Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Results of non-inferiority Phase II Clinical Trial of humoral immunogenicity of a Wuhan- hu-1 monovalent Inactivated Recombinant Newcastle Disease Virus Vaccine Expressing SARS-CoV-2 Spike in comparison to BNT162b2 in Brazil

Érique José Farias Peixoto de Miranda
Instituto Butantan
José Alfredo Sousa Moreira
Instituto Butantan
Ricardo Sobhie Diaz
Azidus Brasil
Carlos Alexandre Antunes Brito
Marcos Carvalho Borges
University of São Paulo
Fábio Eudes Leal
Universidade Municipal de São Caetano do Sul (USCS)
Luis Augusto Tavares Russo
Instituto Brasil de Pesquisa Clínica (IBPClin)
Fernanda Castro Boulos
Instituto Butantan
Maria Aparecida Alves Leite dos Santos Almeida
Instituto Butantan
Marcia Cristina Oliveira da Rocha
Instituto Butantan
Karina Takesaki Miyaji
Instituto Butantan
João Ítalo Dias França
Instituto Butantan
Marcos Alves de Lima
Instituto Butantan
Lucas Ragiotto
Instituto Butantan
Rama Raghunandan
PATH
Adolfo García-Sastre
Icahn School of Medicine at Mount Sinai (ISMMS)

Weina Sun
Icahn School of Medicine at Mount Sinai (ISMMS)
Peter Palese
Icahn School of Medicine at Mount Sinai (ISMMS)
Florian Krammer
Icahn School of Medicine at Mount Sinai (ISMMS)
Esper Georges Kallas
esper.kallas@butantan.gov.br

Instituto Butantan

Article

Keywords: SARS-CoV-2, COVID-19, Newcastle disease virus, recombinant vaccines, omicron strain

Posted Date: May 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9407562/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: Competing interest reported. The A.G.-S. laboratory has received research support from GSK, Pfizer, Senhwa Biosciences, Kenall Manufacturing, Blade Therapeutics, Avimex, Johnson & Johnson, Dynavax, 7Hills Pharma, Pharmamar, ImmunityBio, Accurius, Nanocomposix, Hexamer, N-fold LLC, Model Medicines, Atea Pharma, Applied Biological Laboratories and Merck. A.G.-S. has consulting agreements for the following companies involving cash and/or stock: Castlevax, Amovir, Vivaldi Biosciences, Contrafect, 7Hills Pharma, Avimex, Pagoda, Accurius, Esperovax, Applied Biological Laboratories, Pharmamar, CureLab Oncology, CureLab Veterinary, Synairgen, Paratus, Pfizer and Prosetta. A.G.-S. has been an invited speaker in meeting events organized by Seqirus, Janssen, Abbott, Astrazeneca and Novavax. A.G.-S. is inventor on patents and patent applications on the use of antivirals and vaccines for the treatment and prevention of virus infections and cancer, owned by the Icahn School of Medicine at Mount Sinai, New York. The rest of the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. PP is an inventor on patents and patent applications on the use of vaccines for the treatment and prevention of virus infections, owned by the Icahn School of Medicine at Mount Sinai, New York. WS is listed as co-inventor of NDV-based SARS-CoV-2 vaccine owned by the Icahn School of Medicine at Mount Sinai, New York. WS is co-founder and scientific advisory board member of CastleVax. Mount Sinai has spun out a company, CastleVax, to commercialize SARS-CoV-2 vaccines. FK, PP, WS and AG-S serve on the scientific advisory board of CastleVax and are listed as co-founders of the company. FK has consulted for Merck, Gritstone, Seqirus, Curevac, GSK and Pfizer, and is currently consulting for Third Rock Ventures and Avimex. The FK laboratory has been collaborating with Pfizer on animal models of SARS-CoV-2 and is currently collaborating in the influenza space with Dynavax.

Abstract

Background

Coronavirus disease 2019 (COVID-19) prompted an unprecedented global vaccine development response and accelerated regulatory pathways for clinical vaccine development. Despite the end of the public health emergency, sustained preparedness, including scalable and affordable vaccine platforms remains essential.

Methods

This is a non-inferiority, multicenter, randomized, double-blind and active-controlled Phase II clinical trial, to evaluate the immunogenicity and safety of a fifth- dose booster of NDV-HXP-S 10 μg, an egg-derived, inactivated COVID-19 vectored-vaccine virus using the Newcastle disease virus (NDV) expressing stabilized pre-fusion S protein from Wuhan-hu-1 strain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), versus BNT162b2 30 μg. Healthy participants aged 18–59 years (n = 200) or ≥ 60 years (n = 200) were randomized at a 1:1 ratio to receive a single intramuscular dose on Day 1. Primary immunogenicity endpoints (per-protocol) were the Day 29 geometric mean titer (GMT) ratio of the 50% neutralizing titer (NDV-HXP-S/BNT162b2) against SARS-CoV-2 Wuhan-hu-1 and the difference in seroconversion proportions (≥ 4-fold rise if seropositive at baseline, or NT_{50} ≥40 IU/mL if seronegative). Non-inferiority margins were a point estimate ≥ 0.80 with a 95% confidence interval (CI) lower bound > 0.67 for GMT ratio, and a 95% CI lower bound > − 10% for seroconversion. Primary safety endpoints were solicited vaccine-related adverse events (AEs) through Day 7 and unsolicited and serious vaccine-related AEs through Day 28. Follow-up occurred for 12 and 6 months for safety and immunogenicity evaluation, respectively.

Results

Between January 27th and August 30th 2023, 389 eligible subjects were included in the safety population. Of 389 participants, 263 (67.6%) were female, median age of 60.1 years (IQR, 40.8–65.4) and the majority of them were white, 218 (56.0%). Among the 311 (77.8%), the GMT ratio (NDV-HXP-S/BNT162b2) of neutralizing antibodies against SARS-CoV-2 (Wuhan) was 0.50 (95% CI: 0.40—0.60), measured on Day 29. Meanwhile difference in seroconversion was − 36.7% (-45.3% to -28.0%). Vaccine-related solicited adverse events were 131 (67.2%) in NDV-HXP-S and 148 (77.1%) in BNT162b2 (P = 0.032) within 7 days post-vaccination, while vaccine-related unsolicited adverse events were 46 (23.5%) in NDV-HXP-S *vs*. 51 (26.4%) in BNT162b2 (P = 0.558) within 28 days. There were no vaccine-related serious adverse events (SAEs) () within 28 days post-vaccination.

Conclusion

Inactivated NDV-HXP-S at 10 μg did not meet the criteria for non-inferiority in comparison to BNT162b2 at 30 μg in SARS-CoV-2 Wuhan-hu-1 seroconversion and neutralization as a booster dose. Both vaccines had a favorable safety profile.

Bullet points

1. Inactivated NDV-HXP-S 10 μg did not meet the criteria for non-inferiority in comparison to BNT162b2 30 μg in eliciting neutralizing antibodies against SARS-CoV-2 Wuhan-Hu-1 at Day 29;..
2. Inactivated NDV-HXP-S 10 μg and BNT162b2 30 μg had broadly similar safety; solicited reactogenicity was lower with NDV-HXP-S (67.8% vs. 77.1%).
3. Clinical development of NDV-HXP-S 10 μg as a fifth-dose booster was discontinued after the Phase II Study.

1. Introduction

Coronavirus disease 2019 (COVID-19) vaccines had been demonstrated to be a fundamental public health strategy to provide declining disease incidence rate and its morbidity and mortality, despite the emergence of new variants of concern (VOC), in a fast-changing scenario [1]. Although the COVID-19 public health emergency of international concern (PHEIC) had been declared to be over on May 2023 by the World Health Organization (WHO) [2, 3].

During the COVID-19 pandemic, reactive measures to fight the disease included fast track clinical vaccine development, rapid deployment of national immunization programs and matching to SARS-CoV-2 mutations by providing new strain-adapted vaccines. We witnessed the fast emergence of VOCs resulting in decreased protection after primary immunization with Wuhan-hu-1-based vaccines due to viral immune evasion [4]. Nevertheless, few vaccine manufacturers answered to these antigenic changes, mainly strain-updated booster mRNA vaccines [5, 6]. The effort of regulatory agencies around the world for Emergency Use Authorization (EUA) or full approval resulted in at least 40 COVID-19 vaccines approved since December 2020 [7], out of almost 400 vaccines under clinical development [8]. Furthermore, despite the high overall vaccine coverage during the pandemic, the availability of vaccines doses around the world was unequal [9].

To provide vaccine for low and middle-income countries, a consortium was established in Brazil, Thailand, and Vietnam for the industrial development and clinical testing of a cost-effective, recombinant and inactivated COVID-19 vaccine, NDV-HXP-S, to be manufactured in available embryonated, egg-based influenza vaccine under state of the art good manufacturing practices (GMP). This is an inactivated vectored-vaccine virus resulting from the genetic recombination of Sike protein of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) stabilized in the pre-fusion form with HexaPro technology, in combination of an avian virus (Newcastle disease virus [NDV]) of, cultured in egg allantoic fluid, which could provide higher immunogenicity [10, 11].

After the EUA of a COVID-19 inactivated vaccine (CoronaVac) in Brazil in January 2021, the Butantan Institute planned to evaluate NDV-HXP-S in a dose-escalating Phase I study between 2021 and 2022 [12]. The Phase I study results showed that NDV-HXP-S 10 μg formulation had a favorable immunogenicity and safety profile with a 2-dose primary immunization regimen [12], similar to findings in Phase I studies in Thailand [13] and Vietnam [14]. As the primary immunization and third and fourth booster COVID-19 coverage in Brazil steeply rose between 2021 and 2022, for the Phase II study, we planned to evaluate the NDV-HXP-S 10 μg as a fifth booster dose in comparison to BNT162b2 30 μg, after a background of four doses of COVID-19 vaccines approved by the Brazilian National Immunization Program (PNI). Although there is no gold-standard for comparator against Wuhan strain in clinical trials, BNT162b2 was proven to be the best vaccine not only against Wuhan, but also against Omicron strains BA.1 and BA.4/5, which were circulating in Brazil in 2022 [15].

Thus, the present study is a non-inferiority multicenter, randomized, double-blind and active-controlled Phase II clinical trial aims to evaluate the immunogenicity and safety of a fifth booster dose using NDV-HXP-S 10 µg, in comparison to BNT162b2, against Wuhan-hu-1, Omicron BA.1 and Omicron BA.4/5 strains.

2. Methods

2.1 Study Objectives, Design and Participants

This is a non-inferiority multicenter randomized, double-blind, active-controlled Phase II clinical trial, to evaluate the immunogenicity and safety of Wuhan-hu-1 monovalent NDV-HXP-S 10 µg in comparison to BNT162b2 30 µg as a fifth booster dose (**Figure S1**).

The participants were healthy adults aged 18 or older, at the time of consent, of which 50% aged at least 60 years, with stable comorbidities, regardless of SARS-CoV-2 infection status, with evidence of four doses of any monovalent COVID-19 vaccine provided by the Brazilian National Immunization Program (PNI), of which the last dose was administered at least 120 days to 540 days prior to randomization. Key exclusion criteria were administration of background regimen of COVID-19 vaccine not described in the inclusion criteria up to 30 days prior to the study vaccine dose, use of any other COVID-19 vaccine regimen before the study admission different than the regimen stablished in the inclusion criterion, use of any investigational product within 90 days prior to randomization, the use of vaccine in the last 28 days prior to inclusion in the study, uncontrolled comorbidities, underlying immunosuppression or use of immunosuppressive therapies, human immunodeficiency virus (HIV) infection, hepatitis B or C infection, and pregnancy or breastfeeding. A detailed presentation of the eligibility criteria is available in the **Online Appendix**.

This clinical trial followed the Declaration of Helsinki and Good Clinical Practices guidelines. We obtained written informed consent from all participants, as approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and National Research Ethics Commission (CONEP) (CAAE# 58416122.1.1001.5412). This study was also approved by the Brazilian Health Regulatory Agency (ANVISA), according to the Special Communication in the Clinical Drug Development Dossier number 55/2021.

2.2. Randomization and Masking.

Enrolled subjects were randomly assigned in a 1:1 ratio in sequence to one of two groups: one dose of non-adjuvanted NDV-HXP-S 10 µg/0.5ml or BNT162b2 30 µg/0.3ml, the active control. The liquid injectable solution of the active control vaccine after dilution is slightly opalescent, but it differs from NDV-HXP-S because it corresponds to 30µg/0.3 mL. Therefore, the syringe needed to be masked after preparation by the unblinded pharmacist and handed over to an unblinded administrator.

An independent statistician, without other involvement in the study, generated the randomization sequences through a computer program on a web-based randomization system by a Clinical Research Organization (CRO) (ATCGen, Brazil). This system informed the unblinded team staff which product under investigation or placebo should be randomized to each participant, with correspondent IDs for the stratified randomization sequence according to the age group (18–59 years old or ≥ 60 years old).

The clinical team and volunteers were blinded to randomization codes. Only pharmacists or nurses responsible for the randomization, preparation of product under investigation and blinding, besides the staff responsible for

vaccine administration, had access to unblinded information.

2.3. Investigational product

The vaccine contains inactivated, whole chimeric Newcastle disease virus (NDV) expressing, anchored on its membrane, a trimer of the SARS-CoV-2 virus spike protein (S) (NDV-HXP-S) in pre-fusion conformation by the inclusion of six proline mutations in its ectodomain (HexaPro) [11]. The vaccine was produced by the Instituto Butantan in its influenza vaccine production plant (São Paulo, Brazil), strictly following Good Manufacturing Practices (GMP) guidelines, as described by Peixoto de Miranda *et al.* [12]. NDV-HXP-S were filled in a 10-dose vial (5 mL) and stored at 2–8°C at an individual 0.5 mL volume.

The active control vaccine BNT162b2 30µg (Comirnaty® Wyeth or Pfizer-BioNTech) was presented as a multidose vial (6 doses), that contains 0.45 mL of concentrated suspension for injection (6 doses/vial) in packs of 195 vials with a purple flip cap, that must be diluted with 1.8 mL of saline. BNT162b2 vaccine packs were obtained from the Secretaria de Estado de Saúde de São Paulo (São Paulo, Brazil), stored at a temperature between − 60°C and − 90°C. The product was shipped to the five clinical sites at a -80ºC compartment by road (Valinhos, São Caetano, and Serrana, all in State of São Paulo) or by air (Rio de Janeiro and Recife). At the research site, the multidose vial was stored at 2–8°C and thawed prior to dilution, during the 30-day validity period. After the vaccine extraction, the remaining content returned to the sponsor in a way that ensures that the blinding is not broken.

The recommended administration site for both products under investigation is the deltoid of the upper arm by intramuscular injection. The immunization schedule is one booster dose.

2.4. Clinical procedures

The screening visit included anamnesis, physical examination, vital signs, anthropometry, review of concomitant medications, baseline blood samples drawn for safety analysis, HIV, hepatitis B and hepatitis C serologies, nasal swab for SARS-CoV-2 infection confirmation by reverse transcriptase-polymerase chain reaction (RT-PCR) and chemiluminescence immunoassay (CLIA) for SARS-CoV-2-specific anti-receptor binding domain (RBD) and anti-nucleoprotein (N) IgG detection. Women of childbearing potential (WOCBP) underwent beta-hCG (human chorionic gonadotropin) testing from baseline to Day 29 after the booster.

Eligible subjects were given an intramuscular injection at the deltoid region containing 0.5 mL (NDV-HXP-S) or 0.3 mL (BNT162b2) of investigational product by a staff member unblinded to the randomization arm on Day 1 (visit of vaccination, V1).

Blood samples were collected at baseline, Day 8 (+ 3) (S1), Day 29 (+ 7) (SI1), Day 92 (+ 7) (SI2), Day 182 (+ 14) (SI3) and Day 364 (+ 28) (SI4). Safety samples were drawn at baseline and Day 8 (+ 3). SARS-CoV-2 binding and neutralizing antibody levels were evaluated at baseline, Day 29 (+ 7) (SI1), Day 92 (+ 7) (SI2), Day 182 (+ 14) and Day 364 (+ 28).

Participants underwent 30-minute observations at the study site after each vaccination and registered solicited adverse events (AEs) and their intensity on a card diary for 7 days after each vaccination, including fever, defined as axillary temperature of ≥ 37.8ºC. Severity of adverse events (AE) was graded 1–4 as published elsewhere [16]. On the safety visit (Day 8), diaries were examined by the clinical staff.

Investigators assessed unsolicited AE for causality (related to vaccination or not) [17]. All AEs reported within 28 days after V1 were included on the Case Report Form (CRF) by the investigator, who provided intensity and classification as serious or not. After the safety period, only adverse events of special interest (AESI) [18] and vaccine-related and serious adverse events (SAE) were registered during all the study period of 12 months. The study had a passive surveillance of cases of fever and testing for SARS-CoV-2 infection confirmation by RT-PCR.

2.5. Assessment of immunogenicity against SARS-CoV-2

Serum antibody testing was performed by Nexelis (Laval, Quebec, Canada) using a validated pseudovirus neutralization assay (PNA) to measure the serum neutralizing activity against the Wuhan-hu-1 strain of SARS-CoV-2, variants of concern Omicron BA.1 and Omicron BA.4/5, at biosafety level 2 (BSL2) conditions. Details of the method were published elsewhere [19].

The pseudo-neutralization assay measured particle entry-inhibition at baseline, Day 29 (+ 7), Day 92 (+ 7), Day 182 (+ 14) and Day 364 (+ 28) after the first vaccine dose as a primary immunogenicity evaluation. We used the same assay to measure the 50% neutralization titers (NT_{50}) against pseudotyped virus particles generated for SARS-CoV-2 variants of concern (BA.1 and BA.4/5) at the same timepoints.

The 50% neutralization titer (NT_{50}) of a sample was determined by the reciprocal serum dilution corresponding to the neutralizing antibody titer at 50% inhibition (NT_{50}) for the sample. Values of NT_{50} titers were calculated by using a conversion factor calculated during assay validation (1/1.872), transformed to international units per mL (IU/mL), according to WHO International Standard for anti-SARS-CoV-2 immunoglobulin [20]. PNA titers are strongly correlated with titers in a plaque reduction neutralization test (PRNT).

Enzyme-linked immunosorbent assay (ELISA) (BAU/mL) and PNA (IU/mL) assays were compared to a panel of convalescent human sera obtained 14 days after the onset of symptoms from consecutive mild/moderate cases of COVID-19 in healthcare workers, in Canada, 2020. The NT_{50} lower limit of quantification (LLOQ) were 10 for Wuhan, Omicron BA.1 and Omicron BA.4/5 strains, while the upper limits of quantification (ULOQ) were 15.762, 4.285 and 18.176, respectively

Besides neutralizing antibodies, SARS-CoV-2 spike-specific IgG were measured by using a validated indirect ELISA (binding assay), performed by Nexelis (Laval, Canada) to assess the secondary endpoints at same time points of the PNA test.

Similarly to the PNA, concentrations measured by ELISAs were transformed into binding antibody units per mL (BAU/mL), based on the WHO International Standard for anti-SARS-CoV-2 immunoglobulin, using a conversion factor determined during assay validation (1/7.9815) [20]. The assay lower and upper limit of quantification (LLOQ) were 50.3 ELU/mL and 15797.9 ELU/mL, respectively.

.

2.6. Study outcomes

The primary immunogenicity endpoint was the composite of: 1) geometric mean (GMT) ratios (NDV-HXP-S/BNT162b2) of 50% neutralizing titers (NT_{50}) measured by the pseudo virus neutralization assays against the SARS-CoV-2 Wuhan-hu-1 strain and 2) seroconversion proportion, defined as titers ≥ 4 fold from baseline in

seropositive volunteers at baseline and proportion who reached NT_{50} titer ≥ 40 IU/mL in seronegative volunteers at baseline, both measured on Day 29.

The primary safety outcome of the study was determined as the composite of number and intensity of solicited local and systemic vaccine-related adverse events within 7 days after vaccination, as well as the number and intensity of unsolicited vaccine-related adverse events and vaccine-related serious adverse events within 28 days after vaccination.

Immunogenicity secondary outcomes included the 1) geometric mean titers (GMT), 2) neutralizing geometric mean (GMT) ratio of 50% neutralizing titers (NT_{50}), 3) geometric mean fold rise (GMFR) and 4) seroconversion proportion, defined as an increase of ≥ 4 fold over baseline titers in seropositive or NT_{50} titer ≥ 40 IU/mL increasing in seronegative participants, all measured by pseudo virus neutralization assays against the SARS-CoV-2 Wuhan-hu-1 strain, variants of concern BA.1 and BA.4/5 on Day 29, Day 92, Day 182 and Day 364 after the booster. Finally, immunogenicity secondary outcome included 1) geometric mean concentration (GMC), 2) GMC ratio, 3) GMFR, and seroconversion of SARS-CoV-2 spike-specific antibody (IgG) measured by indirect ELISA, on Day 29, Day 92, Day 182, and Day 364 after the booster.

Safety secondary outcomes included the number, intensity, and summary of all vaccine-related unsolicited AE within the first 28 days after the booster, as well as vaccine-related serious adverse events (SAE), medically attended adverse events (MAAE), and events of special interest (AESI) throughout the study period. Laboratory values for hemoglobin, leukocytes, platelets, alanine aminotransferase, aspartate aminotransferase, and total bilirubin were measured on baseline and on Day 8. Confirmed breakthrough cases of COVID-19 throughout the study period were classified as AESI and described according to WHO Clinical Progression Scale and to SARS-CoV-2 strain by sequencing performed in the sponsor's central laboratory.

2.7. Statistical Analysis

Sample size estimation considered a comparison between NDV-HXP-S and BNT162b2 for evaluating composite primary immunogenicity endpoint as the proportion of seroconversion and GMT ratio measured by PNA against SARS-CoV-2 Wuhan-hu-1 on Day 29. Immunogenicity and safety analysis were stratified by age groups: 18–59 years and ≥ 60 years. The statistical criterion of non-inferiority of GMT ratio (NDV-HXP-S/BNT162b2) requires that: 1) point estimate to be greater to 0.80, 2) the lower bound of the one-tailed 97.5% confidence interval (CI) of the GMT ratio to be greater than 0.67, and while for proportion of seroconversion, 3) the lower bound of 97.5% CI to be greater than − 10.00% [12, 21, 22].

For both tests, we used as power assumptions of 90%, 2.5% alpha error, 10% dropout rate and an allocation ratio of 1:1. The variability of pseudo virus neutralization assay (PNA) was 0.5 as standard deviation on the $\log_{10}$ scale. A pre-specified non-inferiority test for GMT ratio considered the margin of 0.67. Conversely, the assumptions for seroconversion [23] were a p_1 proportion of 0.90 and p_2 of 0.99, 10% delta, and − 10% non-inferiority margin. No correction for multiple comparisons was planned [12].

Descriptive statistics included mean, standard deviation, or median and interquartile range (IQR), as appropriate, for continuous variables, and numbers and proportions per group for categorical variables. Unless otherwise specified, a two-tailed 5% significance value was used for statistical tests and confidence intervals. Exact (Clopper-Pearson) 95% confidence intervals were used for dichotomous variables univariate summaries. Values

of titers (NT_{50}) or concentrations were presented as geometric mean and 95% CI [12]. Values of titers or concentrations categorized with cutoff at increasing of ≥ 4 times in Day 29 after first vaccination were presented as absolute and relative frequencies within the per-protocol population and exact 95% CI. Finally, the frequency of subjects with at least one AE were presented as absolute and relative frequencies within the safety population and exact 95% CI [12].

Safety population was defined in this study as all enrolled (screened, eligible and randomized) participants who have received at least one dose of the study vaccine and had available safety data [12]. The per-protocol population was defined as all randomized participants who did not have a protocol violation affecting the primary immunogenicity, contributing to the follow-up time for per-protocol analyses until the exclusion event. All immunogenicity analyses were performed in the per-protocol population.

2.8. Oversight, role and funding source

The study funders had no role in data collection, data analysis, or writing of the statistical report. The Instituto Butantan was the clinical trial sponsor and approved the study protocol; its members contributed as authors by preparing the investigational vaccine, interpreting data, and writing this report according to the Study Protocol. Data monitoring, management and randomization were delegated to outsourced vendors (Synova Health and ATCGen, Brazil, respectively). All authors had full access to all the study data and had final responsibility for the decision to submit for publication. An independent Data and Safety Monitoring Board was responsible for reviewing the study's unblinded safety data.

3. Results

3.1. Participants

Between January 27th to August 30th, 2023, a total of 608 subjects were screened, 208 (34.2%) of them were considered ineligible. Of the total, 400 (65.8%) were considered eligible and were randomized, but 11 subjects withdrew their consent, resulting in 389 participants available for the intention to treat (ITT) and safety analyses. In the end, 311 (77.8%) subjects were available for the per-protocol (PP) analysis. Subjects' enrollment and their status are described in Fig. 1.

In the safety population, the median age was 60.1 years (IQR, 40.8–65.4), 263 (67.6%) were female, and the majority were described as self-reported white skin color (218, 56.0%). The median body mass index (BMI) was 28.1kg/m² (IQR, 24.8–31.9). Demographic characteristics for the subjects available for safety evaluation are described in Table 1. All demographic and other baseline variables were properly balanced by the randomization.

Table 1
Baseline characteristics of participants (safety population).

Baseline characteristics	NDV-HXP-S 10µg (N = 196)	BNT162b2 30µg (N = 193)	Total (N = 389)
Age (years-old)			
Median (IQR)	60.1 (41.4–65.6)	59.3 (40.5–65.0)	60.1 (40.8–65.4)
Range	18.2–79.1	19.0–80.2	18.2–80.2
Age group			
18–59 years old	97 (49.5)	97 (50.3)	194 (49.9)
60 or more years old	99 (50.5)	96 (49.7)	195 (50.1)
Sex, n (%)			
Female	138 (70.4)	125 (64.8)	263 (67.6)
Race,/Skin color n (%)			
Asian	0 (0.0)	3 (1.6)	3 (0.8)
Black	26 (13.3)	32 (16.6)	58 (14.9)
Pardo	59 (30.1)	49 (25.4)	108 (27.8)
White	110 (56.1)	108 (56.0)	218 (56.0)
Other	1 (0.5)	1 (0.5)	2 (0.5)
Body mass index (kg/m²)			
Median (IQR)	28.3 (25.2–31.6)	27.7 (24.4–32.7)	28.1 (24.8–31.9)
Range	16.7–41.7	17.3–44.4	16.7–44.4
Comorbidities, n (%)			
Cardiovascular	64 (32.7)	67 (34.7)	131 (33.7)
Diabetes mellitus	26 (13.3)	21 (10.9)	47 (12.1)
Neurological	10 (5.1)	4 (2.1)	14 (3.6)
Renal	1 (0.5)	2 (1.0)	3 (0.8)
Respiratory	13 (6.6)	12 (6.2)	25 (6.4)
Hepatic	3 (1.5)	4 (2.1)	7 (1.8)

Notes: CLIA: chemiluminescence immunoassay. CoronaVac - Inactive adsorbed vaccine against COVID-19, AZD1222 (ChAdOx1) - Oxford-AstraZeneca, BNT162b2 - Pfizer-BioNTech or Comirnaty® Wyeth, Ad26.COV2.S - Janssen - JNJ-78436735.

Baseline characteristics	NDV-HXP-S 10µg (N = 196)	BNT162b2 30µg (N = 193)	Total (N = 389)
Age (years-old)			
Other	68 (34.7)	69 (35.8)	137 (35.2)
Primary immunization against COVID-19, n (%)			
CoronaVac	86 (43.9)	75 (38.9)	161 (41.4)
AZD1222 (ChAdOx1)	82 (41.8)	80 (41.5)	162 (41.6)
BNT162b2	27 (13.8)	35 (18.1)	62 (15.9)
Ad26.COV2.S	0 (0.0)	2 (1.0)	2 (0.5)
Other	1 (0.5)	1 (0.5)	2 (0.5)
Interval between the 1st and 2nd dose (days), Median (IQR)	84.0 (28.0–86.0)	84.0 (28.0–86.0)	84.0 (28.0–86.0)
Third dose Booster Vaccination, n (%)			
CoronaVac	35 (17.9)	45 (23.3)	80 (20.6)
AZD1222 (ChAdOx1)	11 (5.6)	18 (9.3)	29 (7.5)
BNT162b2	144 (73.5)	123 (63.7)	267 (68.6)
Ad26.COV2.S	6 (3.1)	7 (3.6)	13 (3.3)
Interval between the 2nd and 3rd doses (days), Median (IQR)	166.5 (129.0–199.3)	166.0 (139.0–232.0)	166.0 (133.0–217.0)
Fourth dose Booster Vaccination, n (%)			
CoronaVac	45 (23.0)	47 (24.4)	92 (23.7)
AZD1222 (ChAdOx1)	43 (21.9)	46 (23.8)	89 (22.9)
BNT162b2	62 (31.6)	57 (29.5)	119 (30.6)
Ad26.COV2.S	46 (23.5)	43 (22.3)	89 (22.9)
Interval between the 3rd and 4th doses (days), Median (IQR)	187.5 (156.8–252.8)	194.0 (154.0–265.0)	191.0 (154.0–262.0)
Time since forth dose (days), Median (IQR)	395.0 (230.8–462.3)	374.0 (211.0–439.0)	393.0 (222.0–446.0)
Past infection (symptomatic or asymptomatic) with SARS-CoV-2, n (%)			

Notes: CLIA: chemiluminescence immunoassay. CoronaVac - Inactive adsorbed vaccine against COVID-19, AZD1222 (ChAdOx1) - Oxford-AstraZeneca, BNT162b2 - Pfizer-BioNTech or Comirnaty® Wyeth, Ad26.COV2.S - Janssen - JNJ-78436735.

Baseline characteristics	NDV-HXP-S 10μg (N = 196)	BNT162b2 30μg (N = 193)	Total (N = 389)
Age (years-old)			
CLIA Anti-RBD positive		191 (99.0)	383 (98.5)
CLIA Anti-Nucleoprotein positive	187 (95.4)	184 (95.3)	371 (95.4)
Missing	4 (2.0)	2 (1.0)	6 (1.5)
Past of symptomatic COVID-19	61 (31.1)	75 (38.9)	136 (35.0)
Notes: CLIA: chemiluminescence immunoassay. CoronaVac - Inactive adsorbed vaccine against COVID-19, AZD1222 (ChAdOx1) - Oxford-AstraZeneca, BNT162b2 - Pfizer-BioNTech or Comirnaty® Wyeth, Ad26.COV2.S - Janssen - JNJ-78436735.			

Of the total, 131 (33.7%) had cardiovascular comorbidities, 47 (12.1%) had diabetes mellitus, 25 (6.4%) had respiratory comorbidities, 14 (3.6%) had neurological comorbidities, 7 (1.8%) had hepatic comorbidities, 3 (0.8%) had renal comorbidities and 137 (34.2%) had other comorbidities. Among all, 248 (63.8%) were under medication. Out of the total population, 136 (35.0%) had a previous history of symptomatic COVID-19; out of them, 371 (95.4%) had CLIA (chemiluminescence immunoassay) IgG anti-nucleoprotein positive serology, and 383 (98.5%) had CLIA IgG anti-RDB positive serology.

Inactivated vaccine was the majority in the background primary immunization regimen: 161 (41.4%) received CoronaVac, 162 (41.6%) received AZD1222 and 62 (15.9%) received BNT162b2 in a two-dose regimen with a median of interval between them of 84.0 days (IQR, 28.0–86.0). Third dose and forth dose were majority of BNT162b2: 267 (68.6%) and 119 (30.6%), with the interval since a primary immunization and a third dose of 166.0 (133.8–217.0), as well as the interval between the third and fourth dose was 191.0 days (IQR, 154.0–262.0), respectively. Median time between the fourth dose and study vaccination was 393.0 days (IQR, 222.0–446.0).

3.2. Vaccine immunogenicity

Clinical development of NDV-HXP-S was terminated early due to futility based on the results of Day 182. Thus, study participants were followed until Day 364 only for safety, but not for immunogenicity.

The GMT ratio (NDV-HXP-S/BNT162b2) of neutralizing antibodies against SARS-CoV-2 (Wuhan) was 0.50 (95% CI 0.40–0.60), measured on Day 29 after the fifth booster vaccination (Table 2 **and** Fig. 2). Seroconversion rates on Day 29 were 5.8% (95% CI 2.7% to 10.7%) in the group receiving NDV-HXP-S 10μg compared with 42.5% (95% CI 34.5% to 50.7%) in the group receiving BNT162b2 30 μg as a booster dose. Therefore, the difference in seroconversion was − 36.7% (-45.3% to -28.0%). Values were similar when stratified by age groups 18–59 years and 60 or more years old (**Table S1**).

Table 2

Summary of immunogenicity by treatment group of 50% neutralizing titters (NT_{50}) of the SARS-CoV pseudovirus neutralization assay (PNA) Wuhan strain, up to Day 182 after vaccination. Per-protocol Population (PP).

Overall population	**NDV-HXP-S 10µg**		**BNT162b2 30µg**		**GMT ratio or Difference seroconversion**
	n	**Estimated (95% CI)**	**n**	**Estimated (95% CI)**	**Estimated (95% CI)**
GMT on Day 1	156	1738.8 (1457.8–2073.9)	154	1249.5 (1056.7–1477.4)	1.4 (1.1–1.8)
GMT on Day 29	156	2371.0 (2040.8–2754.7)	153	4606.2 (4089.5–5188.2)	0.5 (0.4–0.6)
GMT on Day 92	157	1877.2 (1574.2–2238.6)	154	2784.8 (2402.2–3228.4)	0.7 (0.5–0.8)
GMT on Day 182	157	1639.5 (1399.5–1920.5)	152	1703.6 (1494.7–1941.7)	1.0 (0.8–1.2)
Seroconversion on Day 29	9/155	5.8 (2.7–10.7)	65/153	42.5 (34.5–50.7)	-36.7 (-45.3– -28.0)
Seroconversion on Day 92	5/156	3.2 (1.0–7.3)	33/154	21.4 (15.2–28.8)	-18.2 (-25.7– -11.5)
Seroconversion on Day 182	6/156	3.8 (1.4–8.2)	15/152	9.9 (5.6–15.8)	-6.0 (-12.3– -0.4)
GMFR on Day 29	155	1.4 (1.2–1.5)	153	3.7 (3.2–4.3)	0.4 (0.3–0.4)
GMFR on Day 92	156	1.1 (1.0–1.2)	154	2.2 (1.9–2.6)	0.5 (0.4–0.6)
GMFR on Day 182	156	0.9 (0.8–1.1)	152	1.4 (1.2–1.6)	0.7 (0.6–0.8)
GMT: Geometric mean titers; GMFR: Geometric mean fold-rise; CI: Confidence interval					

Results of secondary immunogenicity endpoints were consistent with the primary endpoints findings. Decreasing level of antibodies of both vaccines resulted in convergent values of the GMT ratio of neutralizing antibodies against SARS-CoV-2 (Wuhan): 0.70 (95% CI 0.50–0.80) and 1.0 (95% CI 0.8–1.2), measured on Day 92 and Day 182 after the fifth booster vaccination.

The GMT ratio of neutralizing antibodies against SARS-CoV-2 BA.1 was 0.6 (95% CI 0.5–0.7), 0.8 (95% CI 0.6–1.0) and 0.9 (95% CI 0.7–1.2), measured on Day 29, Day 92 and Day 182 after the fifth booster vaccination (**Table S2 and** Fig. 2). The seroconversion differences were − 22.3% (95% CI -30.5% to -14.4%), -8.5% (95% CI -15.6% to -1.9%) and − 4.9% (95% CI -12.4% to 2.5%) on Day 29, Day 92 and Day 182, respectively.

The neutralizing antibody GMT ratios against SARS-CoV-2 (BA.4/5) were 0.6 (95% CI 0.5–0.7), 0.7 (95% CI 0.6–0.9) and 1.0 (95% CI 0.8–1.3), measured on Day 29, Day 92 and Day 182 after the fifth booster (**Table S3 and** Fig. 2). The differences in seroconversion were − 24.3% (95% CI -32.7% to -16.1%), -13.0% (95% CI -20.1% to -6.8%) and − 8.8% (95% CI -16.6% to -1.2%) on Day 29, Day 92 and Day 182, respectively.

The GMC ratios of binding antibodies against SARS-CoV-2 (Wuhan) measured by ELISA were 0.5 (95% CI 0.4–0.6), 0.7 (95% CI 0.6–0.8) and 0.8 (95% CI 0.7–1.0), on Day 29, Day 92 and Day 182 after the fifth booster vaccination, respectively **(Table S4 and** Fig. 2**)**. The differences in seroconversion were − 11.3% (95% CI -18.3% to -5.0%), -7.9% (95% CI -14.1% to -2.6%) and − 8.0% (95% CI -14.1% to -3.0%) on Day 29, Day 92 and Day 182, respectively.

3.3.Vaccine Safety and Reactogenicity

Briefly, solicited adverse events occurred in 298 (76.6%) participants within 7 days after vaccination, while unsolicited adverse events occurred in 184 (47.3%) participants within 28 days post-vaccination. Throughout the study period, 20 (5.1%) experienced SAE, none of them vaccine-related, and 55 (14.1%) had AESI (Table 3).

Table 3
Overview of adverse events in participants after vaccination. Safety population.

	NDV-HXP-S 10µg (N = 196)	**BNT162b2 30µg (N = 193)**	**Total (N = 389)**
	n (%)	**n (%)**	**n (%)**
Adverse events within 7 days after vaccination	**140 (71.4)**	**158 (81.9)**	**298 (76.6)**
Solicited adverse events within 7 days after vaccination	132 (67.3)	149 (77.2)	281 (72.2)
Vaccine-related	132 (67.3)	149 (77.2)	281 (72.2)
Local	105 (53.6)	134 (69.4)	239 (61.4)
Systemic	82 (41.8)	104 (53.9)	186 (47.8)
Unsolicited adverse events within 28 days after vaccination	**97 (49.5)**	**87 (45.1)**	**184 (47.3)**
Vaccine-related	46 (23.5)	51 (26.4)	97 (24.9)
Medically-attended adverse event	**24 (12.2)**	**17 (8.8)**	**41 (10.5)**
Vaccine-related	2 (1.0)	0 (0.0)	2 (0.5)
Serious adverse events	**10 (5.1)**	**10 (5.2)**	**20 (5.1)**
Vaccine-related	0 (0.0)	0 (0.0)	0 (0.0)
Death	1 (0.5)	1 (0.5)	2 (0.5)
Adverse events special interest	**30 (15.3)**	**25 (13.0)**	**55 (14.1)**
Vaccine-related	2 (1.0)	0 (0.0)	2 (0.5)

NDV-HXP-S had a favorable safety profile in comparison to BNT162b2 for the primary safety endpoint: fewer vaccine-related solicited adverse event (131 [67.2%] *vs*. 148 [77.1%], P = 0.032) within 7 days post-vaccination,

but similar frequency of vaccine-related unsolicited adverse events within 28 days after the booster (46 [23.5%] *vs*. 51 [26.4%], P = 0.558) and no vaccine-related serious adverse events within 28 days post-vaccination in both treatment groups (Table 3). The vast majority of vaccine-related solicited and unsolicited adverse events were grade 1 or 2 (**Table S5, S6 and S7**).

The most frequent local solicited adverse events within 7 days after the booster were pain at the administration site (101 [51.8%] in NDV-HXP-S *vs*. 130 [67.7%] in BNT162b2 group, P = 0.002), followed by oedema (22 [11.3%] in NDV-HXP-S *vs*. 47 [24.5%] in BNT162b2 group, P = 0.002). All except pruritus occurred more in the BNT162b2 group. The most frequent systemic solicited adverse events within 7 days after the booster was headache (44 [22.6%] in NDV-HXP-S *vs*. 58 [30.2%] in BNT162b2 group, P = 0.106), followed by myalgia (32 [16.4%] in NDV-HXP-S *vs*. 55 (28.6) in BNT162b2, P = 0.005). Besides myalgia, pyrexia and arthralgia were more frequent among subjects who received BNT162b2 in comparison to NDV-HXP-S (Table 4).

Table 4
Participants with vaccine-related solicited adverse events within 7 days post-vaccination by vaccination group.

Adverse Reaction	**18–59 years-old**		**≥ 60 years-old**		**Total**		**p****
	NDV-HXP-S 10µg (N = 96)	**BNT162b2 30µg (N = 96)**	**NDV-HXP-S 10µg (N = 99)**	**BNT162b2 30µg (N = 96)**	**NDV-HXP-S 10µg (N = 195)**	**BNT162b2 30µg (N = 192)**	
n (%) (95% CI)	**n (%) (95% CI)**	**n (%) (95% CI)**	**n (%) (95% CI)**	**n (%) (95% CI)**	**n (%) (95% CI)**		
Local*							
General disorders and administration site conditions	**71 (74.0) (64.0 ; 82.4)**	**83 (86.5) (78.0 ; 92.6)**	**33 (33.3) (24.2 ; 43.5)**	**50 (52.1) (41.6 ; 62.4)**	**104 (53.3) (46.1 ; 60.5)**	**133 (69.3) (62.2 ; 75.7)**	**0.002**
Pain	69 (71.9) (61.8 ; 80.6)	83 (86.5) (78.0 ; 92.6)	32 (32.3) (23.3 ; 42.5)	47 (49.0) (38.6 ; 59.4)	101 (51.8) (44.5 ; 59.0)	130 (67.7) (60.6 ; 74.3)	**0.002**
Oedema	16 (16.7) (9.8 ; 25.6)	34 (35.4) (25.9 ; 45.8)	6 (6.1) (2.3 ; 12.7)	13 (13.5) (7.4 ; 22.0)	22 (11.3) (7.2 ; 16.6)	47 (24.5) (18.6 ; 31.2)	**0.001**
Erythema	7 (7.3) (3.0 ; 14.4)	18 (18.8) (11.5 ; 28.0)	2 (2.0) (0.2 ; 7.1)	5 (5.2) (1.7 ; 11.7)	9 (4.6) (2.1 ; 8.6)	23 (12.0) (7.7 ; 17.4)	**0.010**
Induration	6 (6.3) (2.3 ; 13.1)	21 (21.9) (14.1 ; 31.5)	3 (3.0) (0.6 ; 8.6)	12 (12.5) (6.6 ; 20.8)	9 (4.6) (2.1 ; 8.6)	33 (17.2) (12.1 ; 23.3)	**< 0.001**
Pruritus	4 (4.2) (1.1 ; 10.3)	7 (7.3) (3.0 ; 14.4)	3 (3.0) (0.6 ; 8.6)	8 (8.3) (3.7 ; 15.8)	7 (3.6) (1.5 ; 7.3)	15 (7.8) (4.4 ; 12.6)	0.082
Systemic							
Gastrointestinal disorders	**6 (6.3) (2.3 ; 13.1)**	**11 (11.5) (5.9 ; 19.6)**	**4 (4.0) (1.1 ; 10.0)**	**5 (5.2) (1.7 ; 11.7)**	**10 (5.1) (2.5 ; 9.2)**	**16 (8.3) (4.8 ; 13.2)**	0.229

CI: Confidence interval; *Administration site; **Exact Fisher's test

Adverse Reaction	18–59 years-old		≥ 60 years-old		Total		p**
	NDV-HXP-S 10µg (N = 96)	BNT162b2 30µg (N = 96)	NDV-HXP-S 10µg (N = 99)	BNT162b2 30µg (N = 96)	NDV-HXP-S 10µg (N = 195)	BNT162b2 30µg (N = 192)	
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)		
Nauseas	0 (0.0) -	0 (0.0) -	0 (0.0) -	0 (0.0) -	0 (0.0) -	0 (0.0) -	-
Vomiting	1 (1.0) (0.0 ; 5.7)	3 (3.1) (0.6 ; 8.9)	1 (1.0) (0.0 ; 5.5)	0 (0.0) (0.0 ; 3.8)	2 (1.0) (0.1 ; 3.7)	3 (1.6) (0.3 ; 4.5)	0.683
Diarrhea	0 (0.0) -	0 (0.0) -	0 (0.0) -	0 (0.0) -	0 (0.0) -	0 (0.0) -	-
General disorders and administration site conditions	**18 (18.8) (11.5 ; 28.0)**	**32 (33.3) (24.0 ; 43.7)**	**14 (14.1) (8.0 ; 22.6)**	**18 (18.8) (11.5 ; 28.0)**	**32 (16.4) (11.5 ; 22.4)**	**50 (26.0) (20.0 ; 32.9)**	**0.025**
Pyrexia	0 (0.0) (0.0 ; 3.8)	7 (7.3) (3.0 ; 14.4)	0 (0.0) (0.0 ; 3.7)	3 (3.1) (0.6 ; 8.9)	0 (0.0) (0.0 ; 1.9)	10 (5.2) (2.5 ; 9.4)	**0.001**
Fatigue	18 (18.8) (11.5 ; 28.0)	28 (29.2) (20.3 ; 39.3)	10 (10.1) (5.0 ; 17.8)	12 (12.5) (6.6 ; 20.8)	28 (14.4) (9.8 ; 20.1)	40 (20.8) (15.3 ; 27.3)	0.109
Chills	3 (3.1) (0.6 ; 8.9)	10 (10.4) (5.1 ; 18.3)	8 (8.1) (3.6 ; 15.3)	9 (9.4) (4.4 ; 17.1)	11 (5.6) (2.8 ; 9.9)	19 (9.9) (6.1 ; 15.0)	0.131
Decreased appetite	3 (3.1) (0.6 ; 8.9)	6 (6.3) (2.3 ; 13.1)	4 (4.0) (1.1 ; 10.0)	4 (4.2) (1.1 ; 10.3)	7 (3.6) (1.5 ; 7.3)	10 (5.2) (2.5 ; 9.4)	0.468
Immune system disorders	**0 (0.0) -**	**0 (0.0) -**	**0 (0.0) -**	**0 (0.0) -**	**0 (0.0) -**	**0 (0.0) -**	-
Hypersensitivity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-

CI: Confidence interval; *Administration site; **Exact Fisher's test

Adverse Reaction	18–59 years-old		≥ 60 years-old		Total		p**
	NDV-HXP-S 10µg (N = 96)	BNT162b2 30µg (N = 96)	NDV-HXP-S 10µg (N = 99)	BNT162b2 30µg (N = 96)	NDV-HXP-S 10µg (N = 195)	BNT162b2 30µg (N = 192)	
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)		
	-	-	-	-	-	-	
Musculoskeletal and connective tissue disorders	**20 (20.8) (13.2 ; 30.3)**	**36 (37.5) (27.8 ; 48.0)**	**16 (16.2) (9.5 ; 24.9)**	**23 (24.0) (15.8 ; 33.7)**	**36 (18.5) (13.3 ; 24.6)**	**59 (30.7) (24.3 ; 37.8)**	**0.006**
Arthralgia	4 (4.2) (1.1 ; 10.3)	17 (17.7) (10.7 ; 26.8)	7 (7.1) (2.9 ; 14.0)	8 (8.3) (3.7 ; 15.8)	11 (5.6) (2.8 ; 9.9)	25 (13.0) (8.6 ; 18.6)	**0.014**
Myalgia	18 (18.8) (11.5 ; 28.0)	36 (37.5) (27.8 ; 48.0)	14 (14.1) (8.0 ; 22.6)	19 (19.8) (12.4 ; 29.2)	32 (16.4) (11.5 ; 22.4)	55 (28.6) (22.4 ; 35.6)	**0.005**
Nervous system disorders	**25 (26.0) (17.6 ; 36.0)**	**37 (38.5) (28.8 ; 49.0)**	**19 (19.2) (12.0 ; 28.3)**	**21 (21.9) (14.1 ; 31.5)**	**44 (22.6) (16.9 ; 29.1)**	**58 (30.2) (23.8 ; 37.2)**	0.106
Headache	25 (26.0) (17.6 ; 36.0)	37 (38.5) (28.8 ; 49.0)	19 (19.2) (12.0 ; 28.3)	21 (21.9) (14.1 ; 31.5)	44 (22.6) (16.9 ; 29.1)	58 (30.2) (23.8 ; 37.2)	0.106
Respiratory, thoracic and mediastinal disorders	**4 (4.2) (1.1 ; 10.3)**	**9 (9.4) (4.4 ; 17.1)**	**8 (8.1) (3.6 ; 15.3)**	**7 (7.3) (3.0 ; 14.4)**	**12 (6.2) (3.2 ; 10.5)**	**16 (8.3) (4.8 ; 13.2)**	0.438
Cough	4 (4.2) (1.1 ; 10.3)	9 (9.4) (4.4 ; 17.1)	8 (8.1) (3.6 ; 15.3)	7 (7.3) (3.0 ; 14.4)	12 (6.2) (3.2 ; 10.5)	16 (8.3) (4.8 ; 13.2)	0.438
Skin and subcutaneous tissue disorders	**0 (0.0) -**	**0 (0.0) -**	**3 (3.0)**	**2 (2.1) (0.3 ; 7.3)**	**3 (1.5)**	**2 (1.0) (0.1 ; 3.7)**	> 0.999

CI: Confidence interval; *Administration site; **Exact Fisher's test

Adverse Reaction	18–59 years-old		≥ 60 years-old		Total		p**
	NDV-HXP-S 10µg (N = 96)	BNT162b2 30µg (N = 96)	NDV-HXP-S 10µg (N = 99)	BNT162b2 30µg (N = 96)	NDV-HXP-S 10µg (N = 195)	BNT162b2 30µg (N = 192)	
n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)		
			(0.6 ; 8.6)		(0.3 ; 4.4)		
Pruritus	0 (0.0) -	0 (0.0) -	3 (3.0) (0.6 ; 8.6)	2 (2.1) (0.3 ; 7.3)	3 (1.5) (0.3 ; 4.4)	2 (1.0) (0.1 ; 3.7)	> 0.999
Rash	0 (0.0) -	0 (0.0) -	1 (1.0) (0.0 ; 5.5)	0 (0.0) (0.0 ; 3.8)	1 (0.5) (0.0 ; 2.8)	0 (0.0) (0.0 ; 1.9)	> 0.999
TOTAL	**78 (81.3) (72.0 ; 88.5)**	**87 (90.6) (82.9 ; 95.6)**	**53 (53.5) (43.2 ; 63.6)**	**61 (63.5) (53.1 ; 73.1)**	**131 (67.2) (60.1 ; 73.7)**	**148 (77.1) (70.5 ; 82.8)**	**0.032**

CI: Confidence interval; *Administration site; **Exact Fisher's test

All the vaccine related unsolicited adverse events within 28 days after the booster were similarly observed in both treatment groups, including biochemistry (8 [4.1%] in NDV-HXP-S vs. 9 [4.7%] in BNT162b2, P = 0.809) and blood count (16 [8.2%] in NDV-HXP-S vs 12 [6.2%] in BNT162b2, P = 0.557) measurements on Day 8 (**Table S6**). There were no reported vaccine-related unsolicited adverse events from 28 days post-vaccination.

We also did not observe differences in both study groups of medically-attended adverse events (24 [12.2%] participants in NDV-HXP-S *vs*. 17 [8.8%] in BNT162b2, P = 0.323) (**Table S8**), adverse events of special interest (30 [15.3%] in NDV-HXP-S *vs*. 25 [13.0%] in BNT162b2, P = 0.562) (**Table S9**) and serious adverse events (10 [5.1%] in NDV-HXP-S *vs*. 10 [5.2%] in BNT162b2, P > 0.999) (**Table S10**) throughout the study period. Of all AESI, 26 participants had cases of COVID-19 (15 [7.7%] in NDV-HXP-S *vs*. 11 [5.7%] in BNT162b2, P = 0.544), one of them in the BNT162b2 arm was infected two times during the study follow-up. All were WHO progression scale score 2 except one, who was WHO progression scale score 3 (in arm NDV-HXP-S) (**Table S11**). All cases were due to lineages descended from the Omicron B.1.1.529 strain of SARS-CoV-2 (**Table S11**).

4. Discussion

In this multicenter, randomized, active-controlled trial, a single booster dose of NDV-HXP-S 10 µg elicited approximately half the 50% pseudovirus neutralization titer (NT50) against SARS-CoV-2 Wuhan-hu-1 and the Omicron BA.1 and BA.4/5, compared with one dose of BNT162b2 30 µg, at Day 29 post-vaccination. The same

pattern was observed for binding anti-S (Wuhan-Hu-1) IgG measured by ELISA. Antibodies titers waned in both arms over six months, with a steeper decline from Day 29 to Day 92 and a more gradual decrease thereafter, and without discernible differences in the kinetics of waning by age or sex across study groups. Overall, the trajectories of neutralizing and binding responses converged over time, indicating similar decay dynamics for NDV-HXP-S and BNT162b2 (Fig. 2). So, in the per-protocol population, NDV-HXP-S was inferior to BNT162b2 according to the three non-inferiority criteria pre-specified for the primary immunogenicity endpoint.

The booster setting evaluated here was particularly challenging for assessment of non-inferiority. Participants were immunocompetent adults, many aged ≥ 60 years, with mixed-platform four-dose primary/booster histories and a high probability of prior SARS-CoV-2 exposure more than one year before enrollment. As expected in such largely primed population, the proportion achieving seroconversion by Day 29 was substantially lower than in the earlier NDV-HXP-S Phase I study (younger adults, primary immunization) who experienced near-universal seroconversion [12]. Despite detectable responses in both arms, the between-group difference in seroconversion exceeded the pre-specified non-inferiority margin by roughly threefold, and the GMT ratio similarly favored BNT162b2 30 µg. In the absence of a definitive correlate of protection for COVID-19, the magnitude and consistence of these immunogenicity differences supported early discontinuation of NDV-HXP-S development for the fifth-dose booster indication in Brazil. During the study period, Brazilian Ministry of Health provided a fifth-dose booster of a bivalent BNT162b2 (BA.1,BA4/5, Wuhan) for the adult population.

The immune response after the fifth booster dose decreased steeply up to 3 months and dropped slower between 3 and 6 months, similar to the primary immunization using BNT162b2 in a study with 4868 participants [25]. However, contrary to what was observed in the BNT162b2 study, we did not observe waning differences between sex and age groups. Therefore, the waning pattern is similar between NDV-HXP-S and BNT162b2, resulting in convergent levels of neutralizing and binding antibodies.

The mRNA vaccine comparator against COVID-19 was chosen in a context of a lack of gold-standard, as required by regulatory authority. However, it was based on a better performance against Omicron strains in comparison to others, like inactivated COVID-19 vaccine or AZD1222 [15]. Despite this study did not meet the primary endpoint by using an mRNA as comparator, a Phase II/III in Mexico using the same platform (recombinant NDV-HXP-S), administered live intramuscularly as a single booster dose after a background of 2 to 3 doses, resulted in non-inferior values of GMT ratio in comparison to AZD1222 14 days after vaccination [26]. Similarly, the Phase II study of inactivated NDV-HXP-S 6µg in Vietnan was non-inferior to AZD1222 as a primary immunization regimen 14 days after each of two doses [27]. However, our main concern regarding those choices was the fact of AZD1222 was discontinued as a platform for updated strains of SARS-CoV-2 in Brazil. In addition, differences in vaccine administration (live versus inactivated) complicates the comparison between the clinical trials in Mexico and in Brazil.

Several factors likely contributed to the failure of NDV-HXP-S 10 µg to meet non-inferiority criteria. First, an antigenic mismatch was present: a monovalent Wuhan-Hu-1 immunogen was evaluated during Omicron predominance, a period characterized by substantial immune escape; consequently, peak neutralization against heterologous variants was lower than with the mRNA comparator [28, 29]. Second, platform-related differences plausibly favored BNT162b2. Lipid nanoparticle–mRNA vaccines typically elicit robust germinal-center responses and higher peak antibody titers than inactivated virion platforms, particularly in highly primed hosts [30]. Third, the study population- largely middle-aged or older adults with prior antigenic exposure (hybrid

immunity from vaccination and infection) is known to exhibit attenuated fold-rise responses and to emphasize absolute between-arm differences, disadvantaging a lower-dose inactivated booster [31]. Fourth, immune imprinting to ancestral epitopes may have constrained the breadth of responses elicited by a Wuhan-based inactivated construct, further limiting the relative boost versus a high-immunogenicity mRNA dose [32]. Collectively, these elements explain why NDV-HXP-S generated detectable responses yet fell short of the pre-specified non-inferiority margins for GMT ratio and seroconversion. The study, therefore, has limitations, either because it compares two vaccines as a fifth booster dose, or because there is a high prevalence of prior COVID-19 infection or a high frequency of use of at least one prior dose of an mRNA vaccine against COVID-19. However, the study has strengths: it is randomized, stratified by age, with homogeneous background vaccination intervals, a centralized laboratory, and adequate statistical power. Therefore, it is highly unlikely that the biological difference observed in this study would not be reproduced under different conditions.

These findings contribute to the future development and positioning of NDV-based respiratory vaccines. For SARS-CoV-2, inactivated NDV backbones may be better suited for primary immunization in naïve or minimally primed populations, or when paired with contemporary, variant-updated antigens and, if appropriate, with adjuvantation and optimized dosing [33]. Intranasal or mucosal formulations, possibly using as live vaccines if proven to be safe, merit evaluation to promote local IgA and tissue-resident T cells, complementing systemic immunity and potentially reducing transmission [34, 35]. Platform refinement that increases antigen density, preserve prefusion conformation, and manufacturing consistency could further improve humoral performance. Programmatically, the egg-based, GMP-compatible NDV manufacturing remains attractive for rapid, low-cost scale-up; however, as a heterologous booster following mRNA priming, NDV-HXP-S may offer limited incremental benefit relative to available mRNA or protein-subunit options.

Our data are broadly consistent with the literature on heterologous boosting against COVID-19. During Omicron predominance in Brazil [36, 37], mRNA boosters demonstrated superior effectiveness to inactivated or adenoviral platforms, mirroring the immunogenicity advantage observed here. Conversely, NDV-based inactivated vaccines have achieved non-inferiority versus adenoviral-vectored comparators in other settings, underscoring that relative performance is context dependent and influenced by the comparator, antigen update, and priming history [26, 33]. These nuances should inform platform selection for future booster campaigns and pandemic preparedness strategies.

The Butantan Institute's decision to discontinue the clinical development of this vaccine relied on the fact that its immunogenicity fell short. Moreover, there is a certain difficulty in implementing the platform in egg-based influenza vaccine production plants, due to the adaptation of the use of sterilizing filtration and the heterogeneity of the size of the viral particles of the different variants of SARS-CoV-2, which in some cases prevented the rapid updating of the strain as the pandemic developed. While the egg-based platform allows for safe, low-cost, large-scale vaccines to supply low- and middle-income countries during epidemics, there are limitations in the immune response and duration that result in a greater need for boosters.

In conclusion, a fifth booster of inactivated NDV-HXP-S 10μg was inferior to BNT162b2 30μg, both containing monovalent Wuhan spike, in eliciting neutralizing antibodies against Wuhan, BA.1 and BA.4/5 strains of SARS-CoV-2 on Day 29 after vaccination. Values were inferior at peak and during 182-day follow-up, as measured for SARS-CoV-2 Wuhan and variants of concern BA.1 and BA.4/5. Therefore, those results underpin the end of clinical development of NDV-HXP-S by Instituto Butantan in Brazil.

Declarations

Data Availability Statement: The study protocol is provided in the Online Appendix. Individual participant data are made available upon requests directed to Esper Georges Kallas (esper.kallas@butantan.gov.br). After approval of a proposal, data can be shared through a secure online platform.

Contributors: EJFPM is the clinical development medical manager from the sponsor, responsible for the study protocol, informed consent form and study report; RSD, CAAB, MCB, FEL and LATR and are the principal investigators of the study sites; FCB and JASM is the medical director responsible for managing, oversighting the project and reviewing the study protocol and the present manuscript; MAALSA and MCOR are Clinical Operation responsible for procedures of this study; KTM is the safety medical monitor from the Pharmacovigilance; JIDF, MAL and LR performed the statistical analysis of the data; RR is responsible for consultancy for study design; FK, AG-S, WS and PP developed the vaccine seed; and EGK responsible for oversighting and managing the project.

Declaration of interest: The A.G.-S. laboratory has received research support from GSK, Pfizer, Senhwa Biosciences, Kenall Manufacturing, Blade Therapeutics, Avimex, Johnson & Johnson, Dynavax, 7Hills Pharma, Pharmamar, ImmunityBio, Accurius, Nanocomposix, Hexamer, N-fold LLC, Model Medicines, Atea Pharma, Applied Biological Laboratories and Merck. A.G.-S. has consulting agreements for the following companies involving cash and/or stock: Castlevax, Amovir, Vivaldi Biosciences, Contrafect, 7Hills Pharma, Avimex, Pagoda, Accurius, Esperovax, Applied Biological Laboratories, Pharmamar, CureLab Oncology, CureLab Veterinary, Synairgen, Paratus, Pfizer and Prosetta. A.G.-S. has been an invited speaker in meeting events organized by Seqirus, Janssen, Abbott, Astrazeneca and Novavax. A.G.-S. is inventor on patents and patent applications on the use of antivirals and vaccines for the treatment and prevention of virus infections and cancer, owned by the Icahn School of Medicine at Mount Sinai, New York. The rest of the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. PP is an inventor on patents and patent applications on the use of vaccines for the treatment and prevention of virus infections, owned by the Icahn School of Medicine at Mount Sinai, New York. WS is listed as co-inventor of NDV-based SARS-CoV-2 vaccine owned by the Icahn School of Medicine at Mount Sinai, New York. WS is co-founder and scientific advisory board member of CastleVax.

The vaccine candidate administered in this study was developed by faculty members at the Icahn School of Medicine at Mount Sinai including PP, FK, WS and AG-S. Mount Sinai is seeking to commercialize this vaccine; therefore, the institution and its faculty inventors could benefit financially. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-849 CoV-2 serological assays (USA Provisional Application Numbers: 62/994,252, 63/018,457, 63/020,503 and 63/024,436) and NDV-based SARS-CoV-2 vaccines (USA Provisional Application Number: 63/251,020) which list FK as co-inventor. AG-S, WS and PP are a co-inventor in the NDV-based SARS-CoV-2 vaccine patent application. Patent applications were submitted by the Icahn School of Medicine at Mount Sinai.

Mount Sinai has spun out a company, CastleVax, to commercialize SARS-CoV-2 vaccines. FK, PP, WS and AG-S serve on the scientific advisory board of CastleVax and are listed as co-founders of the company. FK has consulted for Merck, Gritstone, Seqirus, Curevac, GSK and Pfizer, and is currently consulting for Third Rock

Ventures and Avimex. The FK laboratory has been collaborating with Pfizer on animal models of SARS-CoV-2 and is currently collaborating in the influenza space with Dynavax.

Acknowledgement: The authors are grateful to the participants of this study and to the members of the DSMB for their work on this study and to the former Director of Instituto Butantan, Dimas Tadeu Covas, due to the valuable contribution to the clinical trial setup. Research and development activities in Brazil were funded by Fundação Butantan. Activities at PATH and Mount Sinai were supported, in part, by the Bill & Melinda Gates Foundation (INV-021239). PATH was also supported by the Coalition for Epidemic Preparedness (CEPI) and Bayer AG. The findings and conclusions contained within this manuscript are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation. Design and generation of reagents used in this project at Mount Sinai laboratories was supported by Centers of Excellence for influenza Research and Response (75N93021C00014) and Collaborative Influenza Vaccine Innovation Centers (75N93019C00051).

References

1. Huang C, Yang L, Pan J, Xu X, Peng R. Correlation between vaccine coverage and the COVID-19 pandemic throughout the world: Based on real-world data. J Med Virol. 2022;94(5):2181-2187.
2. World Health Organization. Coronavirus disease (COVID-19) pandemic (who.int). Available at: Coronavirus disease (COVID-19) pandemic (who.int). Access on June 27th, 2024.
3. COVID-19 National Preparedness Collaborators. Pandemic preparedness and COVID-19: an exploratory analysis of infection and fatality rates, and contextual factors associated with preparedness in 177 countries, from Jan 1, 2020, to Sept 30, 2021. Lancet. 2022;399(10334):1489-1512.
4. Tartof SY, Slezak JM, Puzniak L, Hong V, Frankland TB, Ackerson BK, Xie F, Takhar H, Ogun OA, Simmons S, Zamparo JM, Valluri SR, Jodar L, McLaughlin JM. Effectiveness of BNT162b2 BA.4/5 bivalent mRNA vaccine against a range of COVID-19 outcomes in a large health system in the USA: a test-negative case-control study. Lancet Respir Med. 2023;11(12):1089-1100.
5. Chalkias S, Harper C, Vrbicky K, Walsh SR, Essink B, Brosz A, McGhee N, Tomassini JE, Chen X, Chang Y, Sutherland A, Montefiori DC, Girard B, Edwards DK, Feng J, Zhou H, Baden LR, Miller JM, Das R. A Bivalent Omicron-Containing Booster Vaccine against Covid-19. N Engl J Med. 2022;387(14):1279-1291.
6. Chalkias S, Whatley JL, Eder F, Essink B, Khetan S, Bradley P, Brosz A, McGhee N, Tomassini JE, Chen X, Zhao X, Sutherland A, Shen X, Girard B, Edwards DK, Feng J, Zhou H, Walsh S, Montefiori DC, Baden LR, Miller JM, Das R. Original SARS-CoV-2 monovalent and Omicron BA.4/BA.5 bivalent COVID-19 mRNA vaccines: phase 2/3 trial interim results. Nat Med. 2023;29(9):2325-2333.
7. World Health Organization. Status_COVID_VAX_08AUgust2023.pdf. Available at: https://extranet.who.int/prequal/sites/default/files/document_files/Status_COVID_VAX_08AUgust2023.pdf. Acess on July, 16th, 2025.
8. World Health Organization. COVID-19 vaccine tracker and landscape (who.int). Available at: https://www.who.int. Access on July, 16th, 2025.
9. Our World in Data. Availablet at: https://ourworldindata.org. Access on July 17th, 2025.
10. Sun W, McCroskery S, Liu WC, Leist SR, Liu Y, Albrecht RA, et al. A newcastle disease virus (NDV) expressing a membrane-anchored spike as a cost-effective inactivated SARS-CoV-2 vaccine. Vaccines. 2020;8(4):1–14.

11. Hsieh CL, Goldsmith JA, Schaub JM, DiVenere AM, Kuo HC, Javanmardi K, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science. 2020;369(6510):1501-1505.
12. Peixoto de Miranda ÉJF, Calado RT, Boulos FC, de Sousa Moreira JA, Machado FF, Almeida MAALDS, Da Rocha MCO, Infante V, Mercer LD, Hjorth R, Scharf R, White J, Polyak C, Raghunandan R, García-Sastre A, Sun W, Palese P, Krammer F, Innis B, Pereira CG, Kallas EG. Safety and immunogenicity of an inactivated recombinant Newcastle disease virus vaccine expressing SARS-CoV-2 spike: Results of a randomized vaccine-controlled phase I ADAPTCOV trial in Brazil. Vaccine. 2025;52:126680.
13. Pitisuttithum P, Luvira V, Lawpoolsri S, Muangnoicharoen S, Kamolratanakul S, Sivakorn C, Narakorn P, et al. Safety and Immunogenicity of an Inactivated Recombinant Newcastle Disease Virus Vaccine Expressing SARS-CoV-2 Spike: Interim Results of a Randomised, Placebo-Controlled, Phase 1/2 Trial. EClinicalMedicine. 2022 Mar 08;45:101323.
14. Duc Dang A, Dinh Vu T, Hai Vu H, Thanh Ta V, Thi Van Pham A, Thi Ngoc Dang M, et al. Safety and immunogenicity of an egg-based inactivated Newcastle disease virus vaccine expressing SARS-CoV-2 spike: Interim results of a randomized, placebo-controlled, phase 1/2 trial in Vietnam. Vaccine. 2022;40(26):3621-3632.
15. Ranzani OT, Hitchings MDT, de Melo RL, de França GVA, Fernandes CFR, Lind ML, Torres MSS, Tsuha DH, David LCS, Said RFC, Almiron M, de Oliveira RD, Cummings DAT, Dean NE, Andrews JR, Ko AI, Croda J. Effectiveness of an inactivated Covid-19 vaccine with homologous and heterologous boosters against Omicron in Brazil. Nat Commun. 2022;13(1):5536. doi: 10.1038/s41467-022-33169-0.
16. Food and Drug Administration. Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. Available at: https://www.fda.gov/media/73679/download. Access on June 27th, 2024.
17. UMC/WHO. The use of the WHO-UMC system for standardised case causality assessment [Internet]. Uppsala: The Uppsala Monitoring Centre. 2011.
18. Law B, Sturkenboom M. D2.4 Preliminary guidance on safety data collection for COVID-19 vaccine safety. Available at. https://brightoncollaboration.us Access on June 27th, 2024.
19. Bewley KR, Coombes NS, Gagnon L, McInroy L, Baker N, Shaik I, et al. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. Nat Protoc. 2021;16(6):3114-3140.
20. Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, et al. WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Lancet. 2021;397(10282):1347-1348.
21. Food and Drug Administration. Non-Inferiority Clinical Trials to Establish Effectiveness, Access on https://www.fda.gov/media/78504/download. Access on June 27th, 2024.
22. Donken R, de Melker HE, Rots NY, Berbers G, Knol MJ. Comparing vaccines: a systematic review of the use of the non-inferiority margin in vaccine trials. Vaccine. 2015;33(12):1426-32.
23. Chow SC, Shao J, Wang H. Sample Size Calculation in Clinical Research. New York: Marcel Dekker, 2003.
24. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Raabe V, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes-Garfias C, Shi PY, Türeci Ö, Tompkins KR, Walsh EE, Frenck R, Falsey AR, Dormitzer PR, Gruber WC, Şahin U, Jansen KU. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2021;590(7844):E26. doi: 10.1038/s41586-020-03098-3. Erratum for: Nature. 2020;586(7830):589-593.

25. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, Doolman R, Asraf K, Mendelson E, Ziv A, Rubin C, Freedman L, Kreiss Y, Regev-Yochay G. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med. 2021 Dec 9;385(24):e84.
26. López-Macías C, Torres M, Armenta-Copca B, Wacher NH, Galindo-Fraga A, Castro-Castrezana L, Colli-Domínguez AA, Cervantes-Trujano E, Rucker-Joerg IE, Lozano-Patiño F, Rivera-Alcocer JJ, Simón-Campos A, Sánchez-Campos EA, Aguirre-Rivero R, Muñiz-Carvajal AJ, Del Carpio-Orantes L, Márquez-Díaz F, Rivera-Hernández T, Torres-Flores A, Ramírez-Martínez L, la Rosa GP, Rojas-Martínez O, Suárez-Martínez A, Peralta-Sánchez G, Carranza C, Juárez E, Zamudio-Meza H, Carreto-Binaghi LE, Viettri M, Romero-Rodríguez D, Palencia A, Sarfati-Mizrahi D, Sun W, Chagoya-Cortés HE, Castro-Peralta F, Palese P, Krammer F, García-Sastre A, Lozano-Dubernard B. Phase 2/3 study evaluating safety, immunogenicity, and noninferiority of single booster dose of AVX/COVID-12 vaccine. Sci Adv. 2025;11(26):eadq2887.
27. Thiem VD, Anh DD, Ha VH, Van Thom N, Thang TC, Mateus J, Carreño JM, Raghunandan R, Huong NM, Mercer LD, Flores J, Escarrega EA, Raskin A, Thai DH, Van Be L, Sette A, Innis BL, Krammer F, Weiskopf D. Safety and immunogenicity of an inactivated recombinant Newcastle disease virus vaccine expressing SARS-CoV-2 spike: A randomised, comparator-controlled, phase 2 trial. Vaccine. 2025;44:126542.
28. Cao Y, Yisimayi A, Jian F, et al. BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by vaccination and BA.1 infection. Nature. 2022;608:593-602.
29. Hachmann NP, Miller J, Collier AY, et al. Neutralization Escape by Omicron Subvariants BA.2.12.1, BA.4, and BA.5. N Engl J Med. 2022;387:86-88.
30. Kim W, Zhou JQ, Sturtz AJ, et al. Germinal centre-driven maturation of B-cell response to mRNA vaccination. Nature. 2022;604:141-145.
31. Srivastava K, Koutsakos M, Wheatley AK, et al. SARS-CoV-2 infection- and vaccine-induced antibody repertoire dynamics. Immunity. 2024;57:1371-1386.
32. Wang Q, Bowen A, Valdez R, et al. Antibody Response to Omicron BA.4–BA.5 Bivalent Booster. N Engl J Med. 2023;388:567-569.
33. Thiem VD, Dang AD, Nguyen TT, et al. Safety and immunogenicity of an inactivated recombinant NDV-HXP-S (COVIVAC): a randomised, comparator-controlled phase 2 trial. Vaccine. 2025; (Epub Jan 12).
34. Ponce-de-León S, Torres M, Soto-Ramírez LE, Calva JJ, Santillán-Doherty P, Carranza-Salazar DE, et al. Interim safety and immunogenicity results from an NDV-based COVID-19 vaccine phase I trial in Mexico. NPJ Vaccines. 2023 May 10;8(1):67.
35. Warner BM, Safronetz D, Banadyga L, *et al.* Intranasal vaccination with an NDV-vectored SARS-CoV-2 vaccine protects against Delta and Omicron in rodents. *npj Vaccines.* 2024;9:14.
36. Cerqueira-Silva T, Andrews JR, Boaventura VS, et al. Vaccine effectiveness of heterologous CoronaVac plus BNT162b2 in Brazil. Nat Med. 2022;28:838-843.
37. Costa Clemens SA, Weckx L, et al. Third-dose boosters after CoronaVac: randomized phase-4 study in Brazil. Lancet. 2022;399:521-529.

Figures

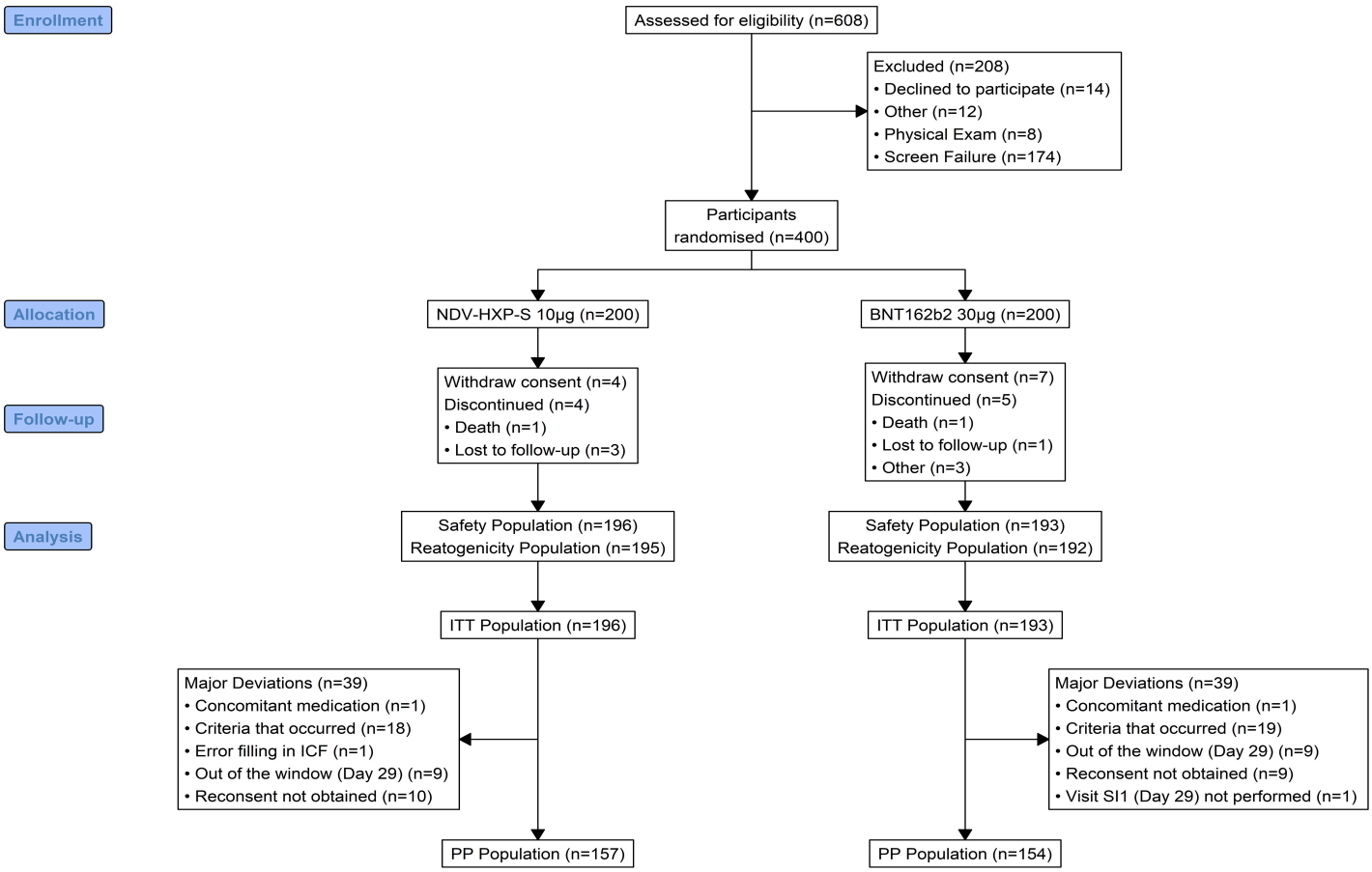


Figure 1

Participant disposition and study design.

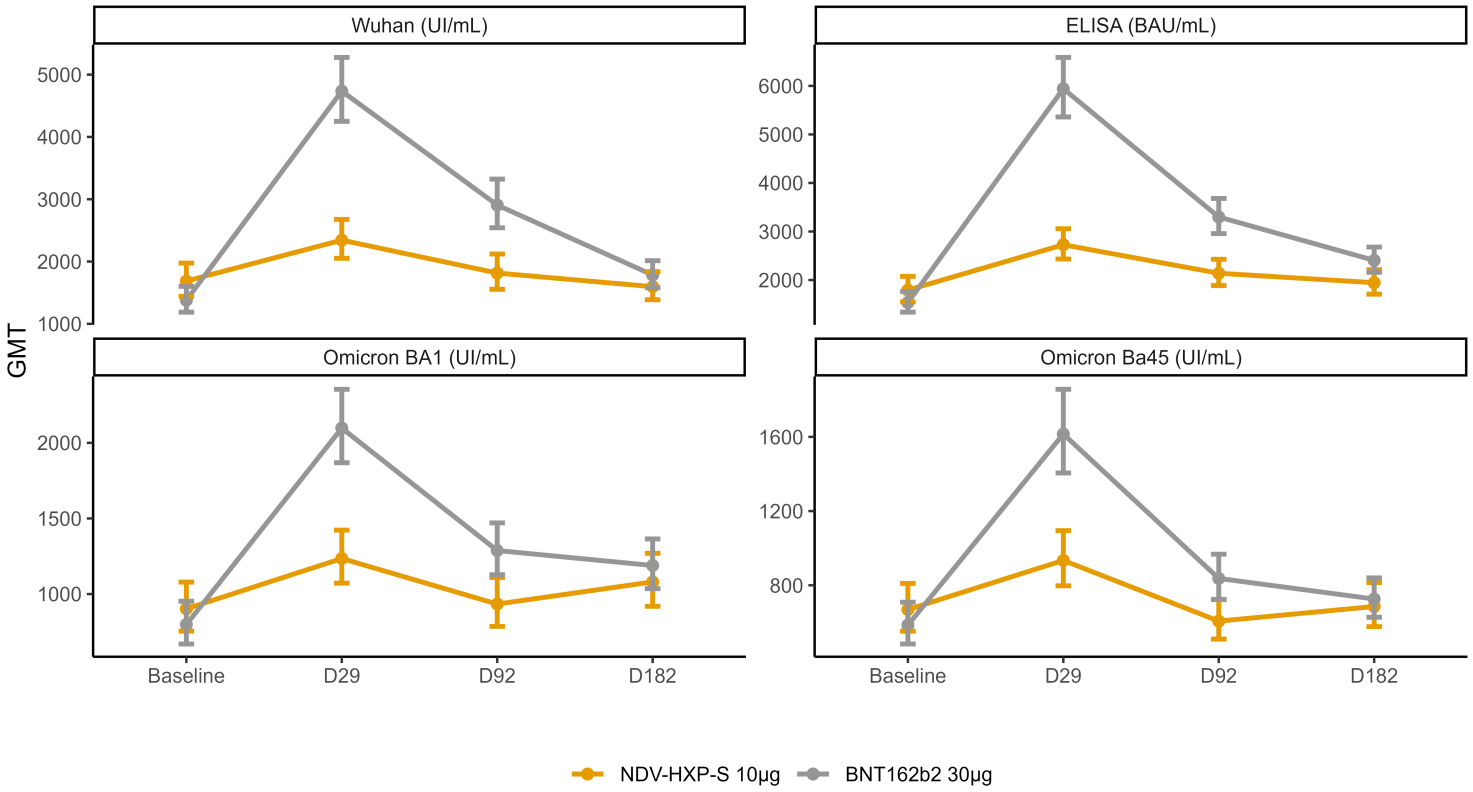


Figure 2

GMT results by time and treatment arm, overall analysis (per-protocol Population).

Supplementary Files

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

- OnlineSupplementButanvacPhase2.docx
- CONSORT2010Checklist.doc