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Short communication

# Phase 1/2 clinical trial of COVID-19 vaccine in Japanese participants: A report of interim findings

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ABSTRACT

We initiated a randomized, placebo-controlled, phase 1/2 trial to evaluate the safety and immunogenicity of the S-268019-b recombinant protein vaccine, scheduled as 2 intramuscular injections given 21 days apart, in 60 randomized healthy Japanese adults. We evaluated 2 regimens of the S-910823 antigen (5 μg [n = 24] and 10 μg [n = 24]) with an oil-in-water emulsion formulation and compared against placebo (n = 12). Reactogenicity was mild in most participants. No serious adverse events were noted. For both regimens, vaccination resulted in robust IgG and neutralizing antibody production at days 36 and 50 and predominant T-helper 1-mediated immune reaction, as evident through antigen-specific polyfunctional CD4+ T-cell responses with IFN-γ, IL-2, and IL-4 production on spike protein peptides stimulation. Based on the interim analysis, the S-268019-b vaccine is safe, produces neutralizing antibodies titer comparable with that in convalescent serum from COVID-19-recovered patients. However, further evaluation of the vaccine in a large clinical trial is warranted.


## 1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a designated global pandemic with more than 270.0 million confirmed cases and 5.3 million deaths reported worldwide as of December 14, 2021 [1]. The World Health Organization (WHO) has identified 136 vaccine candidates against SARS-CoV-2 infection from global clinical trials as of December 2021 [2]. Nonetheless, most countries, including Japan, need domestic vaccines to ensure stable vaccine supply. Development of vaccines effective against mutant strains is imperative, especially if new virus variants emerge and become prevalent. Additionally, the demand for booster vaccine shots is increasing with the goal of staying a step ahead of breakthrough infections among the fully vaccinated people [3]. In Japan, over 1.7 million COVID-19 cases (with 18,374 deaths, 1.04% mortality) were reported, and over 196 million vaccine doses were administered by December 2021 [1]. However, young and middle-aged people were deprioritized and needed to wait for vaccination. In the absence of domestic vaccines, Japan's vaccine supply is dependent on external factors and, thus, limited and unstable. Recently, new mutant strains have been reported in Japan, which may increase the number of patients with severe infection and weaken the effectiveness of existing vaccines [4].

S-268019-b is a recombinant protein prophylactic vaccine comprising the S-910823 antigen, a modified recombinant spike protein of SARS-CoV-2 produced using the baculovirus expression system in rhabdovirus-free insect cells, with a squalene-based adjuvant (A-910823) in an oil-in-water emulsion formulation. Results of primary preclinical immunogenicity studies of S-268019-b in monkeys have been reported (manuscripts in preparation). Here, we report the results of an interim analysis of a phase 1/2, randomized, double-blind, placebo-controlled, parallel-group study evaluating the safety and immunogenicity of the S-268019-b vaccine, scheduled as 2 injections given 21 days apart, in Japanese healthy adult volunteers.

## 2. Methods

### 2.1. Study design and participants

The study population comprised healthy Japanese adults (age 20–64 years) with body mass index ranging between 18.5 and 25.0 kg/m$^2$ at screening. Individuals with SARS-CoV-2 infection

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before the first dose of the intervention, previous SARS-CoV-2 vaccination with an approved or investigational product, or chronic diseases were excluded. The study (jRCT2031210269) was conducted as per study protocol (approved by Institutional Review Board), Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice Guidelines, and other applicable laws and regulations [5,6,7]. All participants provided their written informed consent. Those meeting the eligibility criteria were randomized to receive either 5 μg or 10 μg S-910823 with A-910823 (both 50% v/v) or placebo in saline on day 1. Participants were administered the assigned investigational products intramuscularly twice at a 21-day interval (on day 1 and day 22; Fig. 1) and were evaluated until the data cut-off date (≥day 50 for all participants) with frequent study visits for investigations pertaining to this interim analysis.

### 2.2. Outcomes

Primary endpoints included incidence of adverse events (AEs)/treatment-related adverse events (TRAEs)/serious AEs/solicited AEs (information on systemic and local AEs collected daily for 7 days after each vaccination); changes in vital signs; and changes in laboratory test results and electrocardiograms. Unless otherwise mentioned, analyses were based on treatment-emergent AE (TEAEs; any AEs reported after the initial dose of the study intervention). Secondary endpoints were related to immunogenicity, including geometric mean titer (GMT) for neutralizing antibodies and anti-spike protein immunoglobulin G (IgG) antibodies, and seroconversion rate (defined as a ≥4-fold change from baseline in SARS-CoV-2 neutralizing antibody titer, where titer values reported as below the lower limit of quantification (LLOQ) are replaced by 0.5 × LLOQ). Additional methodology details are described in **Supplementary Appendix**.

### 2.3. Statistical analyses

All analyses were descriptive. Quantitative variables were summarized using mean, standard deviation (SD), median, minimum, and maximum. Categorical variables were summarized using frequency (%). SAS® 9.4 (SAS Institute, Cary, North Carolina, USA) was used for all statistical analyses.

## 3. Results

### 3.1. Trial population

Overall, 133 individuals were screened (**supplementary** Fig. 1). Sixty participants meeting the inclusion criteria were randomized to receive the 5-μg regimen (n = 24), the 10-μg regimen (n = 24), or placebo (n = 12; **supplementary Table 1**). Many participants were ≥30 years old (90.0%) and 51.7% were female. All participants were Asian; most were non-smokers (96.7%), 48.3% had consumed alcohol, and none had a history of COVID-19 infection.

### 3.2. Safety outcomes

The incidence of AEs was 50.0% (6/12) in the placebo group, 95.8% (23/24) in the S-268019-b 5-μg regimen, and 100.0% (24/24) in the S-268019-b 10-μg regimen. Almost all participants receiving S-268019-b experienced at least 1 TRAE, with TRAEs related to injection site reaction and fatigue being the most common (Table 1). A higher proportion of participants reported both

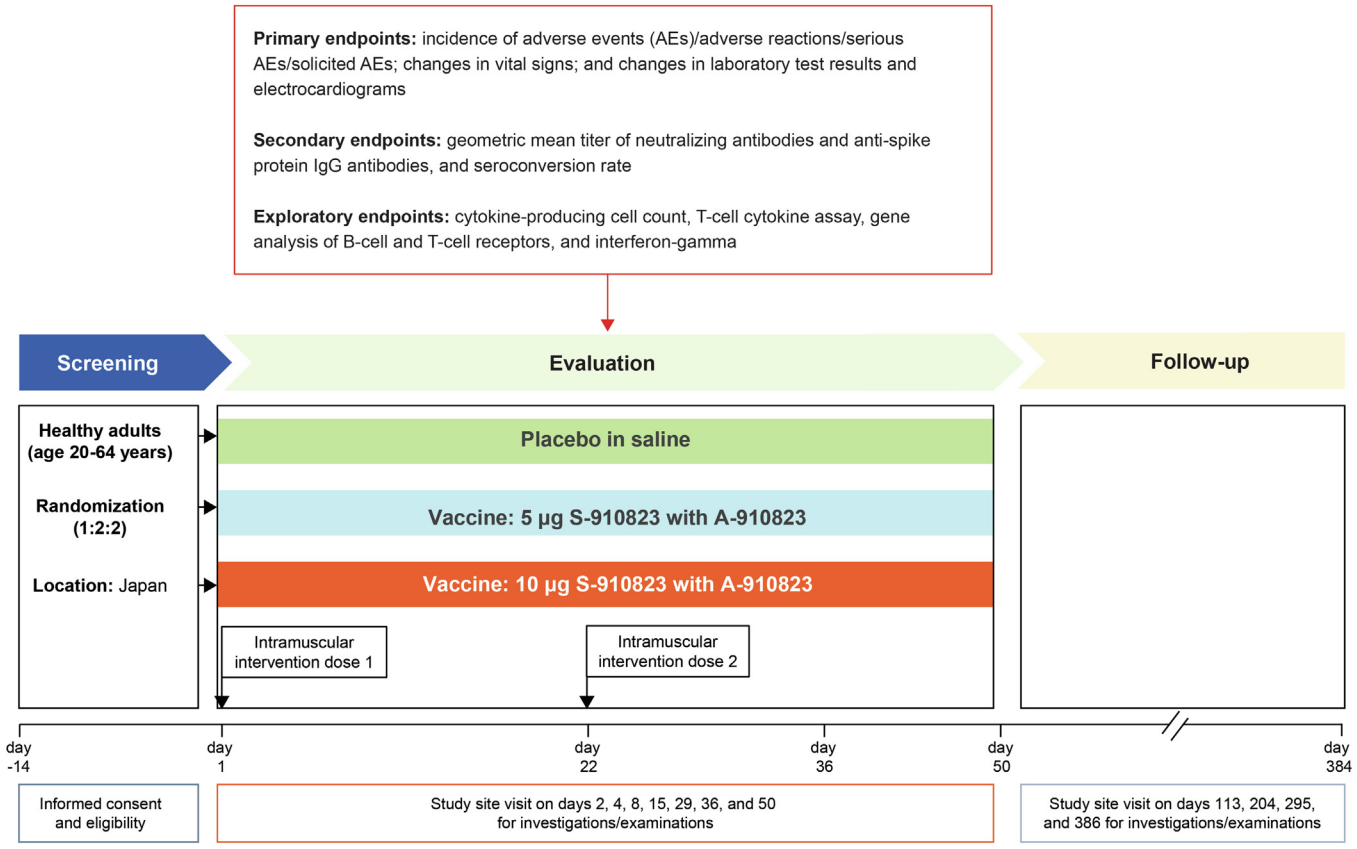


**Fig. 1.** Vaccine regimen and key assessments.

**Table 1**
Treatment-related adverse events.

| | Placebo (n = 12) | 5-μg S-910823 (n = 24) | 10-μg S-910823 (n = 24) |
|---|---|---|---|
| Participants with treatment-related deaths | 0 | 0 | 0 |
| Participants with treatment-related other serious AEs | 0 | 0 | 0 |
| Participants with any treatment-related AEs of special interest | 0 | 0 | 0 |
| Participants with any treatment-related AEs | 5 (41.7) | 23 (95.8) | 24 (100.0) |
| Nervous system disorders | 1 (8.3) | 12 (50.0) | 16 (66.7) |
| Headache | 1 (8.3) | 12 (50.0) | 16 (66.7) |
| Gastrointestinal disorders | 0 | 9 (37.5) | 12 (50.0) |
| Nausea | 0 | 8 (33.3) | 12 (50.0) |
| Diarrhea | 0 | 2 (8.3) | 0 |
| Vomiting | 0 | 0 | 1 (4.2) |
| Musculoskeletal and connective tissue disorders | 1 (8.3) | 8 (33.3) | 13 (54.2) |
| Myalgia | 1 (8.3) | 7 (29.2) | 13 (54.2) |
| Arthralgia | 0 | 1 (4.2) | 0 |
| General disorders and administration site conditions | 5 (41.7) | 23 (95.8) | 24 (100.0) |
| Vaccination site pain | 4 (33.3) | 23 (95.8) | 24 (100.0) |
| Fatigue | 3 (25.0) | 20 (83.3) | 18 (75.0) |
| Vaccination site induration | 0 | 10 (41.7) | 9 (37.5) |
| Pyrexia | 0 | 5 (20.8) | 12 (50.0) |
| Vaccination site swelling | 0 | 10 (41.7) | 5 (20.8) |
| Vaccination site erythema | 0 | 7 (29.2) | 6 (25.0) |
| Vaccination site pruritus | 0 | 0 | 1 (4.2) |

AE, adverse event.
A treatment-related AE is defined as an AE considered to be "related" to the study intervention.
Participants with multiple treatment-related AEs were counted only once within a system organ class and preferred term.
Data are presented as n (%).

solicited systemic and local solicited AEs after S-268019-b administration compared with placebo (**supplementary Table 2**). Further, a higher proportion of participants experienced systemic AEs after the second injection compared to after the first injection or placebo. No serious TEAEs or potential immune-mediated diseases as AEs of special interest were reported, precluding the need to implement vaccination pause rules. No participants experienced grade 3 systemic or local TRAEs after the first injection, whereas 2 participants in the 10-μg regimen experienced systemic grade 3 fever after the second injection. Although pain at the injection site was a common solicited TRAE after both S-910823 regimens (**supplementary Table 2**), only 1 and 3 participants experienced grade 3 local TRAEs in the form of injection site reaction exceeding 21 cm in 5-μg and 10-μg regimens, respectively; importantly, all recovered during the observation period. Overall reactogenicity was generally mild. Unsolicited TRAEs (events other than solicited systemic or local TRAEs) were reported by 0%, 4.2%, and 12.5% of participants receiving placebo, 5-μg regimen, and 10-μg regimen, respectively. No clinically significant abnormalities in vital signs, laboratory tests, and electrocardiograms were observed until day 50.

### 3.3. Immunogenicity

Both vaccine regimens elicited anti-spike protein IgG and neutralizing antibodies responses at days 36 and 50 (Fig. 2**A**). While anti-spike protein IgG levels remained practically unchanged in the placebo group, in both the vaccine regimens (5 μg and 10 μg), they peaked at day 36 and GMTs were 28735 and 38356, respectively. Also, GMTs of neutralizing antibodies remained unchanged in the placebo group during the study period, but were 37.8 and 46.2 in the 5-μg and 10-μg regimens, respectively, at day 36 (Fig. 2**B**). Both vaccine regimens achieved SARS-CoV-2 neutralizing antibody levels that were generally similar with convalescent serum samples from symptomatic outpatients with COVID-19 (n = 59; GMT [95% CI], 28.5 [21.1, 38.4]; **supplementary** Fig. 2). Overall, 91.7% and 100% of participants in the 5-μg and 10-μg regimens, respectively, achieved seroconversion with respect to SARS-CoV-2 neutralizing antibody response at day 36 (**supplementary Table 3**).

T-cell responses showed that both vaccine regimens induced antigen-specific polyfunctional CD4+ T-cell responses that were reflected in interferon-gamma (IFN-γ), interleukin 2 (IL-2), and IL-4 production on spike protein stimulation (Fig. 3**A**). A strong bias toward the T-helper type 1 (Th1) phenotype was noted; Th2 responses, measured through IL-4 and IL-5 cytokine levels were minimal. A substantial increase in IFN-γ levels was also observed on days 36 and 50 in participants receiving the vaccine (Fig. 3**B**).

## 4. Discussion

In this first-in-human study, S-268019-b, a vaccine containing a modified recombinant spike protein of SARS-CoV-2 (formulated with an oil-in-water-based adjuvant) elicited neutralizing antibodies with an acceptable safety profile. This is the first clinical trial of a recombinant protein vaccine made in Japan to report neutralizing antibody induction, specifically in Japanese participants.

The phase 1/2 study was planned on the basis of preclinical data in monkeys. In cynomolgus macaques, S-268019-b exhibited immunogenicity with potent induction of spike-protein-specific antibody, neutralizing antibody, and cellular immunity after the second vaccine injection in a dose-dependent manner, without observational adverse reactions, when intramuscularly administered twice at a 3-week interval (manuscript in preparation).

While AEs were more common in the vaccine groups compared with the placebo group, no serious AEs or AEs of special interest, including narcolepsy, were reported. Absence of any notable safety concerns has led to progression to phase 3 clinical development. Mild physical manifestation of inflammatory response to vaccination, including fatigue, headache, and pain at the injection site, is in alignment with studies assessing reactogenicity of recombinant protein-based vaccines [8,9]. Notably, S-910823 was produced with the baculovirus expression system in insect cells. This mode of antigen production in a rhabdovirus-free cell line may potentially enhance the overall biosafety of the baculovirus expression system [10]. Moreover, high tolerance in both children and adults

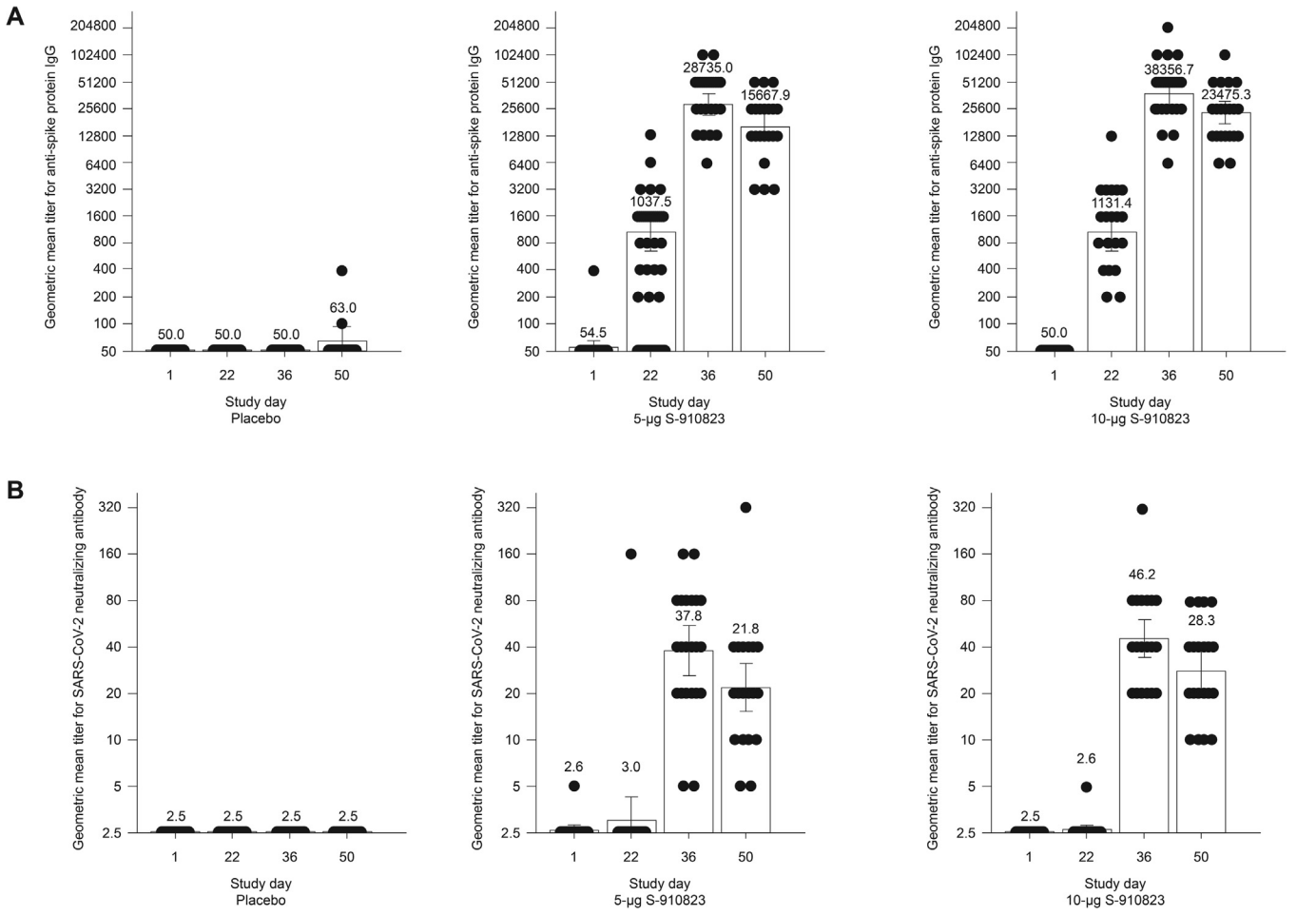


**Fig. 2.** Geometric mean titers in the placebo and vaccine groups for (A) anti-spike protein IgG and (B) neutralizing antibody responses. CI, confidence interval; GMT, geometric mean titer; LLOQ, lower limit of quantification. Data are presented as GMTs and 95% CIs. The bars represent the GMT and 95% CI; closed circles represent individual titers. Titer values reported as below the LLOQ are replaced by 0.5 × LLOQ.

and cumulative safety data for the squalene-based adjuvants from multiple influenza vaccines support the use of squalene-based adjuvants for safe delivery of vaccines [11].

IgG titer and neutralizing antibodies followed similar trends with peaks noted at day 36. This is not surprising—IgG response to the spike protein is considered the best correlate of neutralizing antibodies [12]. Moreover, high anti-spike protein IgG and neutralizing antibody responses were observed in both vaccine doses (5 μg and 10 μg) with higher numerical trends in the 10-μg regimen on days 36 and 50. Cytokine profiling using peripheral blood mononuclear cells (PBMCs) showed a more robust induction of Th1 cell cytokines than of Th2 cytokines. The use of the adjuvant presumably resulted in an enhanced immune response, as evident from the robust CD4+ T-cell response. Additionally, both vaccine regimens elicited a substantial IFN-γ response on days 36 and 50. The critical roles of IFN-γ production in viral clearance and the development of adaptive immune responses are previously documented [13]. We acknowledge the theoretical concern of vaccine potentiating disease pathology through mechanisms including but not limited to the magnitude of immune responses, induction of antibodies with functional characteristics with binding to particular Fc receptors, balance between binding and functional antibodies, and the nature of the Th2 cell response with Th2-polarized cellular responses [14]. However, in our interim analysis, there was no evidence of vaccine-mediated disease development.

S-268019-b vaccine administration induced neutralizing antibodies similar in magnitude to those observed in convalescent serum samples from patients recovered from COVID-19. Although antibodies and neutralizing antibodies against SARS-CoV-2 are found in most confirmed COVID-19 cases and can correlate inversely with viral load, their correlation with conferred protection is unclear owing to paucity of data and the use of heterogeneous serological assays with limited sensitivity and specificity [15]. The role of T cells in producing immune responses to COVID-19 is gradually emerging. In most SARS-infected patients, B-cell and neutralizing antibody responses were relatively short lived (1–2 years) and generally targeted primary homologous strains, which may increase the possibility of reinfection [16,17]. Conversely, T cells are capable of showing robust cross-reactivity between N-proteins of SARS-CoV and SARS-CoV-2 up to 17 years after the first infection with SARS-CoV [18]. Therefore, in addition to neutralizing antibodies, simultaneous recruitment of CD4 and CD8 T cells and the generation of effective T-cell memory may contribute to eliciting broad and long-lasting antiviral immunity.

Along with the findings, the study's limitations should be considered. A small sample size and a short follow-up for the interim analysis in this study may compromise effective capturing of rare serious AEs, AEs of special interest, or late-onset AEs. Although intentional, limited ethnic and racial diversity as a function of the study design could limit the generalizability of these results.

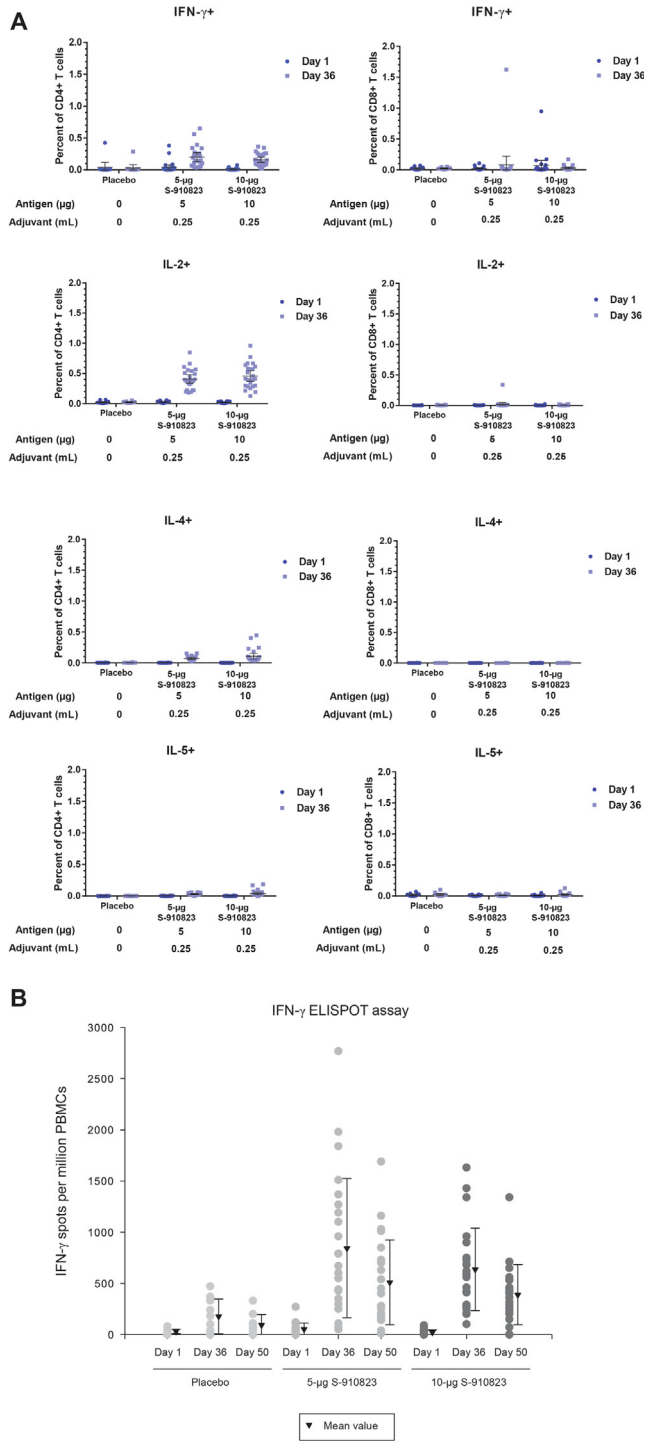


**Fig. 3.** Immunologic assays for (A) Percent CD4/CD8 cells positive for IFN-γ, IL-2, IL-4, and IL-5 at different time points for the study groups with mean (horizontal bar) and 95% confidence interval (vertical bar) and (B) IFN-γ spots per million PBMCs at different time points for the study groups with mean (triangle) and standard deviation (bar). IFN- γ, interferon gamma; IL, interleukin; PBMC, peripheral blood mononuclear cell.

Finally, lack of adjuvant-only control group may have precluded the assessment of specific contribution of the adjuvant to the immune responses. Despite the limitations, the current phase 1/2 study tends to recommend 10 μg S-910823 as the optimal vaccine dose for evaluation in a large-scale phase 2/3 study in Japanese participants.

## 5. Conclusion

S-268019-b vaccine composed of the SARS-CoV-2 spike protein with an oil-in-water-based adjuvant was well tolerated and adequately immunogenic in healthy Japanese participants. The observed induction of neutralizing antibodies at 2 weeks or later of the second vaccine injection, to an extent similar to human convalescent serum obtained from recovered symptomatic outpatients, and the associated safety and well-tolerance to the doses noted in the interim analysis warrant further evaluation in a large-scale clinical trial.

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## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SI received payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing, and educational events from Astellas Pharma Inc., Japan Vaccine Co., Ltd., Meiji Seika Pharma Co., Ltd., Pfizer Japan Inc., and Taisho Toyama Pharmaceutical Co. Ltd. TS, AK, RS, TH, SO, KI, and MA are employees of Shionogi & Co. Ltd..

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.04.054.

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