Dear Editor and Reviewer,

Thank you for the feedback. The responses to the feedback are summarized in the following Table.

Please do not hesitate to contact us if further details are required.

Yours sincerely,

Dr Shyh Poh Teo,

Corresponding Author for the Manuscript.

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| --- | --- | --- |
|  | Feedback | Remarks / Responses |
| 1 | The importance of the Novavax vaccine should be notified. There are no comparison analysis among the COVID-19 vaccines with variant technical platforms. | The following statement has been added to the limitations section:‘Unfortunately, to date, there have been no direct comparison analyses in trials between different COVID-19 vaccines or data evaluating the efficacy of Novavax against the different variants.’While there have been suggestions of comparisons of efficacy between the different vaccine platforms e.g. mRNA vaccines are supposedly more efficacious, there are other factors that need to be taken into account eg type of variant, number of COVID cases etc, which we felt were outside the scope of the paper |
| 2 | Table and figure would be encouraged for the prominent to indicate the development of Novavax vaccine/ | The Figure was added to summarise the different stages of vaccine development.  |
| 3 | The animal study and clinical trials were fractions of the development of the Novavax vaccine. There sections should be rearranged to make the logicclearly. | * Section headings corrected to reflect feedback e.g. ‘Preclinical animal studies’ to clearly emphasise animal studies were preclinical stage of the development of vaccine.
* The Figure to summarise the different stages of vaccine development was added
 |
| 4 | The search database scope should be enriched. | An extra search database was done using Scopus; no further papers were found. The additional database used SCOPUS was added.  |
| 5 | Improper presentation of the conclusions raised among the review, eg.‘Overall, the Novavax vaccine shows great potential in contributing to the immunization approaches to managing the pandemic’ | * Conclusion replaced with: ‘Overall, NVX-CoV2373 is found to be safe and effective, meeting the minimum vaccine efficacy rate of 50% to be considered for COVID-19 vaccine emergency use listing approval.’
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**Title Page:**

**Safety and efficacy of the Novavax vaccine – Narrative review**

Novavax safety and efficacy

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**Safety and efficacy of the Novavax vaccine – Narrative review**

**Abstract**

Mass vaccination programmes are a public health priority to manage the global COVID-19 pandemic. NVX-CoV2373 is a SARS-CoV-2 spike glycoprotein subunit vaccine, which was shown to have structural stability with pH and temperature perturbations. The Novavax vaccine is a combination of NVX-CoV2373 and the saponin-based Matrix M adjuvant, added to enhance B and T-cell immune stimulation. Animal studies in mice, olive baboons and cynomolgus macaques demonstrated the potential of this vaccine in protecting the respiratory tract against COVID-19 infection. The Phase 1 and 2 trials confirmed the safety of the Novavax vaccine and the dose-sparing potential of Matrix M. This confirmed the use of the lower dose (5μg) of NVX-CoV2373 for Phase 3 trials. The Phase 3 trial of 14039 participants identified a vaccine efficacy rate of 89.7% in preventing symptomatic infections. Local and systemic adverse events were mild and self-limiting, with commonly reported symptoms as injection-site pain and tenderness, headache, myalgia and fatigue. A subgroup study confirms the safety and efficacy of co-administration of the Novavax vaccine with seasonal influenza immunization. Overall, NVX-CoV2373 is found to be safe and effective, meeting the minimum vaccine efficacy rate of 50% to be considered for COVID-19 vaccine emergency use listing approval.

**Keywords**

Clinical trials, COVID-19, COVID-19 vaccine, immunology

**Safety and efficacy of the Novavax vaccine – Narrative review**

**Introduction**

The global pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected many lives due to the complications caused by COVID-19 disease. As of 13th October 2021, there were 238,521,855 confirmed cases of COVID-19, with 4,863,818 deaths worldwide. Vaccines offer hope to contain this infectious disease, with 6,364,021,792 vaccine doses administered as of 9th October 2021 [1].Currently, COVID-19 vaccines that have received World Health Organisation Emergency Use Listing (EUL) are the Pfizer-BioNTech, Moderna, AstraZeneca, Sinopharm, Sinovac and Janssen vaccines, which are from the mRNA, adenoviral vector and inactivated virus vaccine platforms [2]. The Novavax vaccine, NVX-CoV2373, is a protein-based vaccine that has completed phase 3 trials. This narrative review summarises the trial findings of the Novavax vaccine, which is hoped to be rolled out in the near future.

**Methods**

The PubMed, MEDLINE, SCOPUS and Google Scholar databases were searched using the search terms ‘COVID-19 vaccine’, ‘protein subunit vaccine’, ‘immunisation’, ‘novavax’ and ‘NVX-CoV2373’ on 1st October 2021. All relevant studies were included in the review.

**Development of NVX-CoV2373**

NVX-CoV2373 is a SARS-CoV2 spike glycoprotein subunit vaccine developed from the full-length spike (S) protein. The SARS-CoV-2 S gene encoding the 1273 amino acid spike protein was mutated at the native furin cleavage site (RRAR to QQAQ) to develop the full-length BV2365 variant, which is protease resistant. Two proline (2P) substitutions at positions K986P and V987P were additionally introduced to provide further stability in the double mutant NVX-CoV2373. These genes were then codon optimized for expression in Sf9 (Spodoptera frugiperda) cells. When purified SARS-COV-2 (wild-type), BV2365 and NVX-CoV2373 proteins were compared in 48-hour tests involving incubation at pH extremes (pH 4 and pH9), prolonged agitation, freeze/thaw cycles or elevated temperatures (25 and 37°C), the NVX-CoV2373 proteins had significantly greater structural stability with minimal effect on hACE2 receptor binding after these stressors [3].

The Novavax vaccine is a combination of NVX-CoV2373 as well as the saponin-based Matrix M adjuvant. Subcutaneous administration of Matrix-M in the absence of an antigen was shown to promote leucocyte recruitment to the nearby lymph nodes and spleen in mice, with improved activation and maturation of immune cells, especially dendritic cells to enhance uptake, processing and presentation of antigens [4]. The supplementation of Matrix M adjuvant to subunit vaccines has been shown to enhance B and T-cell immune stimulation in response to the vaccine, with the advantage of dose-sparing potential [5].

Figure 1 illustrates the published studies in the various stages of development of NVX-CoV2373.

**Pre-Clinical Animal Studies**

* Mice: 0.01μg, 0.1μg, 1μg and 10μg doses with 5 μg Matrix M adjuvant at 14 day intervals
* Cynomolgus macaques: 2.5 μg, 5 μg or 25μg NVX-COV2373 with 25μg or 50μg Matrix-M

**Phase 3 Clinical Trial**

* 14,039 participants in United Kingdom with
* Two groups: NVX-CoV2373 or placebo
* Adverse events and efficacy rate assessed
* Subgroup study assessed adverse events and efficacy rate with co-administration with seasonal influenza vaccine

**Two Studies**:

* 1288 participants in United States and Australia
* Divided into two age groups: 18 to 59 years and 60 to 84 years
* Different groups: one or two intramuscular doses of 5μg and 25μg NVX-CoV2373 or placebo 3 weeks apart
* Reactogenicity and immunogenicity assessed
* 2864 participants in South Africa
* Divided into HIV-negative and HIV-positive adults
* Reactogenicity assessed

**Phase 2 Clinical Trial**

* 131 healthy adults aged 18 to 59 years old
* Two intramuscular injections at 21 days intervals
* Five groups:
* Placebo
* 5μg NVX-CoV2373 + Matrix-M1
* 25μg NVX-CoV2373 + Matrix-M1
* 25μg doses NVX-CoV2373 doses
* One dose of 25μg NVX-CoV2373 doses + Matrix-M1, followed by a placebo dose

**Phase 1-2 Clinical Trial**

Figure 1: Stages of development of NVX-CoV2373 vaccine

**Pre-Clinical Animal Studies**

The immunogenicity of NVX-CoV2373 (0.01μg, 0.1μg, 1μg and 10μg doses) with 5 μg Matrix M adjuvant as a single dose or a prime and boost regimen at 14 day intervals were evaluated in mice. This study showed that in mice, at least 0.1μg doses were required to induce anti-S IgG titres by 21 days. For all doses tested, a prime and boost regimen induced significantly increased anti-S IgG titres at least 7 days after the booster dose. This was enhanced further with the addition of Matrix-M adjuvant (versus 10μg NVX-CoV2373), achieving a ten times or higher dose sparing effect. Across all dose levels, a prime boost approach of NVX-CoV2373/Matrix-M induced high titre antibodies which impaired the binding of hACE2 receptors to S-protein, with subsequent neutralization of SARS-CoV-2 cytopathy on Vero E6 cells.

The immunized mice were then administered 105 plaque forming units(pfu)/mouse of SARS-CoV-2 (WA1 strain). While the prime-only mice immunized with NVX-CoV2373 with Matrix-M had a dose-dependent response to virus titre, with no detectable virus in the 10μg dose, the prime/boost immunization resulted in undetectable lung virus loads at NVX-COV2373 doses of at least 0.1μg. The viral load suppression correlated with protection from weight loss after infection and inflammation in lung histopathology. Cell mediated immune responses were also elicited with a higher proportion of multi-functioning phenotypes within both CD4+ and CD8+ T-cells, with corresponding increased production of TNF-α, IFN-γ, and IL-2 cytokines. These findings in mice were replicated in olive baboons, immunized with two doses of 1μg, 5μg and 25μg of NVX-COV2373 (plus 50μg Matrix-M adjuvant) 21-days apart [3].

Using a non-human primate model, cynomolgus macaques at least 3 years old which received 2.5 μg, 5 μg or 25μg NVX-COV2373 with 25μg or 50μg Matrix-M adjuvants induced anti-S IgG antibodies at day 21 after just one dose. This increased significantly a fortnight after the booster dose with anti-S IgG titres surpassing convalescent human serum by 6.9 to 14.2 times. There was a correspondingly similar increase for the hACE2 receptor inhibition titres and SARS-CoV-2 GMT neutralization antibody titres. When the macaques were exposed to SARS-CoV-2 virus in the lower and upper airways, the placebo recipients had an average of 9131 sgRNA copies/mL in bronchioalveolar lavage two days post-challenge; as opposed to no detectable sgRNA in the immunized group. The control group were found to have moderate to severely inflammed bronchi and alveoli, with minimal inflammation in immunized macaques 7 days post-challenge. This demonstrates the ability of NVX-CoV2373 to prevent viral replication in the upper respiratory tract, and pulmonary disease in the lower respiratory tract, which could be tested in human trials [6].

**Phase 1-2 Clinical Trial**

The phase 1-2 trial recruited 131 healthy adults aged 18 to 59 years old, who were administered two intramuscular injections at 21 day intervals. The participants were randomly assigned into five groups; placebo doses, 5μg NVX-CoV2373 doses with Matrix-M1, 25μg doses NVX-CoV2373 doses with Matrix-M1, 25μg doses NVX-CoV2373 doses without Matrix-M1, and one dose of 25μg NVX-CoV2373 doses with Matrix-M1, followed by a placebo dose. This study was performed at two Australian study sites (Nucleus Network, Herston, Queensland, and Melbourne, Victoria). In terms of reactogenicity, the majority of participants had absent or mild local and systemic side effects after the first and second dose in all five groups. There were eight participants (at most two in each sub-group) who experienced severe systemic events after dose two; the most common being joint pain and fatigue, which was self-limiting. In terms of immunogenicity, the ELISA anti-spike IgG geometric mean fold rises (GMFRs) in all adjuvanted regimens exceeded those without adjuvant by 10 times. This further increased by a factor of 8 a week after the second dose, doubling again by 14 days and exceeding levels from convalescent serum of hospitalized COVID-19 patients. The immunogenicity from two doses of 5μg and 25μg vaccines with adjuvant was similar, confirming dose sparing with Matrix-M1. There were also effective polyfunctional CD4+ T-cell responses with adjuvanted regimens with associated TNF-α, IFN-γ and IL-2 production when stimulated by spike proteins; with a high preference towards Th1 responses and minimal IL-5 and IL-13 cytokines production, or Th2 responses [7].

**Phase 2 Clinical Trial**

After confirming safety and immunogenicity data up to a fortnight after the second dose (day 35), the phase 2 trial randomized 1288 participants between 24th August 2020 and 25th September 2020 at 17 sites in the United States and Australia. There were two groups based on age, 18 to 59 years and 60 to 84 years. Subsequent NVX-CoV2373 doses were all adjuvanted with Matrix-M1. Participants received either one or two intramuscular doses of 5μg and 25μg NVX-CoV2373 or placebo 3 weeks apart. Regardless of age, solicited local adverse events were more common in the NVX-CoV2373 compared to placebo; mostly self-limiting tenderness (up to 59%) and pain (up to 38%). This was more likely in younger participants, with the higher dose, and after dose two. For systemic symptoms, muscle pain was the most frequent symptom (20%). This was similar to the placebo group after one dose, which were mainly Grade 1 and short lasting. Fever occured in less than 2% of vaccine recipients. After dose two, the most common systemic symptoms were fatigue (43%), muscle pain (41%), headache (34%) and malaise (30%); again, these were low grade and self-limiting regardless of age. Both doses of NVX-COV2372 induced anti-spike protein binding IgG geometric mean titres (GMTs) and neutralizing antibody activity, exceeding levels from convalescent sera for outpatient and hospitalized COVID-19 patients. Overall, this demonstrated that NVX-CCoV2373 induced high immunogenicity and was tolerated well by younger and older people, thus 5μg NVX-CoV2373 was progressed to phase 2a/b and phase 3 studies [8].

A separate phase 2 trial in South Africa randomized 2864 seronegative participants between 17th August 2020 and 25th November 2020 at 16 sites. There were two groups, human immunodeficiency virus (HIV)-negative participants between ages 18 and 84 and HIV-positive adults aged 18 to 64, who were medically well. Participants received either two doses of the 5μg  NVX-CoV2373 or placebo 3 weeks apart. In both groups, solicited local adverse events were more common with NVX-CoV2373 compared to placebo, without any significant difference in incidence after the first or second dose. The duration of local adverse events was slightly longer after dose two, but was within 3 days. Severe local adverse events were uncommon but were reported more after dose two in vaccine recipients compared to placebo (4% vs. 1%). Similarly, reported systemic symptoms were more common with NVX-CoV2373 compared to placebo. Headache, muscle pain and fatigue were the most frequent, lasting slightly longer after dose two. There were no differences in reactogenicity found between the HIV-negative and HIV-positive group, although the latter sample size was small. Symptomatic Covid-19 infections occurred in 15 subjects who were vaccinated versus 29 participants given placebo, corresponding to a vaccine efficacy of 49.4%. B.1.351 variant was confirmed in 38 (93%) of the 41 samples sent for whole-genome sequencing. Post hoc analysis identified a vaccine efficacy of 43.0% against B.1.351 variant [9].

**Phase 3 Clinical Trial**

The phase 3 trial was carried out in 33 locations in the United Kingdom with 14,039 participants aged between 18 and 84 years randomised between 28th September 2020 and 29th November 2020. There were 27.9% participants aged 65 years or older, while 44.6% had coexisting illnesses. Similar to the phase 2 trials, local adverse events occurred more in the vaccine group and were more commonly reported after the second dose compared to the first (79.6% vs. 57.6%). The most frequent local adverse events were injection-site pain and tenderness, which were mostly mild to moderate severity and short-lived (< 3 days). There were more reported local adverse events in the younger compared to the older participants (65 years and above). The reported systemic adverse events were higher in vaccine recipients compared to placebo group after dose one (45.7% vs. 36.3%) and dose two (64.0% vs 30.0%). The most frequent solicited systemic symptoms were headache, muscle pain and fatigue, which were mostly grade one or two, and lasted less than 2 days. Systemic adverse events are more commonly reported in younger vaccine recipients, with Grade 4 systemic adverse events reported in three participants from the vaccine group. Two participants reported grade 4 fever (above 40 degrees centigrade); one after dose one, with another after dose two. Myocarditis was diagnosed in a vaccinated participant three days after dose two and fully recovered after 2 days of hospitalisation [10].

Of the 14,039 participants, 10 had symptomatic Covid-19 infections at least one week after dose two of the vaccine versus 96 who received placebo, equating to an efficacy rate of 89.7%. The 10 participants from the vaccine group were all aged 65 years and older. Five participants had severe Covid-19 disease, all of whom received placebo. There were two Covid-19 deaths in this study, one from the vaccine group, and another from the placebo group. The participant who died in the vaccine group developed Covid-19 symptoms a week after the first dose and passed away 8 days later [10].

There were several limitations to this trial. The study was performed in the United Kingdom, with the majority (94.5%) of participants being white. It is unclear whether these efficacy findings could be extrapolated to other ethnic groups. The study also excluded pregnant ladies, those below age 18 years, people receiving immunosuppressants or those with immunodeficiency, thus further studies are required to assess the safety and efficacy of NVX-CoV2373 in these groups. The trial was also conducted when the B.1.351 (Alpha) variant was prevalent, while efficacy of this vaccine on the B.1.617.2  (Delta) variant, currently causing outbreaks in many parts of the world, would be more relevant.

A subgroup study of this phase 3 trial was performed for the first 400 participants meeting the inclusion criteria without contraindications to the influenza vaccine. After randomization to NVX-CoV2373 or placebo, the sub-study participants received an open-label influenza vaccine with their first dose. Compared to the main study, the subgroup participants were younger and had fewer comorbidities. Local reactions were more prevalent in the co-vaccinated group (70.1%), compared to 57.6% in the NVX-CoV2373 alone group and 39.4% in the influenza vaccine (co-administered with placebo) group. Systemic reactions were also more common in those co-vaccinated, 60.1% compared to 45.7% in the NVX-CoV2373 alone group. Commonly reported systemic reactions were myalgia, fatigue and fever; which were mostly mild and self-limiting. The likelihood of severe systemic reactions was increased more than two-fold in the co-vaccinated group compared to the NVX-CoV2373 alone group (2.9% vs. 1.3%).  However, there were no differences in the frequency of all adverse events between the co-vaccinated group and the NVX-CoV2373 alone group (18.4% vs 17.6%). More importantly, the efficacy of NVX-CoV2373 was not affected by the co-administration with seasonal influenza vaccine (87.5% vs 89.7% in the main study). This supports the potential co-administration of COVID-19 and the seasonal influenza vaccine, particularly for at-risk groups, such as older people, pregnancy and multiple comorbidities [11].

**Limitations**

The clinical trials evaluated vaccine effectiveness, mainly with the B.1.351 (Alpha) variant. This efficacy is hoped to be similar against the B.1.617.2 (Delta) variant, the main variant of concern causing outbreaks in many parts of the world. Unfortunately, to date, there have been no direct comparison analyses in trials between different COVID-19 vaccines or data evaluating the efficacy of Novavax against the different variants. Long-term adverse events from the vaccine may not have been identified during the trial, which may only become obvious during post-marketing safety surveillance.

**Conclusion**

The Phase 3 trial for the NVX-CoV2373 vaccine demonstrated its safety and efficacy, meeting the minimum vaccine efficacy rate of 50% to be considered for COVID-19 vaccine emergency use listing approval. Adverse events were mild and self-limiting, occurring more with younger people and after the second dose. Co-administration of this vaccine with seasonal influenza immunization is a practical approach to reach population groups at high risk of developing complications from viral respiratory infections.

**Authors’ Contributions**

Choo SZL and Teo SP were involved in conception of the work, acquisition, analysis and interpretation of data, as well as drafting and finalizing the manuscript.

**Availability of Data and Materials**

Not applicable

**Financial Support and Sponsorship**

Not applicable

**Conflicts of Interest**

All authors declared that there are no conflicts of interest

**Ethical Approval and Informed Consent**

Not applicable

**Consent for Publication**

Not Applicable

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