Immunogenicity and safety of a recombinant fusion protein vaccine (V-01) against coronavirus disease 2019 in healthy adults: a randomized, double-blind, placebo-controlled, phase II trial

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Abstract

Background: Innovative coronavirus disease 2019 (COVID-19) vaccines, with elevated global manufacturing capacity, enhanced safety and efficacy, simplified dosing regimens, and distribution that is less cold chain-dependent, are still global imperatives for tackling the ongoing pandemic. A previous phase I trial indicated that the recombinant COVID-19 vaccine (V-01), which contains a fusion protein (IFN-PADRE-RBD-Fc dimer) as its antigen, is safe and well tolerated, capable of inducing rapid and robust immune responses, and warranted further testing in additional clinical trials. Herein, we aimed to assess the immunogenicity and safety of V-01, providing rationales of appropriate dose regimen for further efficacy study.

Methods: A randomized, double-blind, placebo-controlled phase II clinical trial was initiated at the Gaozhou Municipal Centre for Disease Control and Prevention (Guangdong, China) in March 2021. Both younger ($n = 440$; 18–59 years of age) and older ($n = 440$; ≥60 years of age) adult participants in this trial were sequentially recruited into two distinct groups: two-dose regimen group in which participants were randomized either to follow a 10 or 25 μg of V-01 or placebo given intramuscularly 21 days apart (allocation ratio, 3:3:1, $n = 120$, 120, 40 for each regimen, respectively), or one-dose regimen groups in which participants were randomized either to receive a single injection of 50 μg of V-01 or placebo (allocation ratio, 3:1, $n = 120$, 40, respectively). The primary immunogenicity endpoints were the geometric mean titers of neutralizing antibodies against live severe acute respiratory syndrome coronavirus 2, and specific binding antibodies to the receptor binding domain (RBD). The primary safety endpoint evaluation was the frequencies and percentages of overall adverse events (AEs) within 30 days after full immunization.

Results: V-01 provoked substantial immune responses in the two-dose group, achieving encouragingly high titers of neutralizing antibody and anti-RBD immunoglobulin, which peaked at day 35 (161.9 [95% confidence interval [CI]: 133.3–196.7] and 149.3 [95% CI: 123.9–179.9] in 10 and 25 μg V-01 group of younger adults, respectively; 111.6 [95%CI: 89.6–139.1] and 111.1 [95%CI: 89.2–138.4] in 10 and 25 μg V-01 group of older adults, respectively), and remained high at day 49 after a day-21 second dose; these levels significantly exceed those in convalescent serum from symptomatic COVID-19 patients (53.6, 95%CI: 31.3–91.7). Our preliminary data show that V-01 is safe and well tolerated, with reactogenicity predominantly being absent or mild in severity and only one vaccine-related grade 3 or worse AE being observed within 30 days. The older adult participants demonstrated a more favorable safety profile compared with those in the younger adult group: with AEs percentages of 19.2%, 25.8%, 17.5% in older adults *vs.* 34.2%, 23.3%, 26.7% in younger adults at the 10, 25 μg V-01 two-dose group, and 50 μg V-01 one-dose group, respectively.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001702

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Chinese Medical Journal 2021;134(16)

Received: 23-06-2021 **Edited by:** Jing Ni

Conclusions: The vaccine candidate V-01 appears to be safe and immunogenic. The preliminary findings support the advancement of the two-dose, 10 μg V-01 regimen to a phase III trial for a large-scale population-based evaluation of safety and efficacy.
Trial Registration: http://www.chictr.org.cn/index.aspx (No. ChiCTR2100045107, http://www.chictr.org.cn/showproj.aspx?proj=124702).
Keywords: COVID-19; Phase II; Clinical trial; Recombinant fusion protein vaccine; Safety; Immunogenicity

Introduction

Coronavirus disease 2019 (COVID-19), which is caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been spread all over the world at an unprecedented speed, with more than 171 million confirmed cases, resulting in over 3.7 million deaths, as of June 8, 2021, according to the data released by the World Health Organization (WHO).[1,2] Substantial concerns have been raised about the immune evasion potential of recently emerged SARS-CoV-2 variants of concern, such as alpha strain (B.1.1.7, first identified in United Kingdom), beta strain (B.1.351, first identified in South Africa), gamma strain (P.1, first identified in Brazil) and delta strain (B.1.617.2, first identified in India), which appear to spread from person to person more readily than the prototype strain (enhanced viral infectivity) or have achieved a partial resistance to existing neutralizing antibodies.[3-6] In response to this situation, several vaccines have been validated for WHO emergency use listing since late 2020, including mRNA-based vaccines, recombinant adenoviral vector vaccines, and an inactivated vaccine.[7] Additionally, several more candidate vaccines to combat COVID-19 are currently undergoing testing in clinical trials.[8-11] Among these candidate vaccines, the recombinant protein vaccines represent promising candidates owing to the following strengths: (1) relatively high safety profile, especially in the geriatric population; (2) comparatively simple large-scale production;[12,13] and (3) storage and delivery requirements that facilitate broader usage in developing countries that lack sufficient medical facilities. The above advantages will play an important role in containing the current COVID-19 pandemic. However, only a few recombinant protein vaccine candidates have been approved for marketing or emergency use, and more research and development of the vaccines is needed.

Recently, we reported a vaccine (V-01) containing a dimerized interferon-armed receptor-binding domain (RBD) from the S1 subunit of spike protein; this configuration enhances the immunogenicity of the SARS-CoV-2 spike RBD.[14] V-01 has the protein structure of interferon-α at the N-terminus, followed by the pan human leukocyte antigen-DR-binding epitope (PADRE) sequence and SARS-CoV-2 spike RBD, and this structure is dimerized by a human immunoglobulin (IgG)1 Fc at the C-terminus (named I-P-R-F). The recombinant protein was designed to be co-administered with the conventional alum adjuvant to increase immunogenicity while avoiding severe side effects. This unique vaccine theoretically targets and activates dendritic cells, T-helper cells, and follicular T-helper lymphocytes to enhance antigen processing and presentation.[15-17] A pre-clinical study found that both low- and high-dose V-01 conferred protective immunity against a SARS-CoV-2 challenge in *Rhesus macaques*. In February 2021, we conducted a randomized, double-blind, placebo-controlled phase I clinical trial to evaluate the safety and immunogenicity of V-01 in healthy adults who randomly received a two-dose, 21-day-interval regimen of 10, 25, or 50 μg of V-01 or placebo. In that study, V-01 exhibited promising safety and tolerability in adults, especially in participants over 60 years of age, with overall vaccine-related adverse events (AEs) occurring in approximately 17% of participants and no grade 3 or worse AEs observed within 30 days of vaccine administration. Furthermore, V-01 elicited rapid and strong immune responses, achieved high titers of neutralizing antibody and anti-RBD IgG on day 35 or 49 after the first dose (with a second dose administered on day 21), and even the low-dose (10 μg) subgroup exhibited encouraging evidence of vaccine immunogenicity.

On the basis of these prior successful trials, we carried out a phase II trial with a two-dose (10 or 25 μg of V-01) or one-dose (50 μg of V-01) regimen in both younger (aged 18–59 years) and older (aged over 60 years) adults, aiming to evaluate the immunogenicity and safety of V-01. Here, we report the preliminary findings on the immunogenicity and safety of V-01 from this phase II trial, from which we determined an appropriate dose for a large-scale evaluation of the efficacy and safety of V-01 in a phase III study.

Methods

Ethical approval

The trial was registered at the Chinese Clinical Trial Registry (ChiCTR2100045107). The trial protocol was approved by the Institutional Review Board of the Guangdong Provincial Centre of Disease Control and Prevention (2021V002-E01), and was conducted in accordance with the *Declaration of Helsinki* and good clinical practice. Investigators obtained written informed consent from each participant prior to their screening for eligibility. An independent data safety monitoring board was established, which was composed of multidisciplinary experts that provided safety oversight and advice to the sponsor.

Study design and participants

Beginning on March 30, 2021, we performed a randomized, double-blind, placebo-controlled, phase II trial at the Gaozhou Centre for Disease Control and Prevention (Guangdong, China). Eligible participants were younger adults aged between 18 and 59 years and older adults over 60 years without a history of either traveling in

moderate-to-high risk areas or of contact with confirmed, asymptomatic, or suspected COVID-19 cases. Exclusion criteria were: a history of COVID-19 or positive COVID-19 screening test (reverse transcription polymerase chain reaction or enzyme-linked immunosorbent assay [ELISA]) results; a history of SARS, autoimmune disease, confirmed or suspected immunosuppressive or immunodeficiency disorder, known allergic reactions to vaccines or vaccine components, or any acute febrile disease in the previous 14 days (axillary temperature over 37.3°C); and inability to comply with the study protocol as judged by the investigator. A complete description of the inclusion and exclusion criteria is available in the study protocol.

Randomization and masking

The phase II trial study participants, who included young adults and older adults, were sequentially recruited into two groups that received a two-dose or one-dose regimen. In the two-dose regimen group, participants were randomly allocated at a ratio of 3:3:1 ($n = 120$, 120, 40 for each regimen, respectively) to receive two doses of either 10 μg of V-01, 25 μg of V-01, or placebo administered intramuscularly 21 days apart. In the one-dose regimen group, participants were randomly assigned at a ratio of 3:1 ($n = 120$, 40 for each regimen, respectively) to receive a single injection of either high-dose V-01 (50 μg) or placebo.

An independent statistician used SAS statistical software version 9.4 (SAS Institute Inc., San Diego, CA, USA) to generate a random table, from which random numbers were assigned to eligible participants by investigators, and investigational vaccines and placebos were allocated serial numbers. Participants were assigned to each group by applying a randomized blocking method, with a block of seven and rand of 40 in the two-dose regimen group, and a block of four and rand of 40 in the one-dose regimen group. Statisticians were not allowed to disclose the masking code to any personnel in the clinical trials. The vaccine and placebo were identical in appearance. During the phase II trial, all the participants, investigators, and laboratory staff remained blinded unless formal or emergency unblinding was required.

Procedures

The sponsor was responsible for providing the qualified investigational vaccines and placebos, which were jointly developed by the Institute of Biophysics, Chinese Academy of Sciences and Livzon Bio Inc., and were produced in accordance with good manufacturing practice guidelines. Each vial of vaccine contained 10, 25, or 50 μg per 0.5 mL in a liquid formulation, whereas each vial of the placebo contained only aluminum hydroxide adjuvant in solution buffer. Doses of vaccine or placebo were administered intramuscularly in the deltoid muscle of the arm, either once for the one-dose regimen group (50 μg of V-01 or placebo) or twice 21 days apart for the two-dose regimen group (10 or 25 μg of V-01 or placebo).

When the high-dose group (50 μg) was observed to have a favorable safety profile in the 7-day period following administration of the first V-01 dose in a phase I trial, the phase II trial was initiated immediately. In the phase II trial, participants were monitored for 30 to 60 minutes immediately after each dose as a safety observation. In the 7 days following each dose, AEs were documented daily on diary cards by participants and verified by investigators at day 8. AEs occurring during the 8 to 21 days (for those participants between the first and second immunization in two-dose regimen group) or 8 to 30 days after vaccine administration were reported by participants through contact cards. Regular phone contacts or visits are being made by the investigator for monitoring serious AEs in the 1-year follow-up period. Both solicited AEs and unsolicited AEs within days 0 to 7, as well as days 8 to 21/30, post-vaccination were recorded. Solicited local AEs included pain, pruritus, redness, swelling, rash, and induration, while solicited systemic AEs included fever, diarrhea, constipation, dysphagia, anorexia, vomiting, nausea, muscle pain, arthralgia, joint pain, headache, cough, dyspnea, pruritus (not at the injection site), skin and mucosa abnormities, acute allergic reaction, and fatigue. AEs were observed according to the method described previously in a phase I/II study.[11] Reported AEs were encoded by applying the Medical Dictionary for Regulatory Activities classification and were graded in accordance with the guidelines of the National Medical Products Administration of China.

Blood samples for use in RBD-binding antibody and neutralizing antibody determination were scheduled to be collected from participants on days 0, 28, 35, 49 (two-dose group) or on days 0, 7, 14, 28 (one-dose group) through routine site visits. The binding capacity of IgG to SARS-CoV-2 RBD protein was determined using ELISA kits in accordance with the manufacturer's protocols by the National Institutes for Food and Drug Control (Beijing, China). The neutralizing activity against live SARS-CoV-2 was quantified at the Wuhan Institute of Virology, Chinese Academy of Sciences by conducting micro-dose cytopathogenic effect assays. The limit of detection (LOD) for specific anti-RBD IgG antibodies and neutralizing antibodies were 11 and 10, respectively, and samples with results below the LOD were treated as having 0.5 times the lower limit of quantification, which was 5.5 and 5, respectively. A panel of 38 convalescent human serum samples was obtained from donors aged 18 to 83 years (mean = 45.8 years), with the disease severity of these patients spanning a wide range, from mild ($n = 16$, 42.1%), to moderate ($n = 19$, 50%), severe ($n = 1$, 2.6%), or critical ($n = 2$, 5.3%) [Supplementary Methods and Table 1, http://links.lww.com/CM9/A727].

Outcomes

The primary immunogenicity outcomes were the seroconversion rate, geometric mean titer (GMT) of the RBD-binding antibody and SARS-CoV-2 neutralizing antibody. The seroconversion rate was defined as a seropositive change from seronegative baseline, or an at least four-fold increase if the participant was seropositive at baseline.

The safety outcomes were the frequencies and percentages of AEs, including all AEs (total of solicited local/systemic

AEs in the 7 days after each dose and unsolicited AEs), AEs related to vaccination, AEs classified as grade 3 or worse, AEs leading to participant withdrawal, and AEs of special interest (AESI) in all groups.

Statistical analysis

Participants were recruited in this phase II trial to achieve a sample size of 880, which was estimated to be sufficient for conducting a corresponding immunogenicity assessment: assuming seroconversion rates of 80% and 30% in the V-01 and placebo groups, respectively, a total of 120 participants in each V-01-dose group and 40 participants in the placebo group would be needed to observe a statistically significant difference between the V-01 and placebo groups at 99.99% probability. We performed a safety analysis in all participants who received at least one dose after enrolment. We conducted immunogenicity analyses in participants who had at least one post-vaccination blood sample collected with available antibody results. We present here the frequencies and percentages of participants experiencing each AE post-vaccination and the GMTs with a Clopper-Pearson 95% confidence interval (CI) for the neutralizing and binding antibodies against SARS-CoV-2. We applied a χ^2 test or Fisher exact test to analyze categorical data, analysis of variance to analyze the log-transformed antibody titers, and a Wilcoxon rank-sum test for data that were not normally distributed. The preliminary data were processed using SAS 9.4 (SAS Institute Inc., San Diego, CA, USA), then further analyzed and graphed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). A *P* value less than 0.05 was considered as statistically significant.

Results

Trial population

During the period from March 30 to May 26, 2021, 1189 individuals were screened, and 880 eligible participants (aged 18–59 years and ≥60 years) were enrolled in this phase II trial and were randomly assigned to treatment groups [Figure 1]. All the participants who received at least one dose of V-01 or placebo were included in the safety analysis. A total of 438 younger adult participants and 433 older adult participants were eligible for the immunogenicity evaluation, ie, they had at least one available post-immunization antibody result. In the younger adult group, six participants withdrew owing to unwillingness or inability to complete the subsequent follow-up visits; in the older adult group, four participants withdrew owing to unwillingness or inability to complete the subsequent follow-up visits, and four participants withdrew owing to intolerance of AEs [Figure 1]. The baseline demographic characteristics were comparable across the younger (mean age ranging from 42.0 to 45.4 years) and older (mean age ranging from 66.0 to 67.0 years) adult participants [Table 1], with the mean height ranging from 162.4 to 164.7 cm *vs.* 158.7 to 162.0 cm, respectively, and the mean bodyweight ranging from 62.9 to 66.8 kg *vs.* 58.6–61.1 kg, respectively. The participant groups presented a balanced sex distribution and displayed no ethnic diversity, with the study participants predominantly being Han Chinese.

Safety outcomes

Our preliminary data indicate that the safety profile of V-01 is promising, with overall reactogenicity being largely absent or mild in severity. In participants aged ≥60 years who received V-01, AEs were milder than those in participants aged 18 to 59 years. In younger and older adult participants, respectively, 41 out of 120 (34.2%) *vs.* 23 out of 120 (19.2%) in the two-dose, 10 μg V-01 group; 28 out of 120 (23.3%) *vs.* 31 out 120 (25.8%) in the two-dose, 25 μg V-01 group; 10 out of 40 (25.0%) *vs.* 9 out of 40 (22.5%) in the two-dose, placebo group; 32 out of 120 (26.7%) *vs.* 21 out of 120 (17.5%) in the one-dose, 50 μg V-01 group; and 19 out of 40 (47.5%) *vs.* 10 out of 40 (25.0%) in the one-dose, placebo group reported at least one AE within 30 days after vaccination [Table 2]. The majority of the local and systemic AEs was mild or moderate (grade 1 or 2 AEs) [Figure 2]. The most common solicited local adverse reactions were injection-site pain, with a prevalence of 7.5%, 5.0%, 12.5%, 7.5%, and 30.0% of younger participants and 5.0%, 0, 2.5%, 3.3%, and 15.0% of older participants in the two-dose 10, 25 μg V-01, or placebo groups and one-dose 50 μg V-01 or placebo groups, respectively. Solicited systemic AEs were more frequent compared with solicited local AEs, with the most common types of solicited systemic AEs being fatigue, fever, diarrhea, headache, arthralgia, and muscle pain in younger adults, and the most common types of solicited local AEs being fatigue, fever, cough, headache, muscle pain, and nausea in older adults [Figure 2]. Regarding grade 3 AEs, two, three, and four were reported in the two-dose, 10 μg V-01 group of younger adults and the two-dose, 10 and 25 μg V-01 groups of older adults, respectively, whereas only one (two-dose, 25 μg V-01 group) was assessed as being related to the vaccine by the investigators. No participant receiving any dose regimen reported a vaccine-related life-threatening (grade 4) AE. The overall incidence of vaccine-related AEs within 30 days of immunization was slightly higher in the two-dose, 10 μg V-01 group than that in the two-dose, 25 μg V-01 group of young adults [Table 1], but these incidences were largely the same in older adults and in all groups who received a single dose of V-01 [Supplementary Table 2, http://links.lww.com/CM9/A727]. Notably, no AESIs were reported.

Immunogenicity outcomes

The GMTs of neutralizing antibodies against live SARS-CoV-2 were assessed. Younger adult participants in the two-dose group were predominantly seronegative at baseline [Supplementary Table 3, http://links.lww.com/CM9/A727] and had a modest vaccine-induced immune response at day 28; the levels of neutralizing antibodies against live SARS-CoV-2 peaked at day 35 and remained at high, although slightly declined, at day 49, with GMTs on days 28, 35, and 49 post-immunization of 68.0 (95% CI: 55.3–83.4), 161.9 (95%CI: 133.3–196.7), and 117.0 (95%CI: 97.0–141.2), respectively, in the 10 μg V-01 group and of 77.1 (95%CI: 62.1–95.7), 149.3 (95%CI: 123.9–179.9), and 121.7 (95%CI: 102.9–143.8), respectively, in the 25 μg V-01 group [Supplementary Table 4, http://links.lww.com/CM9/A727]. The GMT pattern for

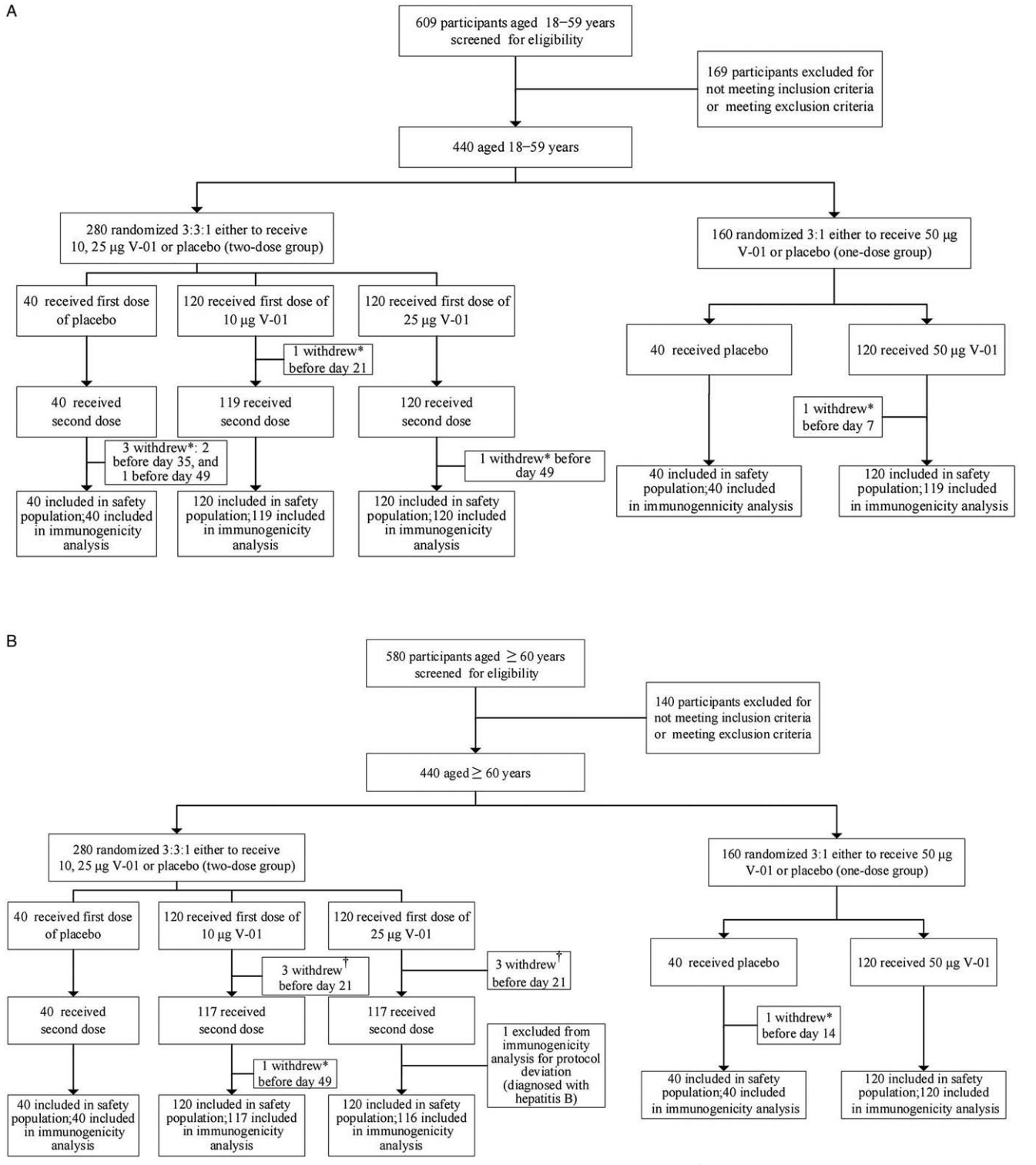

Figure 1: Flow diagram of younger (A) and older (B) participants in phase II trial of a recombinant fusion protein vaccine (V-01). *Participant withdrew due to unwilling or unable to complete the subsequent follow-ups; †One participant withdrew due to unwilling or unable to complete the subsequent follow-ups and two due to intolerance of adverse effects.

older adults in the two-dose group resembled that for younger adults, in that they were largely seronegative at baseline and had a modest vaccine-induced immune response at day 28 that further increased and peaked at day 35 or day 49, with GMTs on days 28, 35, and 49 post-immunization of 28.5 (95%CI: 22.8–35.6), 111.6 (95% CI: 89.6–139.1), and 99.2 (95%CI: 82.2–119.8), respectively, in the 10 μg V-01 group and of 41.2 (95%CI: 32.4–52.5), 111.1 (95%CI: 89.2–138.4), and 118.1 (95%CI: 96.3–144.8), respectively, in the 25 μg V-01 group [Supplementary Table 5, http://links.lww.com/CM9/A727]. Participants in the one-dose group (50 μg V-01)

Table 1: Baseline characteristics of the participants classified by age in phase II trial of a recombinant fusion protein vaccine (V-01).

Characteristics	Younger adults (18–59 years)					Older adults (≥60 years)				
	One-dose		Two-dose			One-dose		Two-dose		
	50 μg (*n* = 120)	Placebo (*n* = 40)	10 μg (*n* = 120)	25 μg (*n* = 120)	Placebo (*n* = 40)	50 μg (*n* = 120)	Placebo (*n* = 40)	10 μg (*n* = 120)	25 μg (*n* = 120)	Placebo (*n* = 40)
Age (years)	43.9 ± 11.3	45.2 ± 10.3	43.5 ± 10.1	45.4 ± 10.0	42.0 ± 8.9	66.4 ± 4.5	66.4 ± 4.6	66.1 ± 4.2	67.0 ± 4.5	66.0 ± 3.9
Ethnicity										
Han Chinese	115 (95.8)	40 (100.0)	119 (99.2)	120 (100.0)	40 (100.0)	120 (100.0)	40 (100.0)	120 (100.0)	119 (99.2)	40 (100.0)
Other	5 (4.17)	0	1 (0.8)	0	0	0	0	0	1 (0.8)	0
Sex										
Male	60 (50.0)	22 (55.0)	56 (46.7)	23 (57.5)	56 (46.7)	76 (63.3)	26 (65.0)	65 (54.2)	70 (58.3)	25 (62.5)
Female	60 (50.0)	18 (45.0)	64 (53.3)	17 (42.5)	64 (53.3)	44 (36.7)	14 (35.0)	55 (45.8)	50 (41.7)	15 (37.5)
Height (cm)	162.4 ± 8.7	162.6 ± 7.7	163.2 ± 8.1	162.6 ± 7.9	164.7 ± 7.8	159.4 ± 7.1	158.9 ± 8.3	158.7 ± 8.5	159.8 ± 8.4	162.0 ± 7.7
Body weight (kg)	63.0 ± 10.4	66.8 ± 10.6	62.9 ± 11.3	64.9 ± 10.0	67.7 ± 10.9	59.7 ± 9.4	58.6 ± 8.5	59.2 ± 11.1	59.9 ± 9.4	61.1 ± 9.4

Data are presented as the mean ± standard deviation or *n* (%).

Table 2: Overall adverse events, solicited local and systemic adverse reactions stratified by age in phase II trial of a recombinant fusion protein vaccine (V-01).

Adverse events	Younger adults (18–59 years)					Older adults (≥60 years)				
	One-dose		Two-dose			One-dose		Two-dose		
	50 μg (*n* = 120)	Placebo (*n* = 40)	10 μg (*n* = 120)	25 μg (*n* = 120)	Placebo (*n* = 40)	50 μg (*n* = 120)	Placebo (*n* = 40)	10 μg (*n* = 120)	25 μg (*n* = 120)	Placebo (*n* = 40)
Overall adverse events within 28/21* days										
Any	32 (26.7)	19 (47.5)	41 (34.2)	28 (23.3)	10 (25.0)	21 (17.5)	10 (25.0)	23 (19.2)	31 (25.8)	9 (22.5)
Vaccination-related	24 (20.0)	16 (40.0)	30 (25.0)	15 (12.5)	6 (15.0)	10 (8.3)	8 (20.0)	12 (10.0)	15 (12.5)	2 (5.0)
Grade ≥3	0	1 (2.5)	1 (0.8)	2 (1.7)	1 (2.5)	0	1 (2.5)	3 (2.5)	4 (3.3)	2 (5.0)
Solicited local adverse reactions										
Pain	9 (7.5)	12 (30.0)	9 (7.5)	6 (5.0)	5 (12.5)	4 (3.3)	6 (15.0)	6 (5.0)	0	1 (2.5)
Induration	0	0	0	0	0	0	0	0	0	0
Swelling	1 (0.8)	0	1 (0.8)	0	0	0	0	0	0	0
Rash	0	0	0	0	0	0	0	0	0	0
Redness	1 (0.8)	0	2 (1.7)	0	0	0	0	0	0	0
Pruritus	2 (1.7)	1 (2.5)	2 (1.7)	1 (0.8)	1 (2.5)	0	0	0	2 (1.7)	0
Solicited systemic adverse reactions										
Fever	4 (3.3)	1 (2.5)	8 (6.7)	1 (0.8)	1 (2.5)	3 (2.5)	0	3 (2.5)	1 (0.8)	0
Diarrhoea	0	1 (2.5)	3 (2.5)	2 (1.7)	1 (2.5)	0	0	0	1 (0.8)	0
Constipation	0	0	0	0	0	0	0	0	0	0
Dysphagia	1 (0.8)	0	0	0	0	0	0	0	0	0
Anorexia	1 (0.8)	0	0	0	0	0	0	0	0	0
Vomiting	0	0	0	0	0	0	0	0	0	0
Nausea	0	0	1 (0.8)	2 (1.7)	0	1 (0.8)	0	1 (0.8)	1 (0.8)	0
Muscle pain	3 (2.5)	2 (5.00)	3 (2.5)	1 (0.8)	0	1 (0.8)	0	1 (0.8)	1 (0.8)	0
Arthralgia	1 (0.8)	1 (2.5)	2 (1.7)	1 (0.8)	0	0	0	0	3 (2.5)	0
Headache	2 (1.7)	0	3 (2.5)	1 (0.8)	1 (2.5)	2 (1.7)	2 (5.0)	2 (1.7)	2 (1.7)	0
Cough	0	1 (2.5)	3 (2.5)	0	0	2 (1.7)	1 (2.5)	2 (1.7)	3 (2.5)	1 (2.5)
Dyspnoea	0	0	0	0	0	0	1 (2.5)	0	0	0
Pruritus	0	0	2 (1.7)	0	0	0	0	1 (0.8)	1 (0.8)	0
Skin and mucosa abnormalities	0	0	1 (0.8)	0	0	0	0	0	0	0
Acute allergic reaction	1 (0.8)	1 (2.5)	0	1 (0.8)	0	0	0	0	0	0
Fatigue	8 (6.7)	3 (7.5)	5 (4.2)	5 (4.2)	3 (7.5)	4 (3.3)	2 (5.0)	5 (4.2)	6 (5.0)	0
Unsolicited adverse events										
Any	16 (13.3)	6 (15.0)	21 (17.5)	18 (15.0)	5 (12.5)	2 (1.7)	2 (5.0)	2 (1.7)	3 (2.5)	0
Vaccination-related	5 (4.2)	0	5 (4.2)	4 (3.3)	0	2 (1.7)	2 (5.0)	2 (1.7)	3 (2.5)	0

Data are presented as the *n* (%). *AEs within 21 days were observed after administration of the first vaccine dose.

demonstrated lower GMTs on days 7, 14, and 28 post-immunization of 5.0 (95%CI: 5.0–5.0), 14.7 (95%CI: 12.1–17.8), and 24.9 (95%CI: 20.6–30.1), respectively, in younger adults and of 5.1 (95%CI: 5.0–5.2), 11.0 (95% CI: 9.2–13.1), and 21.4 (95%CI: 17.9–25.5), respectively, in older adults.

The seroconversion rates for neutralizing antibodies against live SARS-CoV-2 were also assessed. A substantial immune response was observed in the low-dose group (10 μg) of the two-dose regimen. For younger participants following the 10 or 25 μg V-01, 21d-apart two-dose schedule, virtually all were seronegative for SARS-CoV-2

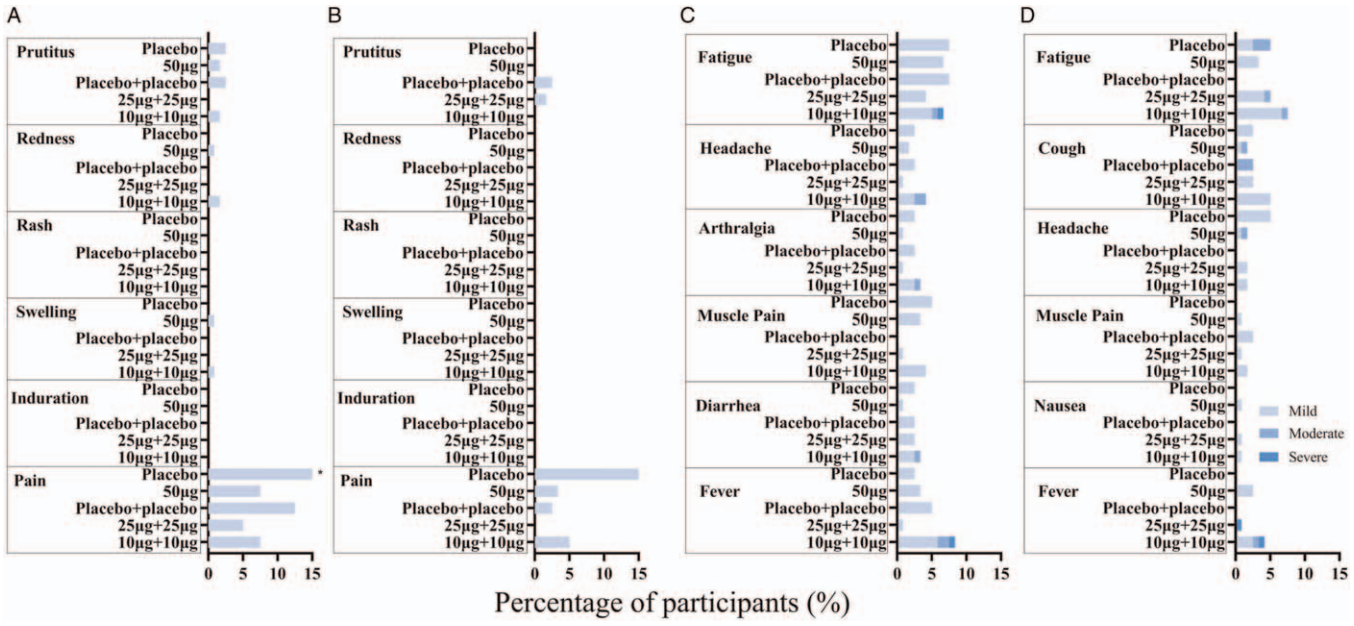

Figure 2: Solicited local and systemic adverse events stratified by age in phase II trial of a recombinant fusion protein vaccine (V-01). The percentage of participants in each vaccine group with six most frequent adverse events, classified according to the scale issued by the National Medical Products Administration (NMPA) of China, within 7 days after each administration of vaccine displayed as solicited local (A for younger, B for older adults), systemic (C for younger, D for older adults) adverse events, respectively. Mild = grade 1, moderate = grade 2, severe = grade 3 or worse. * Percentage of participants was 30%.

neutralizing antibody at day 0 but had seroconversion rates on days 28, 35, and 49 post-immunization of 95.0% (113 out of 119), 98.3% (117 out of 119), and 98.3% (117 out of 119), respectively, in the 10 μg V-01 group and of 95.8% (115 out of 120), 99.2% (119 out of 120), and 99.2% (118 out of 119), respectively, in the 25 μg V-01 group; for older participants, the corresponding seroconversion rates were 78.6% (92 out of 117), 96.6% (113 out of 117), and 97.4% (113 out of 116), respectively, in the 10 μg V-01 group and 85.3% (99 out of 116), 94.8% (110 out of 116), and 96.6% (112 out of 116), respectively, in the 25 μg V-01 group. In contrast, in the 50 μg V-01, one-dose regimen groups, remarkably lower seroconversion rates at days 7, 14, and 21 post-immunization of 0 (0 out of 119), 63.9% (76 out of 119), and 82.4% (98 out of 119), respectively, in younger adults and of 1.7% (2 out of 120), 48.7% (58 out of 119), and 83.3% (100 out of 120), respectively, in older adults were observed. Because the seroconversion rate and GMT of SARS-CoV-2 neutralizing antibody at day 28 post-immunization in the one-dose group were much lower than those in the two-dose group that received a second dose on day 21, it is likely that a second dose of our recombinant fusion protein COVID-19 vaccine is essential for eliciting a robust response. It is noteworthy that the GMTs of SARS-CoV-2 neutralizing antibody after a second dose for both the 10 and 25 μg V-01, two-dose regimen groups were remarkably higher than the GMTs in the convalescent serum panel (53.6, 95%CI: 31.3–91.7) [Figure 3]; specifically, these GMTs at day 35 post-immunization were 3.0 (10 μg V-01) or 2.8 (25 μg V-01) times and 2.1 (10 μg V-01) or 2.0 (25 μg V-01) times greater than that in the convalescent serum from symptomatic COVID-19 patients in younger and older participants, respectively.

A pattern similar to that observed for SARS-CoV-2 neutralizing antibody was noted regarding the RBD-binding antibody. For younger participants following the 10 or 25 μg V-01, 21d-interval two-dose regimen, the GMT titers on days 28, 35, and 49 post-immunization were 1283.5 (95%CI: 1006.6–1636.5), 3861.6 (95%CI: 3094.0–4819.7), and 2732.2 (95%CI: 2230.7–3346.5), respectively, in the 10 μg V-01 group and were 1791.9 (95%CI: 1416.5–2266.8), 3843.5 (95%CI: 3183.3–4640.6), and 2910.12 (95%CI: 2459.2–3443.6), respectively, in the 25 μg V-01 group, with corresponding seroconversion rates predominantly over 98% [Supplementary Table 6, http://links.lww.com/CM9/A727]. Older participants in the two-dose group were predominantly seronegative at the baseline GMT titers [Supplementary Table 7, http://links.lww.com/CM9/A727]; on days 28, 35, and 49 post-immunization GMT titers were 737.6 (95%CI: 559.9–971.9), 3239.2 (95%CI: 2650.8–3958.4), and 2655.8 (95%CI: 2180.8–3234.1), respectively, in the 10 μg V-01 group and were 1257.0 (95%CI: 927.8–1703.0), 3128.1 (95%CI: 2371.5–4126.1), and 2675.4 (95%CI: 2099.8–3408.8), respectively, in the 25 μg V-01 group. For participants following the 50 μg V-01, one-dose regimen, the GMTs on days 7, 14, and 28 post-immunization were 6.5 (95%CI: 5.8–7.3), 147.7 (95%CI: 112.9–193.2), and 734.9 (95%CI: 574.6–940.0), respectively, in the younger adults and were 5.9 (95%CI: 5.6–6.3), 70.7 (95%CI: 53.1–94.1), and 654.2 (95%CI:516.6–828.3), respectively, in the older adults [Supplementary Table 8, http://links.lww.com/CM9/A727].

Discussion

The safety of V-01 was previously assessed in a randomized, double-blind, placebo-controlled phase I trial

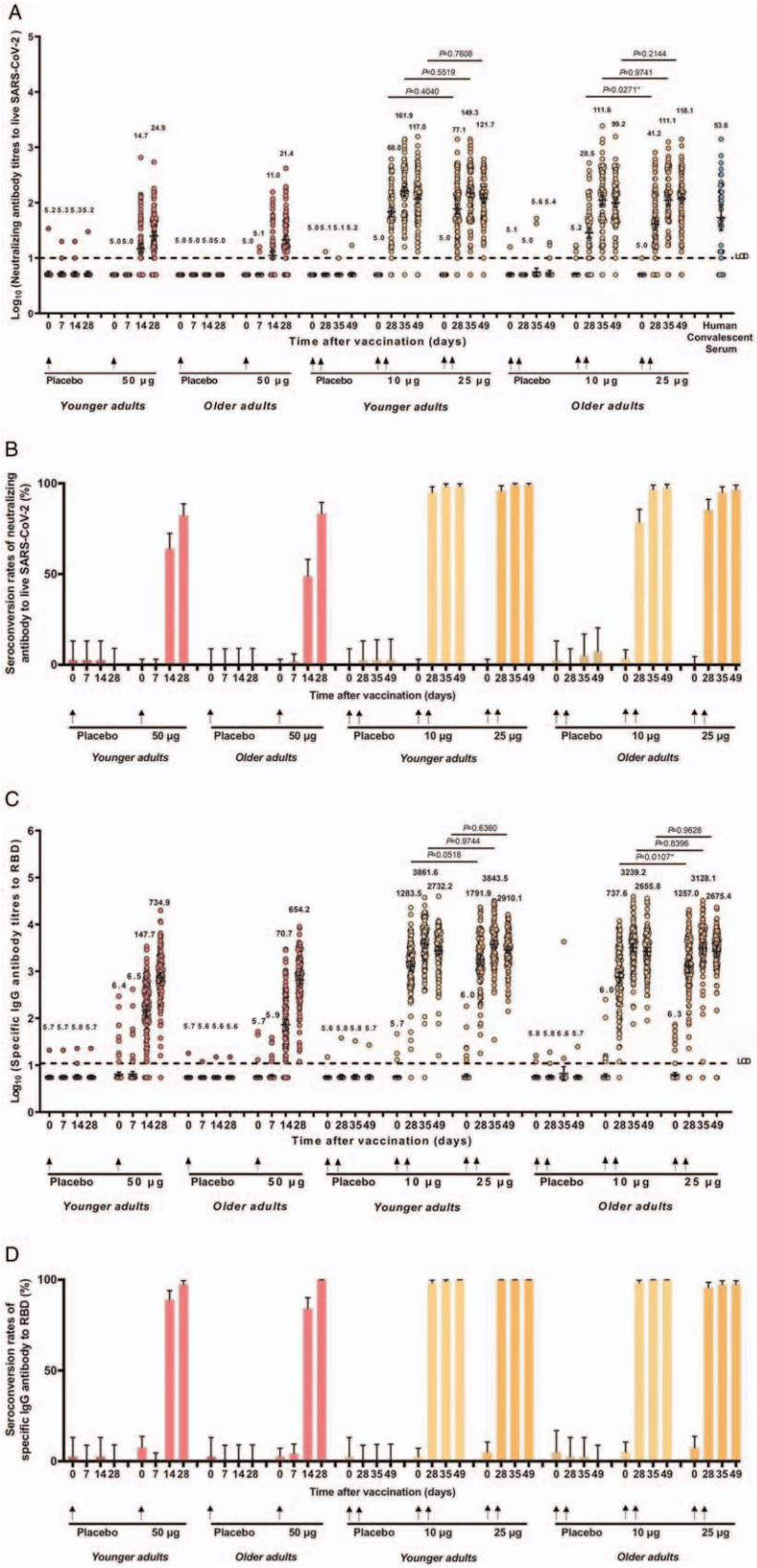

Figure 3: Humoral immune responses in phase II trials. GMTs (A) and seroconversion rates (B) of neutralizing antibodies at different timepoints after administration of the first vaccine dose in the phase II trial. Pink, yellow, blue represents younger adults, older adults and convalescent patients, respectively. GMTs (C) and seroconversion rates (D) of RBD-binding antibodies at different timepoints after administration of the first vaccine dose in the phase II trial. Error bars represent the 95% CIs of the geomeans. Arrows indicate the days of vaccination. The horizontal dashed lines in panels A and C indicate the limit of detection. CI: Confidence interval; GMTs: Geometric mean titers; RBD: Receptor-binding domain.

with a sentinel-observation and dose-escalation design conducted on 180 healthy participants, drawn from both the young adult population and geriatric population, which gave rise to a seamless phase II trial. Eligible younger and older adult participants were assigned to groups receiving a two-dose regimen of 10, 25 μg V-01, or placebo (3:3:1) administered intramuscularly 21 days apart or receiving a one-dose regimen of 50 μg V-01 or placebo via a single injection at day 0. The 50 μg V-01, one-dose regimen was selected for inclusion in this phase II trial owning to the substantially high GMT of neutralizing antibody against SARS-CoV-2 challenge observed in a pre-clinical study and the mounting demand for simplified vaccination, such as single dosing, on the hope that the strategic self-adjuvanted molecular design of V-01 with the conventional alum adjuvant would be able to induce a sufficiently strong immune response.

As was expected, V-01 was observed to have good immunogenicity and an acceptable safety profile in this phase II trial, even in the groups with older participants. The immunogenicity of V-01 had an age-dependent waning decay, owning to the relatively weaker immune response elicited by vaccination with V-01 in older adults. Notably, the immunogenicity represented by the GMTs of neutralizing antibody and anti-RBD IgG were quite different between the single- and two-dose regimens. For example, even before the sharp increase in the GMT of neutralizing antibody against live SARS-CoV-2 to its peak value on day 35 post-immunization, the two-dose, 10 and 25 μg V-01 groups of younger adults achieved GMTs for SARS-CoV-2 neutralizing antibody of 68.0 (95%CI: 55.3–83.4) and 77.1 (95%CI: 62.1–95.7), respectively, on day 28 after the first vaccination with a second dose at day 21, which are approximately three times greater than that observed in the one-dose, 50 μg V-01 group, which had a GMT of 24.9 (95%CI: 20.6–30.1). Therefore, a second dose of V-01 is indispensable for achieving a robust immune response. At present, because the correlation between neutralizing antibody titers and protection efficacy is yet not known, vaccine developers have generally compared post-vaccination immune responses with the immune responses detected in human convalescent serum from symptomatic COVID-19 patients. In our study, after receiving a second dose on day 21, participants in the two-dose, 10 and 25 μg V-01 regimen groups exhibited remarkably higher neutralizing GMTs at levels in excess of those in convalescent serum from patients. It is estimated that the neutralization level for 50% protection against detectable SARS-CoV-2 infection is 20.2% of the mean convalescent level (95%CI: 14.4%–28.4%).[18] The GMTs in the two-dose, 10 and 25 μg V-01 regimen groups ranged from 2.1 to 3.0 times higher than the GMT of convalescent panels, which is highly predictive of sufficient immune protection against COVID-19 in vaccinated individuals. However, the comparison between serum from V-01-vaccinated persons and convalescent serum from symptomatic COVID-19 patients must be interpreted with caution, because the GMT of SARS-CoV-2 neutralizing antibody in COVID-19 patients has not been established as a clinical surrogate endpoint for protective efficacy.

In line with the results of the phase I trial, the safety profile observed in the present trial was also quite favorable, as anticipated for alum-adjuvanted recombinant protein vaccines. The percentages of participants who had AEs were similar between vaccine recipients and placebo recipients. In the younger adult groups, vaccination-related AEs were observed in 12% to 25% of overall V-01-vaccinated participants in both the one-dose and two-dose regimen groups. Two cases of grade 3 AEs (fever and pain) were reported in the two-dose, 10 μg V-01 group, but neither was attributable to receipt of V-01. The overall percentages of vaccine-related AEs were lower in the older adult groups than in the corresponding younger adult groups. Several cases of grade 3 AEs were observed in the older adult groups, but they were primarily classified as unsolicited AEs and vaccination unrelated, except for one case of a vaccine-related grade 3 AE being observed in the two-dose, 25 μg V-01 group. Lower proportions of mild-to-moderate AEs but higher proportions of grade 3 or worse AEs were observed in the older participants, likely because of differences in health care-seeking behavior, but possibly also because of differences in comorbidities. The safety profile of V-01 compares favorably with those of other recombinant protein vaccines, such as ZF001 (Anhui Zhifei Longcom Biopharmaceutical, Hefei, China), SCB-2019 (Clover Biopharmaceuticals, Chengdu, China), and NVX-CoV2373 (Novavax, Gaithersburg, USA).[8,9,11] Like other recombinant protein vaccines, injection site pain was the most frequent solicited adverse reaction. Also in line with previous reports, fatigue, fever, headache, arthralgia, and muscle pain were the most common types of systemic solicited adverse reactions in the present study.

The trial also had several limitations. (1) Human convalescent serum from COVID-19 patients, which is valuable for clarifying the probable correlation between the SARS-CoV-2 neutralizing antibody GMT and COVID-19 protection in healthy adults, was provided in this study; however, comparisons between different vaccine candidates in different studies must be interpreted cautiously and should take into consideration the various characteristics that are difficult to standardize, such as the time since symptom onset and varying degrees of disease severity. In our study, the convalescent serum panels used were collected predominantly from patients who had COVID-19 cases of mild-to-moderate severity. (2) This trial recruited only adults aged over 18 years, and therefore does not provide immunogenicity and safety data for individuals aged less than 18 years. Our study population was not ethnically diverse, with the participants mostly being Han Chinese. A more diverse range of ethnic backgrounds may be included in our multicenter international phase III study. (3) Cellular immune response and immune persistence were not evaluated in our current analysis, and these factors may play a prominent role in protection from severe infection or new variants of SARS-CoV-2. A full analysis of the data to provide a comprehensive immunogenic profile of the V-01-induced T-cell response will be available in the near future. Additionally, participants are instructed for continued site visits (12 months) to collect samples for immune persistence analysis.

In summary, the results from this phase II trial further confirm the favorable immunogenicity and safety profile of V-01. They also demonstrate a much better immunogenicity profile induced by the two-dose regimen in comparison with the one-dose regimen and indicate that the 10 and 25 μg V-01 two-dose schedules produce statistically insignificant immunogenic differences as well as comparably acceptable safety profiles. With the purpose of advancing the V-01 regimen with the best safety and immunogenicity profile, we will test the 10 μg V-01 administered in a two-dose regimen in an upcoming phase III efficacy trial conducted on both younger and older adults.

Acknowledgements

The authors thank all participants who generously volunteered for this study. We extend our gratitude to the People's Hospital of Gaozhou for the tirelessly efforts during execution of clinical trial. We also appreciate National Virus Resource Center of Wuhan Institute of Virology for technical supports in our phase II trial.

Funding

This work was partially supported by a grant from the Emergency Key Program of Guangzhou Laboratory (No. EKPG21-21). The study was funded by Livzon Bio Inc., China.

Conflicts of interest

Bo Wang and Zhong-Hui Xu are the employees of Livzon Bio Inc., China. All other authors declare no competing interests.

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How to cite this article: Shu YJ, He JF, Pei RJ, He P, Huang ZH, Chen SM, Ou ZQ, Deng JL, Zeng PY, Zhou J, Min YQ, Deng F, Peng H, Zhang Z, Wang B, Xu ZH, Guan WX, Hu ZY, Zhang JK. Immunogenicity and safety of a recombinant fusion protein vaccine (V-01) against coronavirus disease 2019 in healthy adults: a randomized, double-blind, placebo-controlled, phase II trial. Chin Med J 2021;134:1967–1976. doi: 10.1097/CM9.0000000000001702