THE LANCET
Microbe

## Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Ahn JY,Lee J, Suh YS, et al. Safety and immunogenicity of two recombinant DNA COVID-19 vaccines containing the coding regions of the spike or spike and nucleocapsid proteins: an interim analysis of two open-label, non-randomised, phase 1 trials in healthy adults. *Lancet Microbe* 2022; published online Feb 8. https://doi.org/10.1016/S2666-5247(21)00358-X.

**Supplementary Appendix**

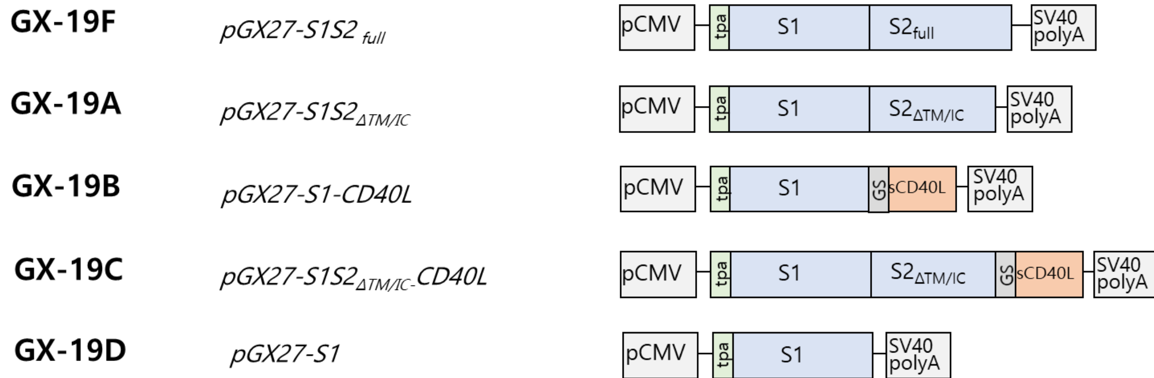
**Safety and immunogenicity of two recombinant DNA COVID-19 vaccines containing the coding regions of the spike or spike/nucleocapsid proteins: An interim analysis of two open-label, non-randomized, phase 1 trials in healthy adults.**

## Appendix

## Content


| Number | content | Title | Page |
|---|---|---|---|
| Appendix 1 | Figure | COVID-19 DNA Vaccine candidates | 2 |
| Appendix 2 | Methods | Description of the pre-clinical development of various prototype vaccines to generate GX-19N | 3 |
| Appendix 3 | Figure | Evaluation of antibody and IFN-g ELISPOT responses induced by GX-19 vaccine candidates in mice | 4 |
| Appendix 4 | Figure | Diagram of the GX-19 and GX-19N DNA vaccines | 5 |
| Appendix 5 | Figure | Determination of GX-19 (pGX27-S1S2ΔTM/IC) and GX-21 (pGX27-SRBD F/NP) ratio for formulation of GX-19N by evaluating binding antibody, surrogate neutralizing antibody and IFN-r ELISPOT responses in mice | 6 |
| Appendix 6 | Figure | GX-19(N)-induced humoral and cellular responses in mice and monkeys | 7 |
| Appendix 7 | Methods | Eligibility criteria of the GX-19 and GX-19N trial protocols | 8 |
| Appendix 8 | Methods | Rationale for dose selection of GX-19N based on preclinical results | 13 |
| Appendix 9 | Methods | Study procedures and assessments of the GX-19 and GX-19N trials | 14 |
| Appendix 10 | Methods | Methods of determination of immunogenicity | 18 |
| Appendix 11 | Table | Incidence of adverse drug reaction (ADR) by system organ class (SOC) and preferred term (PT) | 21 |
| Appendix 12 | Table | Summary of the spike and receptor-binding domain (RBD) binding antibody titers in serum after dosing with GX-19(N) | 22 |
| Appendix 13 | Table | Neutralizing antibody titers of GX-19N (IU/mL) | 23 |
| Appendix 14 | Table | Summary of the specific T-cell response in serum after dosing with GX-19(N) | 24 |
| Appendix 15 | Table | Results of ELISpot and T-SPOT in GX-19N-vaccinated participants | 25 |
| Appendix 16 | Figure | Comparison of amino acids sequence of 15-mer peptides containing NP-specific epitopes for GX-19N-vaccinated participants with those of SARS-CoV-2 variants | 26 |

**Appendix 1. COVID-19 DNA Vaccine candidates**




GX-19F (pGX27-S1S$_{full}$) contains the entire sequence of SARS-CoV-2 spike (S) gene and GX-19A (pGX27-S1S2$_{\Delta TM/IC}$) contains S gene lacking the transmembrane (TM)/intracellular (IC) domain. GX-19D (pGX-27-S1) contains only S1 sequence of SARS-CoV-2. GX-19B and GX-19C contains additionally CD40L gene fused with S1 and S1S2$_{\Delta TM/IC}$ gene, respectively. Each sequence encoding SARS-CoV-2 spike antigen with or without CD40L was inserted into the pGX-27 vector to generate each vaccine candidate.

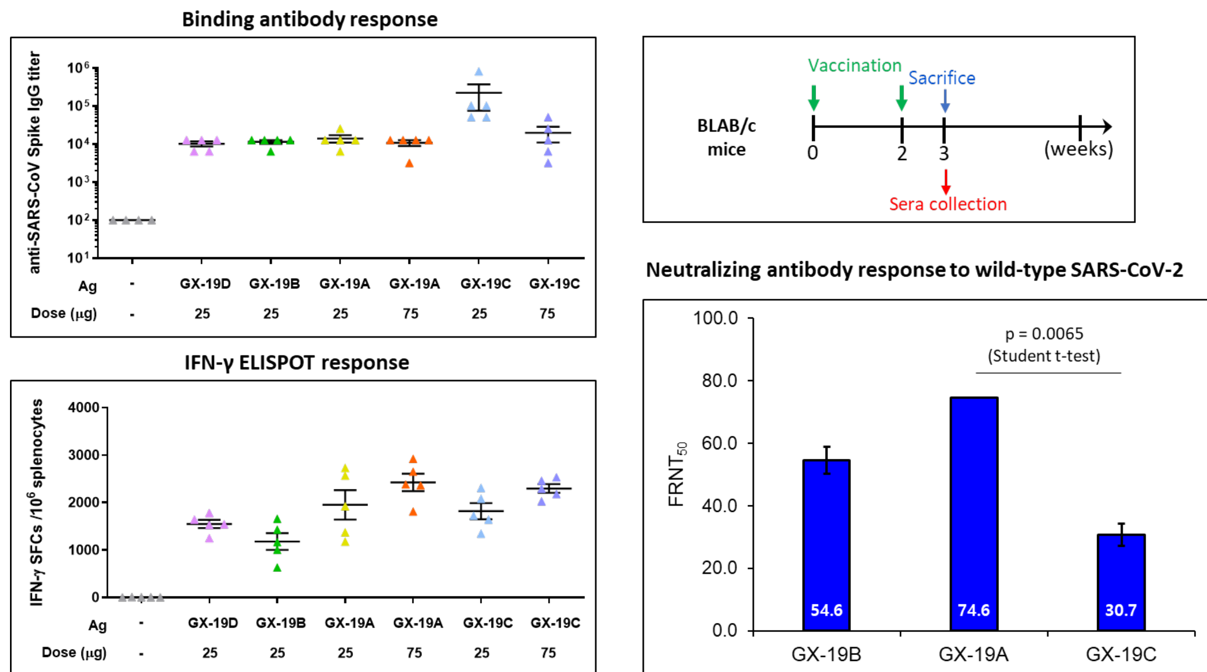
pCMV, cytomegalovirus early enhancer/promoter; tpa, signal sequence of tissue plasminogen activator; GS, Glycine-serine linker sequence; SV40 poly A, Simian virus 40 late polyadenylation sequence.

**Appendix 2. Description of the pre-clinical development of various prototype vaccines to generate GX-19N**

GX-19 is a candidate recombinant DNA vaccine that contains a plasmid encoding the SARS-CoV-2 S proteins (S1, S2). (Vaccines (Basel) 2021; 9(4)) The plasmid vector (pGX27) used for the human papillomavirus DNA vaccine undergoing clinical trials was used to develop GX-19. (Clin Cancer Res 2020; 26(7): 1616-23) In our preclinical trials, we developed five prototype DNA vaccines expressing the SARS-CoV-2 S protein: 1) S1S2full (GX-19F), 2) S1S2ΔTM/IC (GX-19A), 3) S1 (GX-19B), 4) S1S2ΔTM/IC with the CD40 ligand (GX-19C), and 5) S1 with the CD40 ligand (GX-19D) (Appendix 1, pp 2). GX-19F, containing the entire coding region of the gene encoding the SARS-CoV-2 S protein (S1S2full), and GX-19A, containing the S protein gene without the transmembrane domain (TD) and cytoplasmic tail (CT) (S1S2ΔTM/IC), were initially constructed. We observed that GX-19F induced lower antibody responses than GX-19A (Vaccines (Basel) 2021; 9(4)); therefore, the TD/CT-deleted S gene was used for the vaccine candidates. Furthermore, in the MERS-CoV DNA vaccine, the administration of S1 alone induced a stronger immune response than that of S1S2 (Sci Rep 2017; 7: 44875); therefore, we created a construct, GX-19D, encoding the S1 domain only. In addition, the fusion of the antigen gene to the CD40 ligand gene was reported to enhanced the antigen-specific Th1 and antibody responses. (Infect Dis 2019; 220(10): 1558-67, J Immunol 2014; 193(2): 722-34). Considering this, two more constructs, GX-19C, in which the CD40 ligand was fused to S1S2ΔTM/IC, and GX-19B, in which the CD40 ligand was fused to S1, were designed. Subsequently, based on the vaccine-induced binding/ neutralization antibody responses and interferon-γ (IFN-γ) enzyme-linked immunosorbent spot (ELISPOT) assay responses evaluated in mice **(Appendix 3)** and monkeys, GX-19A henceforth referred to as GX-19, was selected as a prototype vaccine for the clinical trial.

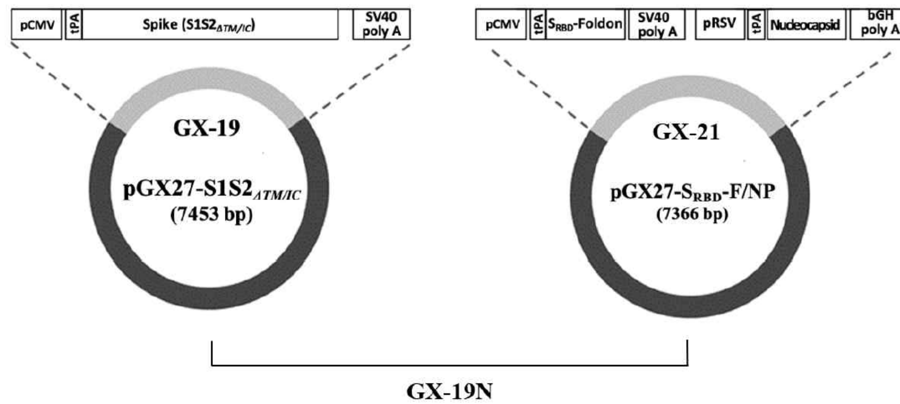
During the phase 1 clinical trial with GX-19, T cell immunity was identified as an important factor for COVID-19 disease protection. (Cell 2020; 183(4):996-1012 e19, Cell 2020; 183(1): 158-68 e14, Trends Immunol 581 2021; 42(1): 18-30, Nature 2021; 590(7847): 630-4, Cell Rep Med 2021; 2(2): 100204) Since the nucleocapsid protein (NP) is more conserved and stable than S protein, (J Virol 2020; 94(13)) and strong NP-specific T cell responses were observed in COVID-19 patients, (Nat Immunol 2020;21(11): 1336-45) the NP gene was chosen to be added to the vaccine to induce T cell response with wider coverage against emerging variants. (Cell 2020; 181(4): 865-76 e12) The RBD gene fused with the foldon (F) gene of T4 fibritin, a trimerization tag, was inserted into the pGX27 vaccine vector, and the NP gene was inserted into the plasmid to be expressed separately under the respiratory syncytial virus promoter **(Appendix 4)**. The developed construct (pGX27-SRBD-F/NP) was named GX-21. Considering the number of expressed antigens (GX-19 expresses the S protein, and GX-21 expresses two proteins, RBD fused with F and NP), the immunogenicity of three mix ratios (2:1, 1:1, and 1:2, weight by weight) of original GX-19 and GX-21 in mice models was assessed **(Appendix 5)**. No significant difference in antibody responses was observed between the mix ratios, and we selected the 1:2 mix ratio (1 mg of GX-19 and 2 mg of GX-21) for the human dose of the next-generation DNA vaccine, GX-19N.

**Appendix 3. Evaluation of antibody and IFN-γ ELISPOT responses induced by GX-19 vaccine candidates in mice**




BALB/c mice were vaccinated intramuscularly at week 0 and 2, via electroporation with each vaccine candidates. Anti-Spike binding antibody and Spike-specific IFN-γ ELISOT responses were evaluated by using sera and splenocytes obtained at week 3, respectively. Neutralizing antibody titer of the sera in the vaccinated mice was determined by Focus Reduction Neutralization Test (FRNT). $FRNT_{50}$ is defined as the reciprocal of serum dilution capable of reducing the formation of SARS-CoV-2 viral foci by 50%.

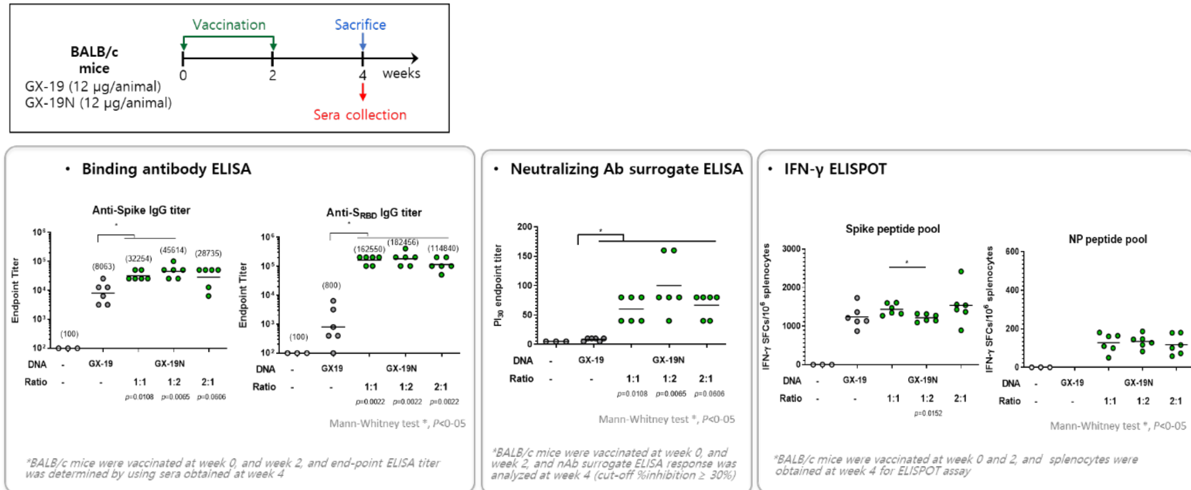
**Appendix 4. Diagram of the GX-19 and GX-19N DNA vaccines.**




GX-19 (pGX27-S1S2ΔTM/IC) contains the SARS-CoV-2 spike (S) gene lacking the transmembrane (TM)/intracellular (IC) domain, constructed by inserting the gene sequence into the pGX27 vector. GX-19N consists of GX-19 and a second plasmid, pGX27-SRBD-F/NP at a 1:2 ratio. pGX27-SRBD-F/NP is designed to express the fusion protein of the receptor binding domain (RBD) of the spike protein and the T4 fibritin C-terminal foldon (SRBD-foldon) and nucleocapsid protein (NP). The S, SRBD-foldon, and NP sequences are preceded by the secretory signal sequence of the tissue plasminogen activator (tPA).

pCMV, cytomegalovirus early enhancer/promoter; SV40 poly A, Simian virus 40 late polyadenylation sequence; pRSV, Rous sarcoma virus promoter; bGH polyA, bovine growth hormone polyadenylation sequence.

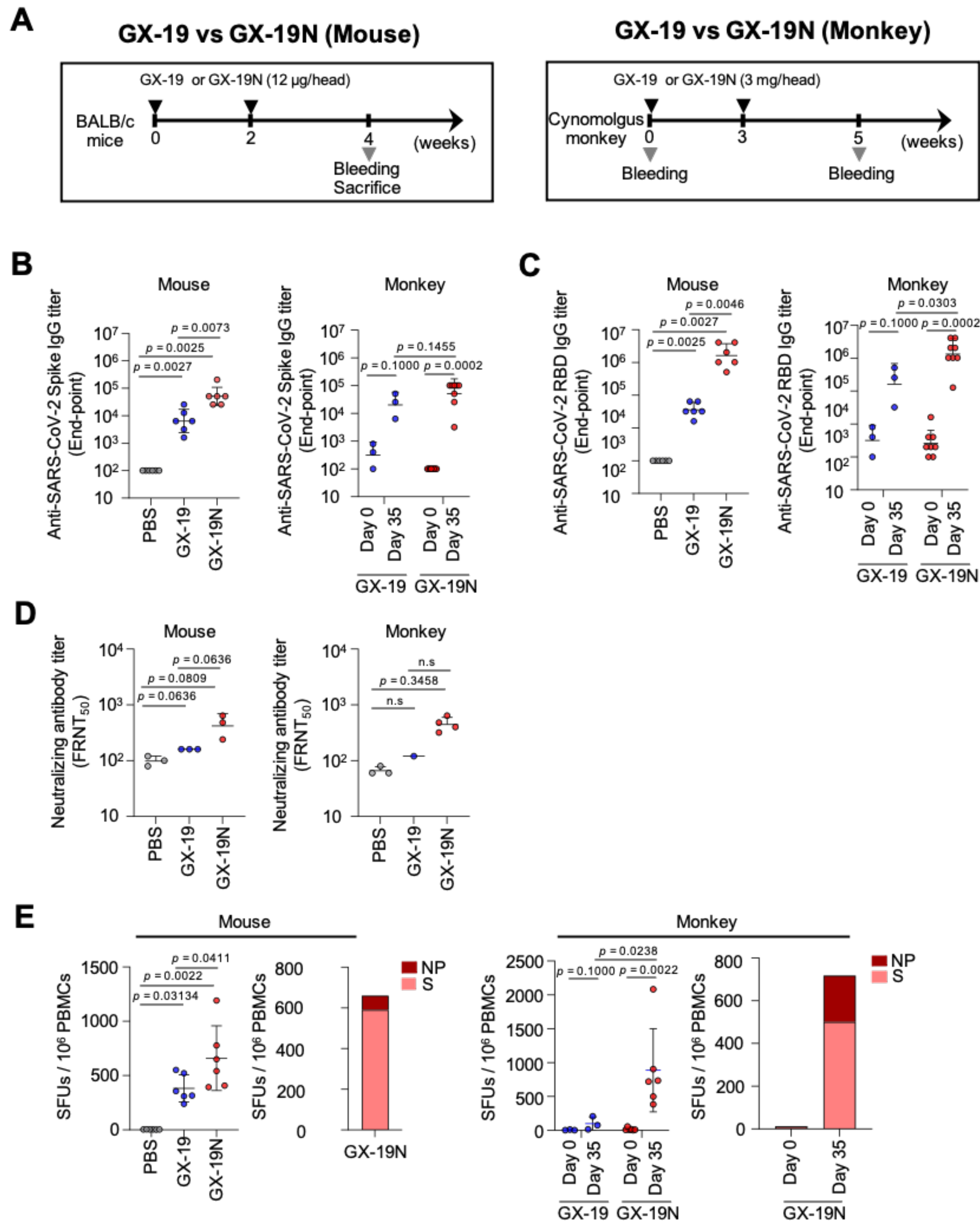
**Appendix 5. Determination of GX-19 (pGX27-S1S2$_{\Delta TM/IC}$) and GX-21 (pGX27-S$_{RBD}$-F/NP) ratio for formulation of GX-19N by evaluating binding antibody, surrogate neutralizing antibody and IFN-γ ELISPOT responses in mice**




BALB/c mice were vaccinated intramuscularly at week 0 and 2, via electroporation with GX-19 or three types of GX-19N vaccines of 1:1, 1:2 or 2:1 GX-19 to GX-21 ratio. Anti-Spike binding antibody titers, surrogate neutralization ELISA titers and Spike-specific IFN-□□ELISOT responses were evaluated after the sacrifice of mice at week 4.

PI30, Surrogate neutralization ELISA titer capable of 30% inhibition of RBD-ACE2 binding.

**Appendix 6. GX-19(N)-induced humoral and cellular responses in mice and monkeys**




Timeline of pre-clinical trial (A), Results of anti-Spike IgG ELISA (B), Results of anti-SRBD IgG ELISA (C), Results of focus reduction neutralization test (D), Results of IFN-γ ELISPOT (E), S: spike, NP: nucleocapsid

**Appendix 7. Eligibility criteria of the GX-19 and GX-19N trial protocols**

**Eligibility criteria of the GX-19 trial**

Inclusion and Exclusion Criteria

1. Inclusion criteria

Subjects who meet all the criteria below will be determined to be eligible for inclusion in the study.

1) Those who have been informed about the study and provide a voluntarily consent to participate in the study

2) Healthy adults (male or female) aged 19years or more and under the 50 years

3) Subjects with a body weight of 50–90 kg and body mass index (BMI) of 18.0–28.0 kg/m2 at the screening visit

4) Subjects who agree for blood and urine samples could be collected throughout the study period, including the EOS visit

2. Exclusion criteria

Subjects who meet any of the criteria below will be determined to be ineligible and excluded from the study

1) Subjects with immune dysfunction, including immunodeficiency disorder, or family history of such conditions

2) Subjects with a history of a malignant disease within the past five years

3) Subjects with any surgery or dental treatment planned during the study period

4) Subjects who have received immunoglobulin or blood-derived products within 3 months prior to the vaccination or are scheduled to receive them during the study period

5) Subjects who have been dependent on antipsychotic drugs and narcotic analgesics within 6 months prior to the vaccination

6) Subjects who test positive on serological test during the screening period [hepatitis B test, hepatitis A test, hepatitis C test; human immunodeficiency virus (HIV) test; and SARS-CoV-2 (COVID-19 IgG Ab) test]

7) Subjects who have a history or are suspected of drug abuse in the last 12 months prior to the IP administration

8) Subjects who continue to drink alcohol (exceeding 21 units/week) or have a history of alcohol abuse

9) Subjects with a history of allergy or SAEs to any antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), or drug or vaccine containing ingredients of GX-19 or same class of ingredients

10) Subjects with a history of hypersensitivity to vaccination, such as those with Guillain–Barre syndrome

11) Subjects with current or previous history of other biliary, renal, nervous (central or peripheral), respiratory (asthma, pneumonia, etc.), endocrine (uncontrolled diabetes, hyperlipidemia, etc.), cardiovascular (congestive heart failure, coronary artery disease, myocardial infarction, uncontrolled hypertension, etc.), hemato-oncologic, urinary, psychological, musculoskeletal, or immune (rheumatic arthritis, systemic lupus erythematosus, immunodeficiency disorder, etc.) disorders, which the principal investigator determines to be clinically significant to make participation in the study impossible

12) Hemophiliacs or people using anticoagulants who are at a risk of serious bleeding from IM injection

13) Subjects who have been in close contact with a COVID-19 patient or have been identified as a confirmed or suspected or symptomatic COVID-19 patient under investigation or those with a previous history of confirmed SARS or MERS infection

14) Subjects with acute fever with temperature above 37.5℃, coughing, breathing difficulty, chills, muscle ache, headache, sore throat, loss of smell, or loss of taste within 72 hours prior to the IP vaccination

15) Subjects who have a history of other vaccination(s) within 28 days prior to the vaccination or are scheduled to receive other vaccinations during the study period

16) Subjects who have taken an immunosuppressant or immune-modifying drug within three months prior to the IP vaccination

① Including but not limited to azathioprine, cyclosporin, interferon, G-CSF, tacrolimus, everolimus, sirolimus, cyclophosphamid, 6-mercaptopurine, methotrexate, rapamycin, and leflunomide

② Subjects who have taken systemic steroids for 14 days or more within three months prior to screening (for prednisolone, continuously taking a maximum dose of ≥10 mg/day for more than 14 days would be considered high dose and participation in this study would be restricted)

17) Subjects who have received other investigational drug(s) while participating in another clinical trial or bioequivalence study within 6 months prior to IP administration

18) Women who are pregnant or breastfeeding; however, participation is possible if breastfeeding is discontinued prior to participation in the study (women of childbearing age† must test negative in serum pregnancy test during the screening period prior to the start of the study)

†Women of childbearing age: Women who have started menarche and have not yet reached menopause (at least 12 months elapsed after non-therapy-induced amenorrhea) and have not received any surgical sterilization procedure (oophorectomy and/or hysterectomy)

19) Women of childbearing age who did not consent to the use of effective contraception† (condoms, contraceptive diaphragms, and/or intrauterine contraceptive devices after completing the consent form) during the study period

†Refer to section 9.2.1 for effective contraception

20) Subjects deemed ineligible by the investigator based on other clinically significant medical or psychiatric findings

2.1. Exclusion criteria: Definition of effective contraception

Women of childbearing age who wish to participate in the study but do not consent to use effective contraception during the study period may not be eligible to participate in the study. Women who have not received any surgical sterilization procedure or are not in menopause (at least 12 months elapsed after non-therapy-induced amenorrhea) must avoid becoming pregnant. Women who have an active sex life with a male partner and could become pregnant must practice abstinence (refraining from intercourse with the opposite sex) or use contraception. While refraining from sexual relationship could be recognized as a form of contraception, temporary abstinence and coitus interruptus are not permissible contraceptive methods. The effective contraceptive methods defined in this study are as follows, and they could be divided broadly into barrier and non-barrier methods. For barrier methods, dual protection must be used.

- Non-barrier methods:

① Intrauterine hormonal device and intrauterine device (IUD): Copper IUD, hormone-releasing IUD, copper T IUD, levonorgestrel-releasing IUD (e.g., Mirena®), ethinyl estradiol/etonogestrel intravaginal ring, etc.

② Oral contraceptives: Regular and low-dose complex oral contraceptives, norelgestromin/ethinyl estradiol transdermal system, Cerazette, etc.

③ Contraceptive injections: Etonogestrel implant, medroxyprogesterone injection, hormone-based methods (e.g., Depo-Provera), etc.

④ Others: Spermicidal foam, sponge, film, hormone patch, etc.

- Barrier methods: Diaphragm, cervical cap, cervical shield, male condom, female condom, etc.

Female subjects of childbearing age and male subjects with a female sex partner of childbearing age must opt for contraception using any of the methods described above from the day of a priming vaccination to 90 days after the boosting vaccination. All subjects must also refrain from egg/sperm donation.

The investigator must be notified immediately if a female subject or the female partner of a male subject becomes pregnant during the study period or after the completion of the study (at least 90 days). If a subject becomes pregnant during the study period, she will receive no additional vaccination and will be dismissed from the study.

When a female subject reports her own pregnancy or a male subject reports the pregnancy of his female partner, the investigator and sub-investigators may collect data regarding the pregnancy and/or childbirth and plan a schedule for follow-up visits when necessary.

**Eligibility criteria of the GX-19N trial**

Inclusion and Exclusion Criteria

1. Inclusion criteria

Candidates who meet all the criteria below will be determined to be eligible for inclusion in the study

1) Those who have been informed about the study and provide a voluntarily consent to participate in the study

2) Healthy adults (male or female) aged 19 years or more and under the 55 years (19-64 years in Phase 2a)

3) Subject with a body mass index (BMI) of 18.0–30.0 kg/m2 at the screening visit

4) Those from whom blood and urine samples could be collected throughout the study period, including the EOS visit

2. Exclusion criteria

Subjects who meet any one of the criteria below will be determined to be ineligible and will be excluded from the study

1) Subjects with immune dysfunction, including immunodeficiency disorder, or family history of such conditions

2) Subjects with a history of a malignant disease within the past five years

3) Subjects with any surgery or dental treatment planned during the study period

4) Subjects who have received immunoglobulin or blood-derived products within three months prior to the vaccination or are scheduled to receive them during the study period

5) Subjects who have been dependent on antipsychotic drugs and narcotic analgesics within six months prior to the vaccination

6) Subjects who test positive on serological test during the screening period [hepatitis B test, hepatitis A test, human immunodeficiency virus test; hepatitis C test; and SARS-CoV-2 (COVID-19 IgG Ab) test]

7) Subjects who have a history or are suspected of drug abuse in the last 12 months prior to the vaccination

8) Subjects who continue to drink alcohol (exceeding 21 units/week) or have a history of alcohol abuse

9) Subjects with a history of allergy or SAEs to any antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), or drug or vaccine containing ingredients of GX-19 or same class of ingredients

10) Subjects with a history of hypersensitivity to vaccination, such as those with Guillain–Barre syndrome

11) Subjects with current or previous history of other biliary, renal, nervous (central or peripheral), respiratory

(asthma, pneumonia, etc.), endocrine (uncontrolled diabetes, hyperlipidemia, etc.), cardiovascular (congestive heart failure, coronary artery disease, myocardial infarction, uncontrolled hypertension, etc.), hemato-oncologic, urinary, psychological, musculoskeletal, or immune (rheumatic arthritis, systemic lupus erythematosus, immunodeficiency disorder, etc.) disorders, which the principal investigator determines to be clinically significant to make participation in the study impossible

12) Hemophiliacs or people using anticoagulants who are at a risk of serious bleeding from IM injection

13) Subjects who have been in close contact with a COVID-19 patient or identified as a confirmed or suspected COVID-19 patient or symptomatic patient under investigation prior to vaccination or those with a previous history of confirmed SARS or MERS infection

14) Subjects with acute fever with temperature above 37·5℃, coughing, breathing difficulty, chills, muscle ache, headache, sore throat, loss of smell, or loss of taste within 72 hours prior to the vaccination

15) Subjects who have a history of other vaccination(s) within 28 days prior to the vaccination or are scheduled to receive other vaccinations during the study period

16) Subjects who have taken an immunosuppressant or immune-modifying drug within three months prior to the vaccination

① Including but not limited to azathioprine, cyclosporin, interferon, G-CSF, tacrolimus, everolimus, sirolimus, cyclophosphamide, 6-mercaptopurine, methotrexate, rapamycin, and leflunomide

② Those who have taken systemic steroids for 14 days or more within three months prior to screening (for prednisolone, continuously taking a maximum dose of ≥10 mg/day for more than 14 days would be considered high dose and participation in this study would be restricted)

17) Subjects who have received other investigational drug(s) while participating in another clinical trial or bioequivalence study within six months prior to vaccination

18) Women who are pregnant or breastfeeding; however, participation is possible if breastfeeding is discontinued prior to participation in the study (women of childbearing age† must test negative in serum pregnancy test during the screening period prior to the start of the study)

†Women of childbearing age: Women who have started menarche and have not yet reached menopause (at least 12 months elapsed after non-therapy-induced amenorrhea) and have not received any surgical sterilization procedure (oophorectomy and/or hysterectomy)

19) Women of childbearing age who did not consent to the use of effective contraception† (condoms, contraceptive diaphragms, and/or intrauterine contraceptive devices after completing the consent form) during the study period

†Refer to below section 2.1 for effective contraception

20) Subjects deemed ineligible by the investigator based on other clinically significant medical or psychiatric findings

2.1. Exclusion criteria: Definition of effective contraception

Women of childbearing age who wish to participate in the study but do not consent to use effective contraception during the study period may not be eligible to participate in the study. Women who have not received any surgical sterilization procedure or are not in menopause (at least 12 months elapsed after non-therapy-induced amenorrhea) must avoid becoming pregnant. Women who have an active sex life with a male partner and could become pregnant must practice abstinence (refraining from intercourse with the opposite sex) or use contraception. While refraining from sexual relationship could be recognized as a form of contraception, temporary abstinence and coitus interruptus are not permissible contraceptive methods. The effective contraceptive methods defined in this study are as follows, and they could be divided broadly into barrier and

non-barrier methods. For barrier methods, dual protection must be used.

- Non-barrier methods:

① Intrauterine hormonal device and intrauterine device (IUD): Copper IUD, hormone-releasing IUD, copper T IUD, levonorgestrel-releasing IUD (e.g., Mirena®), ethinyl estradiol/etonogestrel intravaginal ring, etc.

② Oral contraceptives: Regular and low-dose complex oral contraceptives, norelgestromin/ethinyl estradiol transdermal system, Cerazette, etc.

③ Contraceptive injections: Etonogestrel implant, medroxyprogesterone injection, hormone-based methods (e.g., Depo-Provera), etc.

④ Others: Spermicidal foam, sponge, film, hormone patch, etc.

- Barrier methods: Diaphragm, cervical cap, cervical shield, male condom, female condom, etc.

Female subjects of childbearing age and male subjects with a female sex partner of childbearing age must opt for contraception using any of the methods described above from the day of a priming vaccination to 90 days after the boosting vaccination. All subjects must also refrain from egg/sperm donation.

The investigator must be notified immediately if a female subject or the female partner of a male subject becomes pregnant during the study period or after the completion of the study (at least 90 days). If a subject becomes pregnant during the study period, she will receive no additional vaccination and will be dismissed from the study.

When a female subject reports her own pregnancy or a male subject reports the pregnancy of his female partner, the investigator and sub-investigators may collect data regarding the pregnancy and/or childbirth and plan a schedule for follow-up visits when necessary.

**Appendix 8. Rationale for dose selection of GX-19N based on preclinical results**

This is a first in human (FIH) clinical trial of GX-19N, and as such, there are no clinical pharmacological studies on GX-19N. Therefore, the dose was selected based on the results of a non-clinical trial in mice and monkeys. As shown in mice study, dose range that induced an adequate antibody response and T cell immune response in mice was 6 ~ 24 μg/head, which is equivalent to 1·5 ~ 6 mg/head in humans (Adv Drug Deliv Rev. 2007, 59(11): 1177–1192). Although there was no statistically significant difference, the highest immune response was observed at the dose of 12 μg/head in mice. In addition, a high binding antibody response, T cell immune response, and neutralizing antibody response were observed in monkeys administered twice at 3·0 mg/head. Therefore, 3·0 mg/head was selected as the final dose in the range of 1·5 to 6 mg/head.

The number of doses was set to two based on the results of the nonclinical study on GX-19N, which showed that the antibody response could be increased by boosting after administration of 12 μg/head in mice or 3·0 mg/head in monkeys. Considering two doses of the vaccine were administered at a 2-week interval in mice and at a 21-day interval in monkeys, the dosing interval for humans was set to 28 days based on the fact that humans have a slower metabolism than those of mice and monkeys.

Based on above preclinical results of GX-19N and Phase 1 results of GX-19, we conducted the GX-19N trial with 3·0 mg group only.

**Appendix 9. Study procedures and assessments of the GX-19 and GX-19N trials**

**1. The GX-19 trial**

| Schedule | Screening | First vaccination | Telephone† Visit | Boosting vaccination | Blood Sampling Visit | Blood Sampling Visit | Blood Sampling Visit | End-of-study Visit |
|---|---|---|---|---|---|---|---|---|
| **Day** | **-28 ~ 0** | **1** | **8+3D** | **29±3D** | **43+3D** | **57+3D** | **24W±14D** | **52W±14D** |
| **Item** \ **Visit** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8[13)]** |
| Obtain informed consent | ● | | | | | | | |
| Demographic investigation | ● | | | | | | | |
| Disease/surgery history[1)] | ● | | | | | | | |
| Inclusion/exclusion criteria | ● | ● | | | | | | |
| Physical examination[2)] | ● | ● | ●† | ● | ● | ● | ● | ● |
| Vital signs and body weight[2)] | ● | ● | ●† | ● | ● | ● | ● | ● |
| Chest X-ray examination[3)] | ● | | | | | | | ● |
| ECG[4)] | ● | ● | | ● | | | | ● |
| Randomization[5)] | | ● | | | | | | |
| **Blood Sampling Items and Schedule** | | | | | | | | |
| Hematology and blood chemistry test[6)] | ● | ● | ●† | ● | ● | ● | ● | ● |
| Serology test[7)] | ● | | | | | | | |
| Coagulation test[8)] | ● | ● | | ● | | | | |
| Urinalysis[9)] | ● | ● | ●† | ● | ● | ● | ● | ● |
| Pregnancy test[10)] | ● | | | | | | | ● |
| Immunogenicity blood sampling | ● | ● | | ● | ● | ● | ● | ● |
| **Related to IP Administration** | | | | | | | | |
| IP administration and VAS assessment[11)] | | ● | | ● | | | | |
| Previous and concomitant medication[12)] | ● | ● | ● | ● | ● | ● | ● | ● |
| Distribution of subject diary[14)] | | ● | | ● | | | | |
| Retrieval of subject diary and assessment[14)] | | | | ● | | ● | | |
| AE monitoring[15)] | | ● | ● | ● | ● | ● | ● | ● |
| Telephone monitoring[16)] | | | ● | | | | | |

†The first three subjects in each dose group will undergo safety assessment (examination and tests) through an outpatient visit, instead of a telephone visit, at 8 days (+3D) after the first vaccination.

‡If V1 and V2 take place on the same day, randomization code must be assigned after verifying the screening test results and inclusion/exclusion criteria. In addition, physical examination, vital signs and body weight measurement, electrocardiography (ECG) before the vaccination, hematology and blood chemistry test, coagulation test, and urinalysis results from V1 may be used as results for V2.

1) Previous and current medical history of each subject is obtained by inquiry and from past medical records within one year of the screening visit.

2) Physical examination and measurement of vital signs and body weight are performed prior to the vaccination.

3) Examination may be performed again if deemed necessary by the investigator.

4) ECG is performed during the screening (V1), end-of-study (V8) visits, before the vaccination and within 35 min after the vaccination only for groups of using electroporator during V2 and V4.

5) Randomization and vaccination which is including placebo apply only to phase 2a.

6) Hematology test: WBC differential count (neutrophil, lymphocyte, monocyte, eosinophil, and basophil), RBC,

Hb, Hct and platelet count (including absolute neutrophil counts and absolute lymphocyte counts). If the results of a specific test deviate from the normal level, the investigator may ask the subject for a re-visit to repeat only that test.

Blood chemistry test: Total protein, albumin, creatinine, BUN or urea, total bilirubin, alkaline phosphatase, ALT, AST, GGT, glucose, calcium, magnesium, phosphorus, chloride, sodium, potassium, bicarbonate, LDH, amylase, lipase and CRP. If the results of a specific test deviate from the normal level, the investigator may ask the subject for a re-visit to repeat only that test.

7) HBV (HBsAg), HAV (anti-HAV IgM), HIV antigen/antibody combination assay, HCV (anti-HCV), and SAS-CoV-2 (COVID-19 IgG Ab) will be performed.

8) Coagulation testwill be including aPTT, PT, and INR. The test will be performed during the visits for the vaccination. If screening (V1) and the first vaccination (V2) visit are less than two weeks apart, the test results from V1 could be used for V2.

9) Urinalysis: RBC, WBC, pH, blood, glucose, urobilinogen, ketone, albumin, and bacteria

10) Pregnancy test by blood will be performed only in women in the childbearing age. Women who have received any surgical sterilization procedure (oophorectomy and/or hysterectomy) or menopausal women (at least 12 months elapsed after non-therapy-induced amenorrhea) are exempted.

11) Pain assessment by using VAS is performed immediately after the vaccination and after 10 minutes of the vaccination.

12) Previous medication used within 12 weeks from the screening visit and concomitant medication to be used during the study period must be listed. Concomitant medications other than those prescribed by the principal investigator to treat AEs are not permitted.

13) The end-of-study visit (V8) will be at 52 weeks after the first vaccination. If necessary, follow-up visits may be required for long-term safety and immunogenicity assessment. Moreover, subjects who dropout from the study must go through a procedure equivalent to the end-of-study visit.

14) The subject diary will be distributed at the time of the priming and the boosting vaccination. Each subject will personally list any AEs experienced over approximately four weeks. The diary will be retrieved during V4 and V6 and assessed. Subsequently, telephone or unscheduled visits will be permitted at any time, during which data regarding any AEs that may have occurred could be collected.

15) Information about AEs will be collected during scheduled visits after the vaccination and telephone visit.

16) Within 7–10 days after the IM vaccination, a trained person designated by the principal investigator will call the subject to assess safety and notify the subject of the schedule for the following visit. However, the first three subjects in each dose group will be checked daily for AEs for up to seven days from the day of the first vaccination, and safety-related data will be collected.

## 2. The GX-19N trial

| Schedule | Screening | First vaccination | Telephone† Visit | Second vaccination | Blood Sampling Visit | Blood Sampling Visit | Blood Sampling Visit | Blood Sampling Visit | End-of-study Visit |
|---|---|---|---|---|---|---|---|---|---|
| **Day** | -28 ~ 0 | 1 | 8+3D | 29±3D | 43±3D | 57±3D | 71±3D | 24W±14D | 52W±14D |
| Item / Visit | V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 | V9[17] |
| Obtain informed consent | ● | | | | | | | | |
| Demographic investigation | ● | | | | | | | | |
| Disease/surgery history[1] | ● | | | | | | | | |
| Inclusion/exclusion criteria | ● | ● | | | | | | | |
| Physical examination[2] | ● | ● | ●† | ● | ● | ● | ● | ● | ● |
| Vital signs and body weight[2] | ● | ● | ●† | ● | ● | ● | ● | ● | ● |
| Chest X-ray examination[3] | ● | | | | | | | | ● |
| ECG[4] | ● | ● | | ● | | | | | ● |
| Randomization[5] | | ● | | | | | | | |
| **Blood Sampling Items and Schedule** | | | | | | | | | |
| Hematology and blood chemistry test[6] | ● | ● | ●† | ● | ● | ● | ● | ● | ● |
| Serology test[7] | ● | | | | | | | | |
| Coagulation test[8] | ● | ● | | ● | | | | | |
| Urinalysis[9] | ● | ● | ●† | ● | ● | ● | ● | ● | ● |
| Pregnancy test[10] | ● | | | | | | | | ● |
| Immunogenicity blood Sampling[11] | ● | ● | | | ● | ● | ● | ● | ● |
| **Related to IP Administration** | | | | | | | | | |
| IP administration and VAS assessment[12] | | ● | | ● | | | | | |
| Previous and concomitant medication[13] | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Distribution of subject diary[14] | | ● | | ● | | | | | |
| Retrieval of subject diary and assessment[14] | | | | ● | | ● | | | |
| AE monitoring[15] | | ● | ● | ● | ● | ● | ● | ● | ● |
| Telephone monitoring[16] | | | ● | | | | | | |

† In phase 1, the total subjects will be checked for AEs daily by phone for up to 7 days from the first vaccination by a trained person designated by the principal investigator. They will undergo safety assessment (examination and tests) through an outpatient visit at 8 days (+3D) after the first vaccination.

‡ If V1 and V2 take place on the same day, randomization code must be assigned after verifying the screening test results and inclusion/exclusion criteria. In addition, physical examination, vital signs and body weight measurement, electrocardiography (ECG) before the vaccination, hematology and blood chemistry test, coagulation test, and urinalysis results from V1 may be used as results for V2.

1) Previous and current medical history of each subject is obtained by inquiry and from past medical records within one year of the screening visit.

2) Physical examination and measurement of vital signs and body weight are performed prior to the vaccination.

3) Examination may be performed again if deemed necessary by the investigator.

4) ECG is performed during the screening (V1), end-of-study (V9) visits, and within 35 min before and after the vaccination only for groups of using electroporator during V2 and V4.

5) Randomization and vaccination (including placebo) apply only to phase 2a.

6) Hematology test: WBC differential count (neutrophil, lymphocyte, monocyte, eosinophil, and basophil), RBC, Hb, Hct, and platelet count (including absolute neutrophil counts and absolute lymphocyte counts). If the results of a specific test deviate from the normal level, the investigator may ask the subject for a re-visit to repeat only

that test. Blood chemistry test: Total protein, albumin, creatinine, BUN or urea, total bilirubin, alkaline phosphatase, ALT, AST, GGT, glucose, calcium, magnesium, phosphorus, chloride, sodium, potassium, bicarbonate, LDH, amylase, lipase, and CRP. If the results of a specific test deviate from the normal level, the investigator may ask the subject for a re-visit to repeat only that test.

7) HBV (HBsAg), HAV (anti-HAV IgM), HIV antigen/antibody combination assay, HCV (anti-HCV), and SAS-CoV-2 Ab are performed

8) Coagulation test: Includes aPTT, PT, and INR. The test is performed during the visits for the vaccination. If screening (V1) and the priming vaccination (V2) visit are less than two weeks apart, the test results from V1 could be used for V2.

9) Urinalysis: RBC, WBC, pH, blood, glucose, urobilinogen, ketone, albumin, and bacteria

10) Pregnancy test by blood will be performed only in women in the childbearing age. Women who have received any surgical sterilization procedure (oophorectomy and/or hysterectomy) or menopausal women (at least 12 months elapsed after nontherapy-induced amenorrhea) are exempted.

11) Immunogenicity blood sampling will be performed for isolation of PBMC and serum separation for antibody test on all scheduled visits except for V3 and V4. If screening (V1) and the first vaccination (V2) visit are less than one week apart, at least one blood sampling could be performed before the vaccination. At the visits for vaccination, blood sampling must be performed prior to vaccination.

12) Pain assessment by using VAS is performed immediately after the vaccination and after 10 minutes of the vaccination.

13) Previous medication used within 12 weeks from the screening visit and concomitant medication to be used during the study period must be listed. Concomitant medications other than those prescribed by the principal investigator to treat AEs are not permitted.

14) The subject diary will be distributed at the time of the priming and the boosting vaccination. Each subject will personally list any AEs experienced over approximately four weeks. The diary will be retrieved during V4 and V6 and assessed. Subsequently, telephone or unscheduled visits will be permitted at any time, during which data regarding any AEs that may have occurred could be collected.

15) Information about AEs will be collected during scheduled visits after the vaccination and telephone visit.

16) Within 8–11 days after the IM vaccination, a trained person designated by the principal investigator will call the subject to assess safety and notify the subject of the schedule for the following visit. However, the first three subjects in each dose group will be checked daily for AEs for up to seven days from the day of the first vaccination, and safety-related data will be collected.

17) The end-of-study visit (V9) will be at 52 weeks after the first vaccination. If necessary, follow-up visits may be required for long-term safety and immunogenicity assessment. Moreover, subjects who dropout from the study must go through a procedure equivalent to the EOS visit

**Appendix 10. Methods of determination of immunogenicity**

SARS-CoV-2 Spike protein Ab ELISA

Ninety-six-well ELISA plates (Nunc Immunomodule, Thermo Fisher Scientific, Cat# 468667) were coated overnight at room temperature with 200 ng per well of the purified recombinant SARS-CoV-2 S protein (Acro biosystem, Cat# SPN-C52H4) resuspended in coating buffer (carbonate-bicarbonate buffer pH 9.6, Sigma Aldrich, Cat# C3041). The plates were then blocked with 300 μL of 2% skim milk (BD, Cat# 23210) prepared in PBS (Welgene Cat# LB004-02), and incubated at 37℃ for 2 h. Each serum sample was serially diluted 1:2 in sample dilution buffer (PBS-1% BSA-0.05% Tween 20 (Teknova, Cat# P0234)-10% Skim milk) starting at a dilution of 1:10. Hundred microliters of 100 μL of each dilution was added the plate was incubated for 12~16 hrs at 37℃ incubator. Then, 100 μL of goat-anti-human IgG Fc fragment HRP (Bethyl, Cat# A80-104P), diluted 1:100,000 in PBS-1% BSA buffer (Sigma Aldrich, Cat# P3688), was added the plate was incubated for 1 h at 37℃. Development was performed using the TMB substrate (Surmodics, Cat# TMBW-1000-01). Reactions were stopped with 1N sulfuric acid (Sigma Aldrich, Cat# 285105) and absorbance was measured at 450 nm. Between each step, samples were washed 5 times with 300 μL of PBS-0·05 Tween (Takara, Cat# T9183). The antibody titer was obtained by calculating each plate cutoff level (OD of plate negative control + 0·178). The Ab titers were calculated using Microsoft Excel and SoftMax GxP (version 6.5, Molecular Devices). To evaluate method validation, parameters such as cut point, Titer determination, Specificity, Precision, and Stability were evaluated. The cut point was evaluated by analyzing the serum of 50 normal subjects. After removing outliers from the measured OD values of individual serum, the average and SD of the OD values of the serum were calculated to calculate the titer cut point (3.09 x SD). Titer determination was used to determine the titer by serially diluting the 20 individual naïve samples. For specificity, when the average naïve serum titer in 20 subjects was confirmed, the titer was expected to be as low as 10 or less before vaccine administration. Precision was evaluated by repeating independent surrogate positive control and pooled naïve negative control measurements at least 6 times. Stability samples were prepared and evaluated by comparison with fresh positive control. Short-term temperature stability, freezing/thawing stability, and long-term stability were evaluated.

SARS-CoV-2 RBD Ab ELISA

The binding antibody responses against the receptor binding domain (RBD) with ELISA kit manufactured by Bionote (Lot# T210218). A dilution of 1:1 was the positivity cut off value for ELISA. Samples were serially diluted 1:2 in sample dilution buffer (PBS-1% BSA, Sigma Aldrich, Cat# P3688) starting at a dilution of 1:1. 50 μL of sample dilution buffer was added each well and 50 μL of each dilution was added and incubated for 2 h at a 37℃ incubator. 100 μL of Enzyme conjugate, diluted to 0.1 μg/mL in enzyme conjugate diluent, were added and incubated for 1 h at a 37℃ incubator. Development was performed using TMB substrate and reactions were stopped with 1N sulfuric acid and absorbance was measured at 450 nm. Between each step, samples were washed 5 times with 300 μL of wash buffer including prior to development. The titer was obtained by calculating the plate cutoff level (0·062). The Ab titers were calculated using Microsoft Excel and SoftMax GxP (version 6.5, Molecular Devices). As part of method validation, the following parameters will be investigated: Cut point, Titer determination, and specificity, Precision, Stability. To evaluate method validation, parameters such as cut point, Titer determination, Specificity, Precision, and Stability were performed. The cut point was applied by analyzing the serum of 50 normal subjects. After removing outliers from the measured OD values of individual serum, the average and SD of the OD values of the serum were calculated to calculate the titer cut point (Mean + 3.09 x SD). Titer determination confirmed the titer by serially diluting the 20 individual samples. Specificity was performed by diluting the anti-NP antibody with the same concentration as the high positive control and low positive control of the anti-RBD antibody to confirm the specificity for the test method. Precision was evaluated by repeating independent positive control and negative control measurements at least 6 times. Stability samples were prepared and evaluated by comparison with fresh positive control. Short-term temperature stability, freezing/thawing, and long-term stability were performed.

Pseudovirus neutralization assay

Neutralization efficiency of anti-SARS-CoV-2 spike antibody in sera from vaccinees or convalescent patients was evaluated using SARS-CoV-2 Spike (S)-pseudotyped murine leukemia virus (MLV) retrovirus (SARS2-Spp). The

packaging plasmid pUMVC (Addgene plasmid #8449) (Addgene, Cambridge, MA, USA) and the transfer plasmid pBABE-puro-NanoLuc, which was constructed by inserting the NanoLuc-coding gene into pBABE-puro (Addgene plasmid #1764), were used to prepare the SARS2-Spp by transfection of these plasmids into a HEK293T cell line (HEK293T/Spike) stably expressing a C-terminal 19 amino acid deleted SARS-CoV-2 spike protein (Wuhan-1 strain). SARS2pp titer was determined by real-time RT-PCR using the Takara Retrovirus Titer Set (Takara Bio, Kusatsu, Shiga, Japan) and aliquots were frozen at −80℃ until used for transduction experiments.

Equal volumes (400 µL each) of diluted sera (starting with 1 to 2 with four twofold dilutions for pre-vaccination sera, 1 to 4 with ten twofold dilutions for post-vaccination sera, or 1 to 40 with eight twofold dilutions for convalescent sera from patients) and diluted SARS2pp, which yields ~$10^6$ nanoluciferase reads (relative light units, RLU), were mixed (n = 3) and incubated for 1 h at 37℃. Then HEK293T cells stably expressing hACE2 (HEK293T/hACE2), which were grown for 48 h following seeding at 1 x $10^5$/well on 12-well plates, were transduced with the pseudotyped virus. Following media change at 12 h post-transduction, cells were further cultivated for 36 h and lysed in 115 µL of Glo Lysis Buffer (Promega, Madison, WI, USA). The resulting cell lysates (75 µL) were transferred to white, flat bottom 96-well microplates (Greiner Bio-One, Kremsmünster, Austria) for luciferase assay using the Nano-Glo Luciferase Assay system (Promega). The 50% inhibitory dilution (ID50), which was defined as the serum dilution causing a 50% reduction in luciferase activity compared to the wells displaying no inhibition, was determined by plotting a dose-response curve (log10 nanoluciferase activity vs. dilution) and four-parameter non-linear regression analysis using GraphPad prism 8·0 (GraphPad Software Inc., San Diego, CA, USA). Mann-Whitney U test was used to compare the difference between the neutralizing antibody level in trial participants and in serum samples from convalescent humans.

Live virus neutralization assay.

To assess GX-19 and GX-19N-induced neutralizing Ab titer, focus reduction neutralizing test using wildtype virus was used. Briefly, Vero cells (Korea Cell Line Bank No.10081, Seoul, South Korea) were seeded on 96-well plate (NUNC cat#167008, Thermo Fisher Scientific, MA, USA) and incubate for 16 h at 37°C 5% $CO_2$. Sera from vaccinee were heat-inactivated by incubation at 56°C for 30 min. The sera were serially diluted, mixed with SARS-CoV-2 (450 plaque-forming unit) and the mixture was incubated for 30 min at 37°C 5% $CO_2$. After washing the Vero cells using serum-free DMEM (Invitrogen, cat# 11995065, MA, USA), the virus-sera mixture was treated into the Vero cells and the mixture treated Vero cells were incubated for 5 h at 37°C and 5% $CO_2$. After removal of the treated mixture supernatant, the cells were washed using 100µl of phosphate buffered saline (PBS) (Gibco, cat# 10010-023, MA, USA) and fixed using 300 µL of 10% formalin solution (Sigma, cat# F8775, MO, USA) by incubation for 16 h at 4°C. After washing, the Vero cells were permeabilized by adding 100 µL of ice-cold 100% methanol (Sigma, cat# D7, MO, USA), incubated for 10 min at room-temperature, and washed using 100µl PBS. The cells were blocked by incubation with 100 µL of blocking buffer [0·5% normal goat serum (Abcam, cat# Ab7481, Cambridge, UK) supplemented with 0·1% Tween 20 (GenDEPOT, cat# T9100-100, TX, USA) and 1% (w/v) Bovine serum albumin (Sigma, cat#A3803-100G, MO, USA) in PBS)] for 30 min at room temperature. Then the blocked cells were incubated with 3,000-fold diluted 100 µL of anti-SARS-CoV-2 NP rabbit mAb (Sino Biological, cat# 40143-R001) for 1h at 37°C. After washing the cells using 200 µL of PBS containing 0·1% Tween 20, the Vero cells were incubated with 2,000-fold diluted goat anti-rabbit IgG-HRP (Bio-Rad, cat# 170-6515, CA, USA) solution for 1h at 37°C. After washing, the Vero cells were incubated with 30 µL of TrueBlue solution for 30 min at room temperature. After removal of the TrueBlue solution, the cells were air dried completely. The numbers of focus of each well were read using Cytation7 (Biotek, VA, USA) and the neutralizing Ab titers were calculated using Microsoft Excel and SoftMax (Version 5.4.1. San Diego, CA, USA). P values were calculated using the Student's t test.

ELISPOT assay

The human IFN-γ ELISPOT assay (Mabtech, Nacka Strand, Sweden) was performed as recommended by the manufacturer. PBMCs were isolated from whole blood by Ficoll-Hypaque density gradient centrifugation and resuspended (0.3 x $10^6$ cells per well) in RPMI medium. The PBMCs were stimulated with SARS-CoV-2 S or NP OLP pools for 24 h at 37° C in a 5% $CO_2$ atmosphere. Spots were enumerated using an automated ELISPOT reader (AID GmbH, Strassberg Germany) and the number of specific spots was calculated by subtracting the

number of spots in the negative control wells from the number of spots in the OLP pool-stimulated wells. The subjects with ≥2-fold and ≥100 spots/106 PBMCs increase in IFN-γ ELISPOT assay on day 43, compared to day 1, were regarded as responders. Mann-Whitney U test was used to compare the results of the ELISPOT assay in samples from trial participants and convalescent humans.

To selectively evaluate vaccine-induced SARS-CoV-2-specific T cell responses, the T-SPOT (Oxford Immunotec, Abingdon, UK) was performed as recommended by the manufacturer. In this T-SPOT assay, PBMCs from participants were stimulated with OLPs spanning S and NP that specific sequences with high homology to endemic (non SARS-CoV-2) coronaviruses removed were used as stimulators for 24 h at 37° C in a 5% $CO_2$ atmosphere.

Overlapping peptides

OLPs were synthesized as 15-mers that overlapped by 11 amino acids to cover the whole amino acid sequence of SARS-CoV-2 S and NP proteins encoded by GX19 and GX-19N. Lyophilized peptides were solubilized in 5% dimethyl sulfoxide (DMSO; Sigma Aldrich, Saint Louis, MO, USA). The concentration of each peptide in the pools was 25 µg $mL^{-1}$, which was finally diluted to 1 µg $mL^{-1}$ for stimulation of peripheral blood mononuclear cells (PBMCs) to examine vaccine-induced T cell responses in participants.

**Appendix 11. Incidence of adverse drug reaction (ADR) by system organ class (SOC) and preferred term (PT)**

| Subjects with ADRs | GX-19 1·5mg (N=20) | GX-19 3·0mg (N=20) | GX-19N 3·0mg (N=21) |
|---|---|---|---|
| **ADRs by Severity** | 11 (55·00) [24] | 4 (20·00) [5] | 10 (47·62) [22] |
| Mild | 11 (55·00) [23] | 4 (20·00) [5] | 10 (47·62) [22] |
| Moderate | 1 (5·0) [1] | 0 | 0 |
| Severe | 0 | 0 | 0 |
| **ADRs by SOC and PT** | | | |
| **General disorders and administration site conditions** | 11 (55·00) [21] | 4 (20·00) [5] | 6 (28·57) [16] |
| Chills | 0 | 0 | 1 (4·76) [1] |
| Fatigue | 3 (15·00) [4] | 0 | 0 |
| Injection site erythema | 2 (10·00) [2] | 0 | 1 (4·76) [1] |
| Injection site oedema | 1 (5·00) [1] | 0 | 0 |
| Injection site pain | 6 (30·00) [8] | 3 (15·00) [3] | 6 (28·57) [14] |
| Injection site pruritus | 5 (25·00) [6] | 2 (10·00) [2] | 0 |
| **Skin and subcutaneous tissue disorders** | 0 | 0 | 2 (9·52) [2] |
| Rash | 0 | 0 | 2 (9·52) [2] |
| **Musculoskeletal and connective tissue disorders** | 1 (5·00) [2] | 0 | 1 (4·76) [1] |
| Myalgia | 1 (5·00) [2] | 0 | 1 (4·76) [1] |
| **Nervous system disorders** | 1 (5·00) [1] | 0 | 1 (4·76) [1] |
| Headache | 0 | 0 | 1 (4·76) [1] |
| Paresthesia | 1 (5·00) [1] | 0 | 0 |
| **Investigations** | 0 | 0 | 1 (4·76) [2] |
| Alanine aminotransferase increased | 0 | 0 | 1 (4·76) [1] |
| Aspartate aminotransferase increased | 0 | 0 | 1 (4·76) [1] |

MedDRA version: 23·0
Denominator of percentage is the number of subjects in each cohort.
Adverse events are displayed as number of subjects (percentage of subjects) [number of events]

**Appendix 12. Summary of the spike and receptor-binding domain (RBD) binding antibody titers in serum after dosing with GX-19(N)**

| | Statistic | GX-19 1·5 mg (N=20) | GX-19 3·0 mg (N=20) | GX-19N 3·0 mg (N=21) |
|---|---|---|---|---|
| | | Spike | Spike | Spike/RBD |
| **Day 1** | N | 20 | 20 | 21 |
| | GMT (SD) | 25·49 (2·62) | 33·64 (3·00) | 24·38 (3·67) / 1·14 (1·83) |
| | 95% CI | 16·25, 39·98 | 20·11, 56·25 | 13·49, 44·05 / 0·87, 1·50 |
| | Mean | 42·50 | 61·00 | 97·14 / 1·71 |
| | Median | 20·00 | 30·00 | 20·00 / 1·00 |
| | Min-Max | 10·00-160·00 | 10·00-320·00 | 10·00-1280·00 / 1·00-16·00 |
| **Day 43** | N | 20 | 20 | 21 |
| | GMT (SD) | 60·63 (3·11) | 129·96 (3·58) | 320·00 (4·78) / 8·55 (7·18) |
| | 95% CI | 35·67, 103·05 | 71·59, 235·93 | 156·93, 652·53 / 3·48, 20·97 |
| | ≥4-fold increase, (%) | 35·00 | 65·00 | 80·95 |
| | Mean | 107·50 | 255·00 | 703·33 / 114·90 |
| | Median | 80·00 | 160·00 | 640·00 / 8·00 |
| | Min-Max | 10·00-640·00 | 20·00-1280·00 | 10·00-2560·00 / 1·00-2048·00 |
| | *p*-value [1] vs Day 1 | 0·0010 | <0·0001 | <0·0001 / <0·0001 |
| **Day 57** | N | 20 | 20 | 21 |
| | GMT (SD) | 85·74 (3·21) | 144·20 (3·45) | 201·59 (4·16) / 5·38 (6·31) |
| | 95% CI | 49·67, 148·00 | 80·81, 257·32 | 105·32, 385·83 / 2·33, 12·45 |
| | ≥4-fold increase, (%) | 55·00 | 65·00 | 80·95 |
| | Mean | 166·00 | 286·00 | 433·33 /62·00 |
| | Median | 120·00 | 160·00 | 160·00 / 4·00 |
| | Min-Max | 10·00-1280·00 | 20·00-1280·00 | 10·00-2560·00 / 1·00-1024·00 |
| | *p*-value [1] vs Day 1 | 0·0002 | <0·0001 | 0·0004 / 0·0001 |
| | *p*-value [1] vs Day 43 | 0·0156 | 0·6719 | 0·0009 / 0·0264 |

GMTs = geometric mean titers, SD = Standard deviation, CI = confidence interval, Min = minimum, Max = maximum.

[1] *p*-value was calculated in Wilcoxon matched-pairs signed rank test.
Denominator of percentage is the number of subjects in each cohort.

**Appendix 13. Neutralizing antibody titers of GX-19N (IU/mL)**

| | **Day 1** | **Day 43** | **Day 57** |
|---|---|---|---|
| IU/mL | 10.13 ± 1.66 | 16.91 ± 2.19 | 19.41 ± 2.34 |

Cf.) WHO international standard (NIBSC 20-136): 1,000 IU/mL

**Appendix 14. Summary of the specific T-cell response in serum after dosing with GX-19(N)**

| | Statistic | GX-19 1·5 mg (N=20) | GX-19 3·0 mg (N=20) | GX-19N 3·0 mg (N=21) |
|---|---|---|---|---|
| | | Spike | Spike | Spike / NP |
| **Day 1** | N | 20 | 20 | 21 |
| | Mean (SD) | 152·79 (150·39) | 109·40 (81·54) | 274·90 (216·48) / 156·07 (133·33) |
| | 95% CI | 82·41, 223·17 | 71·23, 147·56 | 176·36, 373·44 / 95·37, 216·76 |
| | Median | 153·45 | 115·50 | 263·33 / 156·67 |
| | Min-Max | 0·00-598·95 | 0·00-306·90 | 9·52-680·00 / 5·00-521·67 |
| **Day 43** | N | 20 | 20 | 21 |
| | Mean (SD) | 291·31 (257·66) | 372·32 (415·47) | 1016·53 (841·96) / 433·53 (347·69) |
| | 95% CI | 170·72, 411·89 | 177·88, 566·77 | 633·27, 1399·78 / 275·26, 591·80 |
| | Median | 228·53 | 183·98 | 725·00 / 335·00 |
| | Min-Max | 39·60-881·10 | 56·10-1546·05 | 31·67-2993·33 / 0·00-1123·33 |
| | *p*-value [1] | 0·0775 | 0·0027 | 0·0005 / 0·0003 |

SD = Standard deviation, CI = confidence interval, Min = minimum, Max = maximum.

T cell responses are presented as spot-forming-unit (SFU) per $10^6$ cells

[1] *p*-value was calculated in Wilcoxon matched-pairs signed rank test.

Denominator of percentage is the number of subjects in each cohort.

**Appendix 15. Results of ELISPOT and T-SPOT in GX-19N-vaccinated participants**

| | ELISPOT (SFUs / $10^6$ PBMCs) | | | T-SPOT (SFUs / $10^6$ PBMCs) | | |
|---|---|---|---|---|---|---|
| **Vaccinee** | **Pre** | **Week 6** | **Ratio** | **Pre** | **Week 6** | **Ratio** |
| **01SN001** | 698·3 | 363·3 | 0·5 | 28·0 | 64.0 | 2·3 |
| **01SN002** | 290·0 | 1550·0 | 5·3 | | N/D | |
| **01SN003** | 270·0 | 3663·3 | 13·67 | | N/D | |
| **01SN004** | 835·0 | 546·67 | 0·7 | 272·0 | 248.0 | 0·9 |
| **01SN005** | 176·7 | 2756·7 | 15·6 | | N/D | |
| **01SN006** | 863·3 | 791·7 | 0·9 | 120·0 | 580.0 | 4·8 |
| **01SN007** | 605·0 | 2006·7 | 3·3 | | N/D | |
| **01SN008** | 1016·7 | 3250·0 | 3·2 | | N/D | |
| **01SN009** | 420·0 | 2463·3 | 5·9 | | N/D | |
| **01SN011** | 1053·3 | 3420·0 | 3·3 | | N/D | |
| **02SN001** | 63·3 | 1040·0 | 16·4 | | N/D | |
| **02SN002** | 330·0 | 985·0 | 3·0 | | N/D | |
| **02SN003** | 113·3 | 1351·7 | 11·9 | | N/D | |
| **02SN004** | 496·7 | 1271·7 | 2·6 | | N/D | |
| **02SN005** | 415·0 | 2520·0 | 6·1 | | N/D | |
| **02SN006** | 523·3 | 695·0 | 1·3 | | N/D | |
| **02SN008** | 113·3 | 135·0 | 1·3 | 28·0 | 196·0 | 7·0 |
| **02SN009** | 61·7 | 516·7 | 8·4 | | N/D | |
| **02SN010** | 326·7 | 756·7 | 2·3 | | N/D | |
| **02SN011** | 358·3 | 293·3 | 0·8 | | N/D | |

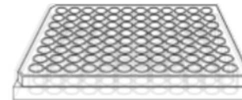
N/D, not determined; SFU, spot-forming units; PBMCs, peripheral blood mononuclear cells.

**Appendix 16. Comparison of amino acids sequence of 15-mer peptides containing NP-specific epitopes for GX-19N-vaccinated participants with those of SARS-CoV-2 variants**

**A** Virtual matrix of OLPs

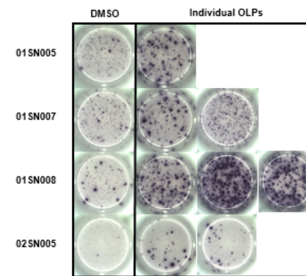
| | A | B | · | · | E | · | · | · | J |
|---|---|---|---|---|---|---|---|---|---|
| Ar | OLP1 | OLP2 | · | · | OLP5 | · | · | · | OLP10 |
| Cr | OLP21 | | | | | | | | |
| Fr | OLP51 | | | | | | | | |
| Jr | OLP91 | | | | | | | | |

OLP mixtures of each row and column of the matrix used as the antigen per well in the IFN-γ ELISPOT assay plate

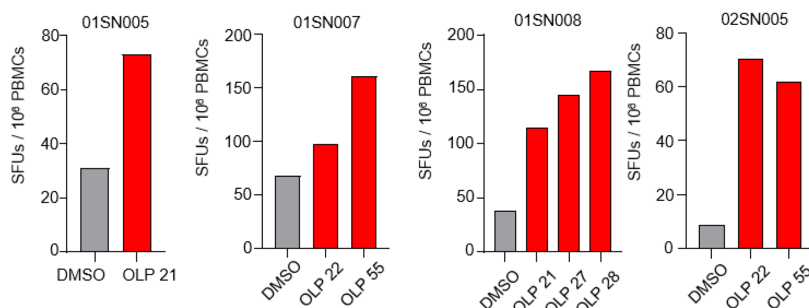


Identification of individual OLPs at the intersection of response-inducing rows and columns in IFN-γ ELISPOT assay

| | A | B | · | · | E | · | · | · | J |
|---|---|---|---|---|---|---|---|---|---|
| Ar | | | | | | | | | |
| Cr | | 22 | | | 25 | | | | |
| Fr | | 52 | | | 55 | | | | |
| Jr | | | | | | | | | |




**B**




**C**

**01SN005**

| OLP-21 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | D | D | Q | I | G | Y | Y | R | R | A | T | R | R | I | R |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**01SN008**

| OLP-21 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | D | D | Q | I | G | Y | Y | R | R | A | T | R | R | I | R |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| OLP-27 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | S | P | R | W | Y | F | Y | Y | L | G | T | G | P | E | A |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| OLP-28 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | Y | F | Y | Y | L | G | T | G | P | E | A | G | L | P | Y |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**01SN007**

| OLP-22 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | G | Y | Y | R | R | A | T | R | R | I | R | G | G | D | G |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| OLP-55 | 217 | 218 | 219 | 220 | 221 | 222 | 223 | 224 | 225 | 226 | 227 | 228 | 229 | 230 | 231 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | A | A | L | A | L | L | L | L | D | R | L | N | Q | L | E |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**02SN005**

| OLP-21 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | D | D | Q | I | G | Y | Y | R | R | A | T | R | R | I | R |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| OLP-55 | 217 | 218 | 219 | 220 | 221 | 222 | 223 | 224 | 225 | 226 | 227 | 228 | 229 | 230 | 231 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GX-19N | A | A | L | A | L | L | L | L | D | R | L | N | Q | L | E |
| B.1.1.7 (Alpha) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.351 (Beta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P.1 (Gamma) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B.1.617.2 (Delta) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C.37 (Lambda) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Strategy to determine 15-mer peptides containing NP-specific epitopes for GX-19-vaccinated participants (A), Results of IFN-γ ELISPOT assay using 15-mer individual peptide (B), Comparison of amino acids sequence of 15-mer peptides containing NP-specific epitopes for GX-19N-vaccinated participants with those of SARS-CoV-2 variants such as B·1·1·7 (Alpha), B·1·351 (Beta), and P·1(Gamma), B·1·617·2 (Delta), and C·37 (Lambda) (C). NP, nucleocapsid protein