Safety and immunogenicity of Nanocovax, a SARS-CoV-2 recombinant spike protein vaccine: Interim results of a double-blind, randomised controlled phase 1 and 2 trial

Thuy P. Nguyen,a,1 Quyet Do,b,1 Lan T. Phan,c,1 Duc V. Dinh,a,1 Hiep Khong,a,1 Luong V. Hoang,b Thuong V. Nguyen,c Hung N. Pham,b Men V. Chu,b Toan T. Nguyen,a Quang D. Pham,c Tri M. Le,a Tuyen N.T. Trang,a Thanh T. Dinh,a Thuong V. Vo,a Thao T. Vu,a Quiynh B.P. Nguyen,a Vuong T. Phan,a Luong V. Nguyen,b Giang T. Nguyen,b Phong M. Tran,b Thuan D. Nghiem,b Tien V. Tran,b Tien G. Nguyen,b Tyungh Q. Tran,b Linh T. Nguyen,c Anh T. Do,c Hung D. Nguyen,b Son A. Ho,b Viet T. Nguyen,b Dung T. Pham,b Hieu B. Tran,b Son T. Vu,b Su X. Hoang,b Trung M. Do,b Xuan T. Nguyen,b Giang Q. Le,b Tien Tran,b Thang M. Cao,c Quan H. Nguyen,c Hieu T. Nguyen,c Hong K. Nguyen,c Vinh T. Tran,c Mai T.N. Tran,c Truc T.T. Nguyen,c Phat T. Ha,a Hieu T. Huynh,a Khanh D. Nguyen,a Ung T. Thuang,a Chung C. Doan,a and Si M. Doa,*

aNanogen Pharmaceutical Biotechnology JSC, Lot I-5C Saigon Hitech Park, Ho Chi Minh City, Viet Nam
bVietnam Military Medical University, 160 Phung Hung, Ha Dong, Ha Noi, Viet Nam
cPasteur Institute, 167 Pasteur, District 3, Ho Chi Minh City, Viet Nam
dNational Institute of Hygiene and Epidemiology (NIHE), Ha Noi, Viet Nam

Summary

Background Nanocovax is a recombinant severe acute respiratory syndrome coronavirus 2 subunit vaccine composed of full-length prefusion stabilized recombinant SARS-CoV-2 spike glycoproteins (S-2P) and aluminium hydroxide adjuvant.

Methods We conducted a dose-escalation, open label trial (phase 1) and a randomized, double-blind, placebo-controlled trial (phase 2) to evaluate the safety and immunogenicity of the Nanocovax vaccine (in 25 mcg, 50 mcg, and 75 mcg doses, aluminium hydroxide adjuvanted (0.5 mg/dose) in 2-dose regime, 28 days apart (ClinicalTrials.gov number, NCT04683484). In phase 1, 60 participants received two intramuscular injection of the vaccine following dose-escalation procedure. The primary outcomes were reactogenicity and laboratory tests to evaluate the vaccine safety. In phase 2, 560 healthy adults received either vaccine doses similar in phase 1 (25 or 50 or 75 mcg S antigen in 0.5 mg aluminium per dose) or adjuvant (0.5 mg aluminium) in a ratio of 2:2:2:1. One primary outcome was the vaccine safety, including solicited adverse events for 7 day and unsolicited adverse events for 28 days after each injection as well as serious adverse event or adverse events of special interest throughout the study period. Another primary outcome was anti-S IgG antibody response (Index unit/ml). Secondary outcomes were surrogate virus neutralisation (inhibition percentage), wild-type SARS-CoV-2 neutralisation (dilution fold), and T-cell responses by intracellular staining for interferon gamma (IFNg). Anti-S IgG and neutralising antibody levels were compared with convalescent serum samples from symptomatic Covid-19 patients.

Findings For phase 1 study, no serious adverse events were observed for all 60 participants. Most adverse events were grade 1 and disappeared shortly after injection. For phase 2 study, after randomisation, 480 participants were assigned to receive the vaccine with adjuvant, and 80 participants were assigned to receive the placebo (adjuvant only). Reactogenicity was absent or mild in the majority of participants and of short duration (mean ≤3 days). Unsolicted adverse events were mild in most participants. There were no serious adverse events related to Nanocovax. Regarding the immunogenicity, Nanocovax induced robust anti-S antibody responses. In general, there humoral responses were similar among vaccine groups which reached their peaks at day 42 and declined afterward. At day 42, IgG levels of vaccine groups were 60.48 [CI95%: 51.12–71.53], 49.11 [41.26–58.46], 57.18 [48.4.67-5] compared to 7.10 [6.32-13.92] of convalescent samples. IgG levels reported here can be converted to WHO international standard binding antibody unit (BAU/ml) by multiplying them to a conversion factor of 21.8. Neutralising antibody titre...
of vaccine groups at day 42 were 89.2 [52.2–152.3], 80.0 [50.8–125.9] and 95.1 [63.1–143.6], compared to 55.1 [33.4–91.0] of the convalescent group.

**Interpretation** Up to day 90, Nanocovax was found to be safe, well tolerated, and induced robust immune responses.

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**Keywords:** SARS-CoV-2; Spike protein; Protein sub-unit vaccine; Immunogenicity; Phase 1 and 2 clinical trial

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**Research in context**

**Evidence before this study**

Nanocovax - an aluminium adjuvanted recombinant Spike protein vaccine - was found to be highly safe and immunogenic in preclinical study. Importantly, the vaccine did not induce antibody dependent enhancement (ADE) on SARS-CoV-2 challenged hamster model, leading to the approval for a clinical trial.

**Added value of this study**

We report here the safety and immunogenicity of Nanocovax in a phase 1/2 trial. The vaccine had excellent safety profile and induced robust immune responses, in terms of anti-Spike protein IgG and neutralising antibody titres.

**Implications of all the available evidence**

Promising data show that Nanocovax is ready for a phase 3 trial for further evaluation on the safety and efficacy in a large population.

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**Introduction**

Global pandemic coronavirus disease 2019 (Covid-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulting in millions of death.¹

SARS-CoV-2 is a member betacoronavirus, named for its corona of spike (S) proteins protruding from the viral envelope.²³ SARS-CoV-2 S, a heavily glycosylated protein, is responsible for the attachment to angiotensin-converting enzyme (ACE2) which helps the virus entry to host cells in human and animals.¹ SARS-CoV-2 S glycoprotein is the antigen of choice for Covid-19 vaccine development due to its highly antigenic property.³

Nanocovax is a subunit vaccine, developed and manufactured at Nanogen Pharmaceutical Biotechnology JSC., containing full-length prefusion stabilized recombinant SARS-CoV-2 S glycoproteins and aluminium hydroxide adjuvant. In rodent and monkey models, Nanocovax induced high levels of anti-S antibody (Ab). Neutralising antibody titres were evaluated by micro-neutralisation (against the original (Wuhan) strain and the Alpha variant) and surrogate virus neutralisation test. Importantly, Nanocovax conferred a remarkable protection against SARS-CoV-2 infection in hamster challenge model.⁶

Here we report the findings of the phase 1 and 2 trials started in December 2020 and February 2021 respectively, to evaluate the safety and immunogenicity of 25 mcg, 50 mcg and 75 mcg dose strengths of recombinant SARS-CoV-2 S glycoprotein with aluminium hydroxide (0.5 mg/dose) as adjuvant control in healthy adults of at least 18 years of age.

**Method**

**Trial design and oversight**

Phase 1 trial was conducted at the Military Medical University, Ha Noi, Vietnam. This was an open-labelled, dose-escalation study with the emphasis on the vaccine safety (Fig. S1). Eligible participants were healthy men and nonpregnant women, 18 to 50 years of age with body-mass index (BMI) of 18 to 27 kg/m² (Table S1). In this phase, 60 participants were allocated into 1:1:1 ratio of 25 mcg, 50 mcg and 75 mcg dose groups (designated as 1.1, 1.2 and 1.3), respectively. All participants were to receive 2 injections of the vaccine by intramuscular injections into the deltoid on day 0 and day 28. For safety measure, the first 3 participants of 25 mcg dose group were vaccinated and monitored for 72 h. at the study site. After no SAE were observed, all remaining participants in this group plus 3 participants in 50 mcg dose group would be vaccinated and monitored for 72 h.. If no SAE were observed among 3 participants of 50 mcg dose group, the remaining participant in this group plus 3 participants in 75 mcg dose group would be vaccinated. If no SAE were observed among 3 participants in 75 mcg dose group, the remaining participant in this group will be vaccinated. For the 2nd injection,
all participants were monitored for 24 h at the study site. Sample size of phase 1 was not based on formal statistical power calculation but on the range of 30–150 recommended in Article 10 of Appendix 10/2020/TT-BYT by the Vietnam Ministry of Health.

Phase 2 trial was conducted at two sites: Military Medical University, Ha Noi and the Pasteur Institute at Ho Chi Minh city, Vietnam. This was a randomized, double-blind, placebo (adjuvant)-controlled study (Figure 1). Eligible participants were healthy men and nonpregnant women, at least 18 years of age with BMI of 17 to 35. They were stratified into 3 age groups: from 18 to 45 years old, 46 to 60 years old, and over 60 years old (Table 1). The 60+ age group accounted for 18% of participants, which was above Vietnam national average of 11.9% (as of 2019). A total of 560 participants would be randomly assigned to 4 groups, into 2:2:2:1 ratio for 25 µg, 50 µg, 75 µg, and adjuvant (designated as 2.1, 2.2, 2.3 and 2.4), respectively. In details, 480 volunteers would receive the vaccine (160 volunteers receiving 25 µg dose; 160 volunteers receiving 50 µg dose and 160 volunteers receiving dose of 75 µg dose) and 80 volunteers would receive the placebo (aluminium adjuvant only). All participants received either 2 doses of vaccine or the placebo on day 0 and day 28. Trial staffs who were responsible for the administration of the vaccine or the placebo, as well as participants were unaware of vaccine or the placebo assignment. Randomisation lists, using block randomisation stratified by the study group and study site, were generated by the study statistician. Computer randomisation was done with full allocation concealment within the secure web platform used for the study electronic case report form (service provided by Medprove company).

All participants were screened by their medical history, clinical and biological examinations, sampling and laboratory tests (complete blood count, biochemistry, urine analysis, testing pregnancy and diagnostic imaging). Participants with a history of Covid-19 or positive results for SARS-CoV-2 at screening period confirmed by real-time reverse transcriptase polymerase-chain-reaction (RT-PCR) were excluded from the trials. All participants provided written consent before being enrolled into the trial.

The trials were designed and funded by Nanogen Pharmaceutical Biotechnology JSC and the Ministry of Science and Technology (MOST) of Vietnam. The trial protocol was approved by the Ethics Committee/Protocol Review Board of the Ministry of Health (Vietnam) and was performed in accordance with the ICH-GCP good clinical practice guidelines, with an ethical policy consistent with the “Declaration of Helsinki” and applicable Vietnamese laws and regulations. The authors take responsibility for the data integrity and the fidelity of the trial to the protocol.

**Trial vaccine and the placebo**

The recombinant SARS-CoV-2 spike (S) glycoprotein in Nanocovax were constructed with two proline substitutions (K986P and V987P) for stabilized prefusion conformation (S-2). The production of the full-length (including the transmembrane domain) recombinant S protein was optimized in the established Chinese
Hamster Ovary (CHO) cell-expression system. Clinical grade aluminium hydroxide was manufactured by Croda (Denmark). Recombinant SARS-CoV-2 S protein were absorbed to aluminium adjuvant in mild shaking condition for 18 h at 2°C to 8°C. Placebo was sterile 0·5 mg aluminium per dose.

Safety assessments

In phase 1, the onsite safety follow-up time after was 72 h after 1st injection and 24 h after the 2nd injection. Participants would return to the study site for follow-up visits at scheduled timepoints (Table S2). In phase 2, the onsite safety follow-up time was 60 min after each vaccination. Follow-up visits to evaluate safety were scheduled on days 28, 35, 42, 90, 180 after vaccination (Table 2). Participants were observed for 60 min after each vaccination for assessment of reactogenicity. In both phases, participants received instruction for self-monitoring and reporting adverse events during 7 days after each vaccination, as facilitated by the use of a diary with predefined reactogenicity. Predefined local (injection site) reactogenicity included pain, tenderness, erythema, and swelling. Predefined systemic reactogenicity included fever, nausea or vomiting, headache, fatigue, malaise, myalgia, and arthralgia. Vaccination pause rules were in place to monitor participants’ safety (supplementary appendix).

The primary safety outcomes were the number and percentage of participants with solicited local and systemic adverse events occurred within 7 days after vaccination and laboratory results (serum biochemistry and haematology) at days 0, 7, 28, 35 according to FDA toxicity scoring. Secondary safety outcomes were occurrence rate and severity rating of unsolicited AE/SAE until the end of the studies and laboratory results at up to 42 (phase 2) and 56 (phase 1).

AE/SAE were recorded and evaluated basing on the Common Terminology Criteria for Adverse Events 5.0 (CTCAE v5.0) and Guidelines for assessing toxicity in healthy volunteers in FDA’s Preventive Vaccine Clinical Trial Study. The procedures for recording and evaluating take place continuously from the time of using the first dose to the end of the last visit in each research volunteer. Adverse events were assessed in terms of severity score (mild, moderate, severe, potentially life-threatening, or fatal), and relatedness to the vaccine. Vital sign measurements were assessed according to FDA toxicity scoring after vaccination. In addition, participants had nasopharyngeal swab tests for SARS-CoV-2 on screening day (before 1st vaccination), day 28 (before 2nd vaccination) and any time that they developed symptoms of possible SARS-CoV-2 infection.

Immunogenicity assessments

The primary outcome was anti-S IgG responses to Nanocovax evaluated by chemiluminescence immunoassay (CLIA). Secondary outcomes were neutralising antibody titre evaluated by 50 percent plaque reduction
neutralisation test (PRNT_{50}) on original strain and Alpha variant (B.1.1.7), competitive enzyme-linked immunosorbent assay (ELISA) based surrogate virus neutralisation test (sVNT), and the T cell response by intracellular cytokine-staining (ICS). The immunogenicity (IgG and neutralising antibody titres) of vaccine groups were compared with convalescent serum specimens from symptomatic Covid-19 patients at the Pasteur Institute at Ho Chi Minh City, Vietnam (Table S3). Detail of immunological assays were provided in the supplementary appendix.

**Statistical analysis**
Descriptive analyses of safety and reactogenicity analysis were shown. For an adverse event occurred more than once, the analysis was based on only the most severe occurrence and the cause of the event. Reactogenicity analyses were presented as counts and percentages. In addition, all serious adverse events were summarized separately. Geometric means (of anti-S IgG concentration and neutralising antibody titre) and associated 95% confidence intervals (CI) were calculated basing on log-transformed data. The estimated vaccine efficacy was calculated basing the predictive model by Khoury and Cromer et al.9

**Role of the funding source**
The Coalition for Epidemic Preparedness Innovations (CEPI) and the Ministry of Science and Technology provided research funding. Nanogen JSC provided research funding, participated in the study design and the writing of the manuscript. Leading authors and the corresponding author had full access to all data and had final responsibility of the decision to submit the manuscript.

**Result**

**Trial population**
The phase 1 trial was started on December 17, 2020. 60 participants were allocated into 3 groups of doses: 20 received 25 mcg (group 1.1), 20 received 50 mcg (group 1.2), and 20 received 75 mcg (group 1.3). There was no placebo group in phase 1.

For phase 2, the study was started on February 26, 2021. 560 participants were recruited and randomly assigned into groups of different doses: 161 received 25 mcg doses of Nanocovax (group 2.1), 160 received 50 mcg doses of Nanocovax (group 2.2), 159 received 75 mcg doses of Nanocovax (group 2.3), and 80 received placebo (group 2.4). Participants were stratified into 2 age groups: from over 18 to below 60 years old and over 60 years old. Demographic characteristics of participants in phase 1 and 2 were shown in Tables 1 and S1.

**Safety outcomes**
In phase 1, no serious adverse events were observed, and vaccination pause rules were not implemented. Overall reactogenicity was largely absent or mild, and second vaccinations were neither withheld nor delayed due to
reactogenicity. The incidences of solicited adverse events in each vaccine group with FDA toxicity grade (grade 1: mild, grade 2: moderate, grade 3: severe, grade 4: potentially life-threatening) were presented in Fig. S2.

In phase 2, 554 out of 560 enrolled participants received 2 doses of vaccine or placebo. 6 withdrew from the study after the 2nd visit, before getting the boosting dose. The incidences of solicited local and systemic AE of each group after the 1st and 2nd injection were shown in Figure 2 and Table 3. After the 1st injection, local and systemic AE of grade 3 or 4 were not observed in group 2.1, 1 case in group 2.2 (0.6%), 1 case in group 2.3 (0.6%) and 1 case in group 2.4 (1.3%). After the 2nd injection, one local or systemic AE of grade 3 or 4 occurred in each group (0.6%) and placebo (1.3%). Incidences of any solicited local AE were 37.7% (211/560) after 1st vaccination and 35.7% (198/554) after 2nd vaccination. The most common AE was local pain with incidence of 32.8% (184/560) after 1st injection and 32.1% (178/554) after 2nd injection. Incidences of mild pain were 32.3% (181/560) after 1st injection and 30.7% (170/554) after 2nd injection. Moderate pain was uncommon, reported by 0.5% participants (3/560) after

![Figure 2. Solicited local adverse event (A) and systemic adverse events (B) within 7 days after vaccination in phase 2.](image-url)
Figure 2 Continued.
## Local events

### Vaccination 1

| Grade       | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Total |
|-------------|---------|---------|---------|---------|-------|
| n           | %       | n       | %       | n       | %     | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       |
| Itching     |         |         |         |         |       |
| 25 mcg      | 155     | 96.30%  | 6       | 3.70%   | 161   | 100.00% |
| 50 mcg      | 157     | 98.10%  | 3       | 1.90%   | 160   | 100.00% |
| 75 mcg      | 155     | 97.50%  | 4       | 2.50%   | 159   | 100.00% |
| Adjuvant    | 79      | 98.80%  | 1       | 1.20%   | 80    | 100.00% |

### Vaccination 2

| Grade       | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Total |
|-------------|---------|---------|---------|---------|-------|
| n           | %       | n       | %       | n       | %     | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       |
| Pain        |         |         |         |         |       |
| 25 mcg      | 157     | 96.30%  | 6       | 3.70%   | 161   | 100.00% |
| 50 mcg      | 157     | 98.10%  | 3       | 1.90%   | 160   | 100.00% |
| 75 mcg      | 155     | 97.50%  | 4       | 2.50%   | 159   | 100.00% |
| Adjuvant    | 79      | 98.80%  | 1       | 1.20%   | 80    | 100.00% |

### Redness

| Grade       | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Total |
|-------------|---------|---------|---------|---------|-------|
| n           | %       | n       | %       | n       | %     | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       |
| Swelling    |         |         |         |         |       |
| 25 mcg      | 157     | 96.30%  | 6       | 3.70%   | 161   | 100.00% |
| 50 mcg      | 157     | 98.10%  | 3       | 1.90%   | 160   | 100.00% |
| 75 mcg      | 155     | 97.50%  | 4       | 2.50%   | 159   | 100.00% |
| Adjuvant    | 79      | 98.80%  | 1       | 1.20%   | 80    | 100.00% |

### Tenderness

| Grade       | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Total |
|-------------|---------|---------|---------|---------|-------|
| n           | %       | n       | %       | n       | %     | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       | n       | %       |
| Adjuvant    | 55      | 68.80%  | 17      | 21.20%  | 8      | 10.00% | 80      | 100.00% |
|                | Vaccination 1 | Vaccination 2 |
|----------------|--------------|--------------|
|                | Grade 0  | Grade 1  | Grade 2  | Grade 3  | Grade 4  | Total  | Grade 0  | Grade 1  | Grade 2  | Grade 3  | Grade 4  | Total |
| Chills         |           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 161 100.00% | 161 100.00% | 157 99.40% | 1 0.60%  | 158 100.00% |
| 50 mcg         | 160 100.00% | 160 100.00% | 159 100.00% |          | 159 100.00% |
| 75 mcg         | 159 100.00% | 159 100.00% | 158 100.00% |          | 158 100.00% |
| Adjuvant       | 79 98.80%  | 79 100.00% | 79 100.00% |          | 79 100.00% |
| Diarrhoea      |           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 160 99.40% | 160 100.00% | 159 100.00% | 1 0.60%  | 159 100.00% |
| 50 mcg         | 156 97.50% | 156 100.00% | 155 100.00% |          | 155 100.00% |
| 75 mcg         | 155 97.50% | 155 100.00% | 154 100.00% |          | 154 100.00% |
| Adjuvant       | 79 98.80%  | 79 100.00% | 79 100.00% |          | 79 100.00% |
| Fatigue        |           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 140 87.00% | 140 100.00% | 139 98.10% | 1 0.60%  | 139 100.00% |
| 50 mcg         | 129 80.60% | 129 100.00% | 127 98.30% | 2 1.70%  | 129 100.00% |
| 75 mcg         | 131 84.20% | 131 100.00% | 129 98.10% | 2 1.90%  | 131 100.00% |
| Adjuvant       | 64 93.50%  | 64 100.00% | 64 100.00% |          | 64 100.00% |
| Headache       |           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 139 86.30% | 140 100.00% | 138 98.10% | 2 1.90%  | 139 100.00% |
| 50 mcg         | 138 86.20% | 138 100.00% | 135 98.70% | 3 1.30%  | 138 100.00% |
| 75 mcg         | 139 86.30% | 139 100.00% | 138 98.10% | 2 1.90%  | 139 100.00% |
| Adjuvant       | 69 95.00%  | 69 100.00% | 69 100.00% |          | 69 100.00% |
| Arthralgia     |           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 144 91.90% | 144 100.00% | 143 98.10% | 3 1.90%  | 144 100.00% |
| 50 mcg         | 149 93.10% | 149 100.00% | 148 98.70% | 2 1.30%  | 149 100.00% |
| 75 mcg         | 147 93.00% | 147 100.00% | 146 98.10% | 3 1.90%  | 147 100.00% |
| Adjuvant       | 71 95.00%  | 71 100.00% | 71 100.00% |          | 71 100.00% |
| Myalgia        |           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 146 90.70% | 146 100.00% | 145 98.10% | 5 1.90%  | 146 100.00% |
| 50 mcg         | 149 93.10% | 149 100.00% | 148 98.10% | 2 1.90%  | 149 100.00% |
| 75 mcg         | 147 92.50% | 147 100.00% | 146 98.10% | 4 2.10%  | 147 100.00% |
| Adjuvant       | 72 95.00%  | 72 100.00% | 72 100.00% |          | 72 100.00% |
| Nausea/Vomiting|           |           |           |           |           |        |           |           |           |           |           |       |
| 25 mcg         | 158 98.10% | 158 100.00% | 157 98.70% | 1 0.60%  | 158 100.00% |
| 50 mcg         | 157 98.10% | 157 100.00% | 156 98.30% | 2 1.70%  | 157 100.00% |
| 75 mcg         | 159 100.00% | 159 100.00% | 158 98.10% | 2 1.90%  | 159 100.00% |
| Adjuvant       | 76 95.00%  | 76 100.00% | 76 100.00% |          | 76 100.00% |

Table 3: Solicited local (A) and systemic (B) AEs in phase 2, followed up to 7 days post each injection.
the 1st injection and 1.4% (8/554) after the 2nd injection. 1 participant (out of 554) experienced severe pain after 2nd injection (0.2%). Grade 1 local sensitivity incidences were 19.1% (107/560) after 1st injection and 14.6% (81/554) after 2nd injection. Grade 2 local sensitivity incidences were 5.9% (33/560) after 1st injection and 4.8% (27/554) after 2nd injection. Grade 3 local redness was observed in 1 participant (0.2%) after 2nd injection. Incidences of any systemic AE were 27.8% (156/560) after 1st injection and 21.8% (121/554) after 2nd injection. Most common systemic AE were fatigue (17.1%-12.9%), headache (13.3%-3.3%) and fever (4.4%/2.5%) after 1st/2nd injections. Fatigue decreased gradually and disappeared within 7 days after injection. Mild fever incidences were 4.4% (25/560) after 1st injection and 2.5% (14/554) after 2nd injection. One participant (0.2%) experienced high fever (grade 3) within 5 days after 2nd injection.

Incidence of any unsolicited AE in phase 2 was 28% (157/560) and most of which was mild to moderate. Unsolicited AE rates were similar among vaccine groups and the placebo (Appendix 7). In details, unsolicited AE incidence of groups 2.1 to 2.4 were 30.4%, 27.5%, 23.3% and 33.8%, respectively. One case of grade 4 AE was dizziness (symptom of anaphylactic reaction). There were 5 AE of grade 3: 1 case of sepsis, 1 case of back pain, 1 case of spondylolisthesis, 1 case of sore throat, 1 case of high blood pressure. The most frequently reported adverse events were sore throat 27 (4.8%) and coughing 11 (2.0%). The most laboratory-related AE were hyperglycaemia 13 cases (2.3%), leucocytosis: 8 cases (1.4%), the most common events related to vital sign were hypothermia with 12 cases (2.1%). In similar to phase 1, vaccination pause rules were not implemented in phase 2. Four SAE were determined unrelated to Nanocovax, including 1 case of angina (history of stent graft), 1 case of fever (determined to be sepsis), 1 case of abscess at axillary lymph nodes occurred on the unvaccinated arm and 1 case of personal injury. One case of SAE grade 1 allergic reaction was undetermined to be related to vaccine or not because the symptoms were unclear (Table S4). The classification of allergic reactions was based on the guidance of the World Allergy Organization (WAO).10 Overall incidences of unsolicited AE of vaccine groups and the placebo were similar: 27.1% (130/480) versus 33.8% (27/80), respectively.

Laboratory abnormalities in phase 2 included increased white blood cell in 3 participants, (0.6%), increased neutrophil in 2 participants (0.4%), elevated ALT (grade 2) in 3 participants (0.6%), and elevated AST (grade 2) in 2 participants (0.4%). The other biochemistry and haematology parameters such as red blood cell (RBC), haemoglobin (HGB), creatinine, bilirubin, prothrombin time (PT) fluctuated within normal limits (Appendix 7).

**Immunogenicity outcomes**

Geometric mean concentration (GMC) of anti-S IgG (U/ml) was reported. Before the 1st injection, anti-S IgG level of the 4 groups were all below the lower limit of detection (0.5 U/ml). Anti-S IgG of the vaccine groups increased remarkably after the 2nd injection (day 28). At day 35, anti-S IgG levels of group 2.1, 2.2 and 2.3 were 6-78 (95% CI: [5-09-9-03]), 9-38 [6-99-12-58], and 13-04 [9-46-17-98] respectively. At day 42, their respective anti-S IgG increased sharply: 60-48 [51-12–71-55], 49-11 [41-26-58-46] and 57-18 [48-4-67-5], respectively. By day 90, their respective anti-S IgG levels dropped to 16-26 [13-76-19-21], 15-73 [13-44-18-41], 18-08 [15-19-21-51] but were still higher than IgG level of convalescent group’s (7-10 [6-32-7-92]). Anti-S IgG levels of the placebo group on days 35, 42 and 90 were 0.29 with respective 95% CI [0-25-0-33], [0-25-0-32], and [0-25-0-33] (Figure 3).

Geometric mean fold rise (GMFR) of anti-S IgG was defined as the fold increase in GMC of a given time-point compared to baseline GMC value of the same group at day 0. GMFR of 2.1, 2.2 and 2.3 groups at day 35 were 25-7 [19-3-34-1], 34-7 [25-7-46-9], and 49-8 [36-0-68-9], respectively. At day 42, the GMFR of 2.1, 2.2 and 2.3 groups were 229-2 [193-2-271-4], 18-18 [152-0-217-4] and 21-83 [185-0-257-7]. By day 90, GMFR of vaccinated groups were 61-54 [52-12-72-67], 58-21 [49-40-68-53] and 69-01 [58-04-82-05]. Meanwhile, GMFR of the group 2.4 on days 35, 42 and 90 were 1-05 [0-97-1-13], 1-04 [0-98-1-09], and 1-04 [0-95-1-13] respectively (Fig. S5).

The seroconversion rate was defined as GMFR ≥4. Based on the GMFR of anti-S IgG, the seroconversion rates of groups 2.1, 2.2 and 2.3 on day 35 were 84%, 84% and 85%. At day 42, the seroconversion rates of groups 2.1, 2.2 and 2.3 were 100%, 99% and 100%. By day 90, seroconversion rates were still very high in vaccine groups: 100%, 100% and 94% (Fig. S4). sVNT results were reported as mean inhibition rate (%). Up to day 28 (before and vaccination), inhibition rates of all groups were below cut-off value of 30%. At day 35, mean inhibition of groups 2.1 to 2.4 were 58.5% [54.1-63.0], 63.8% [59.1-68.5], 70.2% [65.8-74.5], 11.1% [9.3-12.7], respectively. At day 42, their respective mean inhibition rates were 87.5% [85.5-89.5], 86.4% [84.08-88.65], 87.1% [85.06-89.16], and 10.8% [8.9-12.67]. By day 90, respective sVNT were 72.7% [69.4-76.0], 74.2% [71.1-77.3], 74.4 [10-9-77-8] and 19.1% [13.4-24.9]. Meanwhile, mean inhibition rate of convalescent samples was 61.3% [50.1-72.5] (Figure 4A). Individual samples with an inhibition higher than 30% were considered positive for sVNT.11 Accordingly, at day 35, positive rates for sVNT of groups 2.1 to 2.4 were 80.4%, 82.4%, 86.7% and 1.3%, respectively. At day 42, their respective sVNT positive rates were 100%, 100%, 99.4% and 1.3%. By day 90, the respective sVNT positive rates were 94.9, 96.8, 94.9 and 15.2 (Fig. S5).
Neutralising antibody levels were evaluated by plaque reduction neutralisation test with inhibitory dilution greater than 50% (PRNT$_{50}$) and expressed as geometric mean titres (GMT). 112 serum samples of groups 2.1 to 2.4 were randomly selected for PRNT$_{50}$ on the original strain and the Alpha variant. At day 35, GMT of groups 2.1 to 2.3 were 20 $\pm$ 12 $\pm$ 34 $\pm$ 1, 22 $\pm$ 14 $\pm$ 51 $\pm$ 34 $\pm$ 5, and 33 $\pm$ 20 $\pm$ 54 $\pm$ 1, respectively. At day 42, their respective GMT were 89 $\pm$ 52 $\pm$ 152 $\pm$ 3, 80 $\pm$ 50 $\pm$ 125 $\pm$ 9, and 95 $\pm$ 63 $\pm$ 143 $\pm$ 6. These were approximately 1.5 times higher than the GMT of convalescent samples (55 $\pm$ 33 $\pm$ 91 $\pm$ 0). Meanwhile, GMT of group 2.4 (placebo) at days 35 and 42 were 5 $\pm$ 5 (half of limit of detection -LOD) (Figure 4B). Among 112 serum samples (at day 42) tested on original strain, a subset of 21 was randomly selected to evaluate neutralising titre on Alpha variant. GMT in group 2.1 to 2.3 on Alpha variant were 35 $\pm$ 7 $\pm$ 5 $\pm$ 20-3, 56 $\pm$ 14 $\pm$ 220 $\pm$ 6 and 40 $\pm$ 10 $\pm$ 147 $\pm$ 0, an approximately 1.9-fold decrease, compared to the original strain (Fig. S6).

Type 1 helper T cell (Th1) response of 84 randomly selected participants (28 for each vaccine group and 14 for placebo group) were undetectable (data not shown). This was likely due to the nature of aluminium adjuvant which has been well established for Th2 response induction.$^{12}$

Discussion

The results of these phase 1 and phase 2 studies demonstrated an excellent safety profile of Nanocovax at all doses. Most adverse events were grade 1 which disappeared within 48 h after injection. In comparison to similar studies of approved vaccines, Nanocovax appeared to have lower reactogenicity.$^{13}$ The imbalanced male to female ratio in phase 1 could have impacted the data. This issue was addressed in phase 2 with more balance male to female participants. Of note, the occurrence of fever in the 25 mcg group in phase 1 was higher than that of phase 2. A possible explanation is that participants in phase 1 were more closely monitored by study staffs (participants stay at the study site for 72 h after 1st injection and 24 h after 2nd injection). In contrast, participants in phase 2 were monitored for only 4 h by study staffs at the study sites then returned home for self-assessment.

IgG level and neutralising antibody were found to be similar among vaccine groups. The dose-independence immunogenicity of Nanocovax was likely due to the
close range of trial doses: from 25 mcg to 75 mcg antigen. The dose dependence may be observed in a wider dose range (i.e. 5 to 125 mcg antigen) with a 5-fold difference between the doses (i.e. 5, 25, and 125 mcg). An alternative explanation for the observed dose-independence was that the 25 mcg dose was enough to...

Figure 4. Neutralizing antibody responses. A) sVNT inhibition rates of all groups and convalescent specimens. B) PRNT50 on the Wuhan strain was performed on 112 randomly selected serum samples including vaccine groups (n=32 per group), placebo (n=16) and the convalescent group (n=13). Results are expressed as GMT with 95%CI. Error bars represent 95% CI.
stimulated most antigen specific B cells, resulting in similar IgG and neutralising antibody responses. Accordingly, doses higher than 25 mcg may not provide any extra benefit in antibody responses.

We observed negative correlations between the immunogenicity and age. The negative correlation of age versus IgG level and neutralising titre was found to be highest at day 35 and lowest at day 42 (Figs. S7−S9). These observations suggest that age is more likely to influence on the kinetics rather than the peak of antibody response. In other words, antibody responses of high age group may take more time to reach the peak response and contract faster than those of younger groups. The vaccine was found to elicit high level of anti-S IgG which closely correlated with neutralising antibody titres (Fig. S10). Although the efficacy of Nanocovax remains to be seen in phase 3 trial, accumulated evidences have correlated neutralising antibody titre with the immunity against Covid-19. Khoury and Cromer et al. provided a model to predict the vaccine efficacy by comparing the neutralising antibody titres of vaccines to convalescent samples.\(^9\) Essentially, this model predicts the protective efficacy by correlating the fold difference of GMT of neutralising antibody between vaccine and convalescent group. Applying their model on our data, we estimated the initial protection (efficacy) of Nanocovax to be approximately 89.1% [86.5%-91.7%] against the original strain. One should keep in mind that the predicted protection was largely based on the peak immune responses. We therefore project the waning of protection over time of the initial (peak) protection with 95% CI (Figure 5), basing on Khoury and Cromer et al.’s model(9). A significant drop of protection of Nanocovax against new variants of concerns (VOC) including Delta (B.1.617.2) and Omircon (B.1.1.529) is also expected, as observed in other vaccines.\(^{20,21}\)

The cellular immune response, evaluated by ICS for IFNg, was not observable. However, undetectable IFNg signal, a marker of Th1 response, does not guarantee the absence T cell response but rather suggests the Th2 promoting nature of aluminium adjuvant.\(^{12}\) In fact, Th2 cell responses will be re-evaluated in a subset of participants in phase 3 with the addition of Th2 cytokines. The Th2 polarisation may raise a theoretical concern of vaccine-associated enhanced respiratory disease (ERD).\(^{22,23}\) This concern has been partially addressed with vaccine studies of hamster models challenged with SARS-CoV-2 as well as the approval of therapeutic antibodies for Covid-19 treatment.\(^{5,44-46}\) We will further evaluate the risk of ERD on the ongoing trials as well as on the phase 3.

Limitations of these phase 1 and 2 trial report were limited ethnic diversity (mostly Kinh people), short
follow-up duration and small number of convalescent samples.

In conclusion, basing on these phase 1 and 2 studies, Nanocovax is found to be highly safe and immuno-genic.

 Contributors

Study design: TPN, SMD; project management: TNTT, TTD, TTV, QBPN, VTP; data collection: DVD, MVC, LVN, GTN, PGT, TDN, TTTV, TGGT, TTQ, JTN, ATD, DDN, SAH, VTN, DTP, HBT, STV, SXH, TMD, XTN, GQL, UYD, VTT, LPT, NMN, NTN, HTTP, QHN, HLKN, VTT, PTH, HTH, KDN, UTT; data analysis: TVV, TML; investigation and data validation: QD, LTP, TNV, TT, TMC, HMD, TTTN; interpretation of the findings: all authors; original manuscript: HK; revising manuscript: TPN, QD, LTP, HK, SMD. All authors read and approved the final version of the manuscript.

 Data sharing statement

The data is available upon request to the corresponding author. The data requester is required to sign a data access agreement.

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 Declaration of interests

TPN, HK, TML, TTNT, TTD, TVV, TTTV, QBPN, VTP, VTT, MTNT, TTTN, PTH, HTH, KDN, CCD, TTU, SMD are employees of Nanogen Pharmaceutical Biotechnology JSC. MTNT, and SMD are authors of a pending patent for Nanocovax.

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Supplementary materials

Supplementary material associated with this article can be found in the online version, at doi: 10.1016/j.lanwpc.2022.100474.

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