Novavax NVX-COV2373 triggers potent neutralization of Omicron sub-lineages

Jinal N. Bhiman  
National Institute for Communicable Diseases of the National Health Laboratory Services

Simone I. Richardson  
National Institute for Communicable Diseases of the National Health Laboratory Services

Bronwen E. Lambson  
National Institute for Communicable Diseases of the National Health Laboratory Services

Prudence Kgagudi  
National Institute for Communicable Diseases of the National Health Laboratory Services

Nonkululeko Mzindle  
National Institute for Communicable Diseases of the National Health Laboratory Services

Haajira Kaldine  
National Institute for Communicable Diseases of the National Health Laboratory Services

Carol Crowther  
National Institute for Communicable Diseases of the National Health Laboratory Services

Glenda Gray  
The South African Medical Research Council

Linda-Gail Bekker  
University of Cape Town

Vivek Shinde  
Novavax, Inc

Chijioke Bennett  
Novavax, Inc

Gregory M. Glenn  
Novavax, Inc

Shabir Madhi  
University of the Witwatersrand

Penny L. Moore  
National Institute for Communicable Diseases of the National Health Laboratory Services

Anthonet Koen  
University of the Witwatersrand

Lee Fairlie  
Wits RHI

Leon Fouche
Limpopo Clinical Research Initiative

Qasim Bhorat  
Soweto Clinical Trials Centre (SCTC)

Keertan Dheda  
University of Cape Town

Michele Tameris  
South African Tuberculosis Vaccine Initiative

Mduduzi Masilela  
Setshaba Research Centre

Zaheer Hoosain  
Josha Research

Nishanta Singh  
The South African Medical Research Council

Sherika Hanley  
Centre for the AIDS Programme of Research in South Africa

Mohemdran Archary  
Durban International Clinical Research Site, Enhancing Care Foundation

Cheryl Louw  
Madibeng Centre for research

Coert Grobbelaar  
Aurum Institute

Umesh Laloo  
KwaPhila Health Solution

Natasha Joseph  
Peermmed CTC (PTY) - Merk

Gertruida Kruger  
Mzansi Ethical Research Centre

---

**Article**

**Keywords:**

**Posted Date:** October 25th, 2022

**DOI:** [https://doi.org/10.21203/rs.3.rs-2048259/v1](https://doi.org/10.21203/rs.3.rs-2048259/v1)

**License:** [This work is licensed under a Creative Commons Attribution 4.0 International License.](https://creativecommons.org/licenses/by/4.0/)  
[Read Full License](https://creativecommons.org/licenses/by/4.0/)
**Additional Declarations:** Competing interest reported. Dr. Shinde reports being employed by and owning shares in Novavax; Dr. Q. Bhorat, receiving grant support from Wits Health Consortium, Regeneron Pharmaceuticals, GSK, Avillion, Sanofi, Novo Nordisk, and Novavax; Dr. Fouche, receiving grant support from BioNTech; Dr Bennet reports being employed by Novavax; Dr. Glenn, being employed by and owning stock in Novavax and owning stock in RA Capital; and Dr. Madhi, receiving grant support, paid to his institution, from Pfizer and GSK. All remaining authors have not reported any conflicts of interest.

**Version of Record:** A version of this preprint was published at Scientific Reports on January 21st, 2023. See the published version at https://doi.org/10.1038/s41598-023-27698-x.
Abstract

The SARS-CoV-2 Omicron (B.1.1.529) Variant of Concern (VOC) and its sub-lineages (including BA.2, BA.4, BA.5, BA.2.12.1) contain spike mutations that confer high level resistance to neutralizing antibodies. The NVX-CoV2373 vaccine, a protein nanoparticle vaccine, has value in countries with constrained cold-chain requirements. Here we report neutralizing titers following two or three doses of NVX-CoV2373. We show that after two doses, Omicron sub-lineages BA.1 and BA.4/BA.5 were resistant to neutralization by 72% (21/29) and 59% (17/29) of samples. However, after a third dose of NVX-CoV2373, we observed high titers against Omicron BA.1 (GMT: 1,197) and BA.4/BA.5 (GMT: 582), with responses similar in magnitude to those triggered by three doses of an mRNA vaccine. These data are of particular relevance as BA.4/BA.5 is dominating in multiple locations, and highlight the potential utility of the NVX-CoV2373 vaccine as a booster in resource-limited environments.

Introduction

The SARS-CoV-2 Omicron (B.1.1.529) Variant of Concern (VOC) and its sub-lineages (including BA.2, BA.4, BA.5, BA.2.12.1) contain changes to the spike driven by immune escape, and are relatively immune evasive compared with the ancestral-like virus to neutralizing antibodies elicited by coronavirus disease 2019 (COVID-19) vaccines. Similarly, individuals infected with SARS-CoV-2 exhibit reduced neutralizing titers against multiple Omicron sub-lineages, with BA.4 and BA.5 currently detected in over 95 countries and the latter dominating globally. While BA.4 and BA.5 have been classified as distinct sub-lineages, they share the same dominant spike mutations.

Neutralization escape by the Omicron VOC has also been observed following vaccination, regardless of the vaccine type and platform, including with two doses of the NVX-CoV2373 vaccine. However, booster doses, especially using mRNA vaccines, enhance neutralization capacity against Omicron. The NVX-CoV2373 vaccine, which was tested in two phase 3 trials in the US, UK and Mexico demonstrated 90% efficacy against symptomatic and 100% efficacy against severe COVID-19. A Phase 2b trial in South Africa in 2020–2021 demonstrated 48% efficacy against symptomatic infection COVID-19, likely due to relatively antibody-evasive neutralization resistant Beta variant, despite 100% efficacy against severe disease. The vaccine has received authorization for use by the European Medicines Agency, is listed on the World Health Organization’s emergency use listing for COVID-19 vaccines and has received emergency use authorization from the US FDA. This protein-based vaccine is appealing in low-and middle-income countries (LMICs) because of its stability and reduced cold chain requirements. Here, we investigated the effect of a third dose on the neutralizing capacity of NVX-CoV2373 vaccinee sera.

Results

Using a spike-pseudotyped assay, we tested neutralization of the ancestral D614G, Beta, Omicron BA.1 and Omicron BA.4/BA.5 by NVX-CoV2373 vaccinee sera following a 2 dose (n = 29) and 3 dose (n = 48)
regimen. Fourteen days after two doses of NVX-CoV2373, geometric mean titers (GMT) were highest against the D614G variant (GMT: 1,401), with reductions in GMT to 173 (8.1-fold reduction), 34 (41-fold reduction) and 47 (30-fold reduction) against Beta, Omicron BA.1 and Omicron BA.4/BA.5 respectively. For the Omicron sub-lineages BA.1 and BA.4/BA.5, titers were lower than the limit of detection of the assay for 72% (21/29) and 59% (17/29) of samples, respectively, after the 2nd dose of vaccine (Fig. 1, grey).

At one month after the third dose of the NVX-CoV2372 vaccine, neutralizing antibody activity was evident against the Beta and Omicron BA.1 variants in all samples, in contrast to two weeks post the two doses discussed above (Fig. 1, pie charts). The neutralizing antibody titers against the D614G variant were boosted to a GMT of 10,862. Furthermore, we observed a significant 10-, 35- and 12-fold increase in titers against Beta (GMT: 1,733), Omicron BA.1 (GMT: 1,197) and Omicron BA.4/BA.5 (GMT: 582) respectively (Fig. 1, teal), though boosted titers were 6- to 18-fold lower than those against D614G.

We next compared neutralization of Omicron BA.1 and BA.4/BA.5 following multi-dose regimens of adenoviral, mRNA and protein-based vaccines. We tested samples after 2 doses of the adenoviral or 3 doses of the mRNA vaccines, given that these are currently offered as booster regimens in South Africa. As expected, 2 doses of AD26.COV2.S elicited 10- and 14-fold lower GMT against BA.1 than 3 doses of the BNT162b2 and NVX-CoV2373 vaccines respectively (Fig. 2). Similarly the 2 dose AD26.COV2.S vaccine elicited 12- and 11-fold lower GMT against BA.4/BA.5 than 3 doses of either the BNT162b2 and NVX-CoV2373 vaccines. All third dose BNT162b2 and NVX-CoV2373 plasma were able to neutralize Omicron BA.1 and BA.4/BA.5, while only 13–50% of the two dose AD26.COV2.S samples had neutralizing activity against these Omicron sub-lineages. The NVX-CoV2373 third dose plasma GMT against BA.1 and BA.4/BA.5 was comparable to the BNT162b2 titres.

**Discussion**

In summary, we report enhanced neutralization of Omicron BA.1 and BA.4/BA.5 following three doses of the NVX-CoV2373 vaccine with responses comparing well to three dose of an mRNA vaccine. We note that six months after two doses of NVX-CoV2373, increased binding antibodies were reported, and responses may mature further. As durability of vaccine platforms varies, future studies should assess this for NVX-CoV2373 neutralization at later time-points. The two dose NVX-CoV2372 vaccine regimen elicits robust memory CD4+ and CD8+ T cell responses in 100% and 65% of individuals respectively. In addition, the two dose regimen induces antibodies with multiple Fc-mediated functions, which in non-human primate and human cohorts likely contribute to protection from infection. This T cell and Fc effector function data coupled with high titre neutralizing antibodies we have described here, suggests that this vaccine will is likely to limited viral replication to prevent severe disease after SARS-CoV-2 breakthrough infection with currently dominant Omicron sub-lineages.

Limitations of the study include the difference in timing of the sample collection following the second and third dose of the NVX-CoV2373 vaccine, with collection at fourteen days and one month post...
vaccination respectively. Similarly, sample collection following administration of the AD26COV2.S and BNT162b2 booster doses varies between 1–3 months and includes relatively small sample numbers. Despite these limitations, this study highlights the high titres elicited by a third dose of the NVX-CoV2373 against currently circulating Omicron sub-lineages, which supports the use of this vaccine as a booster regimen in countries where mRNA cold chain requirements cannot be met.

**Methods**

**Samples and ethics approvals.**

Individuals vaccinated with two or three doses of the NVX-CoV2373 vaccine were sampled at 14 days after the second dose or 35 days after the third dose. The third NVX-CoV2373 dose was administered 6 months after the first dose. This trial is registered under the ClinicalTrials.gov number, NCT04533399 (registered 17/09/2020), and the protocol was approved by the South African Health Products Regulatory Authority and by the institutional review board at each trial centre as described in detail by Shinde and colleagues. Health care workers vaccinated with two dose of AD26.COV2.S (5 x 10^10 viral particles) as part of the Sisonke implementation trial were sampled at 2 months after vaccination. This trial is registered under the ClinicalTrials.gov number, NCT05148845, and the protocol was approved by the South African Health Products Regulatory Authority. These Sisonke individuals were recruited at the National Institute for Communicable Diseases (NICD), Johannesburg. Individuals vaccinated with two and three doses of the BNT162b2 vaccine were sampled at 2 months after the second dose or 1–3 months after the third dose and were recruited from Johannesburg. This study was given ethics approval by the University of the Witwatersrand Human Research Ethics Committee (Medical) M210465. All individuals provided written informed consent and all research was performed in accordance with the relevant guidelines/regulations and in accordance with the Declaration of Helsinki.

**Lentiviral pseudovirus production and neutralization assay.** The 293T/ACE2. MF cells modified to overexpress human ACE2 were kindly provided by M. Farzan (Scripps Research). Cells were cultured in DMEM (Gibco BRL Life Technologies) containing 10% heat-inactivated fetal bovine serum (FBS) and 3 µg/ml – 1 puromycin at 37°C, 5% CO₂. Cell monolayers were disrupted at confluency by treatment with 0.25% trypsin in 1mM EDTA (Gibco BRL Life Technologies). The SARS-CoV-2, Wuhan-1 spike, cloned into pCDNA3.1 was mutated using the QuikChange Lightning Site-Directed Mutagenesis kit (Agilent Technologies) to include D614G (ancestral D164G) or L18F,D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G, A701V (Beta) or Δ69–70, T915I, Δ143–145, Δ211, L212I, ins 214 EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F (Omicron BA.1) or T19I, L24S, Δ25–27, Δ69–70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K (Omicron BA.4/BA.5). Pseudoviruses were produced by co-transfection with a lentiviral backbone (HIV-1 pNL4.1uc encoding the firefly luciferase gene) and either of the SARS-CoV-2 spike plasmids with PEIMAX (Polysciences). Culture supernatants were clarified of cells by a 0.45 µm filter and
stored at −80°C. Plasma samples were heat-inactivated and clarified by centrifugation. Pseudovirus and serially diluted plasma/sera were incubated for 1h at 37°C, 5% CO₂. Cells were added at 1×10⁴ cells per well after 72h of incubation at 37°C, 5% CO₂, luminescence was measured using PerkinElmer Life Sciences Model Victor X luminometer. Neutralization was measured as described by a reduction in luciferase gene expression after single-round infection of 293T/ACE2.MF cells with spike-pseudotyped viruses. Titers were calculated as the reciprocal plasma dilution (ID₅₀) or monoclonal antibody concentration (IC₅₀) causing 50% reduction of relative light units. Equivalency was established through participation in the SARS-CoV-2 Neutralizing Assay Concordance Survey Concordance Survey 1 run by EQAPOL and VQU, Duke Human Vaccine Institute. Cell-based neutralization assays using live virus or pseudovirus have demonstrated high concordance, with highly correlated 50% neutralization titers (Pearson r = 0.81–0.89).

**Declarations**

**Acknowledgments**

We thank Dr Thandeka Moyo-Gwete, Brent Oosthuysen, Donald Mhlanga, Frances Ayres, Haajira Kaldine, Nelia P. Manamela, Sebotsana Rasebotsa, Sharon Madzorera, Thanusha Naidoo, Thopisang Motloou for technical assistance in generating plasmids and / or proteins for this study.

**Author contributions**

Designed the study, performed analyses and wrote the manuscript: JNB, PLM

Performed experiments and analysed data: SIR, BEL, PK NM, HK

Data curation and project management: CC

PIs for Sisonke (AD26CoV2.S) Trial: GG, LGB

Site PIs for Novavax Trial: AK, LFa, LFo, QB, KD, MT, MM,ZH, NS, SH, MA, CL, CG, UL, NJ, GK

PIs for Novavax Trial: VS, CB, GMG SM

**Funding**

PLM is supported by the South African Research Chairs Initiative of the Department of Science and Innovation and National Research Foundation of South Africa, the SA Medical Research Council SHIP program, and the Centre for the AIDS Programme of Research in South Africa (CAPRISA). We acknowledge funding from the Bill and Melinda Gates Foundation, through the Global Immunology and Immune Sequencing for Epidemic Response (GIISER) program. The phase II clinical trial was funded by Novavax and the Bill and Melinda Gates Foundation. The findings and conclusions contained within are
those of the authors and do not necessarily reflect positions or policies of the Bill and Melinda Gates Foundation.

Data availability

All data reported in this paper will be shared by the lead contacts, Penny L. Moore (pennym@nicd.ac.za) and Shabir Madhi (Shabir.Madhi@wits.ac.za) upon request. This paper does not report original code.

Competing interests

Dr. Shinde reports being employed by and owning shares in Novavax; Dr. Q. Bhorat, receiving grant support from Wits Health Consortium, Regeneron Pharmaceuticals, GSK, Avillion, Sanofi, Novo Nordisk, and Novavax; Dr. Fouche, receiving grant support from BioNTech; Dr Bennet reports being employed by Novavax; Dr. Glenn, being employed by and owning stock in Novavax and owning stock in RA Capital; and Dr. Madhi, receiving grant support, paid to his institution, from Pfizer and GSK. All remaining authors have not reported any conflicts of interest.

References


Figures

Figure 1
Neutralization of SARS-CoV-2 variants by NVX-CoV2373 vaccinee plasma. Neutralization of ancestral D614G, Beta, Omicron BA.1 and Omicron BA.4/BA.5 pseudoviruses by NVX-CoV2373 vaccinee plasma following 2 (grey) or 3 (teal) doses. Samples were collected 14 days after the second dose and 1 month after the third dose. Geometric mean titers (GMT) for each virus are shown above the individual points, and percent of specimens where no neutralization was observed (red) is indicated in the pie charts. Number of vaccinee specimens tested are indicated and p values were calculated using the Mann-Whitney t-test for non-parametric data with p < 0.001 for D614G, Beta, Omicron BA.1 and Omicron BA.4/BA.5. Samples were used at a starting dilution of 1 in 20 (limit of detection) with a seven 3-fold dilutions to create a titration series.

Figure 2

Neutralization of Omicron BA.1 and BA.4/BA.5 by boosted vaccinee plasma. Neutralization of Omicron BA.1 and BA.4/BA.5 by vaccinee plasma following 2 doses of the AD26.COV2.S or 3 doses of the BNT162b2 or NVX-CoV2373 vaccines. Number of doses, number of samples and date of sample collection after boost for each group are indicated. Geometric mean titers (GMT) for each virus are shown above the individual points, P values were calculated using two-way ANOVA with p < 0.001 for AD26CoV2.S versus NXV-CoV2373 and p = 0.0011 for NVX-CoV2373 BA.1 versus BA.4/BA.5). Samples
were used at a starting dilution of 1 in 20 (limit of detection) with a seven 3-fold dilutions to create a titration series.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- BhimanetalNovavaxthirdboostv19supplementaryinfo.docx