubated with peptide pools, phytohemagglutinin (PHA; positive control), or dimethyl sulfoxide (DMSO; negative control) for 16–24 h at 37 °C in a humidified 5% CO_2 incubator. IFN-γ-producing cells were detected according to the manufacturer's protocol. Spot-forming units (SFUs) were developed using BCIP/NBT substrate and quantified with a Mabtech ASTOR ELISpot reader (Mabtech, Nacka Strand, Sweden). Results were expressed as background-subtracted spot-forming units (SFUs) per 2×10^5 PBMCs after subtraction of the DMSO negative control.

2.9.4. Intracellular Cytokine Staining (ICS)

Cell-mediated immune responses were further evaluated by intracellular cytokine staining (ICS) using PBMC samples collected at baseline (Day 0), Day 28, Day 35, and Day 56. ICS assessments included IFN-γ, IL-2, IL-4, and TNF-α responses in CD4+ and CD8+ T cells, together with the Th1/Th2 ratio. The assay was performed at GCCL according to the study-specific Laboratory Instruction Manual (Protocol No. STP2104-101, LIM version 1.0).

2.10. Statistical Analysis

Because this was an exploratory first-in-human Phase I study, no formal sample size calculation was performed. Safety analyses were summarized descriptively using frequencies, percentages, and corresponding 95% confidence intervals (CIs), where appropriate. Immunogenicity endpoints were summarized using geometric mean titers (GMTs), geometric mean concentrations (GMCs), geometric mean fold rises (GMFRs), seroresponse rates (SRRs), and corresponding descriptive statistics, including 95% CIs, where appropriate.

For neutralizing antibody responses, exploratory comparisons among study time points within each dose cohort were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Tukey's test was used to account for multiple pairwise comparisons within each dose-cohort analysis. No multiplicity adjustment beyond the Tukey procedure was applied across the separate analyses. Accordingly, the resulting p-values were interpreted as exploratory rather than confirmatory.

No formal inferential statistical comparisons were performed between the 25 μg and 50 μg dose cohorts because the study was not designed or powered to establish between-dose differences. Between-cohort differences were therefore summarized and interpreted

descriptively. Binding-antibody responses were also summarized descriptively, given the substantial proportion of measurements reaching the assay upper limit of quantification.

Cellular immune responses were summarized using descriptive statistics, including the mean, standard deviation, median, and interquartile range, as appropriate. No formal statistical significance was assigned to differences between dose cohorts or among individual peptide pools. Longitudinal response profiles and reverse cumulative distribution plots were used to visualize vaccine-induced antibody responses over time.

All statistical analyses and graphical presentations were performed using GraphPad Prism version 10.4.0 (GraphPad Software, Boston, MA, USA) and SAS version 9.4 or higher (SAS Institute, Cary, NC, USA). All adverse events were coded using MedDRA version 25.0. For adverse-event summaries, participants were counted once for each system organ class (SOC) and preferred term (PT), regardless of the number of occurrences.

3. Results

3.1. Study Population

Baseline demographic characteristics of the study population are summarized in Table 3. Participants were recruited through competitive recruitment at study sites in South Africa and South Korea; ultimately, all enrolled participants were recruited in South Africa. The median (minimum, maximum) age of the overall study population was 27.5 (18, 41) years, with median ages of 30.0 (20, 41) and 24.0 (18, 38) years in the 25 μg and 50 μg cohorts, respectively. The mean (SD) BMI was 21.11 (2.99) kg/m² overall and was similar between dose cohorts.

Table 3. Demographic and baseline characteristics of participants (Full Analysis Set).

Baseline Characteristics	25 μg (N = 15)	50 μg (N = 15)	Overall (N = 30)
Country of enrollment, n (%)			
South Africa	15 (100.0)	15 (100.0)	30 (100.0)
Age, median (min., max.), years	30.0 (20, 41)	24.0 (18, 38)	27.5 (18, 41)
Female, n (%)	2 (13.3)	2 (13.3)	4 (13.3)
Race, n (%)			
Black or African American [a]	11 (73.3)	15 (100.0)	26 (86.7)
Other [b]	4 (26.7)	0 (0)	4 (13.3)
Ethnicity, n (%)			
Not Hispanic or Latino	15 (100.0)	15 (100.0)	30 (100.0)
Height, mean (SD), cm	169.9 (7.9)	168.7 (7.3)	169.3 (7.5)
Weight, mean (SD), kg	61.0 (7.7)	59.8 (8.8)	60.4 (8.2)
BMI, mean (SD), kg/m²	21.2 (3.2)	21.0 (2.9)	21.1 (3.0)
Close contact with a person infected with COVID-19 within 14 days?			
Yes, n (%)	0 (0)	0 (0)	0 (0)
No, n (%)	15 (100.0)	15 (100.0)	30 (100.0)

Data are presented for the Full Analysis Set (FAS), which included all participants who received at least one dose of STP2104 and had evaluable pre- and post-vaccination immunogenicity data. Characteristics are summarized by dose cohort and for the overall study population. Data are presented as n (%) unless otherwise indicated. Abbreviations: BMI, Body Mass Index; FAS, Full Analysis Set; N, number of participants in the analysis population; SD, standard deviation; n, number of participants in the specified category. Note: [a] "Black or African American" reflects the predefined race category used for demographic data collection in the study database and CSR. For participants enrolled in South Africa, this classification does not imply a U.S.-specific African American identity;

the terminology reflects the standardized category used during study data collection. [b] All four participants categorized as "Other" were recorded as "Other: Coloured" in the study database.

Twenty-six participants (86.7%) were categorized as Black or African American, whereas four participants (13.3%), all in the 25 μg cohort, were categorized as "Other"; the latter were recorded as "Other: Coloured" in the study database. All participants were classified as Not Hispanic or Latino.

3.2. Routine Safety Assessments

Across predefined clinical laboratory, vital sign, physical examination, and electrocardiographic assessments, no persistent safety abnormalities were observed following STP2104 administration. At the individual-participant level, one participant in the 50 μg cohort had a clinically significant decrease in platelet count to 28 × 10^9/L on Day 28 after Dose 2, corresponding to a Grade 3 laboratory abnormality according to conventional CTCAE thresholds. The platelet count returned to the normal range (248 × 10^9/L) 5 days later. The corresponding thrombocytopenia event was recorded in the Clinical Study Report (CSR) as a Grade 1 (mild) unsolicited TEAE under the protocol-defined clinical severity grading criteria. Another participant in the 50 μg cohort had an activated partial thromboplastin time of 29.0 s on Day 28, which was assessed as clinically significant; the participant was reviewed, and D-dimer testing was repeated. After completion of the 25 μg cohort, the DSMB confirmed that no clinically significant safety concerns or protocol-defined temporary halting criteria had been met before authorizing escalation to the 50 μg dose level.

3.3. Participant Disposition and Analysis Populations

All 30 enrolled participants received both scheduled doses of STP2104 and completed the protocol-defined vaccination period. Accordingly, all 30 participants were included in the Safety Set (SS), Safety Set 1 (SS1), and Safety Set 2 (SS2), with 15 participants in each dose cohort (Figure 2; Table 2). The Full Analysis Set (FAS) also comprised all 30 participants.

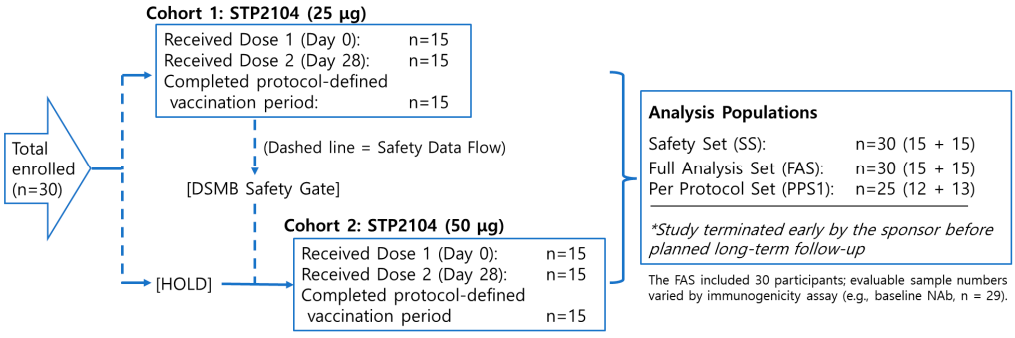


Figure 2. Participant disposition and analysis populations. Thirty participants were enrolled into two separate dose cohorts: 15 participants received STP2104 at 25 μg and 15 received STP2104 at 50 μg. All 30 participants received both scheduled vaccinations and completed the protocol-defined vaccination period. All 30 participants were included in the Safety Set (SS) and Full Analysis Set (FAS), whereas 25 participants met the criteria for Per Protocol Set 1 (PPS1; 12 in the 25 μg cohort and 13 in the 50 μg cohort). The number of evaluable participants for individual immunogenicity assays varied according to sample availability and assay-specific evaluability. Abbreviations: SS, Safety Set; FAS, Full Analysis Set; PPS1, Per Protocol Set 1.

Of the 30 participants in the FAS, 25 (83.3%) met the criteria for PPS1, including 12 of 15 participants (80.0%) in the 25 μg cohort and 13 of 15 participants (86.7%) in the 50 μg cohort. Primary immunogenicity analyses were performed using the PPS1, with the FAS

used for supportive analyses (Figure 2; Table 2). For individual immunogenicity assays, the number of evaluable participants could be smaller because of assay-specific sample availability or evaluability; for example, pre-vaccination neutralizing antibody measurements were available for 29 participants. Assay-specific denominators are reported in the relevant Results sections and figure legends.

3.4. Safety and Tolerability

A total of 311 solicited AEs were reported, including 126 systemic and 185 local events. In the 25 µg cohort, 40 systemic events were reported in 9 participants and 68 local events in 12 participants. In the 50 µg cohort, 86 systemic events were reported in 8 participants and 117 local events in 12 participants (Figure 3a,b). Overall, a greater number of solicited AEs were reported in the 50 µg cohort than in the 25 µg cohort.

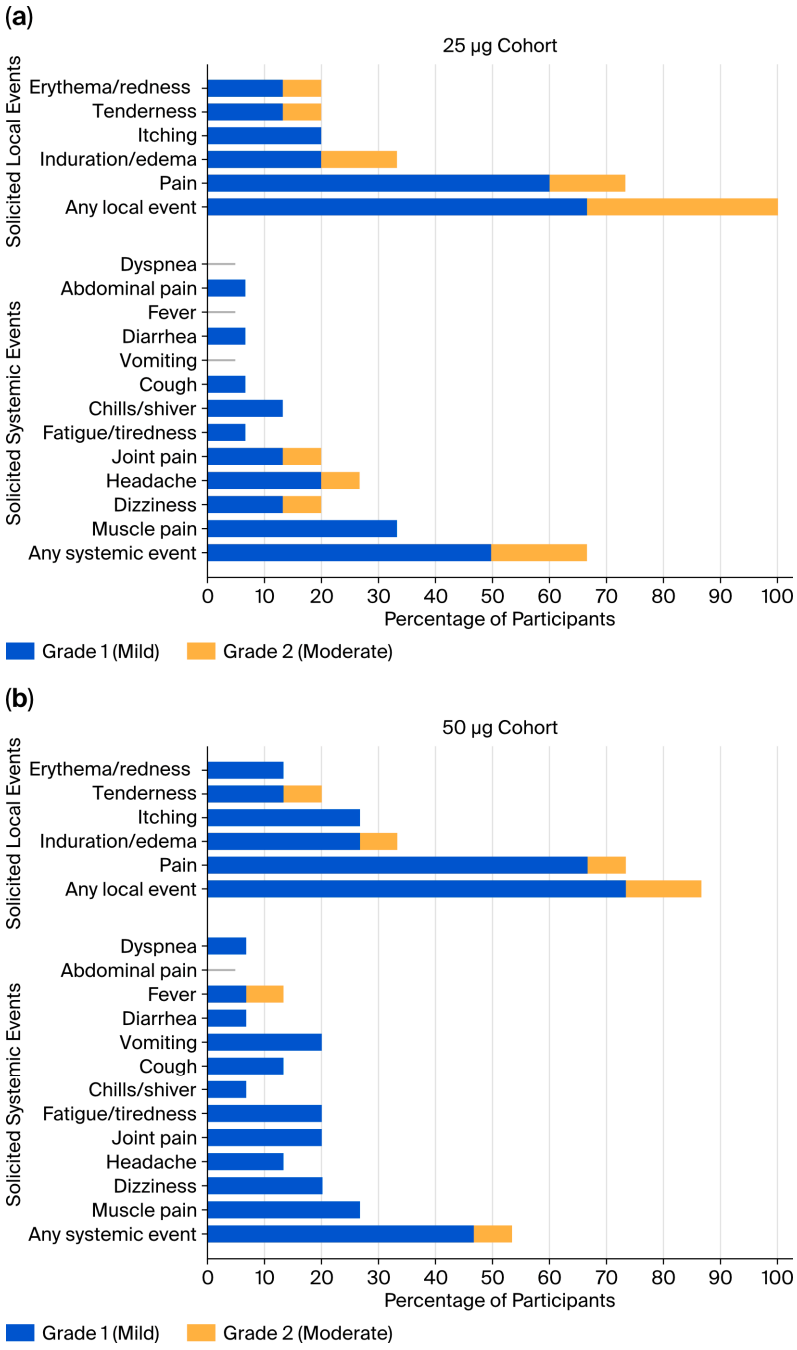


Figure 3. Overall solicited local and systemic adverse events following two administrations of the first-in-human SmartCap® SC101-enabled mRNA vaccine. Percentage of participants reporting

solicited local and systemic adverse events of any grade during the study period. Each participant was counted once per adverse event category, and the highest reported severity grade was used when an event occurred more than once. (**a**) Low-dose cohort (25 μg). (**b**) High-dose cohort (50 μg).

A total of 27 unsolicited TEAEs were reported in 20 participants (66.7%), including 13 events in 9 participants (60.0%) in the 25 μg cohort and 14 events in 11 participants (73.3%) in the 50 μg cohort. Eleven unsolicited treatment-related TEAEs were reported, comprising 2 events in 1 participant (6.7%) in the 25 μg cohort and 9 events in 8 participants (53.3%) in the 50 μg cohort. The most frequently reported unsolicited TEAEs by Preferred Term (PT) were Troponin I increased (2 participants in the 25 μg cohort and 7 participants in the 50 μg cohort) and Fibrin D-dimer increased (2 participants in each cohort), both classified under the System Organ Class (SOC) of Investigations.

Troponin I increased was reported in 9 participants overall. Participant-level review showed that the elevations occurred at different study visits, including Day 7 after Dose 1, the Dose 2 visit, Day 7 after Dose 2, and Day 28 after Dose 2, without a uniform temporal pattern. All events were Grade 1 (Mild) and recovered or resolved, with subsequent troponin I measurements returning to the normal range in the available follow-up assessments. One event in the 25 μg cohort met the protocol-defined criteria for an AESI; this event was considered related to vaccination, was transient and asymptomatic, and did not preclude completion of the vaccination schedule. No associated cardiac symptoms were identified in the available adverse-event records. Participant-level timing, troponin I values and available follow-up measurements, ECG assessments, and outcomes are summarized in Appendix A Table A2.

No SAEs, MAAEs, deaths, or events leading to study discontinuation were reported, and no Grade ≥3 TEAEs were recorded under the protocol-defined clinical severity grading criteria (Table 4). However, one Grade 3 platelet-count laboratory abnormality was identified, with the corresponding thrombocytopenia event recorded as a Grade 1 TEAE in the CSR, as described in Section 3.2.

Table 4. Overall summary of treatment-emergent adverse events (Safety Population).

	25 μg (N = 15)			**50 μg (N = 15)**			**Overall (N = 30)**		
	Events	**Participants**	**Incidence (%) (95% CI)**	**Events**	**Participants**	**Incidence (%) (95% CI)**	**Events**	**Participants**	**Incidence (%) (95% CI)**
TEAEs (Except Solicited AEs)	13	9	60.0 (32.3, 83.7)	14	11	73.3 (44.9, 92.2)	27	20	66.7 (47.2, 82.7)
Related TEAEs (Except Solicited AEs)	2	1	6.7 (0.2, 31.9)	9	8	53.3 (26.6, 78.7)	11	9	30.0 (14.7, 49.4)
Unrelated TEAEs (Except Solicited AEs)	11	8	53.3 (26.6, 78.7)	5	5	33.3 (11.8, 61.6)	16	13	43.3 (25.5, 62.6)
Grade 3 or Higher TEAEs (Except Solicited AEs)	None								
Related Grade 3 or Higher TEAEs (Except Solicited AEs)	None								
TEAEs (Except Solicited AEs) Leading to Discontinuation	None								
Related TEAEs (Except Solicited AEs) Leading to Discontinuation	None								

TEAEs (Except Solicited AEs) Leading to Death	None								
AESIs	1	1	6.7 (0.2, 31.9)	0	0	0	1	1	3.3 (0.1, 17.2)
Related AESIs	1	1	6.7 (0.2, 31.9)	0	0	0	1	1	3.3 (0.1, 17.2)
MAAEs	None								
Related MAAEs	None								
TESAEs	None								
Related TESAEs	None								
Solicited TEAEs	108	12	80.0 (51.9, 95.7)	203	12	80.0 (51.9, 95.7)	311	24	80.0 (61.4, 92.3)
Grade 3 or Higher Solicted TEAEs	None								

Incidence and severity of treatment-emergent adverse events (TEAEs) are summarized by dose cohort in the Safety Population. Data are presented as the number of participants with at least one event, the corresponding percentage, 95% confidence interval (CI), and the total number of events. Abbreviations: TEAE, Treatment-Emergent Adverse Event; AESI, Adverse Event of Special Interest; MAAE, Medically Attended Adverse Event; N, number of participants in the treatment cohort; TESAE, Treatment-Emergent Serious Adverse Event. 95% Confidence Interval (CI) based on the Clopper-Pearson method. TEAE severity grades reflect the protocol-defined clinical severity criteria and are distinct from laboratory-based CTCAE grades.

3.5. Immunogenicity

3.5.1. Neutralizing Antibodies

Neutralizing antibody responses increased following vaccination in both dose cohorts (Figure 4). Pre-vaccination neutralizing antibody titers were available for 25 participants in the PPS1 (25 µg, n = 12; 50 µg, n = 13) and for 29 participants in the FAS (15 and 14 participants, respectively).

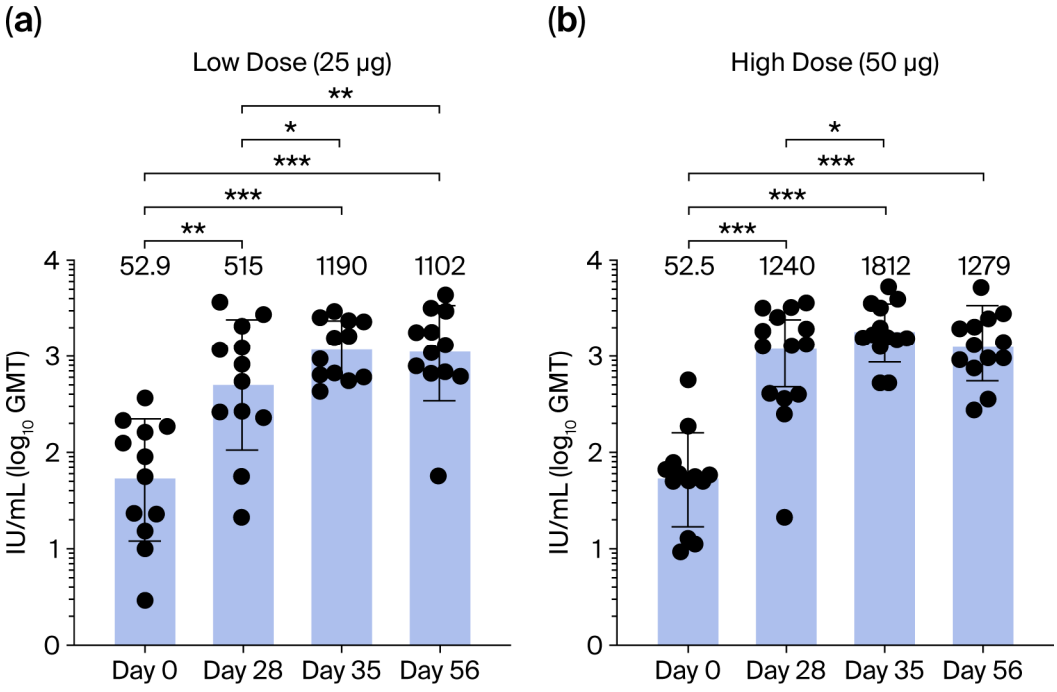


Figure 4. Neutralizing antibody responses following STP2104 vaccination. Neutralizing antibody responses against ancestral SARS-CoV-2 in the Per Protocol Set 1 (PPS1) following two intramuscular doses of STP2104 at 25 µg or 50 µg administered on Days 0 and 28. Neutralizing antibody titers were quantified using a 50% plaque reduction neutralization test (PRNT50) and are expressed as

international units per milliliter (IU/mL). Serum samples were collected at baseline (Day 0), prior to the second vaccination (Day 28), and at 1 and 4 weeks after the second vaccination (Days 35 and 56). (**a**) 25 μg cohort. (**b**) 50 μg cohort. Data are presented as geometric mean titers (GMTs) with 95% confidence intervals (CIs). Individual data points (●) represent neutralizing antibody titers measured in individual participants at each time point. Exploratory within-cohort comparisons among study time points were performed separately for each dose cohort using one-way ANOVA followed by Tukey's multiple comparisons test. Horizontal brackets indicate the specific within-cohort pairwise comparisons analyzed. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

In both the PPS1 and FAS, neutralizing antibody titers increased after vaccination, with GMTs and GMFRs reaching their highest observed values at Day 35 and declining modestly by Day 56. Descriptively, the 50 μg cohort showed an earlier seroresponse and numerically higher GMTs than the 25 μg cohort at several post-vaccination time points. These between-cohort differences were not evaluated by formal inferential statistical testing.

Within the 50 μg cohort, neutralizing antibody titers declined from Day 35 to Day 56, whereas titers in the 25 μg cohort were relatively stable over this period. Reverse cumulative distribution plots showed a numerically greater proportion of participants with higher neutralizing antibody titers in the 50 μg cohort than in the 25 μg cohort at Days 28 and 35. Exploratory within-cohort statistical comparisons across study time points are shown in Figure 4.

3.5.2. Binding Antibodies

Pre-vaccination binding-antibody results were evaluable for 29 of 30 participants in the FAS (15 in the 25 μg cohort and 14 in the 50 μg cohort). At baseline, binding-antibody concentrations were at or above the assay upper limit of quantification (ULOQ; 250 U/mL) in 12 participants, including 7 in the 25 μg cohort and 5 in the 50 μg cohort.

Following vaccination, binding-antibody concentrations increased in both dose cohorts. In the PPS1, the reported GM concentration in the 25 μg cohort increased from 32.13 U/mL at baseline to 156.31 U/mL on Day 28 and was 250 U/mL on Days 35 and 56. In the 50 μg cohort, the reported GM concentration increased from 112.55 U/mL at baseline to 250 U/mL on Day 28 and was 250 U/mL on Days 35 and 56 (Figure 5; Table 5). At Day 28, 10 of 12 participants (83.3%) in the 25 μg cohort and all 13 participants (100%) in the 50 μg cohort had binding-antibody concentrations above the ULOQ. By Days 35 and 56, all participants in both cohorts had concentrations at or above the ULOQ (Table 5). Samples above the ULOQ were not further quantified by dilution and re-assay in the dataset analyzed for this study.

The high proportion of post-vaccination measurements reaching or exceeding the assay ceiling limited quantitative assessment of subsequent binding-antibody kinetics and differences between the two dose cohorts. Reverse cumulative distribution plots of binding-antibody concentrations are provided in Appendix A Figure A1.

Table 5. Summary of binding-antibody responses following STP2104 vaccination in the PPS1.

Time Point	25 μg GM (U/mL)	25 μg ≥ULOQ n/N (%)	50 μg GM (U/mL)	50 μg ≥ULOQ n/N (%)
Baseline	32.13	6/12 (50.0)	112.55	4/13 (30.8)
Day 28	156.31	10/12 (83.3)	250.00	13/13 (100)
Day 35	250.00	12/12 (100)	250.00	13/13 (100)
Day 56	250.00	12/12 (100)	250.00	13/13 (100)

GM, geometric mean; PPS1, Per Protocol Set 1; ULOQ, upper limit of quantification (250 U/mL). Values at or above the ULOQ were reported at the assay ceiling in the dataset analyzed for this study.

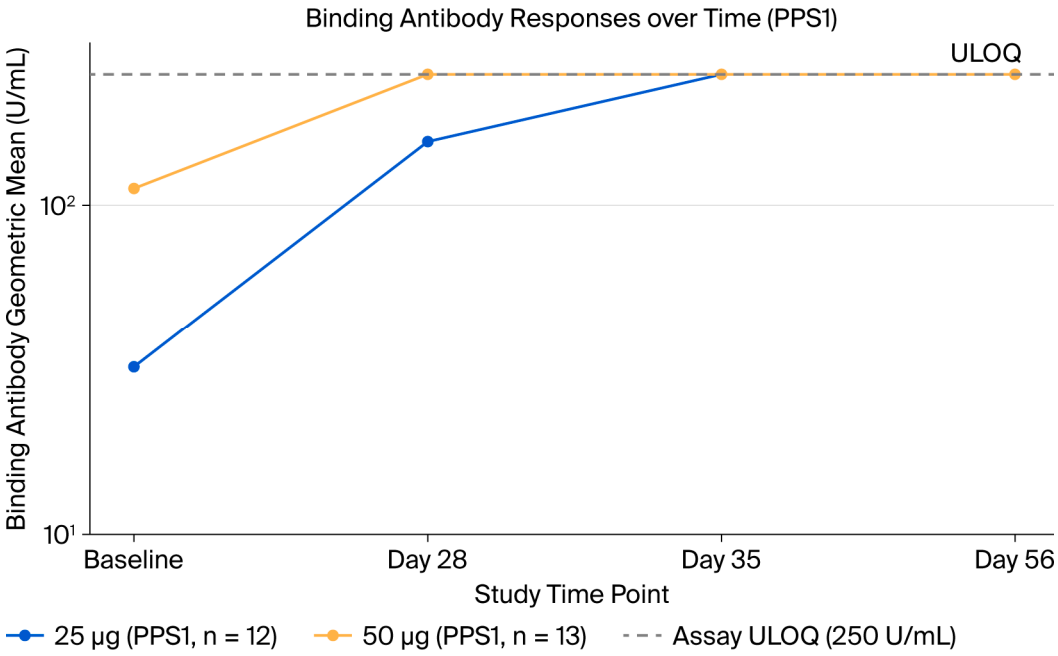


Figure 5. Binding-antibody responses following STP2104 vaccination in the Per Protocol Set 1 (PPS1). Geometric mean binding-antibody concentrations at baseline and on Days 28, 35, and 56 are shown for the 25 µg (n = 12) and 50 µg (n = 13) cohorts. The horizontal dashed line indicates the assay upper limit of quantification (ULOQ; 250 U/mL). Values reported at the assay ceiling should be interpreted in the context of the ULOQ. Data are presented descriptively; no formal statistical comparisons were performed between dose cohorts.

3.5.3. IFN-γ ELISpot Responses

Antigen-specific IFN-γ ELISpot responses were detected following STP2104 vaccination in both dose cohorts. In the PPS1, mean responses across several peptide pools were numerically higher in the 50 µg cohort than in the 25 µg cohort at Days 28 and 35; however, this pattern was not consistent across individual peptide pools or maintained at Day 56 (Figure 6 and Appendix A Figure A2). No formal inferential statistical comparisons were performed between the two dose cohorts.

The magnitude and temporal pattern of responses varied among the individual peptide pools, without a consistent dose-related pattern across PP1–PP5 and study time points. Seven of 29 participants in the FAS had insufficient peripheral blood mononuclear cell (PBMC) counts for one or more ELISpot assessments, resulting in missing data.

3.5.4. Intracellular Cytokine Staining (ICS) Analyses

Exploratory ICS analyses assessed IFN-γ-, IL-2-, IL-4-, and TNF-α-positive CD4+ and CD8+ T cells at baseline and after vaccination. In the PPS1, the mean frequencies of cytokine-positive T cells varied across cytokines, T-cell subsets, study time points, and dose cohorts, without a consistent temporal or dose-dependent pattern (Appendix A Figure A3).

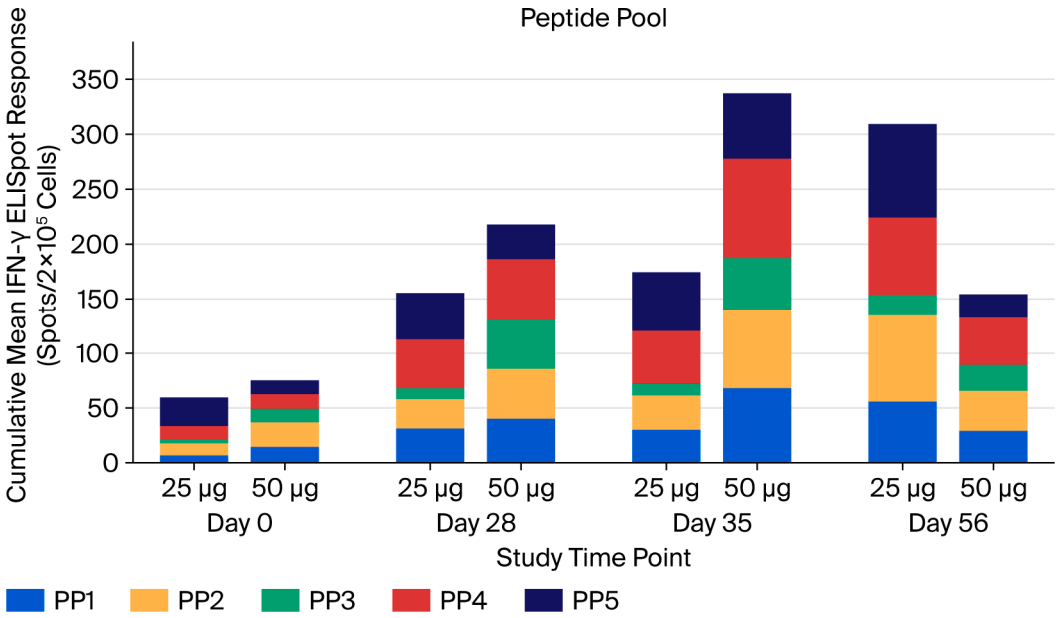


Figure 6. IFN-γ ELISpot responses following STP2104 vaccination in the Per Protocol Set 1 (PPS1). IFN-γ ELISpot responses following stimulation with five overlapping SARS-CoV-2 spike peptide pools (PP1–PP5) are shown at baseline (Day 0), Day 28, Day 35, and Day 56 for participants receiving 25 µg or 50 µg STP2104. Each stacked bar comprises the mean responses obtained separately for PP1–PP5 and is presented to visualize the overall temporal response pattern across the five peptide pools; responses to the individual peptide pools were measured and summarized separately. Results are expressed as background-subtracted spot-forming units (SFUs) per 2 × 10^5 PBMCs. The numbers of evaluable participants at Days 0, 28, 35, and 56 were 10, 11, 12, and 12, respectively, in the 25 µg cohort and 12, 12, 13, and 13, respectively, in the 50 µg cohort. Day 28 corresponds to 28 days after Dose 1, Day 35 to 7 days after Dose 2, and Day 56 to 28 days after Dose 2. Data are presented descriptively for the PPS1. No formal inferential statistical comparisons were performed between dose cohorts. Detailed peptide-pool-specific responses are provided in Appendix A Figure A2. IFN-γ, interferon gamma; PBMC, peripheral blood mononuclear cell; PP, peptide pool; PPS1, Per Protocol Set 1; SFU, spot-forming unit.

4. Discussion

To our knowledge, this study represents the first clinical evaluation of an mRNA vaccine incorporating the novel co-transcriptional 5′ cap analogue SmartCap® SC101. Following two-dose administration, STP2104 showed an acceptable short-term safety and tolerability profile and induced both humoral and antigen-specific IFN-γ-secreting cellular immune responses. No treatment-emergent serious adverse events or events leading to study discontinuation were reported. Although no Grade ≥3 treatment-emergent adverse events were reported under the protocol-defined clinical severity criteria, one clinically significant Grade 3 platelet-count laboratory abnormality was identified.

The present results extend the growing clinical experience with mRNA vaccine platforms to an mRNA construct incorporating SmartCap® SC101, a novel co-transcriptional 5′ cap analogue. In our previous preclinical study, SC101 demonstrated capping efficiency, mRNA synthesis, and protein-expression characteristics that were generally comparable to those achieved with the established co-transcriptional capping reagent CleanCap®, although performance varied depending on the mRNA construct and experimental system [12]. The present study extends these preclinical findings by providing first-in-human safety, tolerability, and immunogenicity data for an mRNA vaccine incorporating SmartCap® SC101.

Troponin I increased was reported as an unsolicited TEAE in 9 participants; however, only one event, occurring in the 25 µg cohort, met the protocol-defined criteria for an AESI

and was considered related to vaccination. This Grade 1 event was transient, asymptomatic, resolved without intervention, and did not interrupt the vaccination schedule. Participant-level review showed that the troponin I elevations occurred at different study visits without a uniform temporal pattern in relation to vaccination. All events were Grade 1 and recovered or resolved, with subsequent troponin I measurements returning to the normal range in the available follow-up assessments. No associated cardiac symptoms were identified in the available adverse-event records. Available ECG assessments for these participants were performed at screening and were either normal or showed abnormalities assessed as not clinically significant; however, ECGs were not obtained contemporaneously with each troponin I elevation. A direct temporal relationship between the troponin I elevations and electrocardiographic changes therefore cannot be determined from this study (Appendix A Table A2).

Prospective studies incorporating serial cardiac troponin measurements after BNT162b2 and mRNA-1273 vaccination have not identified a consistent increase in troponin elevations attributable to vaccination [16,17]. These findings provide clinical context for interpreting isolated troponin elevations after mRNA vaccination; however, direct comparison with the present study is limited by differences in study population, sampling schedule, assay methodology, and the definition of elevated troponin.

Two clinically significant laboratory abnormalities were identified at the individual-participant level in the 50 μg cohort: a decrease in platelet count to 28 × 10^9/L and an activated partial thromboplastin time of 29.0 s, both at Day 28 after Dose 2. The platelet count returned to the normal range 5 days later. Although the corresponding thrombocytopenia event was recorded in the CSR as a Grade 1 (mild) unsolicited TEAE, the platelet count itself corresponds to a Grade 3 laboratory abnormality according to conventional CTCAE thresholds. This distinction between laboratory-based severity grading and protocol-defined clinical AE grading should be considered when interpreting the safety findings. Given the limited sample size of the present Phase I study, these findings should be interpreted cautiously, and the STP2104 results do not exclude uncommon laboratory abnormalities or cardiac adverse events that may become apparent in larger populations.

STP2104 elicited neutralizing antibody (NAb) responses following the two-dose regimen, with descriptively earlier responses and numerically higher post-vaccination titers observed in the 50 μg cohort than in the 25 μg cohort. NAb titers reached their post-boost maximum at Day 35 and declined modestly by Day 56 while remaining above baseline in both dose cohorts. This pattern is compatible with the expected post-peak contraction of vaccine-induced antibody responses; however, the limited duration of follow-up does not permit conclusions regarding long-term persistence or durable protection. Neutralizing antibodies are widely regarded as one of the principal correlates of protection against COVID-19, although cellular immunity and non-neutralizing antibody functions may also contribute to protection [18].

For context, a subsequent analysis of the Phase III COVE trial reported a Day 57 live-virus MN50 GMT of 718 IU50/mL (95% CI, 676–763) in the mRNA-1273 immunogenicity subcohort without subsequent COVID-19, with neutralization results calibrated to the WHO International Standard [19]. In the present study, $PRNT_{50}$ GMTs at Day 56 were 1102 and 1279 IU/mL in the 25 μg and 50 μg STP2104 cohorts, respectively. Although the STP2104 values were numerically higher, direct quantitative comparison is limited by differences in neutralization assay format, virus strain, study population, and laboratory procedures despite the use of WHO-standardized units in both studies.

Binding-antibody concentrations increased after vaccination in both dose cohorts, although interpretation of the response was constrained by the assay quantitative range. Notably, pre-vaccination SARS-CoV-2 spike-specific binding-antibody concentrations measured by ECLIA were at or above the assay ULOQ in 12 participants in the FAS,

although all enrolled participants had satisfied the protocol-specified screening eligibility criteria. The screening assessment and the Day 0 immunogenicity assessment were separate tests: eligibility was determined using the protocol-specified serological screening assessment, whereas pre-vaccination binding antibodies were subsequently quantified by ECLIA. The study protocol had been amended from ELISA alone to include ECLIA because ECLIA was considered more sensitive and to provide a wider range of IgG antibody detection. This difference in assay methodology provides context for the apparently discordant findings but does not establish their cause. Detailed analytical characteristics of the screening assay, including its positivity threshold, analytical sensitivity, and quantitative range, were not available in the study documentation reviewed for the present analysis. Anti-nucleocapsid serology was not available to distinguish prior SARS-CoV-2 infection from assay-related explanations for this discrepancy. The precise reason for the discordance therefore cannot be established retrospectively from the available study documentation.

Interpretation of the post-vaccination binding-antibody response was also limited by the quantitative range of the ECLIA. As an increasing proportion of measurements exceeded the ULOQ after vaccination, the assay had limited ability to quantify the magnitude of binding-antibody responses above this threshold or to distinguish differences between the two dose cohorts. Accordingly, values at the assay ceiling should not be interpreted as evidence of a biological plateau or equivalence between the dose cohorts.

The IFN-γ ELISpot responses did not show a consistent dose-dependent pattern. Although mean responses were generally higher in the 50 μg cohort at Day 28 and Day 35, this pattern was not maintained at Day 56, and responses varied among individual peptide pools. A similar lack of a clear dose-dependent T-cell response has been reported in a clinical dose-ranging study of BNT162b1, despite dose-dependent antibody responses [20]. This provides clinical context for the STP2104 findings but does not establish a common mechanism.

In the present study, inter-individual variability, the small cohort size, peptide-pool-specific differences, and missing ELISpot assessments due to insufficient PBMC counts may have contributed to the observed pattern. Exploratory ICS analyses of IFN-γ, IL-2, IL-4, and TNF-α responses in CD4+ and CD8+ T cells likewise showed no consistent temporal or dose-dependent pattern (Appendix A Figure A3). Because markers of T-cell differentiation and exhaustion, functional avidity, and antigen persistence were not assessed, the biological basis for the observed dose-response pattern cannot be determined from the available data. Appropriately powered dose-ranging studies incorporating additional dose levels and more detailed characterization of T-cell phenotype, function, and durability will be required to define the relationship between STP2104 dose and cellular immune responses.

The clinical development of COVID-19 RNA vaccines has evolved substantially since the introduction of the first ancestral strain-based mRNA vaccines. Early monovalent vaccines such as BNT162b2 and mRNA-1273 established the clinical feasibility and efficacy of the mRNA–LNP platform, whereas subsequent development has progressively shifted toward variant-adapted formulations, broader coverage of circulating variants, improved dose efficiency, and self-amplifying RNA (saRNA) technologies (Appendix A Table A1). This evolution is reflected in current vaccine-composition strategies, in which vaccine antigens are periodically updated to more closely match circulating SARS-CoV-2 variants, as well as in the regulatory emergence of saRNA vaccine technologies with the potential to achieve immunogenicity at lower RNA doses [7,21–23]. In this context, the use of an ancestral SARS-CoV-2 spike antigen in STP2104 limits the direct relevance of its immunogenicity profile to currently circulating variants; however, it does not diminish the value of the study as a first-in-human evaluation of an mRNA construct incorporating SmartCap® SC101. The ancestral spike provides a clinically well-characterized antigenic

framework for evaluating the safety, tolerability, and immunogenicity of an mRNA vaccine construct incorporating a previously untested capping technology.

The specific contribution of SmartCap® SC101 cannot be distinguished from those of other components of the vaccine construct in the present study. These findings nevertheless extend our previous preclinical characterization of this cap analogue [12] and support further clinical evaluation of SC101-containing mRNA constructs. Whether SC101 provides specific clinical advantages over established capping technologies will require appropriately controlled comparative studies. Because SC101 is not intrinsically linked to a specific encoded antigen, SC101-containing mRNA constructs could be evaluated with updated or alternative antigens, an adaptability that may be relevant to rapid-response vaccine development and future pandemic preparedness. More broadly, current mRNA vaccine development increasingly focuses on reducing reactogenicity, improving immune durability and breadth, optimizing dose, and refining both RNA and delivery-system components [18].

Several limitations should be considered when interpreting these findings. Given its exploratory first-in-human Phase I design and small cohort size, this study was not designed to detect rare adverse events, establish clinical efficacy, or provide definitive comparisons between the two dose cohorts. The study was terminated early based on a voluntary business-related decision by the sponsor. Consequently, the planned long-term follow-up was not completed, limiting evaluation of immune durability and delayed safety events. Interpretation of the binding-antibody responses was limited by the assay ceiling, while cellular immune analyses were constrained by missing ELISpot assessments and the lack of a consistent temporal or dose-dependent pattern in the exploratory ICS findings. Although STP2104 encodes the ancestral SARS-CoV-2 spike antigen, its use in this first-in-human study provided a clinically well-characterized antigenic framework for evaluating an mRNA vaccine construct incorporating SmartCap® SC101. Future evaluation of SC101-containing mRNA constructs in larger study populations with extended follow-up will be required to further characterize their safety, immune durability, and applicability to updated or alternative antigens.

5. Conclusions

In conclusion, this first-in-human Phase I study showed that STP2104, an mRNA–LNP vaccine incorporating the novel co-transcriptional 5′ cap analogue SmartCap® SC101, had an acceptable short-term safety and tolerability profile in the small study population and induced both humoral and antigen-specific IFN-γ-secreting cellular immune responses. These findings support further clinical evaluation of SC101-containing mRNA constructs in larger study populations. Controlled comparative studies will be required to determine whether SC101 provides specific clinical advantages over established capping technologies.

6. Patents

The patent entitled “Novel Nucleic Acid Molecule” was granted in the Republic of Korea (Patent No. 10-2553857, 11 July 2023), Japan (Patent No. 07756721, 09 October 2025), China (Patent No. ZL202180071587.3, 11 November 2025), and Hong Kong (Patent No. HK40091261, 24 December 2025). The Korean patent (No. 10-2553857), entitled “Novel Nucleic Acid Molecule,” relates to nucleic acid constructs for enhanced antigen expression and immune induction and is associated with the mRNA vaccine technology underlying the development of STP2104.

Author Contributions: R.K.; Conceptualization, Data curation, Project administration, Supervision of clinical trial, Visualization, Writing—original draft, Writing—review & editing. U.-I.K.;

Conceptualization, Project administration, Supervision. T.G.U.; Conceptualization, Data curation, Formal analysis, Visualization, Writing—review & editing. X.M.; Conceptualization, Project administration. W.G.; Formal analysis, Methodology, Project administration, Supervision, Validation. T.S.; Conceptualization, Writing—review & editing. E.H.; Conceptualization, Project administration, Supervision, Writing—review & editing. S.K.; Methodology, Resources, Supervision, Validation. J.Y.C.; Conceptualization, Methodology, Supervision, Writing—review & editing. K.K.; Conceptualization, Investigation, Methodology, Supervision, Writing—review & editing. J.-S.Y.; Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing—original draft, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board / Ethics Committee of Korea University Guro Hospital (approval code K2021-2812-002, date of approval 1 September 2022, Hallym University Kangnam Sacred Heart Hospital (approval code HKS2022-02-005-001, date of approval 26 August 2022), Inha University Hospital (approval code 2020-01-010, date of approval 22 August 2022), Pusan National University Hospital (approval code 2204-003-124, date of approval 21 June 2022), and the Pharma-Ethics of Republic of South Africa (approval code 220324578, date of approval 3 May 2022 and 25 June 2022). The clinical trial conducted in South Africa was registered with the South African National Clinical Trials Registry (SANCTR) under registration number DOH-27-062022-6527, with a registry approval date of 14 June 2022.

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Abbreviations

The following abbreviations are used in this manuscript:

AE	Adverse event
AESI	Adverse event of special interest
ANOVA	Analysis of variance

CI	Confidence interval
CLS	Capping Library Screening
COVID-19	Coronavirus disease 2019
CTM	Clinical trial material
DSMB	Data and Safety Monitoring Board
ECLIA	Electrochemiluminescence immunoassay
FAS	Full Analysis Set
GMC	Geometric mean concentration
GMFR	Geometric mean fold rise
GMP	Good manufacturing practice
GMT	Geometric mean titer
ICS	Intracellular cytokine staining
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
LNP	Lipid nanoparticle
MAAE	Medically attended adverse event
mRNA	Messenger ribonucleic acid
NAb	Neutralizing antibody
PBMC	Peripheral blood mononuclear cells
PPS1	Per Protocol Set 1
PPS2	Per Protocol Set 2
PRNT	Plaque reduction neutralization test
PT	Preferred term
QC	Quality control
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SFU	Spot-forming unit(s)
SOC	System organ class
SRR	Seroresponse rate
SS	Safety Set
SS1	Safety Set for the first dose
SS2	Safety Set for the second dose
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
ULOQ	Upper limit of quantification
WHO	World Health Organization

Appendix A

Table A1. Selected mRNA and self-amplifying RNA COVID-19 vaccines: platform evolution and clinical/regulatory status through August 2026.

Vaccine	Company/Institution	Vaccine Type	Characteristics	Status
Spikevax (mRNA-1273)	Moderna (USA) & Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID) (USA)	mRNA	Nucleoside-modified mRNA encoding spike protein of SARS-CoV-2, encapsulated in LNP [9]	· Initial U.S. EUA, 18 December 2020 [24] · FDA-approved as SPIKEVAX, 31 January 2022 [25]
Spikevax Bivalent Original/Omicron BA.1 (mRNA-1273.214) (Previously Spikevax Bivalent Zero/Omicron)	Moderna (USA)		Bivalent mRNA booster containing ancestral and Omicron BA.1 components	Investigational in the U.S.; BA.1 bivalent clinical development Australia TGA Provisionally approved, 29 August 2022 [26]
Spikevax Bivalent Original/Omicron BA.4-5			Bivalent mRNA booster containing ancestral and Omicron BA.4/BA.5 components	FDA EUA as a single booster dose in individuals ≥ 18 years [27]

Comirnaty (BNT162b2)	Pfizer/BioNTech (Germany/USA)	mRNA	Nucleoside-modified mRNA encoding mutated form of full-length spike protein of SARS-CoV-2, encapsulated in LNP [2,28]	· Initial FDA EUA, 11 December 2020 [29] · FDA Full Approval as COMIRNATY, 23 August 2021 [30]
Pfizer-BioNTech COVID-19 Vaccine, Bivalent (COMIRNATY Original/Omicron BA.4-5 COVID-19 vaccine)			Booster dose containing tozinameran and famtozinameran	· FDA EUA for Original/Omicron BA.4/BA.5 bivalent booster, 31 August 2022 [31]
Updated COMIRNATY			Omicron XBB.1.5 monovalent mRNA	· FDA approved/authorized, 11 September 2023 [32]
CVnCoV	CureVac (Germany)	mRNA	mRNA	Phase IIb/III [33,34] (12 October 2021 CVnCoV to be withdrawn from regulatory review due to potential overlap with approval timelines for a second-generation candidate, flu+corona combo vaccine with modified nucleoside per CureVac) [35]
PTX-COVID19-B	Providence (Canada)	mRNA	mRNA	Phase III (booster vaccination) [36,37]
CS-2034	Cansino Biologics (China)	mRNA	mRNA	Phase Iib [38]
ChulaCov19	Chulalongkorn University (Thailand)	mRNA	mRNA (LNP encapsulated nucleoside-modified mRNA encoding a prefusion non-stabilised, ectodomain of the SARS-CoV-2 spike protein of the original strain)	Thailand's home-grown mRNA vaccine. Phase II (Australia, Thailand) [39]
DAICHIRONA® (DS-5670)	Daiichi Sankyo (Japan)	mRNA	Monovalent Original Strain and Omicron XBB.1.5-adapted monovalent mRNA vaccine (booster)	· Initial approval for booster vaccination (August 2023) [40] · Approval as an Omicron XBB.1.5-adapted monovalent mRNA vaccine (November 2023) [41]
KOSTAIVE® (ARCT-154)	Arcturus (USA) & CSL (partner) (Australia)	saRNA	saRNA (monovalent JN.1): updated KOSTAIVE strain formulation	· First globally approved in Japan (28 November 2023) [42] · First authorized in EU (12 February 2025) [23]
KOSTAIVE® Bivalent (ARCT-2301)			saRNA (bivalent: Ancenstral/Omicron BA.4/5)	Phase III peer-reviewed paper [43]
KOSTAIVE® XBB.1.5 (ARCT-2303)			saRNA (monovalent: XBB.1.5)	Phase III [44]

Table A2. Participant-level characteristics and temporal profiles of troponin I elevations following STP2104 vaccination.

Subject	Dose	Troponin I Elevation/Timing	Recovery/Kinetics	Cardiac Symptoms/ Clinical Correlation	Screening ECG Assessment	Causality	AESI	Outcome
25-006	25 µg	347 ng/L, Day 7 after Dose 2	<4 ng/L at Day 28 after Dose 2	No associated cardiac symptom identified in available AE listing	Screening: Normal	Not related	No	Recovered/resolved
25-017	25 µg	388 ng/L, Day 7 after Dose 1; 341 ng/L, Day 7 after Dose 2	<4 at unscheduled visits after each episode; <4 at Dose 2 and Day 28 after Dose 2	No associated cardiac symptom identified; available unscheduled vital signs were not clinically abnormal	Screening: Normal	Related	Yes for first episode; No for second	Recovered/resolved

25-022	50 µg	345 ng/L, Day 7 after Dose 2	<4 at Day 28 after Dose 2	No associated cardiac symptom identified	Screening: abnormal NCS bradycardia/RR interval	Related	No	Recovered/resolved
25-023	50 µg	201 ng/L, Day 28 after Dose 2	<4 at unscheduled visit 5 days later	No associated cardiac symptom identified	Screening: Normal	Related	No	Recovered/resolved
25-024	50 µg	128 ng/L, Dose 2 visit	<4 at Day 7 and Day 28 after Dose 2	No associated cardiac symptom identified	Screening: Normal	Related	No	Recovered/resolved
25-025	50 µg	281 ng/L, Dose 2 visit	<4 at Day 7 and Day 28 after Dose 2	No associated cardiac symptom identified	Screening: Normal	Related	No	Recovered/resolved
25-028	50 µg	583 ng/L, Day 7 after Dose 1	<4 at unscheduled follow-up and subsequent scheduled visits	No associated cardiac symptom identified; pulse 101 beats/min at Day 7 was assessed as abnormal NCS	Screening: abnormal NCS PR interval	Related	No	Recovered/resolved
25-030	50 µg	267 ng/L, Day 7 after Dose 1	<4 at unscheduled follow-up; later 6 ng/L at Day 28 after Dose 2 (Normal)	No associated cardiac symptom identified	Screening: abnormal NCS bradycardia	Related	No	Recovered/resolved
25-031	50 µg	125 ng/L, Day 7 after Dose 1	<4 at unscheduled follow-up and subsequent visits	No associated cardiac symptom identified	Screening: Normal	Related	No	Recovered/resolved

Participant-level data are shown for the 9 participants with reported troponin I elevations, including dose cohort, timing relative to vaccination and study visit, troponin I concentration (ng/L) and available follow-up measurements, associated cardiac symptoms or clinical findings, available ECG assessment, investigator-assessed relationship to vaccination, AESI classification, and outcome. ECG assessments shown in the table were obtained at screening and were not performed contemporaneously with each troponin I elevation. Abbreviations: AESI, adverse event of special interest; ECG, electrocardiogram.

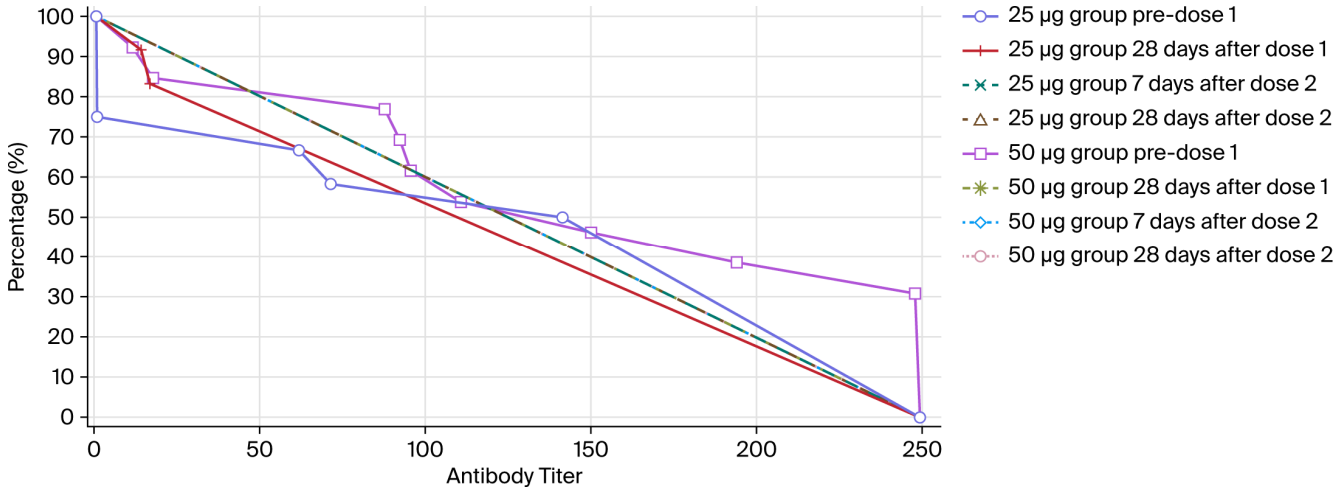


Figure A1. Reverse cumulative distribution of SARS-CoV-2 spike-specific binding-antibody concentrations following STP2104 vaccination in the Per Protocol Set 1 (PPS1). Reverse cumulative

distribution plots of binding-antibody concentrations are shown by dose cohort and study time point. The upper limit of quantification (ULOQ) of the binding-antibody assay was 250 U/mL; measurements above the ULOQ were not further quantified by dilution and re-assay in the dataset analyzed for this study. The distributions are presented descriptively, and no formal inferential statistical comparisons were performed between the 25 µg and 50 µg dose cohorts.

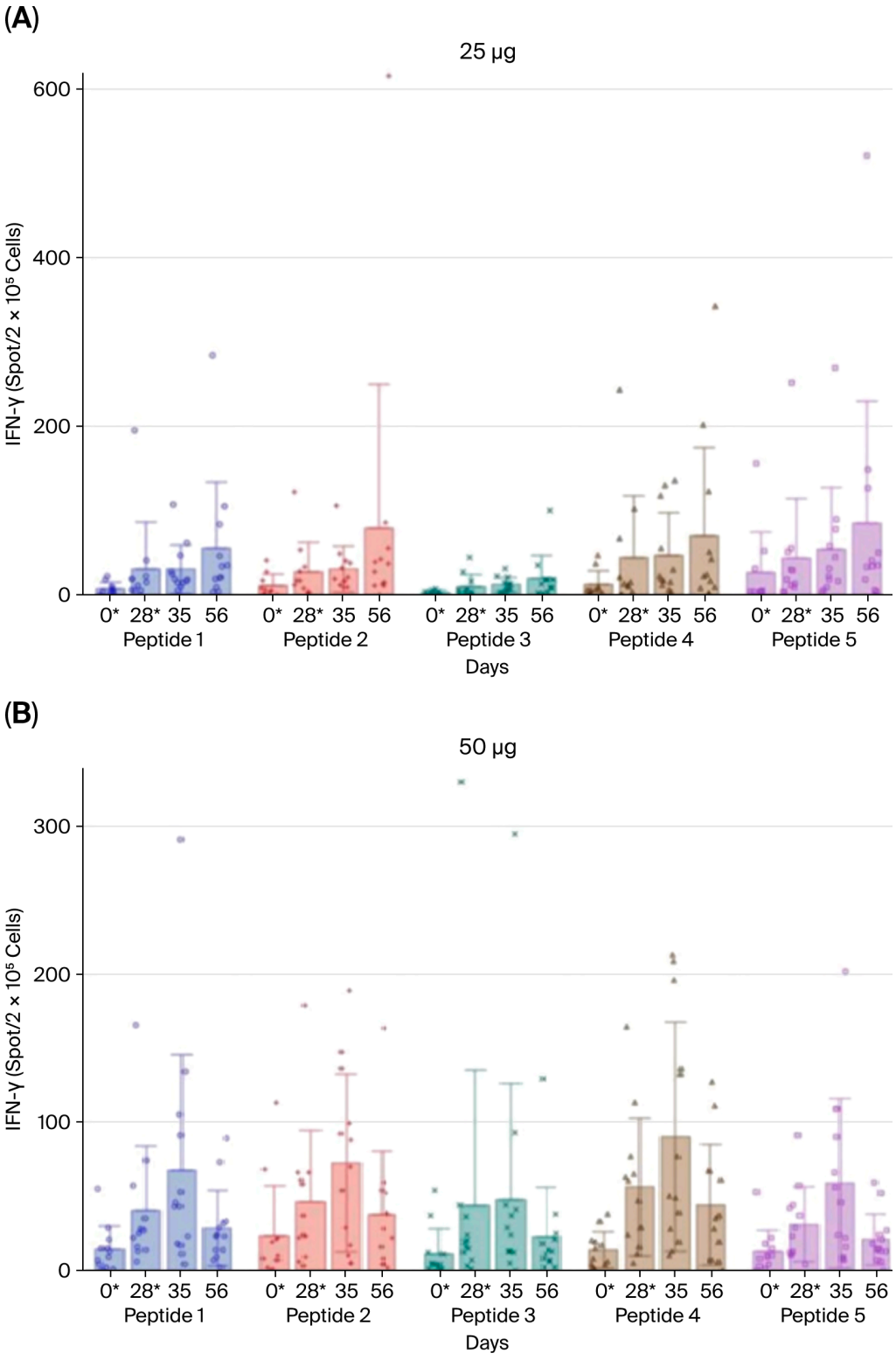


Figure A2. Peptide-pool-specific IFN-γ ELISpot responses following STP2104 vaccination in the Per Protocol Set 1 (PPS1). IFN-γ ELISpot responses following stimulation with individual SARS-CoV-2 spike peptide pools (PP1–PP5) are shown at baseline (Day 0), Day 28, Day 35, and Day 56 for

participants receiving (**A**) 25 µg or (**B**) 50 µg STP2104. Results are expressed as background-subtracted spot-forming units (SFUs) per 2×10^5 PBMCs. The numbers of evaluable participants at Days 0, 28, 35, and 56 were 10, 11, 12, and 12, respectively, in the 25 µg cohort and 12, 12, 13, and 13, respectively, in the 50 µg cohort. Day 28 corresponds to 28 days after Dose 1, Day 35 to 7 days after Dose 2, and Day 56 to 28 days after Dose 2. Data are presented as mean ± standard deviation (SD) and are summarized descriptively. No formal inferential statistical comparisons were performed between the 25 µg and 50 µg dose cohorts. Abbreviations: IFN-γ, interferon gamma; PBMC, peripheral blood mononuclear cell; PP, peptide pool; PPS1, Per Protocol Set 1; SD, standard deviation; SFU, spot-forming unit. * Asterisk indicates days of vaccinations.

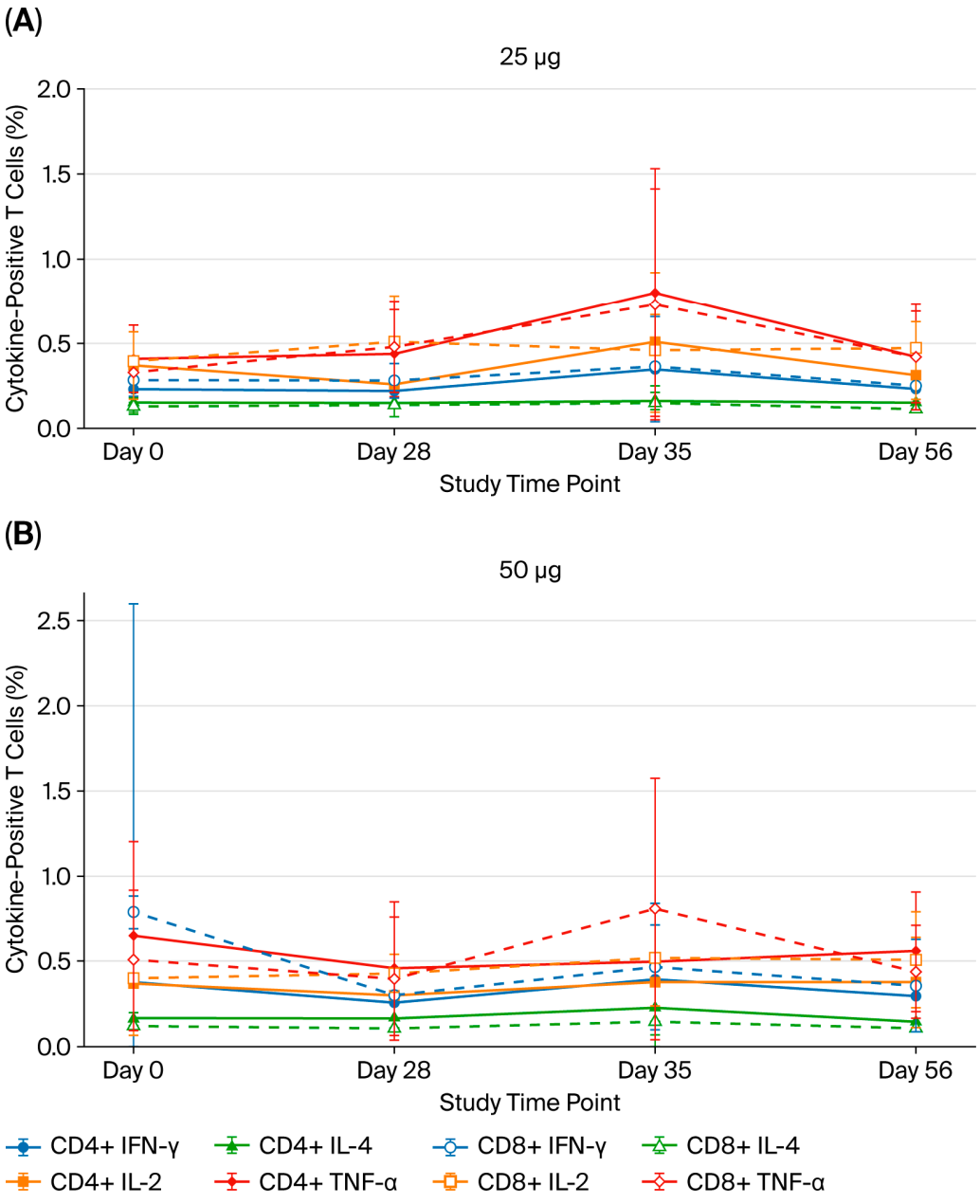


Figure A3. Exploratory intracellular cytokine staining (ICS) responses following STP2104 vaccination in the PPS1. ICS was used to assess IFN-γ-, IL-2-, IL-4-, and TNF-α-positive CD4+ and CD8+ T cells at baseline (Day 0), Day 28, Day 35, and Day 56. (**A**) 25 µg cohort; (**B**) 50 µg cohort. Data are presented as mean ± SD. In the 25 µg cohort, the numbers of evaluable participants were 12, 12, 11, and 12 at Days 0, 28, 35, and 56, respectively; the corresponding numbers in the 50 µg cohort were 13, 13, 12, and 13. Abbreviations: PPS1, Per Protocol Set 1; ICS, intracellular cytokine staining; IFN-γ, interferon gamma; IL, interleukin; TNF-α, tumor necrosis factor alpha; SD, standard deviation.

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