VACCINES AGAINST CORONAVIRUS AND METHODS OF USE

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] The present application claims the benefit of U.S. Provisional Appl. No. 62/981,451, filed February 25, 2020; U.S. Provisional Appl. No. 63/004,380, filed April 2, 2020; U.S. Provisional Appl. No. 63/028,404, filed May 21, 2020; U.S. Provisional Appl. No. 63/033,349, filed June 2, 2020; U.S. Provisional Appl. No. 63/040,865, filed June 18, 2020; U.S. Provisional Appl. No. 63/046,415, filed June 30, 2020; U.S. Provisional Appl. No. 63/062,762, filed August 7, 2020; U.S. Provisional Appl. No. 63/114,858, filed November 17, 2020; U.S. Provisional Appl. No. 63/130,593 filed December 24, 2020; U.S. Provisional Appl. No. 63/136,973 filed January 13, 2021; U.S. Provisional Appl. No. 62/981,168, filed February 25, 2020; U.S. Provisional Appl. No. 63/022,032, filed May 8, 2020; U.S. Provisional Appl. No. 63/056,996, filed July 27, 2020; and U.S. Provisional Appl. No. 63/063,157, filed August 7, 2020. The contents of each of these applications are incorporated herein by reference in the entirety.

[0003] SEQUENCE LISTING

[0004] This application includes a Sequence Listing submitted electronically as a text file named "104409_000596_SL.txt", created on February 24, 2021 with a size of 58,410 bytes. The Sequence Listing is incorporated by reference herein.

[0005] TECHNICAL FIELD

[0006] The present invention relates to a vaccine for Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) and methods of administering the vaccine.

[0007] BACKGROUND

[0008] COVID-19, known previously as 2019-nCoV pneumonia or disease, has rapidly emerged as a global threat to public health, joining severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in a growing number of coronavirus-associated illnesses which have jumped from animals to people. There is an imminent need for medical countermeasures such as vaccines to combat the spread of such emerging coronaviruses. There are at least seven identified coronaviruses that infect humans, including MERS-CoV and SARS-CoV.

[0009] In December 2019, the city of Wuhan in China became the epicenter for a global outbreak of a novel coronavirus. This coronavirus, SARS-CoV-2, was isolated and sequenced from human airway epithelial cells from infected patients (Zhu, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020; Wu, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020). Disease symptoms can range from mild flu-like to severe cases with life-threatening pneumonia (Huang, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020). The global situation is dynamically evolving, and on January 30th, 2020 the World Health Organization declared COVID-19 as a public health emergency of international concern (PHEIC).

[0010] SUMMARY

[0011] Provided herein are nucleic acid molecules encoding a SARS-CoV-2 spike antigen. According to some embodiments, the encoded SARS-CoV-2 spike antigen is a consensus antigen. In some embodiments, the nucleic acid molecule comprises: a nucleic acid sequence having at least about 90% identity over an entire length of the nucleic acid sequence set forth in nucleotides 55 to 3837 of SEQ ID NO: 2; a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 2; the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 2; the nucleic acid sequence of SEQ ID NO: 2; a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 3; the nucleic acid sequence of SEQ ID NO: 3; a nucleic acid sequence having at least about 90% identity over an entire length of nucleotides 55 to 3837 of SEQ ID NO: 5; a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 5; the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 5; the nucleic acid sequence of SEQ ID NO: 5; a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 6; or the nucleic acid sequence of SEQ ID NO: 6. Also provided herein are nucleic acid molecules encoding a SARS-CoV-2 spike antigen, wherein the SARS-CoV-2 spike antigen comprises: an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1; an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 1; the amino acid sequence of SEQ ID NO: 1; an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4; an amino acid sequence having at least about 90%

identity over an entire length of SEQ ID NO: 4; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4, or the amino acid sequence of SEQ ID NO: 4.

[0012] In some embodiments, the nucleic acid molecule encoding the SARS-CoV-2 antigen is incorporated into a viral particle.

[0013] Further provided are vectors comprising the nucleic acid molecule encoding the SARS-CoV-2 antigen. In some embodiments, the vector is an expression vector. The nucleic acid molecule may be operably linked to a regulatory element selected from a promoter and a poly-adenylation signal. The expression vector may be a plasmid or viral vector.

[0014] Immunogenic compositions comprising an effective amount of the vector or viral particle are disclosed. The immunogenic composition may comprise a pharmaceutically acceptable excipient, such as but not limited to, a buffer. The buffer may optionally be saline-sodium citrate buffer. In some embodiments, the immunogenic compositions comprise an adjuvant.

[0015] Also provided herein are SARS-CoV-2 spike antigens. According to some embodiments, the SARS-CoV-2 spike antigen is a consensus antigen. In some embodiments, the SARS-CoV-2 spike antigen comprises: an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1; an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 1; the amino acid sequence of SEQ ID NO: 1; an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4; an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 4; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4; or the amino acid sequence of SEQ ID NO: 4.

[0016] Further provided herein are vaccines for the prevention or treatment of Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) infection. The vaccines comprising an effective amount of any one or combination of the aforementioned nucleic acid molecules, vectors, or antigens. According to some embodiments, the vaccine further comprises a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be a buffer, optionally saline-sodium citrate buffer. According to some embodiments, the vaccine further comprises an adjuvant.

[0017] Methods of inducing an immune response against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) in a subject in need thereof are further provided. In come embodiments, the methods of inducing an immune response comprise administering an effective amount of any one or combination of the aforementioned nucleic acid molecules,

vectors, immunogenic compositions, antigens, or vaccines to the subject. Also provided herein are methods of protecting a subject in need thereof from infection with SARS-CoV-2, the method comprising administering an effective amount of any one or combination of the aforementioned nucleic acid molecules, vectors, immunogenic compositions, antigens, or vaccines to the subject. Further provided are methods of treating SARS-CoV-2 infection in a subject in need thereof, the method comprising administering an effective amount of any one or combination of the aforementioned nucleic acid molecules, vectors, immunogenic compositions, antigens, or vaccines to the subject. In any of these methods, the administering may include at least one of electroporation and injection. According to some embodiments, the administering comprises parenteral administration, for example by intradermal, intramuscular, or subcutaneous injection, optionally followed by electroporation. In some embodiments of the disclosed methods, an initial dose of about 0.5 mg to about 2.0 mg of the nucleic acid molecule is administered to the subject, optionally the initial dose is 0.5 mg, 1.0 mg or 2.0 mg of the nucleic acid molecule. The methods may further involve administration of a subsequent dose of about 0.5 mg to about 2.0 mg of the nucleic acid molecule to the subject about four weeks after the initial dose, optionally wherein the subsequent dose is 0.5 mg,1.0 mg or 2.0 mg of the nucleic acid molecule. In still further embodiments, the methods involve administration of one or more further subsequent doses of about 0.5 mg to about 2.0 mg of the nucleic acid molecule to the subject at least twelve weeks after the initial dose, optionally wherein the further subsequent dose is 0.5 mg, 1.0 mg, or 2.0 mg of the nucleic acid molecule. In any of these embodiments, INO-4800 or a biosimilar thereof is administered.

[0018] Also provided herein are uses of any one or combination of the disclosed nucleic acid molecules, vectors, immunogenic compositions, antigens, or vaccines in a method of inducing an immune response against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) in a subject in need thereof. Further provided are uses of any one or combination of the disclosed nucleic acid molecules, vectors, immunogenic compositions, antigens, or vaccines in a method of protecting a subject from infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). Also provided herein are uses of any one or combination of the disclosed nucleic acid molecules, vectors, immunogenic compositions, antigens, or vaccines in a method of treating a subject in need thereof against SARS-CoV-2 infection. In accordance with any of these uses, the nucleic acid molecule, the vector, the immunogenic composition, the antigen, or the vaccine may be administered to the subject by at least one of electroporation and injection. In some embodiments, the nucleic

acid molecule, the vector, the immunogenic composition, the antigen, or the vaccine is administered parenterally to the subject followed by electroporation. In some embodiments of the disclosed uses, an initial dose of about 0.5 mg to about 2.0 mg of the nucleic acid molecule is administered to the subject, optionally the initial dose is 0.5 mg, 1.0 mg or 2.0 mg of the nucleic acid molecule. The uses may further involve administration of a subsequent dose of about 0.5 mg to about 2.0 mg of the nucleic acid molecule to the subject about four weeks after the initial dose, optionally wherein the subsequent dose is 0.5 mg,1.0 mg or 2.0 mg of the nucleic acid molecule. In still further embodiments, the uses involve administration of one or more further subsequent doses of about 0.5 mg to about 2.0 mg of the nucleic acid molecule to the subject at least twelve weeks after the initial dose, optionally wherein the further subsequent dose is 0.5 mg, 1.0 mg, or 2.0 mg of the nucleic acid molecule. In any of these embodiments, INO-4800 or a biosimilar thereof is administered.

[0019] Further provided herein are uses of any one or combination of the disclosed nucleic acid molecules, vectors, immunogenic compositions, antigens, or vaccines in the preparation of a medicament. In some embodiments, the medicament is for treating or protecting against infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). In some embodiments, the medicament is for treating or protecting against a disease or disorder associated with SARS-CoV-2 infection. In some embodiments, the medicament is for treating or protecting against Coronavirus Disease 2019 (COVID-19), Multisystem inflammatory syndrome in adults (MIS-A), or Multisystem inflammatory syndrome in children (MIS-C).

[0020] The invention further relates to a method of detecting a persistent cellular immune response in a subject, the method comprising the steps of: administering an immunogenic composition for inducing an immune response against a SARS-CoV-2 antigen to a subject in need thereof; isolating peripheral mononuclear cells (PBMCs) from the subject; stimulating the isolated PBMCs with a spike antigen comprising an amino acid sequence selected from the group consisting of: the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1the amino acid sequence of SEQ ID NO: 1; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4; or the amino acid sequence of SEQ ID NO: 4; or a fragment thereof comprising at least 20 amino acids; and detecting at least one of the number of cytokine expressing cells and the level of cytokine expression. In one embodiment, the step of detecting at least one of the number of cytokine expressing cells and the level of cytokine expression is performed using Enzyme-linked immunospot (ELISpot) or Intracellular Cytokine Staining (ICS) analysis using flow cytometry.

[0021] In one embodiment, the subject is administered an immunogenic composition comprising a nucleic acid molecule, wherein the nucleic acid molecule comprises a nucleotide sequence encoding a peptide comprising: an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1; an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 1; the amino acid sequence of SEQ ID NO: 1; an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4; an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 4; the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4; or the amino acid sequence of SEQ ID NO: 4.

[0022] BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **Figures 1A, 1B, 1C, and 1D** illustrate the design and expression of SARS-CoV-2 synthetic DNA vaccine constructs. **Figure 1A** shows a schematic diagram of SARS-CoV-2 synthetic DNA vaccine constructs, pGX9501 (matched) and pGX9503 (outlier (OL)) containing the IgE leader sequence and SARS-CoV-2 spike protein insert ("Covid-19 spike antigen" or "Covid-19 spike-OL antigen"). **Figure 1B** shows results of an RT-PCR assay of RNA extract from COS-7 cells transfected in duplicate with pGX9501 or pGX9503. Extracted RNA was analyzed by RT-PCR using PCR assays designed for each target and for COS-7 β-Actin mRNA, used as an internal expression normalization gene. Delta C_T (ΔC_T) was calculated as the C_T of the target minus the C_T of β-Actin for each transfection concentration and is plotted against the log of the mass of pDNA transfected (Plotted as mean±SD). **Figure 1C** shows analysis of *in vitro* expression of Spike protein after transfection of 293T cells with pGX9501, pGX9503 or MOCK plasmid by Western blot. 293T cell lysates were resolved on a gel and probed with a polyclonal anti-SARS Spike Protein. Blots were stripped then probed with an anti-β-actin loading control. **Figure 1D** shows *in vitro* immunofluorescent staining of 293T cells transfected with 3μg/well of pGX9501, pGX9503 or pVax (empty control vector). Expression of Spike protein was measured with polyclonal anti-SARS Spike Protein IgG and anti-IgG secondary. Cell nuclei were counterstained with DAPI. Images were captured using ImageXpress™ Pico automated cell imaging system.

[0024] **Figure 2** illustrates an IgG binding screen of a panel of SARS-CoV-2 and SARS-CoV antigens using sera from INO-4800-treated mice. BALB/c mice were immunized on Day 0 with 25 μg INO-4800 or pVAX-empty vector (Control) as described in the methods.

Protein antigen binding of IgG at 1:50 and 1:250 serum dilutions from mice at day 14. Data shown represent mean OD450 nm values (mean+SD) for each group of 4 mice.

[0025] **Figures 3A, 3B, 3C, and 3D** demonstrate humoral responses to SARS-CoV-2 S 1+2 and S receptor binding domain (RBD) protein antigen in BALB/c mice after a single dose of INO-4800. BALB/c mice were immunized on Day 0 with indicated doses of INO-4800 or pVAX-empty vector as described in Example 1. SARS-CoV-2 S1+2 (**Figure 3A**) or SARS-CoV-2 RBD (**Figure 3B**) protein antigen binding of IgG in serial serum dilutions from mice at day 14 are shown. Data shown represent mean OD450 nm values (mean+SD) for each group of 8 mice (**Figure 3A and 3B**) and 5 mice (**Figure 3C and 3D**). Serum IgG binding endpoint titers to SARS-CoV-2 S1+2 (**Figure 3B**) and SARS-CoV-2 RBD (**Figure 3D**) protein. Data representative of 2 independent experiments.

[0026] **Figures 4A and 4B** illustrate neutralizing antibody responses after immunization with INO-4800. BALB/c mice (n of 5 per group) were immunized twice on days 0 and 14 with 10 μg of INO-4800. Sera was collected on day 7 post-2nd immunization and serial dilutions were incubated with a pseudovirus displaying the SARS-CoV-2 Spike and co-incubated with ACE2-293T cells. **Figure 4A** shows neutralization ID50 (mean±SD) in naïve and INO-4800 immunized mice. **Figure 4B** shows relative luminescence units (RLU) for sera from naive mice and mice vaccinated with INO-4800 as described in methods.

[0027] **Figures 5A and 5B** show humoral responses to SARS-CoV-2 in Hartley guinea pigs after a single dose of INO-4800. Hartley guinea pigs mice were immunized on Day 0 with 100 μg INO-4800 or pVAX-empty vector as described in Example 1. **Figure 5A** shows SARS-CoV-2 S protein antigen binding of IgG in serial serum dilutions at day 0 and 14. Data shown represent mean OD450 nm values (mean+SD) for the 5 guinea pigs. **Figure 5B** shows serum IgG binding titers (mean±SD) to SARS-CoV-2 S protein at day 14. P values determined by Mann-Whitney test.

[0028] **Figures 6A-6F** demonstrate that INO-4800 immunized mouse and guinea pig sera compete with ACE2 receptor for SARS-CoV-2 Spike protein binding. **Figure 6A** illustrates that soluble ACE2 receptor binds to CoV-2 full-length spike with an EC50 of 0.025 μg/ml. **Figure 6B** illustrates that purified serum IgG from BALB/c mice (n of 5 per group) after second immunization with INO-4800 yields significant competition against ACE2 receptor. Serum IgG samples from the animals were run in triplicate. **Figure 6C** illustrates that IgGs purified from n=5 mice day 7 post second immunization with INO-4800 show significant competition against ACE2 receptor binding to SARS-CoV-2 S 1+2 protein. The soluble ACE2 concentration for the competition assay is ~0.1 μg/ml. Bars represent the mean and

standard deviation of AUC. **Figure 6D** illustrates Hartley guinea pigs immunized on Day 0 and 14 with 100 μg INO-4800 or pVAX-empty vector as described in the methods. Day 28 collected sera (diluted 1:20) was added to SARS-CoV-2 coated wells prior to the addition of serial dilutions of ACE2 protein. Detection of ACE2 binding to SARS-CoV-2 S protein was measured. Sera collected from 5 INO-4800-treated and 3 pVAX-treated animals were used in this experiment. **Figure 6E** illustrates serial dilutions of guinea pig sera collected on day 21 were added to SARS-CoV-2 coated wells prior to the addition of ACE2 protein. Detection of ACE2 binding to SARS-CoV-2 S protein was measured. Sera collected from 4 INO-4800-treated and 5 pVAX-treated guinea pigs were used in this experiment. **Figure 6F** depicts IgGs purified from n=5 mice day 14 post second immunization with INO-4800 show competition against ACE2 receptor binding to SARS-CoV-2 Spike protein compared to pooled naïve mice IgGs. Naïve mice were run in a single column. Vaccinated mice were run in duplicate. If error bars are not visible, error is smaller than the data point.

[0029] **Figures 7A-7D** illustrate detection of SARS-CoV-2 S protein-reactive antibodies in the BAL of INO-4800 immunized animals. BALB/c mice (n of 5 per group) were immunized on days 0 and 14 with INO-4800 or pVAX and BAL collected at day 21 (**Figures 7A and 7B**). Hartley guinea pigs (n of 5 per group) were immunized on days 0, 14 and 21 with INO-4800 or pVAX and BAL collected at day 42 (**Figures 7C and 7D**). Bronchoalveolar lavage fluid was assayed in duplicate for SARS-CoV-2 Spike protein-specific IgG antibodies by ELISA. Data are presented as endpoint titers (**Figures 7A and 7C**), and BAL dilution curves with raw OD 450 nm values (**Figures 7B and 7D**). In **Figures 7A and 7C**, bars represent the average of each group and error bars the standard deviation. **$p<0.01$ by Mann-Whitney U test.

[0030] **Figure 8A-8C** show induction of T cell responses in BALB/c mice post-administration of INO-4800. BALB/c mice (n=5/group) were immunized with 2.5 or 10 μg INO-4800. T cell responses were analyzed in the animals on days 4, 7, 10 (**Figures 8A and 8B**), and day 14 (**Figure 8C**). T cell responses were measured by IFN-γ ELISpot in splenocytes stimulated for 20 hours with overlapping peptide pools spanning the SARS-CoV-2 (**Figure 8A**), SARS-CoV (**Figure 8B**), or MERS-CoV (**Figure 8C**) Spike proteins. Bars represent the mean +SD.

[0031] **Figures 9 and 10** illustrate cellular and humoral immune responses measured in INO-4800-treated New Zealand White (NZW) rabbits. Day 0 and 28 intradermal delivery of pDNA. PBMC IFN-γ ELISpot (**Figure 9**); Serum IgG binding ELISA (**Figure 10**).

[0032] **Figures 11A-11E** illustrate humoral immune responses to SARS-CoV-2 spike protein measured in INO-4800 treated in rhesus monkeys. Day 0 and 28 intradermal delivery of pDNA. Serum IgG binding ELISA.

[0033] **Figures 12A-12G** illustrate humoral immune responses to SARS and MERS spike protein measured in INO-4800 treated rhesus monkeys. Day 0 and 28 intradermal delivery of pDNA. Serum IgG binding ELISA. (**Figure 12A-12G**; left panel, 1 mg INO-4800; right panel, 2 mg INO-4800).

[0034] **Figures 13A-13C** illustrate cellular immune responses measured by PBMC IFN-γ ELISpot in INO-4800-treated in rhesus monkeys following intradermal delivery of pDNA on days 0 and 28 intradermal. Results are shown in **Figure 13A** (SARS CoV-2 Spike peptides); **13B** (SARS CoV Spike peptides); and **13C** (MERS CoV Spike peptides).

[0035] **Figures 14A and 14B** show T cell epitope mapping after INO-4800 administration to BALB/c mice. Splenocytes were stimulated for 20 hours with SARS-CoV-2 peptide matrix mapping pools. **Figure 14A** demonstrates T cell responses following stimulation with matrix mapping SARS-CoV-2 peptide pools. Bars represent the mean +SD of 5 mice. **Figure 14B** shows the map of the SARS-CoV-2 Spike protein and identification of immunodominant peptides in BALB/c mice. A known immunodominant SARS-CoV HLA-A2 epitope is included for comparison. **Figure 14B** discloses SEQ ID NOS 26-35, respectively, in order of appearance.

[0036] **Figures 15A-15H** depict humoral correlates of protection in throat and nasal compartments. (**Figures 15A-15D**) Correlation of throat viral load Log10 cDNA copies mL-1 at day 1 (**Figures 15A, 15B**) and day 3 (**Figures 15C, 15D**) post SARS-CoV-2 challenge with microneutralization titers (**Figs. 15A, 15C**) and RBD IgG binding endpoint titers (**Figs. 15B, 15D**). (**Figs. 15E-15H**) Same analysis for nasal viral loads. P and R values provided for two-sided non-parametric Spearman rank-correlation analyses. Control animals – red filled circles, INO-4800 X1 – green filled circles and INO-4800 X2 – blue filled circles.

[0037] **Figure 16** illustrates the Phase I study flow diagram.

[0038] **Figures 17A, 17B, 17C, and 17D** illustrate the humoral antibody response of the phase I clinical study. The humoral response in the 1.0 mg dose group and 2.0 mg dose group was assessed for the ability to neutralize of live virus (n=18, 1.0mg; n=19, 2.0 mg) (**Figure 17A**); binding to the RBD regions (**Figure 17B**); and binding to whole spike protein (S1 and S2) (**Figure 17C**). End point titers were calculated as the titer that exhibited an OD 3.0 SD above baseline, titers at baseline were set at 1. In **Figure 17D**, the humoral response in the 1.0 mg dose group and 2.0 mg dose group was assessed for the ability to bind whole spike

protein (S1 and S2) (n=19, 1.0mg; n=19, 2.0 mg). End point titers were calculated as the titer that exhibited an OD 3.0 SD above baseline, titers at baseline were set at 1. A response to live virus neutralization was a PRNT IC50 ≥ 10. In all graphs horizontal lines represent the Median and bars represent the Interquartile Range.

[0039] **Figures 18A-18G** illustrate Phase I clinical study cellular immune response analytical results. PBMCs isolated from vaccinated individuals were stimulated *in vitro* with SARS-CoV-2 spike antigen. The number of cells capable of secreting IFN-gamma were measured in a standard ELISpot assay for the 1.0 mg dose group and 2.0 mg dose group (**Figure 18A**). Horizontal lines represent Medians and bars represent Interquartile Ranges. As shown in **Figure 18B**, peptides spanning the entirety of the spike antigen were divided into pools and tested individually in ELISpot, with pools mapped to specific regions of the antigen. Three subjects are shown exemplifying the diversity of pool responses and associated magnitude across subjects. The pie chart represents the diversity of entirety of the 2.0mg dose group. As illustrated in **Figure 18C**, SARS-CoV-2 spike specific cytokine production was measured from CD4+ and CD8+ T cells via flow cytometry. Bars represent Mean response. Cytokine production is additionally broken out in **Figure 18D** using CCR7 and CD45RA into Central Memory (CM), Effector Memory (EM) or Effector (E) differentiation status with data conveying what percentage of the overall cytokine response originates from what differentiated group. Pie charts represent the polyfunctionality of CD4+ and CD8+ T cells for each dose cohort are provided in **Figure 18E**. IL-4 production by CD4+ T cells for each dose cohort is illustrated in **Figure 18F**. Horizontal lines represent Mean response. Graphs represent all evaluable subjects. Statistical analyses were performed on all paired datasets. Those that were significant are noted within the figure, lack of notation in the figure represents lack of statistical significance. **Figure 18G** provides a heat map of each subject in the 2.0mg dose group and the percentage of their ELISpot response dedicated to each pool covering the SARS-CoV-2 spike antigen.

[0040] **Figure 19** illustrates the Phase I Related Systemic and Local Adverse Events in severity of mild (Grade 1), moderate (Grade 2), severe (Grade 3) and life-threatening (Grade 4).

[0041] **Figure 20** provides supplementary data for humoral immune response. Three convalescent samples (all 3 with symptoms but non-hospitalized), tested by the ELISpot assay showed lower T cell responses, with a median of 33, than the 2.0 mg dose group at Week 8.

[0042] **Figure 21** provides supplementary Enzyme-linked immunospot (ELISpot) data.

[0043] **Figures 22A-22F** depict humoral and cellular responses in rhesus macaques vaccinated with INO-4800. Study outline (**Fig. 22A**). Spike-specific IgG (**Fig. 22B**), RBD (**Fig. 22C**) and live virus-neutralising antibodies (**Fig. 22D**) measured in serum from rhesus macaques that received 1 or 2 doses of INO-4800 or were unvaccinated (Control). Lines represent the geometric means. Cellular immune responses in rhesus macaques vaccinated with INO-4800. SARS-CoV-2 Spike-specific interferon gamma (IFNγ) secretion from PBMCs was measured in rhesus macaques that received 1 or 2 doses of INO-4800 or were unvaccinated (Control) pre- (**Fig. 22E**) and post-challenge (**Fig. 22F**). PBMCs were stimulated with 5 separate peptide pools spanning the spike protein and SFU frequencies measured in response to each pool summed. Lines represent the means.

[0044] **Figures 23A-23C** illustrate change in weight, temperature and hemoglobin in the animals through the duration of the study. Animals received one (INO-4800X1) or two (INO-4800X2) doses of INO-4800 or were unvaccinated (control). Percentage change in body weights (**Fig. 23A**), temperature (**Fig. 23B**) and hemoglobin counts (**Fig. 23C**) of individual animals were recorded and plotted at the indicated time points pre- and post-challenge. Lines represent mean (**Fig. 23A**) and geometric mean (**Fig. 23B & Fig. 23C**) value for each group.

[0045] **Figures 24A-24F** illustrate the upper respiratory tract viral loads detected by RT-qPCR following challenge with SARS-CoV-2. Animals received one (INO-4800X1) or two (INO-4800X2) doses of INO-4800 or were unvaccinated (control). Viral load plotted as Log10 cDNA copies/ml for each animal in throat swabs (**Figs. 24A-24C**) and nasal swabs (**Figs. 24D-24F**). (**Figs. 24A&24D**) Lines represent group geometric means with 95% CI. Area under the curve (AUC) of viral loads for throat swabs (**Fig. 24B**) and nasal swabs (**Fig. 24E**) for each experimental group. Peak viral loads measured in each animal during the challenge period for throat swabs (**Fig. 24C**) and nasal swabs (**Fig. 24F**). LLOQ (lower limit of quantification, 3.80 log copies/ml) and LLOD (lower limit of detection, 3.47 log copies/ml). Positive samples detected below the LLOQ were assigned the value of 3.80 log copies/ml. * $p \leq 0.05$ with Mann-Whitney t test.

[0046] **Figures 25A-25F** illustrate the upper respiratory tract subgenomic viral loads detected by RT-qPCR following challenge with SARS-CoV-2. Animals received one (INO-4800X1) or two (INO-4800X2) doses of INO-4800 or were unvaccinated (control). Viral load plotted as Log10 cDNA copies/ml for each animal in throat swabs (**Figs. 25A-25C**) and nasal swabs (**Figs. 25D-25F**). (**Figs. 25A and 25D**) Lines represent group geometric means with 95% CI. Area under the curve (AUC) of viral loads for throat swabs (**Fig. 25B**) and nasal swabs (**Fig. 25E**) for each experimental group. Peak viral loads measured in each

animal during the challenge period for throat swabs (**Fig. 25C**) and nasal swabs (**Fig. 25F**). LLOQ (4.11 log copies/mL) and LLOD (3.06 log copies/mL). Positive samples detected below the LLOQ were assigned the value of 3.81 log copies/ml.

[0047] **Figures 26A-26D** illustrate lower respiratory tract viral loads detected by RT-qPCR following challenge with SARS-CoV-2. Animals received one (INO-4800X1) or two (INO-4800X1) doses of INO-4800 or were unvaccinated (control). SARS-CoV-2 genomic and subgenomic viral loads were measured for individual animals in bronchoalveolar lavage (BAL (**Figs. 26A and 26B**)) and lung tissue (**Figs. 26C and 26D**) samples collected at necropsy (6-8 days post challenge). Bars represent group medians. Assay LLOQ's and LLOD's are provided in the methods section.

[0048] **Figure 27** illustrates viral RNA in animal tissue post challenge. Animals received one (INO-4800X1) or two (INO-4800X2) doses of INO-4800 or were unvaccinated (control). SARS-CoV-2 viral loads were measured for individual animals in tissue samples collected at necropsy (6-8 DPC). Bars represent group median with 95% CI. Positive tissue samples detected below the limit of quantification (LoQ) of 4.76 log copies/ml were assigned the value of 5 copies/µl, this equates to 4.46 log copies/g, whilst undetected samples were assigned the value of < 2.3 copies/µl, equivalent to the assay's lower limit of detection (LoD) which equates to 4.76 log copies/g.

[0049] **Figure 28** shows representative histopathology (H&E stain) and presence of SARS-CoV-2 viral RNA (ISH RNAScope stain) in animals vaccinated with 1 dose (top), 2 doses (middle) or unvaccinated (bottom). Animals vaccinated with 1 dose showed multifocal minimal to mild alveolar and interstitial pneumonia (*), with higher severity in animal 10A. The remaining animals from group 1 show minimal/mild inflammatory infiltrates (*). Mild perivascular cuffing was also observed (arrowheads) and viral RNA was shown by ISH within the lesions (arrows), abundantly in animal 10A, and in small amounts in animals 30A, 24A, 21A and 38A (arrows). Animals vaccinated with 2 doses showed multifocal minimal to mild alveolar and interstitial pneumonia (*) together with minimal perivascular cuffing (arrowheads). Small quantities of viral RNA were observed by ISH within the lesions from animals 9A, 45A, 33A and 13A (arrows). Unvaccinated animals showed moderate multifocal alveolar and interstitial pneumonia (*), with presence of abundant viral RNA within the lesions from all animals (arrows).

[0050] **Figures 29A-29G** illustrate lung disease burden measured by histopathology and CT scan following challenge with SARS-CoV-2. Total histopathology score (**Fig. 29A**), and image analysis of area positively stained area in ISH RNAScope labelled sections for viral

RNA (**Fig. 29B**). **Fig. 29C** provides a heat map illustration of histopathology scoring for each parameter for individual animals. Total CT score (**Fig. 29E**). CT radiology scores for individual animals (**Figs. 29D-29G**). **Fig. 29D**: The extent of abnormality as a percentage of the lung affected. (**Fig. 29E**: COVID disease pattern with scoring based on presence of nodules, ground glass opacity, and consolidation. **Fig. 29F**: Zone classification (lung is divided into 12 zones and each zone showing abnormalities is attributed 1 point). **Fig. 29G**: Total cumulative CT score (Pattern + Zone scores). Line on graphs represent median value of group. * $p \leq 0.05$ with Mann-Whitney t test.

[0051] **Figure 30** illustrates representative example of pulmonary abnormalities identified on images constructed from CT scans. Images represent animals that did not receive a vaccination (control): 8A [A], 25A [B], 28A [C], 14A [D], 50A [E]; animals that received a single dose of INO-4800 vaccine: 10A [F], 21A [G], 38A [H]; animals that received two doses of INO-4800 vaccine: 21A [I], 33A [J]. Arrows indicate areas of ground glass opacification and areas of consolidation. Images from macaques that did not have abnormal features are not shown.

[0052] **Figures 31A through Figure 31F** depict ELISpot images of IFN-γ+ mouse splenocytes after stimulation with SARS-CoV-2 and SARS antigens. Mice were immunized on day 0 and splenocytes harvested at the indicated time points. IFNγ-secreting cells in the spleens of immunized animals were enumerated via ELISpot assay. Representative images show SARS-CoV-2 specific (**Figure 31A through Figure 31C**) and SARS-CoV-specific (**Figure 31D through Figure 31F**) IFNγ spot forming units in the splenocyte population at days 4, 7, and 10 post-immunization. Images were captured by ImmunoSpot CTL reader.

[0053] **Figure 32A and Figure 32B** depict flow cytometric analysis of T cell populations producing IFN-γ upon SARS-CoV-2 S protein stimulation. Splenocytes harvested from BALB/c and C57BL/6 mice 14 days after pVAX or INO-4800 treatment were made into single cell suspensions. The cells were stimulated for 6 hours with SARS-CoV-2 overlapping peptide pools. **Figure 32A**: CD4+ and CD8+ T cell gating strategy; singlets were gated on (i), then lymphocytes (ii) followed by live CD45+ cells (iii). Next CD3+ cells were gated (iv) and from that population CD4+ (v) and CD8+ (vi) T-cells were gated. IFNγ+ cells were gated from each of the CD4+ (vii) and CD8+ (viii) T-cell populations. **Figure 32B**: The percentage of CD4+ and CD8+ T cells producing IFNγ is depicted. Bars represent mean +SD. 4 BALB/c and 4 C57BL/6 mice were used in this study. * $p < 0.05$, Mann Whitney test.

[0054] **Figures 33A through 33H** depict humoral and cellular immune responses in rhesus macaques. **Figure 33A**: The study outline showing the vaccination regimen and blood

collection timepoints. **Figure 33B**: Schematic of SARS-CoV-2 spike protein. **Figure 33C**: SARS-CoV-2 S1+S2 ECD, S1, RBD and S2 protein antigen binding of IgG in serially diluted NHP sera collected on Week 0, 2, 6, 12 and 15. Data represents the mean endpoint titers for each individual NHP. (D&E) Pseudoneutralization assay using NHP sera, showing the presence of SARS-CoV-2 specific neutralizing antibodies against the D614 (**Figure 33D**) and G614 (**Figure 33E**) variants of SARS-CoV-2. **Figure 33F** and **Figure 33G**: Serum collected at Week 6 from INO-4800 vaccinated NHPs inhibited ACE2 binding. **Figure 33F**: Plate-based ACE2 competition assay. **Figure 33G**: Flow-based ACE2 inhibition assay showing that inhibition of ACE2 binding in serially diluted NHP sera. **Figure 33H**: T cell responses were measured by IFN-γ ELISpot in PBMCs harvested at weeks 0, 2, 6 and 15, and stimulated for 20h with overlapping peptide pools spanning the SARS-CoV-2 Spike protein. Bars represent the mean + SD.

[0055] **Figure 34** depicts serum IgG cross-reactivity to SARS-CoV and MERS-CoV spike protein. IgG binding was measured in sera from INO-4800 vaccinated rhesus macaques to SARS-CoV S1 and MERS-CoV S1 protein antigen.

[0056] **Figure 35** depicts bronchoalveolar lavage (BAL) IgG reactive to SARS-CoV-2 S protein antigens. BAL samples collected from vaccinated animals were assessed for SARS-CoV-2 reactive IgG binding to the full length SARS-CoV-2 spike protein and the RBD domain.

[0057] **Figure 36A and Figure 36B** depict exemplary experimental data demonstrating cellular response cross-reactivity to SARS-CoV and MERS-CoV spike protein. PBMC responses were analyzed by IFNγ ELISpot after stimulation with overlapping peptide pools spanning the SARS-CoV-1 spike protein (**Figure 36A**) and MERS-CoV spike protein (**Figure 36B**). Bars represent the mean + SD.

[0058] **Figure 37A through Figure 37C** depict exemplary experiments demonstrating recall of humoral immune responses after viral challenge. **Figure 37A**: Study outline. **Figure 37B**: IgG binding ELISA. SARS-CoV-2 S1+S2 and SARS-CoV-2 RBD protein antigen binding of IgG in diluted NHP sera collected prior to challenge, during challenge and post challenge. **Figure 37C**: Pseudo-neutralization assay using NHP sera, showing the presence of SARS-CoV-2 specific neutralizing antibodies against the D614 and G614 variants of SARS-CoV-2 before and after viral challenge in INO-4800 vaccinated (top panels) and naïve animals (bottom panels).

[0059] **Figure 38** depicts exemplary experiments demonstrating recall of cellular immune responses after viral challenge. T cells responses were analyzed by IFNγ ELISpot in PBMCs

stimulated with overlapping peptide pools spanning the SARS-CoV-2 spike protein. Bars represent the mean + SD. T cell responses analyzed by IFNγ ELISpot in PBMCs isolated pre and post challenge with SARS-CoV-2 virus. Left panel naïve animals, right panel INO-4800 vaccinated animals.

[0060] **Figure 39** depicts exemplary experiments demonstrating recall of cellular immune responses after viral challenge in individual rhesus macaques. Cellular responses were analyzed pre and post viral challenge by IFNγ ELISpot in PBMCs stimulated with overlapping peptide pools spanning the SARS-CoV-2 spike protein. Right panel naïve animals, left panel INO-4800 vaccinated animals.

[0061] **Figures 40A through 40F** depict viral loads in the BAL fluid and Nasal swabs after viral challenge. At week 17 naïve and INO-4800 immunized (5 per group) rhesus macaques were challenged by intranasal and intracheal administration of 1.1 x 10^4 PFU SARS-CoV-2 (US-WA1 isolate). **Figure 40A and Figure 40D**: Log sgmRNA copies/ml in BAL (**Figure 40A**), and NS copies/swab (**Figure 40D**) were measured at multiple timepoints following challenge in naïve (left panels) and INO-4800 vaccinated (right panels) animals. **Figure 40B and Figure 40E**: Peak viral loads (Between days 1 to 7) in BAL (**Figure 40B**) and NS (**Figure 40E**) following challenge. **Figure 40C and Figure 40F**: Viral RNA in BAL and NS at day 7 after challenge. Blue and Red lines reflect median viral loads. Mann-Whitney test P values are provided (**Figure 40B and Figure 40C**).

[0062] DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0063] Definitions

[0064] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0065] The term "comprising" is intended to include examples encompassed by the terms "consisting essentially of" and "consisting of"; similarly, the term "consisting essentially of" is intended to include examples encompassed by the term "consisting of." The present disclosure also contemplates other embodiments "comprising," "consisting of" and

"consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0066] It is to be appreciated that certain features of the disclosed materials and methods which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed materials and methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

[0067] The singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise.

[0068] The term "about" when used in reference to numerical ranges, cutoffs, or specific values is used to indicate that the recited values may vary by up to as much as 10% from the listed value. Thus, the term "about" is used to encompass variations of ± 10% or less, variations of ± 5% or less, variations of ± 1% or less, variations of ± 0.5% or less, or variations of ± 0.1% or less from the specified value. When values are expressed as approximations by use of the antecedent "about," it will be understood that the particular value forms another embodiment. Reference to a particular numerical value includes at least that particular value unless the context clearly dictates otherwise.

[0069] "Adjuvant" as used herein means any molecule added to the vaccine described herein to enhance the immunogenicity of the antigen.

[0070] "Antibody" as used herein means an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab′) 2, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody can be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

[0071] The term "biosimilar" (of an approved reference product/biological drug, i.e., reference listed drug) refers to a biological product that is highly similar to the reference product notwithstanding minor differences in clinically inactive components with no clinically meaningful differences between the biosimilar and the reference product in terms of safety, purity and potency, based upon data derived from (a) analytical studies that demonstrate that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components; (b) animal studies (including the assessment of toxicity); and/or (c) a clinical study or studies (including the assessment of immunogenicity and pharmacokinetics or pharmacodynamics) that are

sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which the reference product is licensed and intended to be used and for which licensure is sought for the biosimilar. The biosimilar may be an interchangeable product that may be substituted for the reference product at the pharmacy without the intervention of the prescribing healthcare professional. To meet the additional standard of "interchangeability," the biosimilar is to be expected to produce the same clinical result as the reference product in any given patient and, if the biosimilar is administered more than once to an individual, the risk in terms of safety or diminished efficacy of alternating or switching between the use of the biosimilar and the reference product is not greater than the risk of using the reference product without such alternation or switch. The biosimilar utilizes the same mechanisms of action for the proposed conditions of use to the extent the mechanisms are known for the reference product. The condition or conditions of use prescribed, recommended, or suggested in the labeling proposed for the biosimilar have been previously approved for the reference product. The route of administration, the dosage form, and/or the strength of the biosimilar are the same as those of the reference product and the biosimilar is manufactured, processed, packed or held in a facility that meets standards designed to assure that the biosimilar continues to be safe, pure and potent. The biosimilar may include minor modifications in the amino acid sequence when compared to the reference product, such as N- or C-terminal truncations that are not expected to change the biosimilar performance.

[0072] "Coding sequence" or "encoding nucleic acid" as used herein means the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to which the nucleic acid is administered.

[0073] "Complement" or "complementary" as used herein means Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

[0074] "Consensus" or "Consensus Sequence" as used herein may mean a synthetic nucleic acid sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple subtypes of a particular antigen. The sequence may be used to induce broad immunity against multiple subtypes, serotypes, or strains of a particular antigen. Synthetic antigens, such as fusion proteins, may be manipulated to generate consensus sequences (or consensus antigens).

[0075] "Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein means the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

[0076] "Fragment" as used herein means a nucleic acid sequence or a portion thereof that encodes a polypeptide capable of eliciting an immune response in a mammal. The fragments can be DNA fragments selected from at least one of the various nucleotide sequences that encode protein fragments set forth below.

[0077] "Fragment" or "immunogenic fragment" with respect to polypeptide sequences means a polypeptide capable of eliciting an immune response in a mammal that cross reacts with a full-length wild type strain SARS-CoV-2 antigen. Fragments of consensus proteins can comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% of a consensus protein. In some embodiments, fragments of consensus proteins can comprise at least 20 amino acids or more, at least 30 amino acids or more, at least 40 amino acids or more, at least 50 amino acids or more, at least 60 amino acids or more, at least 70 amino acids or more, at least 80 amino acids or more, at least 90 amino acids or more, at least 100 amino acids or more, at least 110 amino acids or more, at least 120 amino acids or more, at least 130 amino acids or more, at least 140 amino acids or more, at least 150 amino acids or more, at least 160 amino acids or more, at least 170 amino acids or more, at least 180 amino acids or more, at least 190 amino acids or more, at least 200 amino acids or more, at least 210 amino acids or more, at least 220 amino acids or more, at least 230 amino acids or more, or at least 240 amino acids or more of a consensus protein.

[0078] As used herein, the term "genetic construct" refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

[0079] "Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, means that the sequences have a specified percentage of residues

that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent. Identity can be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[0080] "Immune response" as used herein means the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of antigen. The immune response can be in the form of a cellular or humoral response, or both.

[0081] "Nucleic acid" or "oligonucleotide" or "polynucleotide" or "nucleic acid molecule" as used herein means at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid can be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that can hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

[0082] Nucleic acids can be single stranded or double-stranded or can contain portions of both double-stranded and single-stranded sequence. The nucleic acid can be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods.

[0083] "Operably linked" as used herein means that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter can be positioned 5′ (upstream) or 3′ (downstream) of a gene under its control. The distance between the promoter and a gene can be approximately the same as the distance between that promoter and the gene

it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance can be accommodated without loss of promoter function.

[0084] A "peptide," "protein," or "polypeptide" as used herein can mean a linked sequence of amino acids and can be natural, synthetic, or a modification or combination of natural and synthetic.

[0085] "Promoter" as used herein means a synthetic or naturally derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter can comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter can also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can regulate the expression of a gene component constitutively or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, and CMV IE promoter.

[0086] "Signal peptide" and "leader sequence" are used interchangeably herein and refer to an amino acid sequence that can be linked at the amino terminus of a SARS-CoV-2 protein set forth herein. Signal peptides/leader sequences typically direct localization of a protein. Signal peptides/leader sequences used herein preferably facilitate secretion of the protein from the cell in which it is produced. Signal peptides/leader sequences are often cleaved from the remainder of the protein, often referred to as the mature protein, upon secretion from the cell. Signal peptides/leader sequences are linked at the N terminus of the protein.

[0087] "Subject" as used herein can mean a mammal that wants or is in need of being immunized with a herein described immunogenic composition or vaccine. The mammal can be a human, chimpanzee, guinea pig, dog, cat, horse, cow, mouse, rabbit, or rat.

[0088] "Substantially identical" as used herein can mean that a first and second amino acid sequence are at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% over a region of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100

or more amino acids. Substantially identical can also mean that a first nucleic acid sequence and a second nucleic acid sequence are at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% over a region of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 or more nucleotides.

[0089] "Treatment" or "treating," as used herein can mean protecting of an animal from a disease through means of preventing, suppressing, repressing, or completely eliminating the disease. Preventing the disease involves administering an immunogenic composition or a vaccine of the present invention to an animal prior to onset of the disease. Suppressing the disease involves administering an immunogenic composition or a vaccine of the present invention to an animal after induction of the disease but before its clinical appearance. Repressing the disease involves administering an immunogenic composition or a vaccine of the present invention to an animal after clinical appearance of the disease.

[0090] As used herein, unless otherwise noted, the term "clinically proven" (used independently or to modify the terms "safe" and/or "effective") shall mean that it has been proven by a clinical trial wherein the clinical trial has met the approval standards of U.S. Food and Drug Administration, EMA or a corresponding national regulatory agency. For example, proof may be provided by the clinical trial(s) described in the examples provided herein.

[0091] The term "clinically proven safe", as it relates to a dose, dosage regimen, treatment or method with a SARS-CoV-2 antigen (for example, a SARS-CoV-2 spike antigen administered as pGX9501 or INO-4800 or a biosimilar thereof) refers to a favorable risk:benefit ratio with an acceptable frequency and/or acceptable severity of treatment-emergent adverse events (referred to as AEs or TEAEs) compared to the standard of care or to another comparator. An adverse event is an untoward medical occurrence in a patient administered a medicinal product. One index of safety is the National Cancer Institute (NCI) incidence of adverse events (AE) graded per Common Toxicity Criteria for Adverse Events CTCAE v4.03.

[0092] The terms "clinically proven efficacy" and "clinically proven effective" as used herein in the context of a dose, dosage regimen, treatment or method refer to the effectiveness of a particular dose, dosage or treatment regimen. Efficacy can be measured based on change in the course of the disease in response to an agent of the present invention. For example, a SARS-CoV-2 antigen (for example, a SARS-CoV-2 spike antigen administered as pGX9501

or INO-4800 or a biosimilar thereof) is administered to a patient in an amount and for a time sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder that is being treated. Various indicators that reflect the extent of the subject's illness, disease or condition may be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for example, clinically recognized indicators of disease severity, symptoms, or manifestations of the disorder in question. The degree of improvement generally is determined by a physician, who may make this determination based on signs, symptoms, biopsies, or other test results, and who may also employ questionnaires that are administered to the subject, such as quality-of-life questionnaires developed for a given disease. For example, a SARS-CoV-2 antigen (for example, a SARS-CoV-2 spike antigen administered as pGX9501 or INO-4800 or a biosimilar thereof) may be administered to achieve an improvement in a patient's condition related to SARS-CoV-2 infection. Improvement may be indicated by an improvement in an index of disease activity, by amelioration of clinical symptoms or by any other measure of disease activity.

[0093] "Variant" used herein with respect to a nucleic acid means (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

[0094] Variant can further be defined as a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Representative examples of "biological activity" include the ability to be bound by a specific antibody or to promote an immune response. Variant can also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte *et al.*, *J. Mol. Biol.* 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function.

In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions can be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

[0095] A variant may be a nucleic acid sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The nucleic acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof. A variant may be an amino acid sequence that is substantially identical over the full length of the amino acid sequence or fragment thereof. The amino acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino acid sequence or a fragment thereof.

[0096] "Vector" as used herein means a nucleic acid sequence containing an origin of replication. A vector can be a viral vector, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector can be a DNA or RNA vector. A vector can be a self-replicating extrachromosomal vector, and preferably, is a DNA plasmid.

[0097] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0098] ***Nucleic Acid Molecules, Antigens, and Immunogenic Compositions***

[0099] Provided herein are immunogenic compositions, such as vaccines, comprising a nucleic acid molecule encoding a SARS-CoV-2 antigen, a fragment thereof, a variant thereof, or a combination thereof. Also provided herein are immunogenic compositions, such as vaccines, comprising a SARS-CoV-2 antigen, a fragment thereof, a variant thereof, or a combination thereof. The immunogenic compositions can be used to protect against and treat any number of strains of SARS-CoV-2, thereby treating, preventing, and/or protecting against SARS-CoV-2-based pathologies. The immunogenic compositions can significantly induce an immune response of a subject administered the immunogenic compositions, thereby protecting against and treating SARS-CoV-2 infection.

[0100] The immunogenic composition can be a DNA vaccine, a peptide vaccine, or a combination DNA and peptide vaccine. The DNA vaccine can include a nucleic acid molecule encoding the SARS-CoV-2 antigen. The nucleic acid molecule can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The nucleic acid molecule can also include additional sequences that encode linker, leader, or tag sequences that are linked to the nucleic acid molecule encoding the SARS-CoV-2 antigen by a peptide bond. The peptide vaccine can include a SARS-CoV-2 antigenic peptide, a SARS-CoV-2 antigenic protein, a variant thereof, a fragment thereof, or a combination thereof. The combination DNA and peptide vaccine can include the above described nucleic acid molecule encoding the SARS-CoV-2 antigen and the SARS-CoV-2 antigenic peptide or protein, in which the SARS-CoV-2 antigenic peptide or protein and the encoded SARS-CoV-2 antigen have the same amino acid sequence.

[0101] The disclosed immunogenic compositions can elicit both humoral and cellular immune responses that target the SARS-CoV-2 antigen in the subject administered the immunogenic composition. The disclosed immunogenic compositions can elicit neutralizing antibodies and immunoglobulin G (IgG) antibodies that are reactive with the SARS-CoV-2 spike antigen. The immunogenic composition can also elicit CD8+ and CD4+ T cell responses that are reactive to the SARS-CoV-2 antigen and produce interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin-2 (IL-2).

[0102] The immunogenic composition can induce a humoral immune response in the subject administered the immunogenic composition. The induced humoral immune response can be specific for the SARS-CoV-2 antigen. The induced humoral immune response can be reactive with the SARS-CoV-2 antigen. The humoral immune response can be induced in the subject administered the vaccine by about 1.5-fold to about 16-fold, about 2-fold to about 12-fold, or about 3-fold to about 10-fold. The humoral immune response can be induced in the

subject administered the vaccine by at least about 1.5-fold, at least about 2.0-fold, at least about 2.5-fold, at least about 3.0-fold, at least about 3.5-fold, at least about 4.0-fold, at least about 4.5-fold, at least about 5.0-fold, at least about 5.5-fold, at least about 6.0-fold, at least about 6.5-fold, at least about 7.0-fold, at least about 7.5-fold, at least about 8.0-fold, at least about 8.5-fold, at least about 9.0-fold, at least about 9.5-fold, at least about 10.0-fold, at least about 10.5-fold, at least about 11.0-fold, at least about 11.5-fold, at least about 12.0-fold, at least about 12.5-fold, at least about 13.0-fold, at least about 13.5-fold, at least about 14.0-fold, at least about 14.5-fold, at least about 15.0-fold, at least about 15.5-fold, or at least about 16.0-fold.

[0103] The humoral immune response induced by the immunogenic composition can include an increased level of neutralizing antibodies associated with the subject administered the immunogenic composition as compared to a subject not administered the immunogenic composition. The neutralizing antibodies can be specific for the SARS-CoV-2 antigen. The neutralizing antibodies can be reactive with the SARS-CoV-2 antigen. The neutralizing antibodies can provide protection against and/or treatment of SARS-CoV-2 infection and its associated pathologies in the subject administered the immunogenic composition.

[0104] The humoral immune response induced by the immunogenic composition can include an increased level of IgG antibodies associated with the subject administered the immunogenic composition as compared to a subject not administered the immunogenic composition. These IgG antibodies can be specific for the SARS-CoV-2 antigen. These IgG antibodies can be reactive with the SARS-CoV-2 antigen. The level of IgG antibody associated with the subject administered the immunogenic composition can be increased by about 1.5-fold to about 16-fold, about 2-fold to about 12-fold, or about 3-fold to about 10-fold as compared to the subject not administered the immunogenic composition. The level of IgG antibody associated with the subject administered the immunogenic composition can be increased by at least about 1.5-fold, at least about 2.0-fold, at least about 2.5-fold, at least about 3.0-fold, at least about 3.5-fold, at least about 4.0-fold, at least about 4.5-fold, at least about 5.0-fold, at least about 5.5-fold, at least about 6.0-fold, at least about 6.5-fold, at least about 7.0-fold, at least about 7.5-fold, at least about 8.0-fold, at least about 8.5-fold, at least about 9.0-fold, at least about 9.5-fold, at least about 10.0-fold, at least about 10.5-fold, at least about 11.0-fold, at least about 11.5-fold, at least about 12.0-fold, at least about 12.5-fold, at least about 13.0-fold, at least about 13.5-fold, at least about 14.0-fold, at least about 14.5-fold, at least about 15.0-fold, at least about 15.5-fold, or at least about 16.0-fold as compared to the subject not administered the immunogenic composition.

[0105] The immunogenic composition can induce a cellular immune response in the subject administered the immunogenic composition. The induced cellular immune response can be specific for the SARS-CoV-2 antigen. The induced cellular immune response can be reactive to the SARS-CoV-2 antigen. The induced cellular immune response can include eliciting a CD8+ T cell response. The elicited CD8+ T cell response can be reactive with the SARS-CoV-2 antigen. The elicited CD8+ T cell response can be polyfunctional. The induced cellular immune response can include eliciting a CD8+ T cell response, in which the CD8+ T cells produce interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-2 (IL-2), or a combination of IFN-γ and TNF-α.

[0106] The induced cellular immune response can include an increased CD8+ T cell response associated with the subject administered the immunogenic composition as compared to the subject not administered the immunogenic composition. The CD8+ T cell response associated with the subject administered the immunogenic composition can be increased by about 2-fold to about 30-fold, about 3-fold to about 25-fold, or about 4-fold to about 20-fold as compared to the subject not administered the immunogenic composition. The CD8+ T cell response associated with the subject administered the immunogenic composition can be increased by at least about 1.5-fold, at least about 2.0-fold, at least about 3.0-fold, at least about 4.0-fold, at least about 5.0-fold, at least about 6.0-fold, at least about 6.5-fold, at least about 7.0-fold, at least about 7.5-fold, at least about 8.0-fold, at least about 8.5-fold, at least about 9.0-fold, at least about 9.5-fold, at least about 10.0-fold, at least about 10.5-fold, at least about 11.0-fold, at least about 11.5-fold, at least about 12.0-fold, at least about 12.5-fold, at least about 13.0-fold, at least about 13.5-fold, at least about 14.0-fold, at least about 14.5-fold, at least about 15.0-fold, at least about 16.0-fold, at least about 17.0-fold, at least about 18.0-fold, at least about 19.0-fold, at least about 20.0-fold, at least about 21.0-fold, at least about 22.0-fold, at least about 23.0-fold, at least about 24.0-fold, at least about 25.0-fold, at least about 26.0-fold, at least about 27.0-fold, at least about 28.0-fold, at least about 29.0-fold, or at least about 30.0-fold as compared to the subject not administered the immunogenic composition.

[0107] The induced cellular immune response can include an increased frequency of CD3+CD8+ T cells that produce IFN-γ. The frequency of CD3+CD8+IFN-γ+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold as compared to the subject not administered the immunogenic composition.

[0108] The induced cellular immune response can include an increased frequency of CD3+CD8+ T cells that produce TNF-α. The frequency of CD3+CD8+TNF-α+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, or 14-fold as compared to the subject not administered the immunogenic composition.

[0109] The induced cellular immune response can include an increased frequency of CD3+CD8+ T cells that produce IL-2. The frequency of CD3+CD8+IL-2+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 0.5-fold, 1.0-fold, 1.5-fold, 2.0-fold, 2.5-fold, 3.0-fold, 3.5-fold, 4.0-fold, 4.5-fold, or 5.0-fold as compared to the subject not administered the immunogenic composition.

[0110] The induced cellular immune response can include an increased frequency of CD3+CD8+ T cells that produce both IFN-γ and TNF-α. The frequency of CD3+CD8+IFN-γ+TNF-α+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 85-fold, 90-fold, 95-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, or 180-fold as compared to the subject not administered the immunogenic composition.

[0111] The cellular immune response induced by the immunogenic composition can include eliciting a CD4+ T cell response. The elicited CD4+ T cell response can be reactive with the SARS-CoV-2 antigen. The elicited CD4+ T cell response can be polyfunctional. The induced cellular immune response can include eliciting a CD4+ T cell response, in which the CD4+ T cells produce IFN-γ, TNF-α, IL-2, or a combination of IFN-γ and TNF-α.

[0112] The induced cellular immune response can include an increased frequency of CD3+CD4+ T cells that produce IFN-γ. The frequency of CD3+CD4+IFN-γ+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold as compared to the subject not administered the immunogenic composition.

[0113] The induced cellular immune response can include an increased frequency of CD3+CD4+ T cells that produce TNF-α. The frequency of CD3+CD4+TNF-α+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-

fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 21-fold, or 22-fold as compared to the subject not administered the immunogenic composition.

[0114] The induced cellular immune response can include an increased frequency of CD3+CD4+ T cells that produce IL-2. The frequency of CD3+CD4+IL-2+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 21-fold, 22-fold, 23-fold, 24-fold, 25-fold, 26-fold, 27-fold, 28-fold, 29-fold, 30-fold, 31-fold, 32-fold, 33-fold, 34-fold, 35-fold, 36-fold, 37-fold, 38-fold, 39-fold, 40-fold, 45-fold, 50-fold, 55-fold, or 60-fold as compared to the subject not administered the immunogenic composition.

[0115] The induced cellular immune response can include an increased frequency of CD3+CD4+ T cells that produce both IFN-γ and TNF-α. The frequency of CD3+CD4+IFN-γ+TNF-α+ associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 2.5-fold, 3.0-fold, 3.5-fold, 4.0-fold, 4.5-fold, 5.0-fold, 5.5-fold, 6.0-fold, 6.5-fold, 7.0-fold, 7.5-fold, 8.0-fold, 8.5-fold, 9.0-fold, 9.5-fold, 10.0-fold, 10.5-fold, 11.0-fold, 11.5-fold, 12.0-fold, 12.5-fold, 13.0-fold, 13.5-fold, 14.0-fold, 14.5-fold, 15.0-fold, 15.5-fold, 16.0-fold, 16.5-fold, 17.0-fold, 17.5-fold, 18.0-fold, 18.5-fold, 19.0-fold, 19.5-fold, 20.0-fold, 21-fold, 22-fold, 23-fold 24-fold, 25-fold, 26-fold, 27-fold, 28-fold, 29-fold, 30-fold, 31-fold, 32-fold, 33-fold, 34-fold, or 35-fold as compared to the subject not administered the immunogenic composition.

[0116] The immunogenic composition of the present invention can have features required of effective immunogenic compositions such as being safe so the immunogenic composition itself does not cause illness or death; is protective against illness resulting from exposure to live pathogens such as viruses or bacteria; induces neutralizing antibody to prevent invention of cells; induces protective T cells against intracellular pathogens; and provides ease of administration, few side effects, biological stability, and low cost per dose.

[0117] The immunogenic composition can further induce an immune response when administered to different tissues such as the muscle or skin. The immunogenic composition can further induce an immune response when parenterally administered, for example by subcutaneous, intradermal, or intramuscular injection, optionally followed by electroporation as described herein.

[0118] a. SARS-CoV-2 Antigen and Nucleic Acid Molecules Encoding the Same

[0119] As described above, provided herein are immunogenic compositions comprising a nucleic acid molecule encoding a SARS-CoV-2 antigen, a fragment thereof, a variant thereof, or a combination thereof. Also provided herein are immunogenic compositions comprising a SARS-CoV-2 antigen, a fragment thereof, a variant thereof, or a combination thereof.

[0120] Upon binding cell surface proteins and membrane fusion, the coronavirus enters the cell and its singled-stranded RNA genome is released into the cytoplasm of the infected cell. The singled-stranded RNA genome is a positive strand and thus, can be translated into a RNA polymerase, which produces additional viral RNAs that are minus strands. Accordingly, the SARS-CoV-2 antigen can also be a SARS-CoV-2 RNA polymerase.

[0121] The viral minus RNA strands are transcribed into smaller, subgenomic positive RNA strands, which are used to translate other viral proteins, for example, nucleocapsid (N) protein, envelope (E) protein, and matrix (M) protein. Accordingly, the SARS-CoV-2 antigen can comprise a SARS-CoV-2 nucleocapsid protein, a SARS-CoV-2 envelope protein, or a SARS-CoV-2 matrix protein.

[0122] The viral minus RNA strands can also be used to replicate the viral genome, which is bound by nucleocapsid protein. Matrix protein, along with spike protein, is integrated into the endoplasmic reticulum of the infected cell. Together, the nucleocapsid protein bound to the viral genome and the membrane-embedded matrix and spike proteins are budded into the lumen of the endoplasmic reticulum, thereby encasing the viral genome in a membrane. The viral progeny are then transported by golgi vesicles to the cell membrane of the infected cell and released into the extracellular space by endocytosis.

[0123] Coronaviruses, including SARS-CoV-2, are encapsulated by a membrane and have a type 1 membrane glycoprotein known as spike (S) protein, which forms protruding spikes on the surface of the coronavirus. The SARS-CoV-2 S protein is a class I membrane fusion protein, which is the major envelope protein on the surface of coronaviruses. The spike protein facilitates binding of the coronavirus to proteins located on the surface of a cell, for example, the metalloprotease amino peptidase N, and mediates cell-viral membrane fusion. In particular, the spike protein contains an S1 subunit that facilitates binding of the coronavirus to cell surface proteins. Accordingly, the S1 subunit of the spike protein controls which cells are infected by the coronavirus. The spike protein also contains a S2 subunit, which is a transmembrane subunit that facilitates viral and cellular membrane fusion. Accordingly, the SARS-CoV-2 antigen can comprise a SARS-CoV-2 spike protein, a S1 subunit of a SARS-CoV-2 spike protein, a S2 subunit of a SARS-CoV-2 spike protein, or a fragment of the S1 subunit comprising the SARS-CoV-2 Spike Receptor Binding Domain.

[0124] In some embodiments, the SARS-CoV-2 antigen can be a SARS-CoV-2 spike protein, a SARS-CoV-2 RNA polymerase, a SARS-CoV-2 nucleocapsid protein, a SARS-CoV-2 envelope protein, a SARS-CoV-2 matrix protein, a fragment thereof, a variant thereof, or a combination thereof.

[0125] The SARS-CoV-2 antigen can be a SARS-CoV-2 spike antigen, a fragment thereof, a variant thereof, or a combination thereof. The SARS-CoV-2 spike antigen is capable of eliciting an immune response in a mammal against one or more SARS-CoV-2 strains. The SARS-CoV-2 spike antigen can comprise an epitope(s) that makes it particularly effective as an immunogen against which an anti- SARS-CoV-2 immune response can be induced.

[0126] The SARS-CoV-2 antigen can be a consensus antigen derived from two or more strains of SARS-CoV-2. In some embodiments, the SARS-CoV-2 antigen is a SARS-CoV-2 consensus spike antigen. The SARS-CoV-2 consensus spike antigen can be derived from the sequences of spike antigens from strains of SARS-CoV-2, and thus, the SARS-CoV-2 consensus spike antigen is unique. In some embodiments, the SARS-CoV-2 consensus spike antigen can be an outlier spike antigen, having a greater amino acid sequence divergence from other SARS-CoV-2 spike proteins. Accordingly, the immunogenic compositions of the present invention are widely applicable to multiple strains of SARS-CoV-2 because of the unique sequences of the SARS-CoV-2 consensus spike antigen. These unique sequences allow the vaccine to be universally protective against multiple strains of SARS-CoV-2, including genetically diverse variants of SARS-CoV-2. Nucleic acid molecules encoding the SARS-CoV-2 antigen can be modified for improved expression. Modification can include codon optimization, RNA optimization, addition of a kozak sequence for increased translation initiation, and/or the addition of an immunoglobulin leader sequence to increase the immunogenicity of the SARS-CoV-2 antigen. The SARS-CoV-2 spike antigen can comprise a signal peptide such as an immunoglobulin signal peptide, for example, but not limited to, an immunoglobulin E (IgE) or immunoglobulin (IgG) signal peptide. In some embodiments, the SARS-CoV-2 spike antigen can comprise a hemagglutinin (HA) tag. The SARS-CoV-2 spike antigen can be designed to elicit stronger and broader cellular and/or humoral immune responses than a corresponding codon optimized spike antigen.

[0127] In some embodiments, the SARS-CoV-2 antigen comprises an amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1. In some embodiments the SARS-CoV-2 antigen comprises the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1. In

some embodiments, the SARS-CoV-2 antigen comprises an amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of SEQ ID NO: 1. In some embodiments the SARS-CoV-2 antigen comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments the nucleic acid molecule encoding the SARS-CoV-2 antigen comprises the nucleotide sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the sequence set forth in nucleotides 55 to 3837 of SEQ ID NO:2, SEQ ID NO: 2, or SEQ ID NO: 3.

[0128] In some embodiments the SARS-CoV-2 antigen comprises an amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4 or over an entire length of SEQ ID NO: 4. In some embodiments the SARS-CoV-2 antigen comprises the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4. In some embodiments the SARS-CoV-2 antigen comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments the nucleic acid molecule encoding the SARS-CoV-2 antigen comprises: a nucleic acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of nucleotides 55 to 3837 of SEQ ID NO: 5 or over an entire length of SEQ ID NO: 5; the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 5; the nucleic acid sequence of SEQ ID NO: 5; a nucleic acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of SEQ ID NO: 6; or the nucleic acid sequence of SEQ ID NO: 6.

[0129] In some embodiments the SARS-CoV-2 antigen is operably linked to an IgE leader sequence. In some such embodiments, the SARS-CoV-2 antigen comprises the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the SARS-CoV-2 antigen is encoded by the nucleotide sequence set forth in SEQ ID NO:2 or SEQ ID NO: 3. In some embodiments in which the SARS-CoV-2 antigen includes an IgE leader, the SARS-CoV-2 antigen comprises the amino acid sequence set forth in SEQ ID NO: 4. In some such embodiments, the SARS-CoV-2 antigen is encoded by the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO: 6.

[0130] Immunogenic fragments of SEQ ID NO:1 can be provided. Immunogenic fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least

85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:1. In some embodiments, immunogenic fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, immunogenic fragments are free of a leader sequence.

[0131] Immunogenic fragments of proteins with amino acid sequences homologous to immunogenic fragments of SEQ ID NO:1 can be provided. Such immunogenic fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of proteins that are 95% homologous to SEQ ID NO:1. Some embodiments relate to immunogenic fragments that have 96% homology to the immunogenic fragments of consensus protein sequences herein. Some embodiments relate to immunogenic fragments that have 97% homology to the immunogenic fragments of consensus protein sequences herein. Some embodiments relate to immunogenic fragments that have 98% homology to the immunogenic fragments of consensus protein sequences herein. Some embodiments relate to immunogenic fragments that have 99% homology to the immunogenic fragments of consensus protein sequences herein. In some embodiments, immunogenic fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, immunogenic fragments are free of a leader sequence.

[0132] Some embodiments relate to immunogenic fragments of SEQ ID NO:1. Immunogenic fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:1. Immunogenic fragments can be at least 95%, at least 96%, at least 97% at least 98% or at least 99% homologous to fragments of SEQ ID NO:1. In some embodiments, immunogenic fragments include sequences that encode a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of coding sequences that encode a leader sequence.

[0133] Immunogenic fragments of SEQ ID NO:4 can be provided. Immunogenic fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:4. In some embodiments, immunogenic fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, immunogenic fragments are free of a leader sequence.

[0134] Immunogenic fragments of proteins with amino acid sequences homologous to immunogenic fragments of SEQ ID NO:4 can be provided. Such immunogenic fragments can

comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of proteins that are 95% homologous to SEQ ID NO:4. Some embodiments relate to immunogenic fragments that have 96% homology to the immunogenic fragments of consensus protein sequences herein. Some embodiments relate to immunogenic fragments that have 97% homology to the immunogenic fragments of consensus protein sequences herein. Some embodiments relate to immunogenic fragments that have 98% homology to the immunogenic fragments of consensus protein sequences herein. Some embodiments relate to immunogenic fragments that have 99% homology to the immunogenic fragments of consensus protein sequences herein. In some embodiments, immunogenic fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, immunogenic fragments are free of a leader sequence.

[0135] Some embodiments relate to immunogenic fragments of SEQ ID NO:4. Immunogenic fragments can be at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of SEQ ID NO:4. Immunogenic fragments can be at least 95%, at least 96%, at least 97% at least 98% or at least 99% homologous to fragments of SEQ ID NO:4. In some embodiments, immunogenic fragments include sequences that encode a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of coding sequences that encode a leader sequence.

[0136] b. Vector

[0137] The immunogenic compositions can comprise one or more vectors that include a nucleic acid molecule encoding the SARS-CoV-2antigen. The one or more vectors can be capable of expressing the antigen. The vector can have a nucleic acid sequence containing an origin of replication. The vector can be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. The vector can be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

[0138] The one or more vectors can be an expression construct, which is generally a plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the protein that is encoded by the gene is produced by the cellular-transcription and translation machinery ribosomal complexes. The plasmid is frequently engineered to contain regulatory sequences that act as enhancer and promoter regions and

lead to efficient transcription of the gene carried on the expression vector. The vectors of the present invention express large amounts of stable messenger RNA, and therefore proteins.

[0139] The vectors may have expression signals such as a strong promoter, a strong termination codon, adjustment of the distance between the promoter and the cloned gene, and the insertion of a transcription termination sequence and a PTIS (portable translation initiation sequence).

[0140] (1) Expression Vectors

[0141] The vector can be a circular plasmid or a linear nucleic acid. The circular plasmid and linear nucleic acid are capable of directing expression of a particular nucleotide sequence in an appropriate subject cell. The vector can have a promoter operably linked to the antigen-encoding nucleotide sequence, which may be operably linked to termination signals. The vector can also contain sequences required for proper translation of the nucleotide sequence. The vector comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development.

[0142] (2) Circular and Linear Vectors

[0143] The vector may be a circular plasmid, which may transform a target cell by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication).

[0144] The vector can be pVAX, pcDNA3.0, pGX-0001, or provax, or any other expression vector capable of expressing DNA encoding the antigen and enabling a cell to translate the sequence to an antigen that is recognized by the immune system.

[0145] Also provided herein is a linear nucleic acid immunogenic composition, or linear expression cassette ("LEC"), that is capable of being efficiently delivered to a subject via electroporation and expressing one or more desired antigens. The LEC may be any linear DNA devoid of any phosphate backbone. The DNA may encode one or more antigens. The LEC may contain a promoter, an intron, a stop codon, and/or a polyadenylation signal. The expression of the antigen may be controlled by the promoter. The LEC may not contain any

antibiotic resistance genes and/or a phosphate backbone. The LEC may not contain other nucleic acid sequences unrelated to the desired antigen gene expression.

[0146] The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the antigen. The plasmid can be pNP (Puerto Rico/34) or pM2 (New Caledonia/99). The plasmid may be WLV009, pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing DNA encoding the antigen and enabling a cell to translate the sequence to an antigen that is recognized by the immune system.

[0147] The LEC can be perM2. The LEC can be perNP. perNP and perMR can be derived from pNP (Puerto Rico/34) and pM2 (New Caledonia/99), respectively.

[0148] (3) Promoter, Intron, Stop Codon, and Polyadenylation Signal

[0149] The vector may have a promoter. A promoter may be any promoter that is capable of driving gene expression and regulating expression of the isolated nucleic acid. Such a promoter is a cis-acting sequence element required for transcription via a DNA dependent RNA polymerase, which transcribes the antigen sequence described herein. Selection of the promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter may be positioned about the same distance from the transcription start in the vector as it is from the transcription start site in its natural setting. However, variation in this distance may be accommodated without loss of promoter function.

[0150] The promoter may be operably linked to the nucleic acid sequence encoding the antigen and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. The promoter may be a CMV promoter, SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or another promoter shown effective for expression in eukaryotic cells.

[0151] The vector may include an enhancer and an intron with functional splice donor and acceptor sites. The vector may contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

[0152] c. Excipients and Other Components of the Immunogenic compositions

[0153] The immunogenic compositions may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient can be functional molecules such as vehicles, carriers, buffers, or diluents. As used herein. "buffer" refers to a buffered

solution that resists changes in pH by the action of its acid-base conjugate components. The buffer generally has a pH from about 4.0 to about 8.0, for example from about 5.0 to about 7.0. In some embodiments, the buffer is saline-sodium citrate (SSC) buffer. In some embodiments in which the immunogenic composition comprises a nucleic acid molecule encoding a SARS-CoV-2 spike antigen as described above, the immunogenic composition comprises 10 mg/ml of vector in buffer, for example but not limited to SSC buffer. In some embodiments, the immunogenic composition comprises 10 mg/mL of the DNA plasmid pGX9501 or pGX9503 in buffer. In some embodiments, the immunogenic composition is stored at about 2°C to about 8°C. In some embodiments, the immunogenic composition is stored at room temperature. The immunogenic composition may be stored for at least a year at room temperature. In some embodiments, the immunogenic composition is stable at room temperature for at least a year, wherein stability is defined as a supercoiled plasmid percentage of at least about 80%. In some embodiments, the supercoiled plasmid percentage is at least about 85% following storage for at least a year at room temperature.

[0154] The pharmaceutically acceptable excipient can be a transfection facilitating agent, which can include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

[0155] The transfection facilitating agent may be a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and the poly-L-glutamate may be present in the immunogenic composition at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the genetic construct. The DNA plasmid immunogenic compositions may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example WO9324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the immunogenic composition is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less

than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

[0156] The pharmaceutically acceptable excipient can be an adjuvant. The adjuvant can be other genes that are expressed in an alternative plasmid or are delivered as proteins in combination with the plasmid above in the immunogenic composition. The adjuvant may be selected from the group consisting of: α-interferon(IFN-α), β-interferon (IFN-β), γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15, MHC, CD80, CD86 including IL-15 having the signal sequence deleted and optionally including the signal peptide from IgE. The adjuvant can be IL-12, IL-15, IL-28, CTACK, TECK, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, or a combination thereof.

[0157] Other genes that can be useful as adjuvants include those encoding: MCP-1, MIP-1a, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, IL-22, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof.

[0158] The immunogenic composition may further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

[0159] The immunogenic composition can be formulated according to the mode of administration to be used. According to some embodiments, the immunogenic composition is formulated in a buffer, optionally saline-sodium citrate buffer. For example, the immunogenic composition may formulated at a concentration of 10 mg nucleic acid molecule per milliliter of a sodium salt citrate buffer. An injectable immunogenic pharmaceutical composition can be sterile, pyrogen free and particulate free. An isotonic formulation or solution can be used. Additives for isotonicity can include sodium chloride, dextrose,

mannitol, sorbitol, and lactose. The immunogenic composition can comprise a vasoconstriction agent. The isotonic solutions can include phosphate buffered saline. Immunogenic compositions can further comprise stabilizers including gelatin and albumin. The stabilizers can allow the formulation to be stable at room or ambient temperature for extended periods of time, including LGS or polycations or polyanions.

[0160] Also provided herein are articles of manufacture comprising the immunogenic composition. In some embodiments, the article of manufacture is a container holding the immunogenic composition. The container may be, for example but not limited to, a syringe or a vial. The vial may have a stopper piercable by a syringe.

[0161] The immunogenic composition can be packaged in suitably sterilized containers such as ampules, bottles, or vials, either in multi-dose or in unit dosage forms. The containers are preferably hermetically sealed after being filled with a vaccine preparation. Preferably, the vaccines are packaged in a container having a label affixed thereto, which label identifies the vaccine, and bears a notice in a form prescribed by a government agency such as the United States Food and Drug Administration reflecting approval of the vaccine under appropriate laws, dosage information, and the like. The label preferably contains information about the vaccine that is useful to a health care professional administering the vaccine to a patient. The package also preferably contains printed informational materials relating to the administration of the vaccine, instructions, indications, and any necessary required warnings.

[0162] Methods of Vaccination

[0163] Also provided herein are methods of treating, protecting against, and/or preventing disease in a subject in need thereof by administering the immunogenic composition to the subject. Administration of the immunogenic composition to the subject can induce or elicit an immune response in the subject. The induced immune response can be used to treat, prevent, and/or protect against disease, for example, pathologies relating to SARS-CoV-2 infection. The induced immune response in the subject administered the immunogenic composition can provide resistance to one or more SARS-CoV-2 strains.

[0164] The induced immune response can include an induced humoral immune response and/or an induced cellular immune response. The humoral immune response can be induced by about 1.5-fold to about 16-fold, about 2-fold to about 12-fold, or about 3-fold to about 10-fold. The induced humoral immune response can include IgG antibodies and/or neutralizing antibodies that are reactive to the antigen. The induced cellular immune response can include

a CD8+ T cell response, which is induced by about 2-fold to about 30-fold, about 3-fold to about 25-fold, or about 4-fold to about 20-fold.

[0165] The vaccine dose can be between 1 μg to 10 mg active component/kg body weight/time, and can be 20 μg to 10 mg component/kg body weight/time. The vaccine can be administered every 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or more days or every 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more weeks. The number of vaccine doses for effective treatment can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

[0166] In one embodiment, the total vaccine dose is 1.0 mg of nucleic acid. In one embodiment, the total vaccine dose is 2.0 mg of nucleic acid, administered as 2x1.0mg nucleic acid.

[0167] a. Administration

[0168] The immunogenic composition can be formulated in accordance with standard techniques well known to those skilled in the pharmaceutical art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical arts taking into consideration such factors as the age, sex, weight, and condition of the particular subject, and the route of administration. The vaccine may be administered, for example, in one, two, three, four, or more injections. In some embodiments, an initial dose of about 0.5 mg to about 2.0 mg of the nucleic acid molecule is administered to the subject. The initial dose may be administered in one, two, three, or more injections. The initial dose may be followed by administration of one, two, three, four, or more subsequent doses of about 0.5 mg to about 2.0 mg of the nucleic acid molecule about one, two, three, four, five, six, seven, eight, ten, twelve or more weeks after the immediately prior dose. Each subsequent dose may be administered in one, two, three, or more injections. In some embodiments, the immunogenic composition is administered to the subject before, with, or after the additional agent. In some embodiments, the immunogenic composition is administered as a booster following administration of an agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection. In one embodiment, the disease or disorder associated with SARS-CoV-2 infection includes, but is not limited to, to Coronavirus Disease 2019 (COVID-19). In some embodiments, the disease or disorder associated with SARS-CoV-2 infection is Multisystem inflammatory syndrome in adults (MIS-A) or Multisystem inflammatory syndrome in children (MIS-C).

[0169] The subject can be a mammal, such as a human, a horse, a nonhuman primate, a cow, a pig, a sheep, a cat, a dog, a guinea pig, a rabbit, a rat, or a mouse.

[0170] The vaccine can be administered prophylactically or therapeutically. In prophylactic administration, the vaccines can be administered in an amount sufficient to induce an immune response. In therapeutic applications, the vaccines are administered to a subject in need thereof in an amount sufficient to elicit a therapeutic effect. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the particular composition of the vaccine regimen administered, the manner of administration, the stage and severity of the disease, the general state of health of the patient, and the judgment of the prescribing physician.

[0171] The vaccine can be administered by methods well known in the art as described in Donnelly et al. (Ann. Rev. Immunol. 15:617-648 (1997)); Felgner et al. (U.S. Pat. No. 5,580,859, issued Dec. 3, 1996); Felgner (U.S. Pat. No. 5,703,055, issued Dec. 30, 1997); and Carson et al. (U.S. Pat. No. 5,679,647, issued Oct. 21, 1997), the contents of all of which are incorporated herein by reference in their entirety. The DNA of the vaccine can be complexed to particles or beads that can be administered to an individual, for example, using a vaccine gun. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the expression vector.

[0172] The vaccine can be delivered via a variety of routes. Typical delivery routes include parenteral administration, e.g., intradermal, intramuscular or subcutaneous delivery. Other routes include oral administration, intranasal, and intravaginal routes. For the DNA of the vaccine in particular, the vaccine can be delivered to the interstitial spaces of tissues of an individual (Felgner et al., U.S. Pat. Nos. 5,580,859 and 5,703,055, the contents of all of which are incorporated herein by reference in their entirety). The vaccine can also be administered to muscle, or can be administered via intradermal or subcutaneous injections, or transdermally, such as by iontophoresis. Epidermal administration of the vaccine can also be employed. Epidermal administration can involve mechanically or chemically irritating the outermost layer of epidermis to stimulate an immune response to the irritant (Carson et al., U.S. Pat. No. 5,679,647, the contents of which are incorporated herein by reference in its entirety). Parenteral administration may optionally be followed with electroporation as described herein.

[0173] The vaccine can also be formulated for administration via the nasal passages. Formulations suitable for nasal administration, wherein the carrier is a solid, can include a

coarse powder having a particle size, for example, in the range of about 10 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. The formulation can be a nasal spray, nasal drops, or by aerosol administration by nebulizer. The formulation can include aqueous or oily solutions of the vaccine.

[0174] The vaccine can be a liquid preparation such as a suspension, syrup or elixir. The vaccine can also be a preparation for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as a sterile suspension or emulsion.

[0175] The vaccine can be incorporated into liposomes, microspheres or other polymer matrices (Felgner et al., U.S. Pat. No. 5,703,055; Gregoriadis, Liposome Technology, Vols. I to III (2nd ed. 1993), the contents of which are incorporated herein by reference in their entirety). Liposomes can consist of phospholipids or other lipids, and can be nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

[0176] The vaccine can be administered via electroporation, such as by a method described in U.S. Pat. No. 7,664,545, the contents of which are incorporated herein by reference. The electroporation can be by a method and/or apparatus described in U.S. Pat. Nos. 6,302,874; 5,676,646; 6,241,701; 6,233,482; 6,216,034; 6,208,893; 6,192,270; 6,181,964; 6,150,148; 6,120,493; 6,096,020; 6,068,650; and 5,702,359, the contents of which are incorporated herein by reference in their entirety. The electroporation may be carried out via a minimally invasive device.

[0177] The minimally invasive electroporation device ("MID") may be an apparatus for injecting the vaccine described above and associated fluid into body tissue. The device may comprise a hollow needle, DNA cassette, and fluid delivery means, wherein the device is adapted to actuate the fluid delivery means in use so as to concurrently (for example, automatically) inject DNA into body tissue during insertion of the needle into the said body tissue. This has the advantage that the ability to inject the DNA and associated fluid gradually while the needle is being inserted leads to a more even distribution of the fluid through the body tissue. The pain experienced during injection may be reduced due to the distribution of the DNA being injected over a larger area.

[0178] The MID may inject the vaccine into tissue without the use of a needle. The MID may inject the vaccine as a small stream or jet with such force that the vaccine pierces the surface of the tissue and enters the underlying tissue and/or muscle. The force behind the

small stream or jet may be provided by expansion of a compressed gas, such as carbon dioxide through a micro-orifice within a fraction of a second. Examples of minimally invasive electroporation devices, and methods of using them, are described in published U.S. Patent Application No. 20080234655; U.S. Pat. Nos. 6,520,950; 7,171,264; 6,208,893; 6,009,347; 6,120,493; 7,245,963; 7,328,064; and 6,763,264, the contents of each of which are herein incorporated by reference.

[0179] The MID may comprise an injector that creates a high-speed jet of liquid that painlessly pierces the tissue. Such needle-free injectors are commercially available. Examples of needle-free injectors that can be utilized herein include those described in U.S. Pat. Nos. 3,805,783; 4,447,223; 5,505,697; and 4,342,310, the contents of each of which are herein incorporated by reference.

[0180] A desired vaccine in a form suitable for direct or indirect electrotransport may be introduced (e.g., injected) using a needle-free injector into the tissue to be treated, usually by contacting the tissue surface with the injector so as to actuate delivery of a jet of the agent, with sufficient force to cause penetration of the vaccine into the tissue. For example, if the tissue to be treated is mucosa, skin or muscle, the agent is projected towards the mucosal or skin surface with sufficient force to cause the agent to penetrate through the stratum corneum and into dermal layers, or into underlying tissue and muscle, respectively.

[0181] Needle-free injectors are well suited to deliver vaccines to all types of tissues, particularly to skin and mucosa. In some embodiments, a needle-free injector may be used to propel a liquid that contains the vaccine to the surface and into the subject's skin or mucosa. Representative examples of the various types of tissues that can be treated using the invention methods include pancreas, larynx, nasopharynx, hypopharynx, oropharynx, lip, throat, lung, heart, kidney, muscle, breast, colon, prostate, thymus, testis, skin, mucosal tissue, ovary, blood vessels, or any combination thereof.

[0182] The MID may have needle electrodes that electroporate the tissue. By pulsing between multiple pairs of electrodes in a multiple electrode array, for example set up in rectangular or square patterns, provides improved results over that of pulsing between a pair of electrodes. Disclosed, for example, in U.S. Pat. No. 5,702,359 entitled “Needle Electrodes for Mediated Delivery of Drugs and Genes” is an array of needles wherein a plurality of pairs of needles may be pulsed during the therapeutic treatment. In that application, which is incorporated herein by reference as though fully set forth, needles were disposed in a circular array, but have connectors and switching apparatus enabling a pulsing between opposing pairs of needle electrodes. A pair of needle electrodes for delivering recombinant expression

vectors to cells may be used. Such a device and system is described in U.S. Pat. No. 6,763,264, the contents of which are herein incorporated by reference. Alternatively, a single needle device may be used that allows injection of the DNA and electroporation with a single needle resembling a normal injection needle and applies pulses of lower voltage than those delivered by presently used devices, thus reducing the electrical sensation experienced by the patient.

[0183] The MID may comprise one or more electrode arrays. The arrays may comprise two or more needles of the same diameter or different diameters. The needles may be evenly or unevenly spaced apart. The needles may be between 0.005 inches and 0.03 inches, between 0.01 inches and 0.025 inches; or between 0.015 inches and 0.020 inches. The needle may be 0.0175 inches in diameter. The needles may be 0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm, 2.5 mm, 3.0 mm, 3.5 mm, 4.0 mm, or more spaced apart.

[0184] The MID may consist of a pulse generator and a two or more-needle vaccine injectors that deliver the vaccine and electroporation pulses in a single step. The pulse generator may allow for flexible programming of pulse and injection parameters via a flash card operated personal computer, as well as comprehensive recording and storage of electroporation and patient data. The pulse generator may deliver a variety of volt pulses during short periods of time. For example, the pulse generator may deliver three 15 volt pulses of 100 ms in duration. An example of such a MID is the Elgen 1000 system by Inovio Biomedical Corporation, which is described in U.S. Pat. No. 7,328,064, the contents of which are herein incorporated by reference.

[0185] The MID may be a CELLECTRA® (Inovio Pharmaceuticals, Blue Bell Pa.) device and system, which is a modular electrode system, that facilitates the introduction of a macromolecule, such as a DNA, into cells of a selected tissue in a body or plant. The modular electrode system may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The macromolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the macromolecule into the cell between the plurality of electrodes. Cell death due to overheating of cells is minimized by limiting the power dissipation in the tissue by virtue of constant-

current pulses. The Cellectra® device and system is described in U.S. Pat. No. 7,245,963, the contents of which are herein incorporated by reference. The CELLECTRA® device may be the CELLECTRA 2000® device or CELLECTRA® 3PSP device.

[0186] The MID may be an Elgen 1000 system (Inovio Pharmaceuticals). The Elgen 1000 system may comprise device that provides a hollow needle; and fluid delivery means, wherein the apparatus is adapted to actuate the fluid delivery means in use so as to concurrently (for example automatically) inject fluid, the described vaccine herein, into body tissue during insertion of the needle into the said body tissue. The advantage is the ability to inject the fluid gradually while the needle is being inserted leads to a more even distribution of the fluid through the body tissue. It is also believed that the pain experienced during injection is reduced due to the distribution of the volume of fluid being injected over a larger area.

[0187] In addition, the automatic injection of fluid facilitates automatic monitoring and registration of an actual dose of fluid injected. This data can be stored by a control unit for documentation purposes if desired.

[0188] It will be appreciated that the rate of injection could be either linear or non-linear and that the injection may be carried out after the needles have been inserted through the skin of the subject to be treated and while they are inserted further into the body tissue.

[0189] Suitable tissues into which fluid may be injected by the apparatus of the present invention include tumor tissue, skin or liver tissue but may be muscle tissue.

[0190] The apparatus further comprises needle insertion means for guiding insertion of the needle into the body tissue. The rate of fluid injection is controlled by the rate of needle insertion. This has the advantage that both the needle insertion and injection of fluid can be controlled such that the rate of insertion can be matched to the rate of injection as desired. It also makes the apparatus easier for a user to operate. If desired means for automatically inserting the needle into body tissue could be provided.

[0191] A user could choose when to commence injection of fluid. Ideally however, injection is commenced when the tip of the needle has reached muscle tissue and the apparatus may include means for sensing when the needle has been inserted to a sufficient depth for injection of the fluid to commence. This means that injection of fluid can be prompted to commence automatically when the needle has reached a desired depth (which will normally be the depth at which muscle tissue begins). The depth at which muscle tissue begins could for example be taken to be a preset needle insertion depth such as a value of 4 mm which would be deemed sufficient for the needle to get through the skin layer.

[0192] The sensing means may comprise an ultrasound probe. The sensing means may comprise a means for sensing a change in impedance or resistance. In this case, the means may not as such record the depth of the needle in the body tissue but will rather be adapted to sense a change in impedance or resistance as the needle moves from a different type of body tissue into muscle. Either of these alternatives provides a relatively accurate and simple to operate means of sensing that injection may commence. The depth of insertion of the needle can further be recorded if desired and could be used to control injection of fluid such that the volume of fluid to be injected is determined as the depth of needle insertion is being recorded.

[0193] The apparatus may further comprise: a base for supporting the needle; and a housing for receiving the base therein, wherein the base is moveable relative to the housing such that the needle is retracted within the housing when the base is in a first rearward position relative to the housing and the needle extends out of the housing when the base is in a second forward position within the housing. This is advantageous for a user as the housing can be lined up on the skin of a patient, and the needles can then be inserted into the patient's skin by moving the housing relative to the base.

[0194] As stated above, it is desirable to achieve a controlled rate of fluid injection such that the fluid is evenly distributed over the length of the needle as it is inserted into the skin. The fluid delivery means may comprise piston driving means adapted to inject fluid at a controlled rate. The piston driving means could for example be activated by a servo motor. However, the piston driving means may be actuated by the base being moved in the axial direction relative to the housing. It will be appreciated that alternative means for fluid delivery could be provided. Thus, for example, a closed container which can be squeezed for fluid delivery at a controlled or non-controlled rate could be provided in the place of a syringe and piston system.

[0195] The apparatus described above could be used for any type of injection. It is however envisaged to be particularly useful in the field of electroporation and so it may further comprises means for applying a voltage to the needle. This allows the needle to be used not only for injection but also as an electrode during, electroporation. This is particularly advantageous as it means that the electric field is applied to the same area as the injected fluid. There has traditionally been a problem with electroporation in that it is very difficult to accurately align an electrode with previously injected fluid and so users have tended to inject a larger volume of fluid than is required over a larger area and to apply an electric field over a higher area to attempt to guarantee an overlap between the injected substance and the electric

field. Using the present invention, both the volume of fluid injected and the size of electric field applied may be reduced while achieving a good fit between the electric field and the fluid.

[0196] Use in Combination

[0197] In some embodiments, the present invention provides a method of treating SARS-CoV-2 infection, or treating, protecting against, and/or preventing a disease or disorder associated with SARS-CoV-2 infection in a subject in need thereof by administering a combination of a nucleic acid molecule encoding a SARS-CoV-2 antigen, or fragment or variant thereof in combination with one or more additional agents for the treatment of SARS-CoV-2 infection or the treatment or prevention of disease or disorder associated with SARS-CoV-2 infection. In some embodiments, the disease or disorder associated with SARS-CoV-2 infection is Coronavirus Disease 2019 (COVID-19), Multisystem inflammatory syndrome in adults (MIS-A), or Multisystem inflammatory syndrome in children (MIS-C).

[0198] The nucleic acid molecule encoding a SARS-CoV-2 antigen and additional agent may be administered using any suitable method such that a combination of the nucleic acid molecule encoding a SARS-CoV-2 antigen and the additional agent are both present in the subject. In one embodiment, the method may comprise administration of a first composition comprising an agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of disease or disorder associated with SARS-CoV-2 infection and administration of a second composition comprising a nucleic acid molecule encoding a SARS-CoV-2 antigen less than 1, less than 2, less than 3, less than 4, less than 5, less than 6, less than 7, less than 8, less than 9 or less than 10 days following administration of the first composition comprising the agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of disease or disorder associated with SARS-CoV-2 infection. In one embodiment, the method may comprise administration of a first composition comprising a nucleic acid molecule encoding a SARS-CoV-2 antigen and administration of a second composition comprising an agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of disease or disorder associated with SARS-CoV-2 infection less than 1, less than 2, less than 3, less than 4, less than 5, less than 6, less than 7, less than 8, less than 9 or less than 10 days following administration of the nucleic acid molecule encoding a SARS-CoV-2 antigen. In one embodiment, the method may comprise administration of a first composition comprising an agent for the treatment of SARS-CoV-2 infection or the treatment

or prevention of disease or disorder associated with SARS-CoV-2 infection and a second composition comprising a nucleic acid molecule encoding a SARS-CoV-2 antigen concurrently. In one embodiment, the method may comprise administration of a single composition comprising an agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of disease or disorder associated with SARS-CoV-2 infection and a nucleic acid molecule encoding a SARS-CoV-2 antigen.

[0199] In some embodiments, the agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of disease or disorder associated with SARS-CoV-2 infection is a therapeutic agent. In one embodiment, the therapeutic agent is an antiviral agent. In one embodiment, the therapeutic agent is an antibiotic agent.

[0200] Non-limiting examples of antibiotics that can be used in combination with the a nucleic acid molecule encoding a SARS-CoV-2 antigen of the invention include aminoglycosides (e.g., gentamicin, amikacin, tobramycin), quinolones (e.g., ciprofloxacin, levofloxacin), cephalosporins (e.g., ceftazidime, cefepime, cefoperazone, cefpirome, ceftobiprole), antipseudomonal penicillins: carboxypenicillins (e.g., carbenicillin and ticarcillin) and ureidopenicillins (e.g., mezlocillin, azlocillin, and piperacillin), carbapenems (e.g., meropenem, imipenem, doripenem), polymyxins (e.g., polymyxin B and colistin) and monobactams (e.g., aztreonam).

[0201] Administration as a booster

[0202] In one embodiment, the immunogenic composition is administered as a booster vaccine following administration of an initial agent or vaccine for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection, including, but not limited to COVID-19, Multisystem inflammatory syndrome in adults (MIS-A), or Multisystem inflammatory syndrome in children (MIS-C). In one embodiment, the booster vaccine is administered at least once, at least twice, at least 3 times, at least 4 times, or at least 5 times following administration of an initial agent or vaccine for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection, including, but not limited to COVID-19, Multisystem inflammatory syndrome in adults (MIS-A), or Multisystem inflammatory syndrome in children (MIS-C). In one embodiment, the booster vaccine is administered at least 8 hours, at least 12 hours, at least 16 hours, at least 20 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 60 hours, at least 72 hours, at least 4 days, at least 5 days, at least 6 days, at least 1 week at least 2 weeks, at least 3 weeks, at least 4

weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year or greater than 1 year following administration of an initial agent or vaccine for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection, including, but not limited to COVID-19, Multisystem inflammatory syndrome in adults (MIS-A), or Multisystem inflammatory syndrome in children (MIS-C).

[0203] Use in assays

[0204] In some embodiments, the nucleic acid molecules, or encoded antigens, of the invention can be used in assays *in vivo* or *in vitro*. In some embodiments, the nucleic acid molecules, or encoded antigens can be used in assays for detecting the presence of anti-SARS-CoV-2 spike antibodies. Exemplary assays in which the nucleic acid molecules or encoded antigens can be incorporated into include, but are not limited to, Western blot, dot blot, surface plasmon resonance methods, Flow Cytometry methods, various immunoassays, for example, immunohistochemistry assays, immunocytochemistry assays, ELISA, capture ELISA, enzyme-linked immunospot (ELISpot) assays, sandwich assays, enzyme immunoassay, radioimmunoassay, fluorescent immunoassay, and the like, all of which are known to those of skill in the art. See e.g. Harlow et al., 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Harlow et al., 1999, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY.

[0205] In one embodiment, the SARS-CoV-2 spike antigen, or fragments thereof, of the invention can be used in an assay for intracellular cytokine staining combined with flow cytometry, to assess T-cell immune responses. This assay enables the simultaneous assessment of multiple phenotypic, differentiation and functional parameters pertaining to responding T-cells, most notably, the expression of multiple effector cytokines. These attributes make the technique particularly suitable for the assessment of T-cell immune responses induced by the vaccine of the invention.

[0206] In one embodiment, the SARS-CoV-2 spike antigen, or fragments thereof, of the invention can be used in an ELIspot assay. The ELISpot assay is a highly sensitive immunoassay that measures the frequency of cytokine-secreting cells at the single-cell level. In this assay, cells are cultured on a surface coated with a specific capture antibody in the presence or absence of stimuli. In one embodiment, the SARS-CoV-2 spike antigen, or fragments thereof, of the invention can be used as the stimulus in the ELISpot assay.

[0207] Diagnostic Methods

[0208] In some embodiments, the invention relates to methods of diagnosing a subject as having SARS-CoV-2 infection or having SARS-CoV-2 antibodies. In some embodiments, the methods include contacting a sample from a subject with a SARS-CoV-2 antigen of the invention, or a cell comprising a nucleic acid molecule for expression of the SARS-CoV-2 antigen, and detecting binding of an anti-SARS-CoV-2 spike antibody to the SARS-CoV-2 antigen of the invention. In such an embodiment, binding of an anti-SARS-CoV-2 spike antibody present in the sample of the subject to the antigen, or fragment thereof, of the invention would indicate that the subject is currently infected or was previously infected with SARS-CoV-2.

[0209] Kits and Articles of Manufacture

[0210] Provided herein is a kit, which can be used for treating a subject using the method of vaccination described above. The kit can comprise the immunogenic composition described herein.

[0211] The kit can also comprise instructions for carrying out the vaccination method described above and/or how to use the kit. Instructions included in the kit can be affixed to packaging material or can be included as a package insert. While instructions are typically written or printed materials, they are not limited to such. Any medium capable of storing instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges), optical media (e.g., CD ROM), and the like. As used herein, the term "instructions" can include the address of an internet site which provides instructions.

[0212] Further provided herein are articles of manufacture containing the immunogenic composition described herein. In some embodiments, the article of manufacture is a container, such as a vial, optionally a single-use vial. In one embodiment, the article of manufacture is a single-use glass vial equipped with a stopper, which contains the immunogenic composition described herein to be administered. In some embodiments, the vial comprises a stopper, pierceable by a syringe, and a seal. In some embodiments, the article of manufacture is a syringe.

[0213] The present invention has multiple aspects, illustrated by the following non-limiting examples.

[0214] EXAMPLES

[0215] Example 1

[0216] Materials & Methods:

[0217] Cell lines. Human embryonic kidney (HEK)-293T (ATCC® CRL-3216™) and African green monkey kidney COS-7 (ATCC® CRL-1651™) cell lines were obtained from ATCC (Old Town Manassas, VA). All cell lines were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin.

[0218] *In vitro* protein expression (Western blot). Human embryonic kidney cells, 293T were cultured and transfected as described previously (Yan, et al. Enhanced cellular immune responses elicited by an engineered HIV-1 subtype B consensus-based envelope DNA vaccine. *Mol Ther*. 2007;15(2):411-421.). 293T cells were transfected with pDNA using TurboFectin8.0 (OriGene) transfection reagent following the manufacturer's protocol. Forty-eight hours later, cell lysates were harvested using modified RIPA cell lysis buffer. Proteins were separated on a 4-12% BIS-TRIS gel (ThermoFisher Scientific). Following transfer, blots were incubated with an anti-SARS-CoV spike protein polyclonal antibody (Novus Biologicals), and then visualized with horseradish peroxidase (HRP)-conjugated anti-mouse IgG (GE Amersham).

[0219] Immunofluorescence of transfected 293T cells. For *in vitro* staining of Spike protein expression, 293T cells were cultured on 4-well glass slides (Lab-Tek) and transfected with 3 μg/well of pDNA using TurboFectin8.0 (OriGene) transfection reagent following the manufacturer's protocol. Cells were fixed 48hrs after transfection with 10% Neutral-buffered Formalin (BBC Biochemical, Washington State) for 10 min at room temperature (RT) and then washed with PBS. Before staining, chamber slides were blocked with 0.3% (v/v) Triton-X (Sigma), 2% (v/v) donkey serum in PBS for 1hr at RT. Cells were stained with a rabbit anti-SARS-CoV spike protein polyclonal antibody (Novus Biologicals) diluted in 1% (w/v) BSA (Sigma), 2% (v/v) donkey serum, 0.3% (v/v) Triton-X (Sigma) and 0.025% (v/v) 1g/ml Sodium Azide (Sigma) in PBS for 2hrs at RT. Slides were washed three times for 5 min in PBS and then stained with donkey anti-rabbit IgG AF488 (Life Technologies, A21206) for 1hr at RT. Slides were washed again and mounted and covered with DAPI-Fluoromount (SouthernBiotech).

[0220] *In vitro* RNA expression (qRT-PCR). *In vitro* mRNA expression of the plasmid was demonstrated by transfection of COS-7 with serially diluted plasmids followed by analysis of the total RNA extracted from the cells using reverse transcription and PCR. Transfections of

four concentrations of the plasmid were performed using FuGENE® 6 transfection reagent (Promega) which resulted in final masses ranging between 80 and 10 ng/well. The transfections were performed in duplicate. Following 18 to 26 hours of incubation, the cells were lysed with RLT Buffer (Qiagen). Total RNA was isolated from each well using the Qiagen RNeasy kit following the kit instructions. The resulting RNA concentration was determined by $OD_{260/280}$, and samples of the RNA were diluted to 10 ng/µL. One hundred nanograms of RNA was then converted to cDNA using the High Capacity cDNA Reverse Transcription (RT) kit (Applied Biosystems) following the kit instructions. RT reactions containing RNA but no reverse transcriptase (minus RT) were included as controls for plasmid DNA or cellular genomic DNA sample contamination. Eight µL of sample cDNA were then subjected to PCR using primers and probes that are specific to the target sequence (pGX9501 Forward – CAGGACAAGAACACACAGGAA (SEQ ID NO: 7); pGX9501 Reverse – CAGGCAGGATTTGGGAGAAA (SEQ ID NO: 8); pGX9501 Probe – ACCCATCAAGGACTTTGGAGG (SEQ ID NO: 9); and pGX9503 Forward – AGGACAAGAACACACAGGAAG (SEQ ID NO: 10); pGX9503 Reverse – CAGGATCTGGGAGAAGTTGAAG (SEQ ID NO: 11); pGX9503 Probe – ACACCACCCATCAAGGACTTTGGA (SEQ ID NO: 12)). In a separate reaction, the same quantity of sample cDNA was subjected to PCR using primers and a probe designed for COS-7 cell line β-actin sequences (β-actin Forward – GTGACGTGGACATCCGTAAA (SEQ ID NO: 13); β-actin Reverse – CAGGGCAGTAATCTCCTTCTG (SEQ ID NO: 14); β-actin Probe – TACCCTGGCATTGCTGACAGGATG (SEQ ID NO: 15)). The primers and probes were synthesized by Integrated DNA Technologies, Inc. and the probes were labeled with 56-FAM and Black Hole Quencher 1. The reaction used ABI Fast Advance 2X (Cat. No. 4444557), with final forward and reverse primer concentrations of 1 µM and probe concentrations of 0.3 µM. Using a QuantStudio™ 7 Flex Real Time PCR Studio System (Applied Biosystems), samples were first subjected to a hold of 1 minute at 95°C and then 40 cycles of PCR with each cycle consisting of 1 second at 95°C and 20 seconds at 60°C. Following PCR, the amplifications results were analyzed as follows. The negative transfection controls (NTCs), the minus RT controls, and the NTC were scrutinized for each of their respective indications. The threshold cycle (C_T) of each transfection concentration for the INO-4800 SARS-CoV-2 target mRNA and for the β-actin mRNA was generated from the QuantStudio™ software using an automatic threshold setting. The plasmid was considered to be active for mRNA expression if the expression in any of the plasmid-transfected wells compared to the negative transfection controls were greater than 5 C_T.

Animals. Female, 6 week old C57/BL6 and BALB/c mice were purchased from Charles River Laboratories (Malvern, PA) and The Jackson Laboratory (Bar Harbor, ME). Female, 8 week old Hartley guinea pigs were purchased from Elm Hill Labs (Chelmsford, MA). All animals were housed in the animal facility at The Wistar Institute Animal Facility or Acculab Life Sciences (San Diego, CA). All animal testing and research complied with all relevant ethical regulations and studies received ethical approval by the Wistar Institute or Acculab Institutional Animal Care and Use Committees (IACUC). For mouse studies, on day 0, doses of 2.5, 10 or 25 μg pDNA were administered to the tibialis anterior (TA) muscle by needle injection followed by CELLECTRA® *in vivo* electroporation (EP). The CELLECTRA® EP delivery consists of two sets of pulses with 0.2 Amp constant current. Second pulse sets is delayed 3 seconds. Within each set there are two 52 ms pulses with a 198 ms delay between the pulses. On days 0 and 14, blood was collected. Parallel groups of mice were serially sacrificed on days 4, 7, and 10 post-immunization for analysis of cellular immune responses. For guinea pig studies, on day 0, 100 μg pDNA was administered to the skin by Mantoux injection followed by CELLECTRA® *in vivo* EP.

[0221] Antigen binding ELISA. ELISAs were performed to determine sera antibody binding titers. Nunc ELISA plates were coated with 1 μg/ml recombinant protein antigens in Dulbecco's phosphate-buffered saline (DPBS) overnight at 4°C. Plates were washed three times, then blocked with 3% bovine serum albumin (BSA) in DPBS with 0.05% Tween 20 for 2 hours at 37°C. Plates were then washed and incubated with serial dilutions of mouse or guinea pig sera and incubated for 2 hours at 37°C. Plates were again washed and then incubated with 1:10,000 dilution of horse radish peroxidase (HRP)-conjugated anti-guinea pig IgG secondary antibody (Sigma-Aldrich, cat. A7289) or HRP-conjugated anti-mouse IgG secondary antibody (Sigma-Aldrich) and incubated for 1 hour at RT. After final wash, plates were developed using SureBlue™ TMB 1-Component Peroxidase Substrate (KPL, cat. 52-00-03), and the reaction stopped with TMB Stop Solution (KPL, cat. 50-85-06). Plates were read at 450 nm wavelength within 30 minutes using a Synergy™ HTX plate reader (BioTek Instruments, Highland Park, VT). Binding antibody endpoint titers (EPTs) were calculated as previously described (Bagarazzi ML, Yan J, Morrow MP, et al. Immunotherapy against HPV16/18 generates potent TH1 and cytotoxic cellular immune responses. Sci Transl Med. 2012;4(155):155ra138). Binding antigens tested included, SARS-CoV-2 antigens: S1 spike protein (Sino Biological 40591-V08H), S1+S2 ECD spike protein (Sino Biological 40589-V08B1), RBD (University of Texas, at Austin (McLellan Lab.)); SARS-COV antigens: Spike

S1 protein (Sino Biological 40150-V08B1), S (1-1190) (Immune Tech IT-002-001P) and Spike C-terminal (Meridian Life Science R18572).

[0222] ACE2 Competition ELISA. For mouse studies, ELISAs were performed to determine sera IgG antibody competition against human ACE2 with a human Fc tag. Nunc ELISA plates were coated with 1 µg/mL rabbit anti-His6X in 1X PBS for 4-6 hours at room temperature (RT) and washed 4 times with washing buffer (1X PBS and 0.05% Tween® 20). Plates were blocked overnight at 4°C with blocking buffer (1X PBS, 0.05% Tween® 20, 5% evaporated milk and 1% FBS). Plates were washed four times with washing buffer then incubated with full length (S1+S2) spike protein containing a C-terminal His tag (Sino Biologics, cat. 40589-V08B1) at 10 µg mL-1 for 1 hour at RT. Plates were washed and then serial dilutions of purified mouse IgG mixed with 0.1 µg mL-1 recombinant human ACE2 with a human Fc tag (ACE2-IgHu) were incubated for 1-2 hours at RT. Plates were again washed and then incubated with 1:10,000 dilution of horse radish peroxidase (HRP) conjugated anti-human IgG secondary antibody (Bethyl, cat. A80-304P) and incubated for 1 hour at RT. After final wash plates were developed using 1-Step Ultra TMB-ELISA Substrate (Thermo, cat. 34029) and the reaction stopped with 1 M Sulfuric Acid. Plates were read at 450 nm wavelength within 30 minutes using a SpectraMax Plus 384 Microplate Reader (Molecular Devices, Sunnyvale, CA). Competition curves were plotted and the area under the curve (AUC) was calculated using Prism 8 analysis software with multiple t-tests to determine statistical significance.

[0223] For guinea pig studies, 96 well half area assay plates (Costar) were coated with 25 µl per well of 5 µg/mL of SARS-CoV-2 spike S1+S2 protein (Sino Biological) diluted in 1x DPBS (Thermofisher) overnight at 4°C. Plates were washed with 1xPBS buffer with 0.05% TWEEN® 20 (Sigma). 100 µl per well of 3% (w/v) BSA (Sigma) in 1x PBS with 0.05% TWEEN® 20 were added and incubated for 1 hr at 37°C. Serum samples were diluted 1:20 in 1% (w/v) BSA in 1x PBS with 0.05% TWEEN. After washing the assay plate, 25 µl/well of diluted serum was added and incubated 1 hr at 37°C. Human recombinant ACE2-Fc-tag (Sinobiological) was added directly to the diluted serum, followed by 1 hr of incubation at 37°C. Plates were washed and 25 µl per well of 1:10,000 diluted goat anti-hu Fc fragment antibody HRP (Bethyl, A80-304P) was added to the assay plate. Plates were incubated 1 hr at RT. For development the SureBlue/TMB Stop Solution (KPL, MD) was used and O.D. was recorded at 450 nm.

[0224] SARS-CoV-2 Pseudovirus neutralization assay. SARS-CoV-2 pseudotyped viruses were produced using HEK293T cells transfected with GeneJammer (Agilent) using IgE-

SARS-CoV-2 S plasmid (Genscript) and pNL4-3.Luc.R-E- plasmid (NIH AIDS reagent) at a 1:1 ratio. Forty-eight hours post transfection, transfection supernatant was collected, enriched with FBS to 12% final volume, steri-filtered (Millipore Sigma), and aliquoted for storage at -80°C. SARS-CoV-2 pseudotyped viruses were titered and yielded greater than 50 times the relative luminescence units (RLU) to cells alone after 72h of infection. Mouse sera from INO-4800 vaccinated and naive groups were heat inactivated for 15 minutes at 56°C and serially diluted three fold starting at a 1:10 dilution for assay. Sera were incubated with a fixed amount of SARS-CoV-2 pseudotyped virus for 90 minutes. HEK293T cells stably expressing ACE2 were added after 90 minutes and allowed to incubate in standard incubator (37% humidity, 5% CO_2) for 72 hours. Post infection, cells were lysed using britelite™ plus luminescence reporter gene assay system (Perkin Elmer Catalog no. 6066769) and relative luminescence units (RLU) were measured using the Biotek plate reader. Neutralization titers (ID_{50}) were calculated as the serum dilution at which RLU were reduced by 50% compared to RLU in virus control wells after subtraction of background RLU in cell control wells.

[0225] SARS-CoV-2 wildtype virus neutralization assays. SARS-CoV-2/Australia/VIC01/2020 isolate neutralization assays were performed at Public Health England (Porton Down, UK). Neutralizing virus titers were measured in serum samples that had been heat-inactivated at 56°C for 30 minutes. SARS-CoV-2 (Australia/VIC01/2020 isolate) (Caly et al., Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. *Med. J. Aust.* (2020) doi: 10.5694/mja2.50569; Published online: 13 April 2020) was diluted to a concentration of 933 pfu/ml and mixed 50:50 in 1% FCS/MEM containing 25mM HEPES buffer with doubling serum dilutions from 1:10 to 1:320 in a 96-well V-bottomed plate. The plate was incubated at 37 °C in a humidified box for 1 hour before the virus was transferred into the wells of a twice DPBS-washed 24-well plate that had been seeded the previous day at 1.5 x 10^5 Vero E6 cells per well in 10% FCS/MEM. Virus was allowed to adsorb at 37°C for a further hour and overlaid with plaque assay overlay media (1X MEM/1.5% CMC/4% FCS final). After 5 days incubation at 37°C in a humidified box, the plates were fixed, stained and plaques counted. Median neutralizing titers (ND50) were determined using the Spearman-Karber formula relative to virus only control wells.

[0226] SARS-CoV-2/WH-09/human/2020 isolate neutralization assays were performed at the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (CAMS) approved by the National Health Commission of the People's Republic of China. Seed SARS-CoV-2 (SARS-CoV-2/WH-09/human/2020) stocks and virus isolation studies were

performed in Vero E6 cells, which are maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 μg/ml streptomycin, and incubated at 36.5°C, 5% CO_2. Virus titer were determined using a standard 50% tissue culture infection dose (TCID50) assay. Serum samples collected from immunized animals were inactivated at 56°C for 30 minutes and serially diluted with cell culture medium in two-fold steps. The diluted samples were mixed with a virus suspension of 100 TCID50 in 96-well plates at a ratio of 1:1, followed by 2 hours incubation at 36.5°C in a 5% CO_2 incubator. 1-2 × 10^4 Vero cells were then added to the serum-virus mixture, and the plates were incubated for 3-5 days at 36.5°C in a 5% CO_2 incubator. Cytopathic effect (CPE) of each well was recorded under microscopes, and the neutralizing titer was calculated by the dilution number of 50% protective condition.

[0227] Bronchoalveolar lavage collection. Bronchoalveolar lavage (BAL) fluid was collected by washing the lungs of euthanized and exsanguinated mice with 700-1000ul of ice-cold PBS containing 100μm EDTA, 0.05% sodium azide, 0.05% Tween® 20, and 1x protease inhibitor (Pierce) (mucosal prep solutions (MPS)) with a blunt-ended needle. Guinea pig lungs were washed with 20 ml of MPS via 16G catheter inserted into the trachea. Collected BAL fluid was stored at -20 °C until the time of assay.

[0228] IFN-γ ELISpot. Mice: Spleens from mice were collected individually in RPMI1640 media supplemented with 10% FBS (R10) and penicillin/streptomycin and processed into single cell suspensions. Cell pellets were re-suspended in 5 mL of ACK lysis buffer (Life Technologies, Carlsbad, CA) for 5 min at room temperature, and PBS was then added to stop the reaction. The samples were again centrifuged at 1,500 g for 10 min, cell pellets re-suspended in R10, and then passed through a 45 μm nylon filter before use in ELISpot assay. ELISpot assays were performed using the Mouse IFN-γ ELISpot^{PLUS} plates (MABTECH). 96-well ELISpot plates pre-coated with capture antibody were blocked with R10 medium overnight at 4°C. 200,000 mouse splenocytes were plated into each well and stimulated for 20 hours with pools of 15-mer peptides overlapping by 9 amino acid from the SARS-CoV-2, SARS-CoV, or MERS-CoV Spike proteins (5 peptide pools per protein). Additionally, matrix mapping was performed using peptide pools in a matrix designed to identify immunodominant responses. Cells were stimulated with a final concentration of 5μL of each peptide/well in RPMI + 10% FBS (R10). The spots were developed based on manufacturer's instructions. R10 and cell stimulation cocktails (Invitrogen) were used for negative and positive controls, respectively. Spots were scanned and quantified by ImmunoSpot™ CTL

reader. Spot-forming unit (SFU) per million cells was calculated by subtracting the negative control wells.

[0229] Flow cytometry. Intracellular cytokine staining was performed on splenocytes harvested from BALB/c and C57BL/6 mice stimulated with the overlapping peptides spanning the SARS-CoV-2 S protein for 6 hours at 37 °C, 5% CO_2. Cells were stained with the following antibodies from BD Biosciences, unless stated, with the dilutions stated in parentheses: FITC anti-mouse CD107a (1:100), PerCP-Cy5.5 anti-mouse CD4 (1:100), APC anti-mouse CD8a (1:100), ViViD Dye (1-40) (LIVE/DEAD® Fixable Violet Dead Cell Stain kit; Invitrogen, L34955), APC-Cy7 anti-mouse CD3e (1:100), and BV605 anti-mouse IFN-γ (1:75) (eBiosciences). Phorbol Myristate Acetate (PMA) were used as a positive control, and complete medium only as the negative control. Cells were washed, fixed and, cell events were acquired using an FACS CANTO (BD Biosciences), followed by FlowJo software (FlowJo LLC, Ashland, OR) analysis.

[0230] Statistics. All statistical analyses were performed using GraphPad Prism 7 or 8 software (La Jolla, CA). These data were considered significant if $p < 0.05$. The lines in all graphs represent the mean value and error bars represent the standard deviation. No samples or animals were excluded from the analysis. Randomization was not performed for the animal studies. Samples and animals were not blinded before performing each experiment.

[0231] Results

[0232] Design and synthesis of SARS-CoV-2 DNA vaccine constructs

[0233] Four spike protein sequences were retrieved from the first four available SARS-CoV-2 full genome sequences published on GISAID (Global Initiative on Sharing All Influenza Data). Three Spike sequences were 100% matched and one was considered an outlier (98.6% sequence identity with the other sequences). After performing a sequence alignment, the SARS-CoV-2 spike glycoprotein sequence ("Covid-19 spike antigen"; SEQ ID NO: 1) was generated and an N-terminal IgE leader sequence was added. The highly optimized DNA sequence encoding SARS-CoV-2 IgE-spike was created as described elsewhere herein to enhance expression and immunogenicity. SARS-CoV-2 spike outlier glycoprotein sequence ("Covid-19 spike-OL antigen"; SEQ ID NO: 4) was generated and an N-terminal IgE leader sequence was added. The optimized DNA sequence was synthesized, digested with BamHI and XhoI, and cloned into the expression vector pGX0001 under the control of the human cytomegalovirus immediate-early promoter and a bovine growth hormone polyadenylation signal. The resulting plasmids were designated as pGX9501 and

pGX9503, designed to encode the SARS-CoV-2 S protein from the 3 matched sequences and the outlier sequence, respectively (**Figure 1A**).

[0234] ***In vitro*** **characterization of synthetic DNA vaccine constructs**

[0235] Expression of the encoded SARS-CoV-2 spike transgene at the RNA level in COS-7 cells transfected with pGX9501 and pGX9503 was measured. Using the total RNA extracted from the transfected COS-7 cells, expression of the spike transgene was confirmed by RT-PCR (**Figure 1B**). *In vitro* spike protein expression in 293T cells was measured by Western blot analysis using a cross-reactive antibody against SARS-CoV S protein on cell lysates. Western blots of the lysates of HEK-293T cells transfected with pGX9501 or pGX9503 constructs revealed bands approximate to the predicted S protein molecular weight, 140-142 kDa, with slight shifts likely due to the 22 potential N-linked glycans in the S protein (**Figure 1C**). In immunofluorescent studies, the S protein was detected in 293T cells transfected with pGX9501 or pGX9503 (**Figure 1D**). In summary, *in vitro* studies revealed the expression of the Spike protein at both the RNA and protein level after transfection of cell lines with the candidate vaccine constructs.

[0236] **Humoral immune responses in mice.** pGX9501 was selected as the vaccine construct to advance to immunogenicity studies, due to the broader coverage it would likely provide compared to the outlier, pGX9503. pGX9501 was subsequently termed INO-4800. The immunogenicity of INO-4800 was evaluated in BALB/c mice, post-administration to the tibialis anterior muscle using the CELLECTRA® delivery device. (Sardesai & Weiner, Curr. Opin. Immunol., 23, 421-429 (2011). The reactivity of the sera from a group of mice immunized with INO-4800 was measured against a panel of SARS-CoV-2 and SARS-CoV antigens (**Figure 2**). Analysis revealed IgG binding against SARS-CoV-2 S protein antigens, with limited cross-reactivity to SARS-CoV S protein antigens in the sera of INO-4800-immunized mice. The serum IgG binding endpoint titers in mice immunized with pDNA against recombinant SARS-CoV-2 spike protein S1+S2 regions (**Figure 3A and 3B**) and recombinant SARS-CoV-2 spike protein receptor binding domain (RBD) (**Figures 3C and 3D**) were measured. Endpoint titers were observed in the sera of mice at day 14 after immunization with a single dose of INO-4800 (**Figures 3B, 3C, 3D**).

[0237] **Neutralization assay.** A neutralization assay with a pNL4-3.Luc.R-E-based pseudovirus displaying the SARS-CoV-2 Spike protein was developed. Neutralization titers were detected by a reduction in relative luciferase units (RLU) compared to controls which had no decrease in RLU signal. BALB/c mice were immunized twice with INO-4800, on days 0

and 14, and sera was collected on day 7 post-2nd immunization. The pseudovirus was incubated with serial dilutions of mouse sera and the sera-virus mixture was added to 293T cells stably expressing the human ACE2 receptor (ACE2-293T) for 72 hours. Neutralization ID50 average titers of 92.2 were observed in INO-4800 immunized mice **(Figures 4A and 4B)**. No reduction in RLU was observed for the control animals. Neutralizing titers were additionally measured against two wildtype SARS-CoV-2 virus strains by plaque reduction neutralization test (PRNT) assay. Sera from INO-4800 immunized BALB/c mice neutralized both SARS-CoV-2/WH-09/human/2020 and SARS-CoV-2/Australia/VIC01/2020 virus strains with average ND50 titers of 97.5 and 128.1, respectively **(Table 1)**. Live virus neutralizing titers were also evaluated in C57BL/6 mice following the same INO-4800 immunization regimen. Sera from INO-4800 immunized C57BL/6 mice neutralized wildtype SARS-CoV-2 virus with average ND50 titer of 340 **(Table 1)**.

[0238] Table 1. Sera neutralizing activity after INO-4800 administration to mice and guinea pigs.

Model	Vaccine	N	Immunization Regimen	Sample Time point	Neutralization Assay	Serum ND50 (Reciprocal Dilution)
BALB/c Mouse	pVAX	4	25 µg Days 0, 14	Day 21	SARS-CoV-2 (WH-09/human/2020)	<20, <20, <20, <20
	INO-4800	4	25 µg Days 0, 14	Day 21	SARS-CoV-2 (WH-09/human/2020)	30, 40, 80, 240
	pVAX	8	25 µg Days 0, 14	Day 21	SARS-CoV-2 (Australia/VIC01/2020)	<10, 12, 13, 15, 16, 17, 19, 24
	INO-4800	8	25 µg Days 0, 14	Day 21	SARS-CoV-2 (Australia/VIC01/2020)	27, 46, 91, 108, 130, 161, 221, 241
	pVAX	5	10 µg Days 0, 14	Day 21	SARS-CoV-2 Pseudovirus	8, 8, 8, 8, 8
	INO-4800	5	10 µg Days 0, 14	Day 21	SARS-CoV-2 Pseudovirus	43, 55, 87, 129, 147
	pVAX	4	25 µg Days 0, 14	Day 21	SARS-CoV-2	<20, <20, <20, <20

C57BL/6 Mouse					(WH-09/human/2020)	
	INO-4800	4	25 µg Days 0, 14	Day 21	SARS-CoV-2 (WH-09/human/2020)	240, 240, 240, 640
Guinea pig	pVAX	5	100 µg Days 0, 14, 28	Day 42	SARS-CoV-2 (Australia/VIC01/2020)	<10, 14, 20, 21, 25
	INO-4800	5	100 µg Days 0, 14, 28	Day 42	SARS-CoV-2 (Australia/VIC01/2020)	>320, >320, >320, >320, >320
	pVAX	5	100 µg Days 0, 14, 28	Day 35	SARS-CoV-2 Pseudovirus	<20, <20, <20, <20, <20
	INO-4800	5	100 µg Days 0, 14, 28	Day 35	SARS-CoV-2 Pseudovirus	527, 532, 579, 614, 616
New Zealand White Rabbit	SSC	5	Days 0, 28	Day 42	SARS-CoV-2 Pseudovirus	<10, <10, <10, <10, <10
	INO-4800	5	1 mg, Days 0, 28	Day 42	SARS-CoV-2 Pseudovirus	12, 23, 32, 148, 178
	INO-4800	5	2mg, Days 0. 28	Day 42	SARS-CoV-2 Pseudovirus	202, 237, 252, 455, 995
Non-human primates	INO-4800	5	1 mg, Days 0, 28	Day 42	SARS-CoV-2 Pseudovirus	15, 27, 55, 61, 1489
	INO-4800	5	2mg, Days 0. 28	Day 42	SARS-CoV-2 Pseudovirus	78, 23, 13, 48, <10

[0239] The immunogenicity of INO-4800 in the Hartley guinea pig model, an established model for intradermal vaccine delivery (Carter, et al. The adjuvant GLA-AF enhances human intradermal vaccine responses. Sci Adv. 2018;4(9):eaas9930; Schultheis, et al. Characterization of guinea pig T cell responses elicited after EP-assisted delivery of DNA vaccines to the skin. Vaccine. 2017;35(1):61-70), was assessed. 100 µg of pDNA was administered by Mantoux injection to the skin and followed by CELLECTRA® device on day as described in the methods section above. On day 14, anti-spike protein binding of serum antibodies was measured by ELISA. Immunization with INO-4800 revealed an

immune response in respect to SARS-CoV-2 S1+2 protein binding IgG levels in the sera (**Figures 5A and 5B**). The endpoint SARS-CoV-2 S protein binding titer at day 14 was 10,530 and 21 in guinea pigs treated with 100 μg INO-4800 or pVAX (control), respectively (**Figure 5B**). Antibody neutralizing activity following intradermal INO-4800 immunization in the guinea pig model was evaluated. Guinea pigs were treated on days 0, 14, and 28 with pVAX or INO-4800, and sera samples were collected on days 35 or 42 to measure sera neutralizing activity against pseudovirus or wildtype virus, respectively. SARS-CoV-2 pseudovirus neutralizing activity with average ND50 titers of 573.5 was observed for the INO-4800 immunized guinea pigs (Table 1). Wildtype SARS-CoV-2 virus activity was also observed for the INO-4800 immunized guinea pigs with ND50 titers >320 by PRNT assay observed in all animals (Table 1). The functionality of the serum antibodies was further measured by assessing their ability to inhibit ACE2 binding to SARS-CoV-2 spike protein. Serum (1:20 dilution) collected from INO-4800 immunized guinea pigs after 2nd immunization inhibited binding of SARS-CoV-2 Spike protein over range of concentrations of ACE-2 (0.25 μg/ml through 4 μg/ml) (**Figure 6E**). Furthermore, serum dilution curves revealed serum collected from INO-4800 immunized guinea pigs blocked binding of ACE-2 to SARS-CoV-2 in a dilution-dependent manner (**Figure 6F**). Serum collected from pVAX-treated animals displayed negligible activity in the inhibition of ACE-2 binding to the virus protein, the decrease in OD signal at the highest concentration of serum is considered a matrix effect in the assay.

[0240] Inhibition of SARS-CoV-2 S protein binding to ACE2 receptor. The receptor inhibiting functionality of INO-4800-induced antibody responses was examined. An ELISA-based ACE2 inhibition assay was developed as a surrogate for neutralization. As a control in the assay, ACE2 is shown to bind to SARS-CoV-2 Spike protein with an EC50 of 0.025 μg/ml (**Figure 6A**). BALB/c mice were immunized on Days 0 and Day 14 with 10 μg of INO-4800, and serum IgG was purified on Day 21 post-immunization to ensure inhibition is antibody-mediated. Inhibition of the Spike-ACE2 interaction using serum IgG from a naïve mouse and from an INO-4800 vaccinated mouse were compared (**Figure 6B**). The receptor inhibition assay was repeated with a group of five immunized mice, demonstrating that INO-4800-induced antibodies competed with ACE2 binding to the SARS-CoV-2 Spike protein (**Figure 6C and 6F**). ACE2 binding inhibition was further evaluated in the guinea pig model. Sera collected from INO-4800 immunized guinea pigs inhibited binding of SARS-CoV-2 Spike protein over range of concentrations of ACE2 (0.25 μg/ml through 4 μg/ml) (**Figure 6D**). Furthermore, serum dilution curves revealed sera collected from INO-4800 immunized

guinea pigs blocked binding of ACE2 to SARS-CoV-2 in a dilution-dependent manner (**Figure 6E**). Sera collected from pVAX-treated animals displayed negligible activity in the inhibition of ACE2 binding to the virus protein, the decrease in OD signal at the highest concentration of serum is considered a matrix effect in the assay. **Figure 6F** depicts IgGs purified from n=5 mice day 14 post second immunization with INO-4800 show competition against ACE2 receptor binding to SARS-CoV-2 Spike protein compared to pooled naïve mice IgGs.

[0241] In summary, immunogenicity testing in both mice and guinea pigs revealed the SARS-CoV-2 vaccine candidate, INO-4800, was capable of eliciting antibody responses to SARS-CoV-2 spike protein. ACE2 is considered to be the primary receptor for SARS-CoV-2 cellular entry, blocking this interaction suggests INO-4800-induced antibodies may prevent host infection.

[0242] **Biodistribution of SARS-CoV-2 reactive IgG to the lung.** Lower respiratory disease (LRD) is associated with severe cases of COVID-19. The presence of antibodies at the lung mucosa targeting SARS-CoV-2 could potentially mediate protection against LRD. The presence of SARS-CoV-2 specific antibody in the lungs of immunized mice and guinea pigs was evaluated. BALB/c mice and Hartley guinea pigs were immunized, on days 0 and 14 or 0, 14 and 28, respectively, with INO-4800 or pVAX control pDNA. Bronchoalveolar lavage (BAL) fluid was collected following sacrifice, and SARS-CoV-2 S protein ELISAs were performed. In both BALB/c and Hartley guinea pigs which received INO-4800, a statistically significant increase in SARS-CoV-2 S protein binding IgG in BAL fluid compared to animals receiving pVAX control was measured (**Figures 7A-7D**). Taken together, these data demonstrate the presence of anti-SARS-CoV-2 specific antibody in the lungs following immunization with INO-4800.

[0243] **Coronavirus cross-reactive cellular immune responses in mice.** T cell responses against SARS-CoV-2, SARS-CoV, and MERS-CoV S antigens were assayed by IFN-γ ELISpot. Groups of BALB/c mice were sacrificed at days 4, 7, or 10 post-INO-4800 administration (2.5 or 10 μg of pDNA), splenocytes were harvested, and a single-cell suspension was stimulated for 20 hours with pools of 15-mer overlapping peptides spanning the SARS-CoV-2, SARS-CoV, and MERS-CoV spike protein. Day 7 post-INO-4800 administration, T cell responses of 205 and 552 SFU per 10^6 splenocytes against SARS-CoV-2 were measured for the 2.5 and 10 μg doses, respectively (**Figure 8A**). Higher magnitude responses of 852 and 2,193 SFU per 10^6 splenocytes against SARS-CoV-2 were observed on Day 10 post-INO-4800 administration. Additionally, the cross-reactivity of the cellular

response elicited by INO-4800 against SARS-CoV was assayed, showing detectable, albeit lower, T cell responses on both Day 7 (74 [2.5 μg dose] and 140 [10 μg dose] SFU per 10^6 splenocytes) and Day 10 post-administration (242 [2.5 μg dose] and 588 [10 μg dose] SFU per 10^6 splenocytes) (**Figure 8B**). Interestingly, no cross-reactive T cell responses were observed against MERS-CoV peptides (**Figure 8C**). Representative images of the IFN-γ ELISpot plates are provided in **Figure 31**. The T cell populations which were producing IFN-γ were identified. Flow cytometric analysis on splenocytes harvested from BALB/c mice on Day 14 after a single INO-4800 immunization revealed the T cell compartment to contain 0.04% CD4+ and 0.32% CD8+ IFN-γ+ T cells after stimulation with SARS-CoV-2 antigens (**Figure 32**).

[0244] BALB/c SARS-CoV-2 epitope mapping. Epitope mapping was performed on the splenocytes from BALB/c mice receiving the 10 μg INO-4800 dose. Thirty matrix mapping pools were used to stimulate splenocytes for 20 hours and immunodominant responses were detected in multiple peptide pools (**Figure 14A**). The responses were deconvoluted to identify several epitopes (H2-Kd) clustering in the receptor binding domain and in the S2 domain (**Figure 14B**). Interestingly, one SARS-CoV-2 H2-Kd epitope, PHGVVFLHV (SEQ ID NO: 16), was observed to be overlapping and adjacent to the SARS-CoV human HLA-A2 restricted epitope VVFLHVTVYV (SEQ ID NO: 17).

[0245] In summary, T cell responses against SARS-CoV-2 S protein epitopes were detected in mice immunized with INO-4800.

[0246] Example 2 - Cellular and humoral immune responses measured in INO-4800-treated New Zealand White (NZW) rabbits.

[0247] Day 0 and 28 intradermal delivery of pDNA. PBMC IFN-γ ELISpot (**Figure 9**); Serum IgG binding ELISA (**Figure 10**).

[0248] Example 3

[0249] Humoral immune responses to SARS-CoV-2 spike protein measured in INO-4800 treated in rhesus monkeys. Day 0 and 28 intradermal delivery of pDNA. Serum IgG binding ELISA. (**Figures 11A-11E**.)

[0250] Humoral immune responses to SARS and MERS spike protein measured in INO-4800 treated rhesus monkeys. Day 0 and 28 intradermal delivery of pDNA. Serum IgG binding ELISA. (**Figures 12A-12G**; left panel, 1 mg INO-4800; right panel, 2 mg INO-4800).**Cellular immune responses measured by PBMC IFN-γ ELISpot in INO-4800-treated in rhesus monkeys following intradermal delivery of pDNA on days 0 and 28.**

Results are shown in **Figure 13A** (SARS CoV-2 Spike peptides); **13B** (SARS CoV Spike peptides); and **13C** (MERS CoV Spike peptides).

[0251] Example 4 INO-4800 SARS-CoV-2 Spike ELISA Assay

[0252] The SARS-CoV-2 spike protein is coated onto wells of a 96-well microplate by incubating over night or for up to three days. Blocking buffer is then added to block remaining free binding sites. Human serum samples containing antibodies to SARS-COV-2 spike protein and assay controls are added to the blocked plate and incubated for 1 hour. During the incubation, anti-spike protein antibodies present in the samples and positive controls bind to spike protein immobilized onto the plate. Plates are then washed to remove unbound serum components. Next, a horseradish peroxidase (HRP) labeled anti-human IgG antibody is added to allow for detection of antibody bound to the spike protein. After a one hour incubation, plates are washed to remove unbound HRP detection antibody, and TMB substrate is added to plates. In the presence of horseradish peroxidase, the TMB substrate turns deep blue, proportional to the amount of HRP present in the well. After allowing the reaction to proceed for approximately 10 minutes, an acid-based stop solution is added, which halts the enzymatic reaction and turns the TMB yellow. The yellow color is proportional to the amount of bound anti-spike protein antibodies in each well and is read at 450 nm. The magnitude of the assay response is expressed as titers. Titer values are defined as the greatest serial dilution at which the assay signal is greater than a cutoff value based on the assay background levels for a panel of serum from normal human donors.

[0253] ELISA Assay Method Qualification

[0254] The INO-4800 SARS-CoV-2 Spike ELISA assay has been qualified and has been found suitable for the its intended use to measure the humoral response in subjects participating in clinical trials involving INO-4800. The formal qualification consisted of 18 plates and was conducted by two operators over the course of four days. The qualification determined the assay sensitivity, specificity, selectivity, and precision. At the time the assay was developed convalescent sera was not available. A monoclonal antibody was therefore used in development. The monoclonal antibody diluted in normal human sera was used to test all parameters in this assay. The overall assay sensitivity was found to be 16.1 ng/mL for 1/20-diluted serum, which is 322 ng/mL for undiluted serum. Specificity was assessed by pre-incubating anti-spike protein antibody with recombinant spike protein prior to assay. Preincubation with the recombinant spike protein resulted in greater than 60% signal

reduction, indicating that the antibody was binding specifically to the spike protein coated to the plate and not to a different assay component. Selectivity was investigated by spiking individual human serum samples with positive control anti-spike antibody at a concentration near the limit of detection. Seven out of 10 individuals had signal above the cutoff, and eight out of the ten individuals had assay signal within 20% of the mean signal for the ten individuals, demonstrating that matrix effects are expected to be minor for most human serum samples when diluted 1/20. Assay precision was assessed by assaying a high, low, and medium anti-spike protein antibody positive control six times on each of six plates. Results indicated low intra-assay raw signal variation but high raw signal inter-assay variation. Since each individual plate cutoff is based on the signal of negative controls on each plate, inter-assay variation in raw signal is not expected to influence the precision of final titer calculations. To test this, the precision of plate cutoffs was evaluated in this qualification by titering the HPC (high positive control) six times on each of six plates for a total of thirty-six titer evaluations. Thirty-five out of the thirty-six values were identical (titer of 180), while one of the titer determinations was one step lower than the rest (60 instead of 180). This resulted in an inter-assay CV of 4.6%.

[0255] Example 5 INO-4800 SARS-CoV-2 Spike ELISPOT Assay

[0256] The enzyme-linked immunospot (ELISPOT) assay is a highly sensitive immunoassay that measures the frequency of cytokine-secreting cells at the single-cell level. In this assay, cells are cultured on a surface coated with a specific capture antibody in the presence or absence of stimuli. After an appropriate incubation time, cells are removed and the secreted molecule is detected using a detection antibody in a similar procedure to that employed by the ELISA. The detection antibody is biotinylated and followed by a streptavidin-enzyme conjugate. By using a substrate with a precipitating rather than a soluble product, the end result is visible spots on the surface. Each spot corresponds to an individual cytokine-secreting cell. The IFN-γ ELISPOT assay qualification was successfully completed with an assessment of assay specificity, reproducibility and precision (intra-assay precision and inter-assay precision), dynamic range, linearity, relative accuracy, limit of detection and quantitation and assay robustness. The assay has been tested and qualified under GLP/GCLP laboratory guidelines.

[0257] ELISPOT Assay Method Qualification. Specificity readings gave a mean value of <10 spot-forming units (SFU) for the assay negative control (medium with DMSO), a mean of 565 SFU for the positive control peptide pool CEF and a mean of 593 SFU in response to stimulation with mitogen (Phorbol Myristate Acetate + Ionomycin). The highest reported

%CV for intra-assay variation was 7.37%. The highest reported %CV for inter-assay variation was 17.23%. The highest observed %CV for inter-operator variability was 8.11 %. These values fall below the FDA-recommended standard acceptance criteria of 20%.

[0258] Linearity of the dilution curve was demonstrated with a slope of 0.15 and an R2 value of 0.99. Assay accuracy was >90% over the listed dynamic range (156-5000 cells/well), falling within the acceptance criteria of 80-120%. Limit of detection was determined to be 11 SFU/1x10^6 PBMCs, limit of quantitation was observed at 20 SFU/1x10^6 PBMCs. Robustness of the assay was evaluated by varying (i) peptide concentration; (ii) secondary antibody concentration; (iii) incubation times, and (iv) drying-out of plate membranes.

[0259] Based on the results of this qualification, the IFN-γ ELISPOT is considered qualified and ready for use in clinical trials.

[0260] Example 6 Phase 1 Open-label Study to Evaluate the Safety, Tolerability and Immunogenicity of INO-4800, a Prophylactic Vaccine Against SARS-CoV-2, Administered Intradermally Followed by Electroporation in Healthy Volunteers

[0261] This is a Phase 1, open-label, multi-center trial (clinicaltrials_gov identifier NCT04336410) to evaluate the safety, tolerability and immunological profile of INO-4800 (pGX9501) administered by intradermal (ID) injection followed by electroporation (EP) using CELLECTRA® 2000 device in healthy adult volunteers. Approximately 40 healthy volunteers will be evaluated across two (2) dose levels: Study Group 1 and Study Group 2 as shown in Table 2. A total of 20 subjects will be enrolled into each Study Group.

[0262] Table 2: COVID19-001 Base Study Dose Groups

Study Group	Number of Subjects	Dosing Weeks	Number of Injections + EP per Dosing Visit	INO-4800 (mg) per injection	INO-4800 (mg) per Dosing Visit	Total Dose of INO-4800 (mg)
1	20	0, 4	1	1.0	1.0	2.0
2	20	0, 4	2[a]	1.0	2.0	4.0
Total	**40**					

[a]INO-4800 will be injected ID followed by EP in an acceptable location on two different limbs at each dosing visit

[0263] All subjects will be followed for 24 weeks following the last dose. Week 28 will be the End of Study (EOS) visit.

[0264] Primary Objectives:

- Evaluate the tolerability and safety of INO-4800 administered by ID injection followed by EP in healthy adult volunteers
- Evaluate the cellular and humoral immune response to INO-4800 administered by ID injection followed by EP

[0265] Primary Safety Endpoints:

- Incidence of adverse events by system organ class (SOC), preferred term (PT), severity and relationship to investigational product
- Administration (i.e., injection) site reactions (described by frequency and severity)
- Incidence of adverse events of special interest

[0266] Primary Immunogenicity Endpoints:

- SARS-CoV-2 Spike glycoprotein antigen-specific antibodies by binding assays
- Antigen-specific cellular immune response by IFN-γ, ELISpot and/or flow cytometry assays

[0267] Exploratory Objective:

- Evaluate the expanded immunological profile by assessing both T and B cell immune response

[0268] Exploratory Endpoint:

- Expanded immunological profile which may include (but not limited to) additional assessment of T and B cell numbers, neutralization response and T and B cell molecular changes by measuring immunologic proteins and mRNA levels of genes of interest at all weeks as determined by sample availability

[0269] Safety Assessment:

[0270] Subjects will be followed for safety for the duration of the trial through the end of study (EOS) or the subject's last visit. Adverse events will be collected at every visit (and a Day 1 phone call). Laboratory blood and urine samples will be drawn at Screening, Day 0 (pregnancy test only), Week 1, Week 4 (pregnancy test only), Week 6, Week 8, Week 12 and Week 28, according to the Schedule of Events (Table 3). All adverse events, regardless of relationship, will be collected from the time of consent until EOS. All serious adverse events,

adverse events of special interest and treatment-related adverse events will be followed to resolution or stabilization.

[0271] Table 3. Clinical Trial Schedule of Events

Tests and assessments	Screen[a]	Day 0		Day 1 (+1d)	Week 1 (± 3d)	Week 4 (± 5d)		Week 6 (± 5d)	Week 8 (± 5d)	Week 12 (± 5d)	Week 28 (± 5d)
		Pre	Post			Pre	Post				
Informed Consent	X										
Inclusion/Exclusion Criteria	X										
Medical History	X	X									
Demographics	X										
Concomitant Medications	X	X			X	X		X	X	X	X
Physical Exam[b]	X	X			X	X		X	X	X	X
Vital Signs	X	X			X	X		X	X	X	X
Height and Weight	X										
CBC with Differential	X				X			X	X	X	X
Chemistry[c]	X				X			X	X	X	X
Serology[d]	X										
12-lead ECG	X										
Urinalysis Routine[e]	X				X			X	X	X	X
Pregnancy Test[f]	X	X				X					
INO-4800 + EP[g]		X[h]				X[h]					
Download EP Data[i]			X				X				
Adverse Events[j]	X	X	X	X[k]	X	X	X	X	X	X	X
Immunology (Whole blood)[l]	X	X				X		X	X	X	X
Immunology (Serum)[m]	X	X				X		X	X	X	X

Table 3 (cont.)

a. Screening assessment occurs from -30 days to -1 day prior to Day 0.
b. Full physical examination at screening and Week 28 (or any other study discontinuation visit) only. Targeted physical exam at all other visits.
c. Includes Na, K, Cl, HCO3, Ca, PO4, glucose, BUN, and Cr.
d. HIV antibody or rapid test, HBsAg, HCV antibody.
e. Dipstick for glucose, protein, and hematuria. Microscopic examination should be performed if dipstick is abnormal.
f. Serum pregnancy test at screening. Urine pregnancy test at other visits.
g. All doses delivered via intradermal injection followed by EP.
h. For Study Group 1, one injection in skin preferably over deltiod muscle at Day 0 and Week 4. For Study Group 2, two injections in skin with each injection over a different deltoid or lateral quadriceps; preferably over the deltoid muscles, at Day 0 and Week 4.
i. Following administration of INO-4800, EP data will be downloaded from the CELLECTRA® 2000 device and provided to Inovio.
j. Includes AEs from the time of consent and all injection site reactions that qualify as an AE.
k. Follow-up phone call to collect AEs.
l. 4 x 8.5 mL (34 mL) whole blood in 10 mL Acid Citrate Dextrose (ACD, Yellow top) tubes per time point. Note: Collect a total of 68 mL whole blood prior to 1st dose (screening and prior to Day 0 dosing).
m. 1 x 8 mL blood in 10 mL red top serum collection tube per time point. Note: Collect four aliquots of 1 mL each (total 4 mL) serum at each time point prior to 1st dose (Screening and prior to Day 0 dosing).

[0272] Immunogenicity Assessment:

[0273] Immunology blood samples will be collected at Screening, Day 0 (prior to dose), Week 4 (prior to dose), Week 6, Week 8, Week 12 and Week 28. Determination of analysis of collected samples for immunological endpoints will be determined on an ongoing basis throughout the study.

[0274] Clinical Trial Population:

[0275] Healthy adult volunteers between the ages of 18-50 years, inclusive.

[0276] Inclusion Criteria:

a. Adults aged 18 to 50 years, inclusive;

b. Judged to be healthy by the Investigator on the basis of medical history, physical examination and vital signs performed at Screening;

c. Able and willing to comply with all study procedures;

d. Screening laboratory results within normal limits or deemed not clinically significant by the Investigator;

e. Negative serological tests for Hepatitis B surface antigen (HBsAg), Hepatitis C antibody and Human Immunodeficiency Virus (HIV) antibody screening;

f. Screening electrocardiogram (ECG) deemed by the Investigator as having no clinically significant findings (e.g. Wolff-Parkinson-White syndrome);

g. Use of medically effective contraception with a failure rate of < 1% per year when used consistently and correctly from screening until 3 months following last dose, be post-menopausal, be surgically sterile or have a partner who is sterile.

[0277] Exclusion Criteria:

a. Pregnant or breastfeeding, or intending to become pregnant or father children within the projected duration of the trial starting with the screening visit until 3 months following last dose;

b. Is currently participating in or has participated in a study with an investigational product within 30 days preceding Day 0;

c. Previous exposure to SARS-CoV-2 (laboratory testing at the Investigator's discretion) or receipt of an investigational vaccine product for prevention of COVID-19, MERS or SARS;

d. Current or history of the following medical conditions:

- Respiratory diseases (e.g., asthma, chronic obstructive pulmonary disease);
- Hypertension, sitting systolic blood pressure >150 mm Hg or a diastolic blood pressure >95 mm Hg;
- Malignancy within 5 years of screening;
- Cardiovascular diseases (e.g., myocardial infarction, congestive heart failure, cardiomyopathy or clinically significant arrhythmias);

e. Immunosuppression as a result of underlying illness or treatment including:

- Primary immunodeficiencies;
- Long term use (≥7 days) of oral or parenteral glucocorticoids;
- Current or anticipated use of disease modifying doses of anti-rheumatic drugs and biologic disease modifying drugs;
- History of solid organ or bone marrow transplantation;
- Prior history of other clinically significant immunosuppressive or clinically diagnosed autoimmune disease.

f. Fewer than two acceptable sites available for ID injection and EP considering the deltoid and anterolateral quadriceps muscles;

g. Any physical examination findings and/or history of any illness that, in the opinion of the study investigator, might confound the results of the study or pose an additional risk to the patient by their participation in the study.

[0278] Clinical Trial Treatment: The INO-4800 drug product contains 10 mg/mL of the DNA plasmid pGX9501 in 1X SSC buffer (150 mM sodium chloride and 15 mM sodium citrate). A volume of 0.4 mL will be filled into 2-mL glass vials that are fitted with rubber stoppers and sealed aluminum caps. INO-4800 is stored at 2–8°C.

[0279] Study Group 1 is administered one 1.0 milligram (mg) intradermal (ID) injection of INO-4800 followed by electroporation (EP) using the CELLECTRA® 2000 device per dosing visit at Day 0 and Week 4. Study Group 2 is administered two 1.0 mg ID injections (total 2.0 mg per dosing visit) (in an acceptable location on two different limbs) of INO-4800 followed by EP using the CELLECTRA® 2000 device at Day 0 and Week 4.

[0280] Peripheral Blood Immunogenicity Assessments

[0281] Whole blood and serum samples are obtained. Immunology blood and serum samples are collected at Screening and at visits specified in the Schedule of Events (Table 2). Both Screening and Day 0 immunology samples are required to enable all immunology testing. The T and B cell immune responses to INO-4800 are measured using assays that may include but are not limited to ELISA, neutralization, assessment of immunological gene expression, assessment of immunological protein expression, flow cytometry and ELISPOT. The ELISA binding assay is a standard plate-based ELISA using 96-well ELISA plates. Plates are coated with SARS-CoV-2 spike protein and blocked. Following blocking, sera from vaccinated subjects are serially diluted and incubated on the plate. A secondary antibody that is able to bind human IgG is used to assess the level of vaccine specific antibodies in the sera. T-cell response is assessed by an IFN-gamma ELISPOT assay. PBMCs isolated from study volunteers are incubated with peptide fragments of the SARS-CoV-2 spike protein. The cells and peptides are placed in a MabTech plates coated with an antibody that captures IFN-gamma. Following 24 hours of stimulation, cells are washed out and a secondary antibody that binds IFN-gamma is added. Each vaccine specific cell creates a spot that can be counted to determine the level of cellular responses induced. In addition, humoral responses to SARS-CoV-2 Nucleocapsid Protein (NP) may also be assessed to rule out potential infection by wild-type SARS-CoV-2 post INO-4800 treatment during the study. Determination of analysis of collected samples for immunological endpoints are determined on an ongoing basis throughout the study.

[0282] Primary Outcome Measure:

1.Percentage of Participants with Adverse Events (AEs) [Time Frame: Baseline up to Week 28]

2. Percentage of Patients with Administration (Injection) Site Reactions [Time Frame: Day 0 up to Week 28]

3. Incidence of Adverse Events of Special Interest (AESIs) [Time Frame: Baseline up to Week 28]

4. Change from Baseline in Antigen-Specific Binding Antibody Titers [Time Frame: Baseline up to Week 28] A subject is considered to have a positive antibody response if the optical density post vaccine is 2.0 SD higher than the optical density at day 0 and above the ELISA specific cut off.

5. Change from Baseline in Antigen-Specific Interferon-Gamma (IFN-ɣ) Cellular Immune Response [Time Frame: Baseline up to Week 28] A subject is considered to have a positive cellular response if the number of IFN-gamma producing cells (spots) post vaccine is 2.0 SD higher than the number of spots at day 0 and above the assay LOD.

[0283] The safety of INO-4800 is measured and graded in accordance with the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", issued September 2007 (Appendix A). An adverse event of special interest (AESI) (serious or non-serious) is one of scientific and medical concern specific to the product or program. AESIs include those listed in Table 4.

[0284] Table 4.

Body System	**AESI**
Respiratory	Acute respiratory distress syndrome (ARDS)
	Pneumonitis/Pneumonia
Neurologic	Generalized convulsion
	Aseptic meningitis
	Guillain-Barré Syndrome (GBS)
	Encephalitis/Myelitis
	Acute disseminated encephalomyelitis (ADEM)
	CNS vasculopathy (stroke)
Hematologic	Thrombocytopenia
	Disseminated intravascular coagulation (DIC)
Immunologic	Anaphylaxis
	Vasculitides
	Enhanced disease following immunization
Other	Local/systemic SAEs
	Acute cardiac injury
	Acute kidney injury
	Septic shock-like syndrome

[0285] DOSE LIMITING TOXICITY (DLT)

[0286] For the purpose of this clinical trial, the following are dose limiting toxicities:

- Grade 3 or greater local injection site erythema, swelling and/or induration observed ≥ 1 day after INO-4800 administration (see Table 5);
- Pain or tenderness at the injection site that requires hospitalization despite proper use of non-narcotic analgesics;

- Grade 4 or greater non-injection site adverse event assessed by the PI as related to INO-4800 administration;
- Grade 4 or greater clinically significant laboratory abnormalities assessed by the PI as related to INO-4800 administration.

[0287] Table 5. Grading Scale for Injection Site Reactions

Local Reaction to Injectable Product (Grade)	**Mild (1)**	**Moderate (2)**	**Severe (3)**	**Potentially Life Threatening (4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness[a]	2.5-5 cm	5.1-10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling[b]	2.5-5 cm and no interference w/ activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis

September 2007 "FDA Guidance for Industry-Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials"
[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable
[b] Should be evaluated and graded using the functional scale as well as the actual measurement.

[0288] ANALYTICAL POPULATIONS

[0289] Analysis populations are:

- The modified intention to treat (mITT) population includes all subjects who receive at least one dose of the INO-4800. Subjects in this sample are analyzed by their assigned dose group of INO-4800. The mITT population are used to analyze co-primary and exploratory immunological endpoints.

- The per-protocol (PP) population is comprised of mITT subjects who receive all their planned administrations and who have no Medical Monitor-assessed important protocol violations. Analyses on the PP population are considered supportive of the corresponding mITT analyses.

[0290] The safety analysis population includes all subjects who receive at least one dose of INO 4800 administered by ID injection. Subjects for this population are grouped in accordance with the dose of INO-4800 that they received. This population is used for all safety analyses in the study.

[0291] PRIMARY SAFETY ANALYSES

[0292] The primary analyses for this trial are safety analyses of treatment emergent adverse events (TEAEs), administration site reactions and clinically significant changes in safety laboratory parameters from baseline.

[0293] TEAEs are defined for this trial as any adverse events, adverse events of special interest, or serious adverse events that occur on or after Day 0 following IP administration. All TEAEs will be summarized by frequency, percentage and associated 95% Clopper-Pearson confidence interval. The frequencies are also presented separately by dose number and are depicted by system order class and preferred term. Additional frequencies are presented with respect to maximum severity and relationship to IP. Multiple occurrences of the same AE in a single subject are counted only once following a worst-case approach with respect to severity and relationship to IP. All serious TEAEs are summarized as above. AE duration is calculated as AE stop date – AE start date + 1 day. AEs and SAEs that are not TEAEs or serious TEAEs are presented in listings.

[0294] All of these primary safety analyses are conducted on the subjects in the safety population.

[0295] PRIMARY IMMUNOGENICITY ANALYSES

[0296] SARS-CoV-2 Spike glycoprotein antigen specific binding antibody titers, and specific cellular immune responses are analyzed by Study Group within age strata. Binding antibody titer is analyzed for each Study Group using the geometric mean and associated 95% confidence intervals. Antigen specific cellular immune response increases are analyzed for each Study Group using medians, inter-quartile range and 95% confidence intervals.

Change from baseline for both binding antibody titer and antigen specific cellular response increases are analyzed using Geometric Mean Fold Rise and 95% confidence intervals. Binding antibody titers are analyzed between each Study Group pair within age strata using the geometric mean ratio and associated 95% confidence intervals. Antigen specific cellular immune responses are analyzed between each Study Group pair within age strata using median differences and associated 95% confidence intervals. All of these primary immunogenicity analyses are conducted on the subjects in the mITT and PP populations.

[0297] EXPLORATORY ANALYSES

[0298] T and B post baseline cell number will be analyzed descriptively by Study Group with means/medians and associated 95% confidence intervals. Percent neutralizing antibodies will be analyzed for each Study Group using medians, inter-quartile range and 95% confidence intervals.

[0299] The safety and immunogenicity of the optional booster dose of INO-4800 following a prior two-dose regimen will be analyzed as described below. Live neutralization reciprocal antibody titer and pseudoneutralization reciprocal antibody titer will be analyzed for each Study Group within age strata using the geometric mean and associated 95% confidence intervals. Fold rise from baseline will tabulated for each immunogenic biomarker. If there is sufficient data for analysis, exploratory between group immunogenic comparisons between subjects who opt for just 2 administrations and subjects who opt for 2 administrations plus the booster administration will be undertaken.

[0300] Further exploration of the effect of age and other potential confounders on the relationship between immune biomarkers and INO-4800 dose may involve the use of ANCOVA and/or Logistic regression models.

[0301] PRELIMINARY BASE STUDY RESULTS

[0302] All 8 adverse events reported were Grade 1; 5 due to local injection site reactions. No serious adverse events, adverse events of special interest, or dose limiting toxicities were reported.

[0303] Preliminary Binding ELISA Analysis demonstrated 7/9 (78%) subjects had positive antibody responses. Responders had a four-fold increase in titer.

[0304] At week six, multiple immunology assays, including those for humoral and cellular immune response, were conducted for both 1.0 mg and 2.0 mg dose cohorts after two doses. Analyses at that point showed that 94% (34 out of 36 total trial participants) demonstrated overall immunological response rates based on preliminary data assessing humoral (binding and neutralizing) and T cell immune responses. One participant in the 1 mg dose cohort and two participants in the 2 mg dose cohort were excluded from the immune analyses as they tested positive for COVID-19 immune responses at study entry, indicating prior infection. One participant in the 2 mg dose cohort discontinued the study for reasons unrelated to safety or tolerability.

[0305] Through week eight, INO-4800 was generally safe and well-tolerated in all participants in both cohorts. All ten reported adverse events (AEs) were grade 1 in severity, with most being injection site redness. There were no reported serious adverse events (SAEs).

[0306] INITIAL PHASE I RESULTS

[0307] Study Population Demographics

[0308] A total of 55 participants were screened and 40 participants were enrolled into the initial two groups (**Figure 16**). The median age was 34.5 years (range 18 to 50 years). Participants were 55% male (Table 6). Most participants were white (82.5%).

[0309] Table 6

Variable	Statistic	Group 1, 1 mg (N=20)	Group 2, 2 mg (N-20)	Overall (N=40)
Gender				
Male	n (%)	11 (55.0)	11 (55.0)	22 (55.0)
Female	n (%)	9 (45.0)	9 (45.0)	18 (45.0)
Race				
White	n (%)	18 (90.0)	15 (75.0)	33 (82.5)
Black or African American	n (%)	1 (5.0)	1 (5.0)	2 (5.0)
Asian	n (%)	1 (5.0)	4 (20.0)	5 (12.5)
Ethnicity				
Hispanic or Latino	n (%)	0	0	0
Not Hispanic or Latino	n (%)	20 (100.0)	20 (100.0)	40 (100.0)
Age (years)	n	20	20	40
	Mean (SD)	35.0 (10.69)	35.6 (9.18)	35.3 (9.84)

	Median	33.0	38.0	34.5
	Min, Max	18, 50	19, 50	18, 50
Baseline Height (cm)	n	20	19	39
	Mean (SD)	172.59 (10.853)	172.16 (8.631)	172.38 (9.707)
	Median	169.75	170.10	170.10
	Min, Max	155.9, 195.6	158.0, 188.0	155.9, 195.6
Baseline Weight (kg)	n	20	19	39
	Mean (SD)	74.13 (14.701)	71.35 (12.611)	72.77 (13.615)
	Median	70.45	69.00	69.60
	Min, Max	58.5, 110.0	55.0, 92.5	55.0, 110.0

[0310] The vaccine was administered in 0.1 ml intradermal injections followed by EP at the site of vaccination. EP was performed using CELLECTRA® 2000 with four 52-msec pulses at 0.2A (40 to 200 V, depending on tissue resistance) per season. The first two pulses were spaced 0.2 seconds apart followed by a 3-second pause before the final two pulses that were also spaced by 0.2 seconds. The dose groups were enrolled sequentially with a safety run-in for each. Participants were and will be evaluated clinically and for safety on Day 1 and at Weeks 1, 4 (Dose 2), 6, 8, 12, 28, 40 and 52. Safety laboratory testing (complete blood count, comprehensive metabolic panel and urinalysis) were and will be conducted on all follow-up visits except for Day 0, Day 1 and Week 4. Immunology specimens were obtained at all time points post-dose 1 except Day 1 and Week 1. Local and systemic AEs, regardless of relationship to the vaccine, were recorded and graded by the investigator. AEs were graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines that were issued by the Food and Drug Administration in September 2007.

[0311] Vaccine Safety and Tolerability

[0312] 39 (97.5%) completed both doses and 1 subject in the 2.0 mg group discontinued trial participation prior to receiving the second dose due to lack of transportation to the clinical sites, unrelated to the study or the dosing. All 39 remaining subjects completed the visit 8 weeks post-dose 1. There were a total of 11 local and systemic AEs reported by 8 weeks post-dose 1, six of these were deemed related to vaccine. All AEs were mild or Grade 1 in severity. The most frequent AEs were injection site reactions including injection site pain (3) and erythema (2). One systemic AE related to the vaccine was nausea. There were no

febrile reactions. No subjects discontinued the trial due to an AE. No serious adverse events (SAEs) nor AESIs were reported. There were no abnormal laboratory values of clinical concern throughout the initial 8-week follow-up period. There was no increase in the number of participants who experienced AEs related to the vaccine in the 2.0 mg group (10% of subjects), compared to that in the 1.0 mg group (15% of subjects). In addition, there was no increase in frequencies of AEs with the second dose over the first dose in both dose level groups. The INO-4800 Phase 1 safety data thus suggests that the vaccine is likely a safe booster as there was no increase frequency of side effects after the second vaccine administration compared to the first dose.

[0313] Immunogenicity: Thirty-eight subjects were included in the immunogenicity analysis. In addition to one subject in the 2.0 mg group who discontinued prior to completing dosing, one subject in the 1.0 mg group was deemed seropositive at baseline and was excluded.

[0314] Humoral Immune Responses: Serum samples were used to measure neutralizing antibody titers against SARS-CoV-2/Australia/ VIC01/2020 isolate and binding antibodies to RBD and whole spike S1 +S2 protein.

[0315] S1+S2 Enzyme-Linked Immunosorbent Assay (ELISA): A standard binding ELISA was used to detect serum binding anti-SARS-CoV-2 spike antibodies. ELISA plates were coated with recombinant S1+S2 SARS-CoV-2 spike protein (Sino Biological) and incubated overnight and blocked. Samples were serially diluted and incubated on the blocked assay plates for one hour. The magnitude of the assay response was expressed as titers which were defined as the greatest serial dilution at which the optical density 3 SD above background Day 0. 68% of participants in the 1.0 mg group and 70% of participants in the 2.0 mg group had at least an increase in serum IgG binding titers to S1+S2 spike protein when compared to their pre-vaccination time point (Day 0), with the responder GMT of 320.0 (95% CI: 160.5, 638.1) and 508.0 (95% CI: 243.6, 1059.4) in the 1.0 mg and 2.0 mg groups, respectively (Figure 17C). In Figure 17D, the humoral response in the 1.0 mg dose group and 2.0 mg dose group was assessed for the ability to bind whole spike protein (S1 and S2) (n=19, 1.0mg; n=19, 2.0 mg). End point titers were calculated as the titer that exhibited an OD 3.0 SD above baseline, titers at baseline were set at 1. A response to live virus neutralization was a PRNT IC50 $\geq$ 10. In all graphs horizontal lines represent the Median and bars represent the Interquartile Range.

[0316] Sera was also tested for the ability to neutralize live virus in SARS-CoV-2 wildtype virus neutralization assays. SARS-CoV-2/Australia/ VIC01/2020 isolate neutralization assays were performed at Public Health England (Porton Down, UK). Neutralizing virus titers were measured in serum samples that had been heat-inactivated at 56 °C for 30 min. SARS-CoV-2 (Australia/VIC01/2020 isolate44) was diluted to a concentration of 933 pfu ml−1 and mixed 50:50 in 1% FCS/MEM containing 25mM HEPES buffer with doubling serum dilutions. After 5 days incubation at 37 °C in a humidified box, the plates were fixed, stained and plaques counted. Virus titer were determined using a standard 50% tissue culture infection dose (TCID50) assay. After the second vaccination at week 6, the responder geometric mean titer (GMT) by live virus PRNT IC50 neutralization assay were 82.4 and 63.5 in the 1.0 mg and 2.0 mg groups, respectively. The percentage of responders (post vaccination PRNT IC50 ≥ 10) were 83% and 84% in the 1.0 mg and 2.0 mg groups, respectively (**Figure 17A** and Table 7).

[0317]

Table 7. Live SARS-CoV-2 Neutralization

	1.0mg N=18*	2.0mg N=19
Overall Week 6 GMT Reciprocal Titer (95% CI) Range	44.4 (14.6, 134.8) 1, 11647	34.9 (15.8, 77.2) 1, 652
Responders** n (%) Week 6 GMT Reciprocal Titer (95% CI) Range	15 (83%) 82.4 (29.1, 233.3) 4, 11647	16 (84%) 63.5 (39.6, 101.8) 13, 652

* Excludes one subject with baseline positive NP ELISA
** Week 6 PRNT $IC_{50} \geq 10$, or ≥4 if binding ELISA activity is seen

[0318] RBD Enzyme-Linked Immunosorbent Assay (ELISA): MaxiSorp 96-well plates (ThermoFisher, 439454) were coated with 50ul/well of 1ug/ml of SARS-CoV-2 RBD (SinoBiological, 40592-V08H), protein diluted in PBS and incubated at 4oC overnight. Plates were washed 4 times with PBST (PBS with 0.05% Tween-20) and blocked with 200ul/well of blocking buffer (PBS with 5% non-fat dry milk and 0.1% Tween-20) at room temperature for 2hr. After washing with PBST, 50ul/well of sera sample serially diluted in blocking buffer was added to the plate in duplicate and incubated at room temperature for

2hr. After washing with PBST, 50ul/well of anti-human-IgG-HRP detection antibody (BD Pharmingen, 555788) diluted 500-fold in blocking buffer was added and incubated at room temperature for 1hr. After washing with PBST, 50ul/well of 1-Step Ultra TMB (Thermo, 34028) was added and incubated at room temperature for 5min. 50ul/well of 2M sulfuric acid was added to stop the color change reaction and optical absorbance was measured at 450 and 570nm on a Synergy 2 microplate reader (Biotek). Endpoint titers were defined as the greatest serial dilution at which the OD450-570 values were 3 standard deviations above the matched Day 0 signal. At week 6, the responder GMT were 385.6 (95% CI: 69.0, 2154.9) and 222.1 (95% CI: 87.0, 566.8) in the 1.0 mg and 2.0 mg groups, respectively (**Figure 17B**).

[0319] Overall seroconversion (defined as those participants who respond with neutralization or binding antibodies to S protein or RBD) after 2 vaccine doses in 1.0 mg and 2.0 mg dose group were 89% and 95%, respectively.

[0320] Cellular Responses: Peripheral Blood Mononuclear Cells (PBMCs) were isolated from blood samples, frozen and stored in liquid nitrogen for subsequent analyses.

[0321] *INO-4800 SARS-CoV-2 Spike ELISPOT.* Peripheral mononuclear cells (PBMCs) were isolated pre- and post-vaccination. Cells were stimulated *in vitro* with a pool of 15-mer peptides (overlapping by 9 residues) spanning the full-length consensus spike protein sequence. Cells were incubated overnight (18-22h, 37C, 5% CO2) with peptide pools (225µg/ml), DMSO alone (0.5 %, negative control) or PMA and Ionomycin (positive controls). The next day, cells were washed off, and the plates were developed: The detection antibody is biotinylated and followed by a streptavidin-enzyme conjugate. By using a substrate with a precipitating rather than a soluble product, resulting in visible spots. Each spot corresponds to an individual cytokine-secreting cell. After plates were developed, spots were scanned and quantified using the CTL S6 Micro Analyzer (CTL) with ImmunoCapture™ and ImmunoSpot™ software. Values are shown as background-subtracted average of measured triplicates.

[0322] The percentage of responders at week 8 was 74% in the 1.0 mg dose group, and 100% in the 2.0 mg dose group (Table 8). The Median SFU per 10^6 PBMC was 46 and 71 for the responders in 1.0 mg and 2.0 mg dose groups, respectively. In each group, there were statistically significant increases in the numbers of interferon-γ–secreting cells (SFU) obtained per million PBMCs over baseline ($P=0.001$ and $P<0.0001$, respectively, Wilcoxon matched-pairs signed rank test, post-hoc analysis), Figure 18A. Interestingly, 5 non-

responders in 1.0 mg group by T cell ELISPot assay showed strong reactivity by live virus neutralization assay. It is also interesting to note that 3 convalescent samples tested by the ELISpot assay showed lower T cell responses, with a median of 33, than the 2.0 mg dose group at Week 8. INO-4800 generated strong T cell responses that were more frequent and a higher responder median response (45.6 vs 71.1) in the 2.0 mg dose group. The 2.0 mg group's T cell responses were mapped to 5 epitope pools as shown in Figure 18B. Interestingly T cell responses in the all regions of the Spike protein were observed.

[0323] Table 8. Immune Responses

	1.0 mg Cohort		2.0 mg Cohort	
Immune Assay	Output Value	Responders‡ n (%)	Output Value	Responders‡ n (%)
Neutralization Week 6 GMT Reciprocal Titer [95% CI] (Range)	44.4 [14.6, 134.8] (1, 11647)	15/18 (83%)	34.9 [15.8, 77.2] (1, 652)	16/19 (84%)
RBD Binding Antibody Week 6 GMT Reciprocal Titer [95% CI] (Range)	27.3 [4.8, 156.8] (1, 15625)	10/18 (56%)	66.8 [17.4, 257.5] (1, 3125)	14/18 (78%)
S1+S2 Binding Antibody Week 6 GMT Reciprocal Titer [95% CI] (Range)	174.4 [59.9, 507.3] (1, 2560)	17/19 (89%)	136.8 [34.5, 543.1] (1, 2560)	15/19 (79%)
IFN-gamma ELISpot Week 8 Median SFU per [95% CI] (Range)	26.2 SFU [10-64] (1, 374.4)	14/19 (74%)[µ]	71 SFU [32-194] (8.9, 615.6)	19/19 (100%)[µ]

1.0mg Cohort excludes one subject with baseline ELISA titer of 1280

‡ Response criteria: Neutralization -Week 6 PRNT $IC_{50} \geq 10$, or ≥4 if binding ELISA activity is seen RBD Binding -Week 6 value >1 ELISpot – Value ≥12 SFU over Week 0

µ - Responders generated using Week 6 and Week 8 data

[0324] INO-4800 SARS-CoV-2 Spike Flow Cytometry Assay: The contribution of CD4+ and CD8+ T cells to the cellular immune response against INO-4800 was assessed by

intracellular cytokine staining (ICS). PBMCs were also used for Intracellular Cytokine Staining (ICS) analysis using flow cytometry. One million PMBCs in 200uL complete RPMI media were stimulated for six hours (37°C, 5% CO_2) with DMSO (negative control), PMA and Ionomycin (positive control, 100ng/mL and 2μg/mL, respectively), or with the indicated peptide pools (225ug/mL). After one hour of stimulation, Brefeldin A and Monensin (BD GolgiStop and GolgiPlug, 0.001% and 0.0015%, respectively) were added to block secretion of expressed cytokines. After stimulation the cells were moved to 4°C overnight. Next, cells were washed in PBS for live/dead staining (Life Technologies Live/Dead aqua fixable viability dye, as previously described), and then resuspended in FACS buffer (0.5%BSA, 2mM EDTA, 20mM HEPES). Next, cells were stained for extracellular markers, fixed and permeabilized, and then stained for the indicated cytokines (Table 9) for antibodies used for flow cytometry.

[0325] Table 9: Flow Cytometry Panel

Tube	Channel	Marker/Cytokine
1	Unstained	NA
2	BV510	Live/Dead Fix Aqua
3	BUV737	CD8
4	APC-Cy7	IL-2
5	BV650	CD45RA
6	APC	CD3
7	BV786	CD14/CD16/CD19
8	BV711	IFN-gamma
9	BV421	CCR7
10	PE-Cy7	IL-17
11	FITC	FITC
12	PE Dazzle (PE-CF594)	IL-4
13	PE	CD107a
14	PerCP-eFluor710 (PerCP-Cy5.5)	CD4

[0326] CD8+ T cells producing IFN-γ, TNF-α and/or IL-2 (any response) were statistically significantly increased post vaccination in the 2.0 mg dose group (**Figure 18C**, P=0.0181, Wilcoxon matched-pairs signed rank test, post-hoc analysis). CD4+T cells producing TNF- α were also statistically significantly increased in the 2.0 mg dose group (**Figure 18C,** P=0.0020, Wilcoxon matched-pairs signed rank test, post-hoc analysis).

[0327] CD4+ and CD8+ T cells were explored following vaccination. Nearly half (47%) of the CD8+T cells in the 2.0 mg dose group were dual producing IFN-ɣ and TNF-α (**Figure 18E**). CD8+ T cells producing cytokine in the 1.0 mg dose group were primarily monofunctional IFN-ɣ producing cells. The CD4+ T cell compartment was highly polyfunctional with 6% and 9% (in the 1.0 mg and 2.0 mg dose groups, respectively) producing all 3 cytokines, IFN-ɣ, TNF-α, and IL-2.

[0328] The composition of CD4+ or CD8+ T cells producing any cytokine (any response, IFN-ɣ or TNF-α or IL-2 following vaccination) was also assessed for surface markers CCR7 and CD45RA to characterize effector (CCR7-CD45RA+), effector memory (CCR7-CD45RA-), and central memory (CCR7+CD45RA-) cells (**Figure 18D**). In both dose groups, CD8+T cells making cytokine in response to stimulation with spike peptides were balanced across the three populations, whereas CD4+ T cells were predominantly of the central memory phenotype (**Figure 18D**).

[0329] Th2 responses were also measured by assessing IL-4 production, and no statistically significant increases (Wilcoxon matched-pairs signed rank test, post-hoc analysis) were observed in either group post vaccination (**Figure 18F**).

[0330] In this Phase 1 trial, INO-4800 vaccination led to potent T cell responses with increased Th1 phenotype, demonstrated by both IFN-ɣ ELISpot as well as multiparametric flow cytometry, as evidenced by increased expression of Th1-type cytokines IFN-ɣ, TNF-α. and IL-2 (**Figure 18C**). Assessment of polyfunctionality of T cells induced by INO-4800 suggested the presence of SARS-CoV-2 specific CD4+ and CD8+ T cells exhibiting hallmarks of memory status suggest that a persistent cellular response has been established (Figure 18D). Importantly, this was accomplished while minimizing induction of IL-4, a prototypical Th2 cytokine (**Figure 18F**).

[0331] PHASE 1 UPDATE

[0332] This was designed as a Phase 1, open-label, multicenter trial (NCT04336410) to evaluate the safety, tolerability and immunogenicity of INO-4800 administered intradermally (ID) followed by electroporation using the CELLECTRA 2000 device. Healthy participants 18 to 50 years of age without a known history of COVID-19 illness received either a 1.0 mg or 2.0 mg dose of INO-4800 in a 2-dose regimen (Weeks 0 and 4).

[0333] DNA vaccine INO-4800. The vaccine was produced according to current Good Manufacturing Practices. INO-4800 contains plasmid pGX9501 expressing a synthetic, optimized sequence of the SARS-CoV-2 full length spike glycoprotein which was optimized as previously described at a concentration of 10 mg/ml in a saline sodium citrate buffer.

[0334] Endpoints. Safety endpoints included systemic and local administration site reactions up to 8 weeks post-dose 1. Immunology endpoints include antigen-specific binding antibody titers, neutralization titers and antigen-specific interferon-gamma (IFN-g) cellular immune responses after 2 doses of vaccine. For Live Virus Neutralization, a responder is defined as Week 6 PRNT IC50 > 10, or > 4 if a subject is a responder in ELISA. For S1+S2 ELISA, a responder is defined as a Week 6 value > 1. For the ELISpot assay, a responder is defined as a Week 6 or Week 8 value that is > 12 spot forming units per 10^6 PBMCs above Week 0.

[0335] Study procedures.

[0336] Forty participants were enrolled into two groups; 20 participants in each of 1.0 mg and 2.0 mg dose groups that received their doses on Weeks 0 and 4. The vaccine was administered in 0.1 ml intradermal injections in the arm followed by EP at the site of vaccination. Subjects in the 1.0 mg dose group received one injection on each dosing visit. The second dose of the vaccine could be injected in the same arm or a different arm relative to the first dose. Subjects in the 2.0 mg dose group received one injection in each arm at each dosing visit. EP was performed using CELLECTRA® 2000 as previously described. The device delivers total four electrical pulses, each 52 ms in duration at strengths of 0.2 A current and voltage of 40-200 V per pulse. The dose groups were enrolled sequentially with a safety run-in for each. The 1.0 mg dose group enrolled a single participant per day for 3 days. An independent Data Safety Monitoring Board (DSMB) reviewed the Week 1 safety data and based on a favorable safety assessment, made a recommendation to complete enrollment of the additional 17 participants into that dose group. In a similar fashion, the 2.0 mg dose group was subsequently enrolled. Participants were assessed for safety and concomitant medications at all time points, including screening, Week 0 (Dose 1), post dose next day phone call, Week 1, 4 (dose 2), 6, 8, 12, 28, 40 and 52 post-dose 1. Local and systemic AEs, regardless of relationship to the vaccine, were recorded and graded by the investigator. Safety laboratory testing (complete blood count, comprehensive metabolic panel and urinalysis)

were and will continue to be conducted at screening, Week 1, 6, 8, 12,28 and 52 post-dose 1. Immunology specimens were obtained at all time points post-dose 1 except at Day 1 and Week 1. AEs were graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines that were issued by the Food and Drug Administration in September 2007. The DSMB reviewed laboratory and AE data for the participants up to 8 weeks included in this report. There were protocol-specified safety stopping rules and adverse events of special interest (AESIs). For the purpose of this report, clinical and laboratory safety assessments up to 8 weeks post the first dose are presented.

[0337] Protocol eligibility. Eligible participants must have met the following criteria: healthy adults aged between 18 and 50 years; able and willing to comply with all study procedures; Body Mass Index of 18-30 kg/m^2 at screening; negative serological tests for Hepatitis B surface antigen, Hepatitis C antibody and Human Immunodeficiency Virus antibody; screening electrocardiogram (ECG) deemed by the Investigator as having no clinically significant findings; use of medically effective contraception with a failure rate of <1% per year when used consistently be post-menopausal, or surgically sterile or have a partner who is sterile. Key exclusion criteria included the following: individuals in a current occupation with high risk of exposure to SARS-CoV-2;previous known exposure to SARS-CoV-2 or receipt of an investigational product for the prevention or treatment of COVID-19; autoimmune or immunosuppression as a result of underlying illness or treatment; hypersensitivity or severe allergic reactions to vaccines or drugs; medical conditions that increased risk for severe COVID-19;reported smoking, vaping, or active drug, alcohol or substance abuse or dependence; and fewer than two acceptable sites available for intradermal injection and electroporation.

[0338] Clinical Trial Population:

[0339] Healthy adult volunteers between the ages of 18-50 years, inclusive.

[0340] Inclusion Criteria:

a. Adults aged 18 to 50 years, inclusive;

b. Judged to be healthy by the Investigator on the basis of medical history, physical examination and vital signs performed at Screening;

c. Able and willing to comply with all study procedures;

d. Screening laboratory results within normal limits or deemed not clinically significant by the Investigator;

e. Negative serological tests for Hepatitis B surface antigen (HBsAg), Hepatitis C antibody and Human Immunodeficiency Virus (HIV) antibody screening;

f. Screening electrocardiogram (ECG) deemed by the Investigator as having no clinically significant findings (e.g. Wolff-Parkinson-White syndrome);

g. Use of medically effective contraception with a failure rate of < 1% per year when used consistently and correctly from screening until 3 months following last dose, be post-menopausal, be surgically sterile or have a partner who is sterile.

[0341] Exclusion Criteria:

a. Pregnant or breastfeeding, or intending to become pregnant or father children within the projected duration of the trial starting with the screening visit until 3 months following last dose;

b. Is currently participating in or has participated in a study with an investigational product within 30 days preceding Day 0;

c. Previous exposure to SARS-CoV-2 (laboratory testing at the Investigator's discretion) or receipt of an investigational vaccine product for prevention of COVID-19, MERS or SARS;

d. Current or history of the following medical conditions:

- Respiratory diseases (e.g., asthma, chronic obstructive pulmonary disease);
- Hypertension, sitting systolic blood pressure >150 mm Hg or a diastolic blood pressure >95 mm Hg;
- Malignancy within 5 years of screening;
- Cardiovascular diseases (e.g., myocardial infarction, congestive heart failure, cardiomyopathy or clinically significant arrhythmias);

e. Immunosuppression as a result of underlying illness or treatment including:

- Primary immunodeficiencies;
- Long term use (≥7 days) of oral or parenteral glucocorticoids;
- Current or anticipated use of disease modifying doses of anti-rheumatic drugs and biologic disease modifying drugs;

- History of solid organ or bone marrow transplantation;
- Prior history of other clinically significant immunosuppressive or clinically diagnosed autoimmune disease.

f. Fewer than two acceptable sites available for ID injection and EP considering the deltoid and anterolateral quadriceps muscles;

g. Any physical examination findings and/or history of any illness that, in the opinion of the study investigator, might confound the results of the study or pose an additional risk to the patient by their participation in the study.

[0342] Immunogenicity assessment methods.

[0343] Samples collected at screening, Week 0 (prior to dose) and at Weeks 6 and 8 were analyzed. Peripheral Blood Mono-nuclear Cells (PBMCs) were isolated from blood samples by a standard overlay on ficoll hypaque followed by centrifugation. Isolated cells were frozen in 10% DMSO and 90% fetal calf serum. The frozen PBMCs were stored in liquid nitrogen for subsequent analyses. Serum samples were stored at -80°C until used to measure binding and neutralizing antibody titers.

[0344] SARS-CoV-2 wildtype virus neutralization assays.

[0345] SARS-CoV-2/Australia/VIC01/2020 isolate neutralization assays were performed at Public Health England (Porton Down, UK). Neutralizing virus titers were measured in serum samples that had been heat-inactivated at 56°C for 30 min. SARS-CoV-2 (Australia/VIC01/2020 isolate44) was diluted to a concentration of 933 pfu/m land mixed 50:50 in 1% FCS/MEM containing 25 mM HEPES buffer with doubling serum dilutions. After a 1 h incubation at 37 °C, the virus-antibody mixture was transferred to confluent monolayers of Vero E6 cells (ECACC 85020206; PHE, UK). Virus was allowed to adsorb onto cells at 37 °C for a further hour in an incubator, and the cell monolayer was overlaid with MEM/4% FBS/1.5% CMC. After 5 days incubation at 37 °C, the plates were fixed, stained, with 0.2% crystal violet solution (Sigma) in 25% methanol (v/v). Plaques were counted.

[0346] S1+S2 enzyme-linked immunosorbent assay (ELISA).

[0347] ELISA plates were coated with 2.0mg/mL recombinant SARS-CoV-2 S1+S2 spike

protein (Acro Biosystems; SPN-C52H8) and incubated overnight at 2-8 °C. The S1+S2 contains amino acids residues Val 16-Pro 1213 of the full length spike protein, GenBank # QHD43416.1.It contains two mutations to stabilize the protein to the trimeric pre-fusion state (R683A, R685A) and also contains a C-terminal 10xHis tag (SEQ ID NO: 24). The plates were then washed with PBS with 0.05% Tween-20 (Sigma; P3563) and blocked (Starting Block, Thermo Scientific;37,538) for 1-3 h at room temperature. Samples were serially diluted using blocking buffer and were added in duplicate, along with prepared controls, to the washed and blocked assay plates. The samples were incubated on the blocked assay plates for one hour at room temperature. Following sample and control incubation, the plates were washed and a 1/1000 preparation of anti-human IgG HRP con-jugate (BD Pharmingen; 555,788) in blocking buffer was then added to each well and allowed to incubate for 1 h at room temperature. The plates were washed and TMB substrate (KPL; 5120-0077) was then added and allowed to incubate at room temperature for approximately 10 min. TMB Stop Solution (KPL; 5150-0021) was next added and the plates read at 450 nm and 650 nm on a Synergy HTX Micro-plate Reader (BioTek). The magnitude of the assay response was expressed as titers which were defined as the greatest reciprocal dilution factor of the greatest dilution serial dilution at which the plate corrected optical density is 3 SD above background a subject's corresponding Week 0.

[0348] SARS-CoV-2 spike ELISpot assay.

[0349] Peripheral mononuclear cells (PBMCs) pre- and post-vaccination were stimulated in vitro with 15-mer peptides (overlapping by 9 residues) spanning the full-length consensus spike protein sequence. Cells were incubated overnight in an incubator with peptide pools at a concentration of 5mg per ml in a precoated ELISpot plate, (Mab-Tech, Human IFN-g ELISpot Plus). The next day, cells were washed off, and the plates were developed via a biotinylated anti-IFN-g detection antibody followed by a streptavidin-enzyme conjugate resulting in visible spots. Each spot corresponds to an individual cytokine-secreting cell. After plates were developed, spots were scanned and quantified using the CTL S6 Micro Analyzer (CTL) with Immuno-Capture and ImmunoSpot software. Values are shown as the background-subtracted average of measured triplicates. The ELISpot assay qualification determined that 12 spot forming units was the lower limit of detection. Thus, anything above this cutoff is considered to be a signal of an antigen specific cellular response.

[0350] INO-4800 SARS-CoV-2 spike flow cytometry assay.

[0351] PBMCs were also used for Intracellular Cytokine Staining (ICS)analysis using flow cytometry. One million PMBCs in 200mL complete RPMI media were stimulated for six hours (37 °C, 5% CO_2) with DMSO (negative control), PMA and Ionomycin (positive control,100 ng/mL and 2mg/mL, respectively), or with the indicated peptide pools (225 μg/mL). After one hour of stimulation, Brefeldin A and Monensin (BD GolgiStop and GolgiPlug, 0.001% and 0.0015%, respectively) were added to block secretion of expressed cytokines. After stimulation the cells were moved to 4 °C overnight. Next, cells were washed in PBS for live/dead staining (Life Technologies Live/Dead aqua fixable viability dye), and then resuspended in FACS buffer (0.5%BSA, 2 mM EDTA, 20 mM HEPES). Next, extracellular markers were stained, the cells were fixed and permeabilized (eBioscience™ Foxp3Kit) and then stained for the indicated cytokines (Table 9) using fluorescently conjugated antibodies. Figs. 22A and B show representative gating strategies for CD4+ and CD8+ T cells as well as examples of positive expression of IFNγ, TNFα, IL-2 and IL-4.

[0352] Statistical analysis.

[0353] No formal power analysis was applicable to this trial. Descriptive statistics were used to summarize the safety end-points: proportions with AEs, administration site reactions, and AESIs through 8 weeks. Descriptive statistics were also used to summarize the immunogenicity endpoints: median responses (with 95% confidence intervals) and percentage of responders for cellular results, and geometric mean titers (with 95% confidence intervals) and percentage of responders for humoral results. Post-hoc analyses of post-vaccination minus pre-vaccination paired differences in SARS-CoV-2 neutralization responses (on the natural log-scale, with a paired t-test), ELISpot responses (with Wilcoxon signed-rank tests), and Intracellular Flow Assay responses (with Wilcoxon signed-rank tests) were performed.

[0354] Results

[0355] Study population demographics.

[0356] A total of 55 participants were screened and 40 participants were enrolled into the initial two groups (**Figure 16**). The median age was 34.5 years (range 18 to 50 years). Participants were 55% (22/40) male (Table 6). Most participants were white (82.5%, 33/40).

[0357] Vaccine safety and tolerability.

[0358] A total of 39 of 40 (97.5%) participants completed both doses; one participant in the 2.0 mg group discontinued trial participation prior to receiving the second dose due to lack of transportation to the clinical sites, and discontinuation was unrelated to the study or the dosing (**Figure 16**). All 39 remaining subjects completed the visit 8 weeks post-dose 1. There was a total of 11 local and systemic adverse events (AEs) reported by 8 weeks post-dose 1; six of these were deemed related to vaccine (Table 10). All AEs were Grade 1 (mild) in severity. Five of the six related AEs were injection site reactions including injection site pain (3) and erythema (2). One Grade 1 systemic AE related to the vaccine was nausea. All related AEs occurred on the dosing day when the subjects received the first or second vaccination. There were no febrile reactions and no antipyretic medicine was used post vaccination. No subject discontinued the trial due to an AE. No serious adverse events (SAEs) nor adverse events of special interest (AESIs) were reported. There were no abnormal laboratory values that were deemed clinically significant by the Investigators throughout the initial 8-week follow-up period. There was no increase in the number of participants who experienced AEs related to the vaccine in the 2.0 mg group (10%, 2/20), compared to that in the 1.0 mg group (15%, 3/20) (**Figure 19**). In addition, there was no increase in frequencies of AEs with the second dose over the first dose in both dose groups.

[0359] Table 10. Number of Adverse Events classified by MedDRA® System Organ Class, severity, and investigator assigned relationship to study vaccination

MedDRA® System Organ Class	Severity	Not related to vaccination	Related to vaccination	Total number
Any system Organ Class	Mild	5	6	11
	Moderate	-	-	-
	Severe	-	-	-
Gastrointestinal Disorders	Mild	1	1	2
	Moderate	-	-	-
	Severe	-	-	-
	Mild	-	5	5

General Disorders and Administration Site Conditions	Moderate	-	-	-
	Severe	-	-	-
Injury, Poisoning, and Procedural Complications	Mild	2	-	2
	Moderate	-	-	-
	Severe	-	-	-
Neoplasm Benign, Malignant and Unspecified	Mild	1	-	1
	Moderate	-	-	-
	Severe	-	-	-
Nervous System Disorders	Mild	1	-	1
	Moderate	-	-	-
	Severe	-	-	-

[0360] Immunogenicity.

[0361] Thirty-eight subjects were included in the immunogenicity analyses. In addition to one subject in the 2.0 mg group who discontinued prior to completing dosing, one subject in the 1.0 mg group was deemed seropositive at baseline and was excluded. Data for this subject can be found in Table 11.

[0362] Table 11. Immune Responses for subject who was Sero-positive at enrollment, INO-4800 1.0 mg Dose Group

Immune Assay	Output at Week 0	Output at Week 6
Neutralization Week 6 Reciprocal Titer	785	1089
RBD Binding Antibody Week 6 Reciprocal Titer	1	1
S1+S2 Binding Antibody Week 6 Reciprocal Titer	1	14580

IFN-gamma ELISpot Week 6 SFU/10^6 PBMC	55.6	27.8

[0363] Humoral immune responses.

[0364] Sera was tested for the ability to bind S1+S2 spike protein. 89%(17/19) of participants in the 1.0 mg group and 95% (18/19) of participants in the 2.0 mg group had an increase in serum IgG binding titers to S1+S2 spike protein when compared to their pre-vaccination timepoint (Week 0), with the responder GMT of 655.5 (95% CI:255.6,1681.0) and 994.2 (95% CI: 395.3, 2500.3) in the 1.0 mg and 2.0 mg groups, respectively (**Figure 17B, Figure 20** and Table 13). Sera was also tested for the ability to neutralize live virus by live virus PRNTIC50 neutralization assay. The geometric mean fold-rise at Week 6 relative to baseline was 10.8 with a 95% CI of (4.4, 27.0) and 11.5 with a 95% CI of (5.3, 24.9) in the 1.0 mg and 2.0 mg groups, respectively. In each group, there was a statistically significant increase at Week 6 over baseline ($P<0.0001$ paired t-test, post-hoc analysis), **Figure 17A**. At Week 6, the percentage of responders were 78% (14/18) and 84% (16/19) in the 1.0 mg and 2.0 mg groups, respectively (**Figure 17A** and Table 13), and the responder geometric mean titer (GMT) were 102.3 (95% CI: 37.4, 280.3) and 63.5 (95% CI: 39.6, 101.8) in the 1.0 mg and 2.0 mg groups, respectively. Overall seroconversion (defined as those participants who respond with neutralization and/or binding anti-bodies to S protein) at Week 6 in 1.0 mg and 2.0 mg dose group were 95% (18/19) for each group (Table 13).

[0365] Enzyme-linked immunospot (ELISpot).

[0366] The percentage of responders at week 8 was 74% (14/19) in the1.0 mg dose group, and 100% (19/19) in the 2.0 mg dose group. These data taken with the seroconversion data result in a 100% (19/19) overall immune response in each group (Table 13, **Figures 18A and 21**).The Median SFU per 10^6 PBMC was 46 (95% CI: 21.1, 142.2) and 71 (95% CI: 32.2-194.4) for the responders in 1.0 mg and 2.0 mg dose groups, respectively. The median change at week 8 relative to base-line was 22.3 (95% CI: 2.2, 63.4) and 62.8 (95% CI: 22.2, 191.1) in the respective groups, and in each group, there were statistically significant increases over baseline ($P= 0.001$ and $P<0.0001$, respectively, Wilcoxon matched-pairs signed rank test, post-hoc analysis), Fig. 18A. It is also interesting to note that 3 convalescent samples (all 3 with symptoms but non-hospitalized), tested by the ELISpot assay showed lower T cell

responses, with a median of 33, than the 2.0 mg dose group at Week 8 (**Figure 20**). As shown in **Figures 18B and 18G**, the 2.0 mg group's T cell responses were mapped to 5 epitope pools. Encouragingly, T cell responses were seen in all regions of the spike protein, with the dominant pool encompassing the Receptor Binding Domain region, followed by pools covering the N Terminal Domain, as well as the Fusion Peptide, Heptad Repeat 1 and the Central Helix.

[0367] Intracellular flow assay.

[0368] The contribution of CD4+and CD8+T cells to the cellular immune response against INO-4800 was assessed by intracellular cytokine staining (ICS). In the 2.0 mg dose group, the median change from baseline to Week 6 in CD8+T cells producing IFN-γ, TNF-α and/or IL-2 (Any Response) was 0.11 with a 95% CI of (-0.02, 0.23); the change was significantly increased (P= 0.0181, Wilcoxon matched-pairs signed rank test, post-hoc analysis). owing chiefly to significant increases in IFN-γ as well as TNF-α production (**Figure 18C**). Also in the 2.0 mg dose group, the median change from baseline to Week 6 in CD4+T cells producing TNF-α was 0.02 with a 95% CI of (0.01 to 0.09); the change was also significantly increased (P= 0.0020, Wilcoxon matched-pairs signed rank test, post-hoc analysis, **Figure 18C**).The composition of CD4+or CD8+T cells producing any cytokine (IFN-γ or TNF-α or IL-2 following vaccination) was also assessed for surface markers CCR7 and CD45RA to characterize effector(CCR7-CD45RA+), effector memory (CCR7-CD45RA-), and central memory (CCR7+CD45RA-) cells (**Figure 18D**). In both dose groups, CD8+T cells producing cytokine in response to stimulation with SARS-CoV-2spike peptides were generally balanced across the three populations, whereas CD4+T cells were predominantly of the central memory phenotype (**Figure 18D**). CD4+and CD8+T cells following vaccination were further explored for their ability to produce more than one cytokine at a time and were encouraged to note that nearly half (41%) of the CD8+T cells in the 2.0 mg dose group were dual producing IFN-γ and TNF-α (**Figure 18E**). CD8+T cells producing cytokine in the 1.0 mg dose group were primarily monofunctional IFN-γ producing cells (57%). The CD4+T cell compartment was also polyfunctional in nature with 6%and 9%, in the 1.0 mg and 2.0 mg dose groups, respectively, producing all 3 cytokines, IFN-γ, TNF-α, and IL-2 (Table 12).Th2 responses were also measured by assessing IL-4 production, and no statistically significant increases (Wilcoxon matched-pairs signed rank test, post-hoc analysis) were observed in either group

post vaccination (**Figure 18F**).

[0369] INO-4800 was well tolerated with a frequency of product-related Grade 1 AEs of 15% (3/20 subjects) and 10% (2/20 subjects) of the participants in 1.0 mg and 2.0 mg dose group, respectively. Only Grade 1 AEs were noted in the study, which compares favorably with existing licensed vaccines. The safety profile of a successful COVID-19 vaccine is important and supports broad development of INO-4800 in at-risk populations who are at more serious risk of complications from SARS-CoV-2 infection, including the elderly and those with comorbidities.INO-4800 also generated balanced humoral and cellular immune responses with all 38 evaluable participants displaying either or both antibody or T cell responses following two doses of INO-4800. Humoral responses measured by binding or neutralizing antibodies were observed in 95% (18/19) of the participants in each dose group. The neutralizing antibodies, measured by live virus neutralization assay, were seen in 78% (14/18) and 84% (16/19) of participants, and the corresponding GMTs were 102.3 [95% CI (37.4, 280.3)] and 63.5 [95% CI (39.6, 101.8)] for the 1.0 mg and 2.0 mg dose groups, respectively. The range overlaps that of the PRNT IC50 titers reported from convalescent patients as well as the PRNT IC50 titers in NHPs which were protected in a SARS-CoV-2 challenge. Furthermore, there was a statistically significant increase in titers. It is important to note that all but one vaccine recipient that did not develop neutralizing antibody titers responded positively in the T cell ELISpot assay, suggesting that the immune responses generated by the vaccine are registering differentially in these assays. Cellular immune responses were observed in 74% (14/19) and 100% (19/19) of 1.0 mg and 2.0 mg dose groups, respectively. Importantly, INO-4800 generated T cell responses that were more frequent and with higher responder median responses (46 [95% CI (21.1, 142.2)] vs. 71 [95% CI (32.2,194.4)] SFU 10^6 PBMC) in the 1.0 mg and 2.0 mg dose groups respectively. These T cell responses in the 2.0 mg dose group were higher in magnitude than convalescent samples tested (**Figure 18A**). Furthermore, there was a statistically significant increase in SFU. In the flow cytometric assays, both the 1.0 mg and 2.0 mg Dose Groups showed increases in cytokine production from both the CD4+ and CD8+ T cell compartments, especially in the 2.0 mg group. The 2.0 mg group exhibited a number of statistically significant cytokine outputs, including IFN-ɣ and TNF-α and "any cytokine" from the CD8+T cell compartment and TNF-α from the CD4+T cell compartment (**Figure 18C**).Of considerable importance is that CD8+T cell responses in the 2.0 mg dose group were dominated by cells expressing both

IFN-ɣ and TNF-α with or without IL-2 (**Figure 18E** and Table 12). In total, these cells amounted to nearly half of the total CD8+T cell response (42.7%, Table 12).

[0370] In this Phase 1 trial, INO-4800 vaccination led to substantial T cell responses with increased Th1 phenotype, measured by both IFN-ɣ ELISpot as well as multiparametric flow cytometry, as evidenced by increased expression of Th1-type cytokines IFN-ɣ, TNF-α, and IL-2 (**Figure 18C**). Assessment of cellular responses induced by INO-4800 displayed the presence of SARS-CoV-2 specific CD4+ and CD8+ T cells exhibiting hallmarks of differentiation into both central and effector memory cells, suggesting that a persistent cellular response has been established (**Figure 18D**). Importantly, this was accomplished while minimizing induction of IL-4, a prototypical Th2 cytokine (**Figure 18F**), supporting that this vaccine has an immune phenotype, along with induction of protection in preclinical models, which makes it unlikely to be a risk for induction of enhanced disease.

[0371] Table 12. Flow Cytometry Polyfunctionality

	1.0 mg Cohort		2.0 mg Cohort	
Parameter Output	CD4 Cytokine Frequency (%)	CD8 Cytokine Frequency (%)	CD4 Cytokine Frequency (%)	CD8 Cytokine Frequency (%)
IFN-gamma only	31.2	56.7	29.5	27.1
TNF-alpha only	20.4	14	20.9	11.2
IL-2 only	22.3	16.5	20.1	16.5
IFN-gamma and TNF-alpha only	8.0	9.7	6.7	40.6
IFN-gamma and IL-2 only	2.1	0.9	0.6	1.3
IL-2 and TNF-alpha only	9.6	0.7	13.5	1.2
IFN-gamma and IL-2 and TNF-alpha	6.4	1.5	8.7	2.1

Percents listed are the contributions of each output to the total cytokine response

[0372] Table 13.

	1.0 mg Cohort			2.0 mg Cohort		
Immune Assay	Overall Value	Responder Value	Responders‡ n (%)	Overall Value	Responder Value	Responders‡ n (%)
Neutralization Week 6 GMT Reciprocal Titer [95% CI] (Range)	44.4 [14.6, 134.8] (1, 11647)	102.3 [37.4, 280.3] (13, 11647)	14/18 (78%)	34.9 [15.8, 77.2] (1,652)	63.5 [39.6, 101.8] (13652)	16/19 (84%)
S1+S2 Binding Antibody Week 6 GMT Reciprocal Titer [95% CI] (Range)	331.2 [91.2, 1203.2] (1, 14580)	655.5 [255.6, 168.1] (20, 14580)	17/19 (89%)	691.4 [217.5, 2197.2] (1, 14580)	994.2 [395.3, 2500.3] (20, 14580)	18/19 (95%)
Total Seroconversion (Response in S1+S2 or Neutralization)	N/A	N/A	18/19 (95%)	N/A	N/A	18/19 (95%)
IFN-gamma ELISpot Week 8 Median SFU per [95% CI] (Range)	26.2 SFU [10.0-64.4] (1, 374.4)	45.6 [21.1, 142.2] (16.7, 374.4)	14/19 (74%)µ	71 SFU [32.2-194.4] (8.9, 615.6)	71 SFU [32.2-194.4] (8.9, 615.6)	19/19 (100%)µ
Overall Immune Response Rate (Seroconversion or ELISpot)	N/A	N/A	19/19 (100%)	N/A	N/A	19/19 (100%)

1.0mg Cohort excludes one subject with baseline positive NP ELISA

‡ Response criteria: Live Neutralization -Week 6 PRNT $IC_{50} \geq 10$, or ≥4 if binding ELISA activity is seen; S1+S2 Binding – Week 6 Value>1; RBD Binding -Week 6 value >1; ELISpot – Value ≥12 SFU over Week 0

μ - Responders generated using Week 6 or Week 8 data

[0373] EXPANDED PHASE I STUDY

[0374] Approximately 120 healthy volunteers will be evaluated across three (3) dose levels (Study Groups). A total of 40 subjects will be enrolled into each Study Group. Enrollment into each Study Group will be stratified by age; n=20 for 18-50 years, n=10 for 51-64 years, and n=10 ≥ 65 years (Table 14).

[0375] Subjects will be adults aged at least 18 years; judged to be healthy by the Investigator on the basis of medical history, physical examination and vital signs performed at Screening; able and willing to comply with all study procedures; screening laboratory results within normal limits for testing laboratory or deemed not clinically significant by the Investigator; Body Mass Index of 18-30 kg/m^2, inclusive, at Screening; negative serological tests for Hepatitis B surface antigen (HBsAg), Hepatitis C antibody and Human Immunodeficiency Virus (HIV) antibody at screening; screening ECG deemed by the Investigator as having no clinically significant findings (e.g. Wolff-Parkinson-White syndrome); and must meet one of the following criteria with respect to reproductive capacity: women who are post-menopausal as defined by spontaneous amenorrhea for ≥ 12 months; surgically sterile or have a partner who is sterile; use of medically effective contraception. Exclusion criteria are as follows: pregnant or breastfeeding, or intending to become pregnant or father children within the projected duration of the trial starting with the screening visit until 3 months following last dose; positive serum pregnancy test during screening or positive urine pregnancy test prior to dosing; currently participating in or has participated in a study with an investigational product within 30 days preceding Day 0; previous exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or receipt of an investigational product for the prevention or treatment of COVID-19, middle east respiratory syndrome (MERS), or severe acute respiratory syndrome (SARS); in a current occupation with high risk of exposure to SARS-CoV-2 (e.g., health care workers or emergency response personnel having direct interactions with or providing direct care to patients); current or history of respiratory disease, hypersensitivity or severe allergic reactions to vaccines or drugs,

diagnosis of diabetes mellitus, hypertension, malignancy within 5 years of screening, or cardiovascular disease; immunosuppression as a result of underlying illness or treatment, including primary immunodeficiencies, long term use (≥7 days) of oral or parenteral glucocorticoids, current or anticipated use of disease-modifying doses of anti-rheumatic drugs and biologic disease-modifying drugs, history of solid organ or bone marrow transplantation, and prior history of other clinically significant immunosuppressive or clinically diagnosed autoimmune disease; fewer than two acceptable sites available for ID injection and EP considering the deltoid and anterolateral quadriceps muscles; or reported smoking, vaping, or active drug, alcohol or substance abuse or dependence; or any physical examination findings and/or history of any illness that, in the opinion of the study investigator, might confound the results of the study or pose an additional risk to the patient by their participation in the study.

[0376] All subjects will receive dosing on Day 0 and Week 4 (Table 15). Subjects who consent to receive the booster dose (Table 16) will receive the booster dose no earlier than Week 12 in their dosing schedule with the same dose previously received for their two-dose regimen (Day 0 and Week 4). Safety and immunogenicity will be evaluated at 2 weeks following the booster dose.

[0377] Table 14:

Study Group	Number Total Subjects	Number Subjects by Age	Age (years)	Dosing Weeks	INO-4800 Dose per injection	No. Injections/EP per Dosing Visit	INO-4800 Dose per Dosing Visit	Total INO-4800 Dose
1	40	20*	18-50	0, 4 (± 5 days), Optional Booster[b]	1.0 mg	1	1.0 mg	3.0 mg
		10	51-64					
		10	≥65					
2	40	20*	18-50	0, 4 (± 5 days), Optional Booster[b]	1.0 mg	2[a]	2.0 mg	6.0 mg
		10	51-64					
		10	≥65					
3	40	20	18-50	0, 4 (± 5 days),	0.5 mg	1	0.5 mg	1.5 mg
		10	51-64					

		10	≥65	Optional Booster[b]				
Total	120		* Base Study (Others in expanded study)					

[a]INO-4800 will be injected ID followed by EP in an acceptable location on two different limbs at each dosing visit

[b]Optional booster dose delivered no earlier than Week 12 in their dosing schedule with the same dose previously received for their two-dose regimen.

[0378] Subjects not receiving an optional booster dose will be followed to the End of Study (EOS) visit at Week 52 will be the End of Study (EOS) visit (Table 15). For subjects receiving an optional booster dose, the 48 Week Post-Booster Dose Visit will be the EOS visit (Table 16).

[0379] Primary Objectives:

- Evaluate the tolerability and safety of INO-4800 administered by ID injection followed by EP in healthy adult volunteers
- Evaluate the cellular and humoral immune response to INO-4800 administered by ID injection followed by EP

[0380] Primary Safety Endpoints:

- Incidence of adverse events by system organ class (SOC), preferred term (PT), severity and relationship to investigational product. Percentage of Participants with Adverse Events (AEs) [Time Frame: Baseline up to Week 52 (if not receiving an optional booster dose) or the 48 Week Post-Booster Dose Visit (if receiving an optional booster dose)].
- Administration (i.e., injection) site reactions (described by frequency and severity). Percentage of Participants with Administration (Injection) Site Reactions [Time Frame: Day 0 up to Week 52 (if not receiving an optional booster dose) or the 48 Week Post-Booster Dose Visit (if receiving an optional booster dose)].
- Incidence of adverse events of special interest. Percentage of Participants with Adverse Events of Special Interest (AESIs). [Time Frame: Baseline up to Week 52 (if not receiving an optional booster dose) or the 48 Week Post-Booster Dose Visit (if receiving an optional booster dose)].

[0381] Primary Immunogenicity Endpoints:

- SARS-CoV-2 Spike glycoprotein antigen-specific antibodies by binding assays. Change from Baseline in SARS-CoV-2 Spike Glycoprotein Antigen-Specific Binding Antibody Titers [Time Frame: Baseline up to Week 52 (if not receiving an optional booster dose) or the 48 Week Post-Booster Dose Visit (if receiving an optional booster dose)].
- Antigen-specific cellular immune response by IFN-gamma ELISpot and/or flow cytometry assays. Change from Baseline in Antigen-Specific Cellular Immune Response [Time Frame: Baseline up to Week 52 (if not receiving an optional booster dose) or the 48 Week Post-Booster Dose Visit (if receiving an optional booster dose)].

[0382] Exploratory Objectives:

- Evaluate the expanded immunological profile by assessing both T and B cell immune response
- Evaluate the safety and immunogenicity of an optional booster dose of INO-4800 administered by ID injection followed by EP subsequent to a two-dose regimen

Exploratory Endpoints:

- Expanded immunological profile which may include (but not limited to) additional assessment of T and B cell numbers, neutralization response and T and B cell molecular changes by measuring immunologic proteins and mRNA levels of genes of interest at all weeks as determined by sample availability
- Incidence of all adverse events subsequent to an optional booster dose of INO-4800 administered by ID injection followed by EP
- SARS-CoV-2 Spike glycoprotein antigen-specific neutralizing and binding antibodies subsequent to an optional booster dose of INO-4800 administered by ID injection followed by EP
- Antigen-specific cellular immune response by IFN-ɤ ELISpot and/or flow cytometry subsequent to an optional booster dose of INO-4800 administered by ID injection followed by EP

[0383] Safety Assessment. Subjects will be followed for safety for the duration of the trial through EOS or the subject's last visit. Adverse events will be collected at every visit (including the Day 1 and 36 Week Post-Booster Dose phone calls). Laboratory blood and urine samples will be drawn according to the Schedule of Events (Table 15 and Table 16).

[0384] TABLE 15 – NON-BOOSTER CLINICAL TRIAL SCHEDULE OF EVENTS

Tests and assessments	Screen[a]	Day 0		Day 1 (+1d)	Week 1 (± 3d)	Week 4 (± 5d)		Week 6 (± 5d)	Week 8 (± 5d)	Week 12 (± 5d)	Week 28 (± 5d)	Week 40 (+5d)	Week 52 (± 5d)
		Pre	Post			Pre	Post						
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Medical History	X	X											
Demographics	X												
Concomitant Medications	X	X			X	X		X	X	X	X	X	X
Physical Exam[b]	X	X			X	X		X	X	X	X	X	X
Vital Signs	X	X			X	X		X	X	X	X	X	X
Height and Weight	X												
CBC with Differential	X				X			X	X	X	X	X	X
Chemistry[c]	X				X			X	X	X	X	X	X
HIV, HBV, HCV Serology[d]	X												
SARS-CoV-2 Serology	X												
12-lead ECG	X												
Urinalysis Routine[e]	X				X			X	X	X	X	X	X
Pregnancy Test[f]	X	X				X							
INO-4800 + EP[g]		X[h]				X[h]							
Download EP Data[i]			X				X						
Adverse Events[j]	X	X	X	X[k]	X	X	X	X	X	X	X	X	X
Immunology (Whole blood)[l]	X	X				X		X	X	X	X	X	X
Immunology (Serum)[m]	X	X				X		X	X	X	X	X	X

Table 15 (continued)

a. Screening assessment occurs from -30 days to -1 day prior to Day 0.
b. Full physical examination at screening and Week 52 (or any other study discontinuation visit) only. Targeted physical exam at all other visits.
c. Includes Na, K, Cl, HCO_3, Ca, PO_4, glucose, BUN, Cr, AST, ALT and TBili.
d. HIV antibody or rapid test, HBsAg, HCV antibody.
e. Dipstick for glucose, protein, and hematuria. Microscopic examination should be performed if dipstick is abnormal.
f. Serum pregnancy test at screening. Urine pregnancy test at other visits.
g. All doses delivered via intradermal injection followed by EP.
h. For Study Groups Groups 1 and 3, one injection in skin preferably over deltiod muscle at Day 0 and Week 4. For Study Group 2, two injections in skin with each injection over a different deltoid or lateral quadriceps; preferably over the deltoid muscles, at Day 0 and Week 4.
i. Following administration of INO-4800+EP, EP data will be downloaded from the CELLECTRA® 2000 device and provided to Inovio.
j. Includes AEs from the time of consent and all injection site reactions that qualify as an AE.
k. Follow-up phone call to collect AEs.
l. 4 x 8.5 mL (34 mL) whole blood in 10 mL Acid Citrate Dextrose (ACD, Yellow top) tubes per time point. Note: Collect a total of 68 mL whole blood prior to 1st dose (screening and prior to Day 0 dosing).
m. 1 x 8 mL blood in 10 mL red top serum collection tube per time point. Note: Collect four aliquots of 1 mL each (total 4 mL) serum at each time point prior to 1st dose (Screening and prior to Day 0 dosing).

[0385] Table 16 – Booster Clinical Trial Schedule of Events

Tests and assessments	Booster Dose Visit		2 Week Post-Booster Dose Visit (± 5d)	12 Week Post-Booster Dose Visit (± 5d)	24 Week Post-Booster Dose Visit (± 5d)	36 Week Post-Booster Dose Phone Call (+5d)	48 Week Post-Booster Dose Visit (± 5d)
	Pre	Post					
Concomitant Medications	X		X	X	X		X
Physical Exam[a]	X		X	X	X		X
Vital Signs	X		X	X	X		X
CBC with Differential	X		X	X	X		X
Chemistry[b]	X		X	X	X		X
Urinalysis Routine[c]	X		X	X	X		X
Pregnancy Test[d]	X						
INO-4800 + EP[e]	X[f]						
Download EP Data[g]		X					
Adverse Events[h]	X	X	X	X	X	X[i]	X
Immunology (Whole blood)[j]	X		X	X	X		X
Immunology (Serum)[k]	X		X	X	X		X

a. Full physical examination at the 48 Week Post-Booster Dose Visit (or any other study discontinuation visit) only. Targeted physical exam at all other visits.
b. Includes Na, K, Cl, HCO_3, Ca, PO_4, glucose, BUN, Cr, AST, ALT and TBili.
c. Dipstick for glucose, protein, and hematuria. Microscopic examination should be performed if dipstick is abnormal.
d. Urine pregnancy test must be negative prior to receiving booster dose.
e. All doses delivered via intradermal injection followed by EP.
f. For Study Groups 1 and 3, one injection in skin preferably over deltiod muscle (or alternatively, lateral quadriceps) at the Booster Dose Visit. For Study Group 2, two injections in skin with each injection over a different deltoid or lateral quadriceps; preferably over the deltoid muscles, at the Booster Dose Visit.
g. Following administration of INO-4800+EP, EP data will be downloaded from the CELLECTRA® 2000 device and provided to Inovio.
h. Includes AEs from the time of consent and all injection site reactions that qualify as an AE.
i. Follow-up phone call to collect AEs.
j. 4 x 8.5 mL (34 mL) whole blood in 10 mL Acid Citrate Dextrose (ACD, Yellow top) tubes per time point.
k. 1 x 8 mL blood in 10 mL red top serum collection tube per time point.

[0386] Immunogenicity Assessment. Immunology blood samples will be collected according to the Schedule of Events (Table 15 and Table 16). Determination of analysis of collected samples for immunological endpoints will be determined on an ongoing basis throughout the study.

[0387] INO-4800 delivered ID followed by EP using CELLECTRA® 2000 in healthy volunteers is expected to be well tolerated, exhibit an acceptable safety profile, and result in generation of immune responses to SARS-CoV-2 Spike glycoprotein.

[0388] Example 7 Phase 2/3 Randomized, Blinded, Placebo-Controlled Trial to Evaluate the Safety, Immunogenicity, and Efficacy of INO-4800, A Prophylactic Vaccine Against COVID-19 Disease, Administered Intradermally Followed by Electroporation (EP) in Healthy Seronegative Adults at High Risk of SARS-CoV-2 Exposure

[0389] This is a Phase 2/3, randomized, placebo-controlled, multi-center trial to evaluate the safety, immunogenicity and efficacy of INO-4800 administered by intradermal (ID) injection followed by electroporation (EP) using CELLECTRA® 2000 device to prevent COVID-19 disease in participants at high risk of exposure to SARS-CoV-2. The Phase 2 segment will evaluate immunogenicity and safety in approximately 400 participants at two dose levels across three age groups. Safety and immunogenicity information from the Phase 2 segment will be used to determine the dose level for the Phase 3 efficacy segment of the study involving approximately 6178 participants.

[0390] Table 17.

Arm	Intervention/treatment
Experimental Phase 2: INO-4800 Dose Group 1 Participants will receive one intradermal (ID) injection of 1.0 milligram (mg) of INO-4800 followed by electroporation (EP) using the CELLECTRA® 2000 device on Day 0 and Day 28.	Drug: INO-4800 INO-4800 will be administered ID on Day 0 and Day 28. Device: CELLECTRA® 2000 EP using the CELLECTRA® 2000 device will be administered following ID delivery of INO-4800 on Day 0 and Day 28.
Experimental: Phase 2: INO-4800 Dose Group 2	Drug: INO-4800 INO-4800 will be administered

Participants will receive two ID injections of 1.0 mg (total 2.0 mg per dosing visit) of INO-4800 followed by EP using the CELLECTRA® 2000 device on Day 0 and Day 28.	ID on Day 0 and Day 28. Device: CELLECTRA® 2000 EP using the CELLECTRA® 2000 device will be administered following ID delivery of INO-4800 on Day 0 and Day 28.
Placebo Comparator: Phase 2: Placebo Dose Group 1 Participants will receive one ID injection of placebo followed by EP using the CELLECTRA® 2000 device on Day 0 and Day 28.	Drug: Placebo Sterile saline sodium citrate (SSC) buffer (SSC-0001) will be administered ID on Day 0 and Day 28. Other Names: • SSC-0001 Device: CELLECTRA® 2000 EP using the CELLECTRA® 2000 device will be administered following ID delivery of sterile saline sodium citrate (SSC) buffer (SSC-0001) on Day 0 and Day 28.
Placebo Comparator: Phase 2: Placebo Dose Group 2 Participants will receive two ID injections of placebo followed by EP using the CELLECTRA® 2000 device on Day 0 and Day 28.	Drug: Placebo Sterile saline sodium citrate (SSC) buffer (SSC-0001) will be administered ID on Day 0 and Day 28. Other Names: • SSC-0001 Device: CELLECTRA® 2000 EP using the CELLECTRA® 2000 device will be administered following ID delivery of sterile saline sodium citrate (SSC) buffer (SSC-0001) on Day 0 and Day 28.
Experimental: Phase 3: INO-4800 Optimum Dose Participants will receive either one or two 1.0 mg ID injections of INO-4800 based on results from Phase 2 segment, followed by EP using the CELLECTRA® 2000 device on Day 0 and Day 28.	Drug: INO-4800 INO-4800 will be administered ID on Day 0 and Day 28. Device: CELLECTRA® 2000 EP using the CELLECTRA® 2000 device will be administered following ID delivery of INO-4800 on Day 0 and Day 28.

Placebo Comparator: Phase 3: Placebo Optimum Dose Participants will receive either one or two ID injections of placebo based on results from Phase 2 segment, followed by EP using the CELLECTRA® 2000 device on Day 0 and Day 28.	Drug: Placebo Sterile saline sodium citrate (SSC) buffer (SSC-0001) will be administered ID on Day 0 and Day 28. Other Names: • SSC-0001 Device: CELLECTRA® 2000 EP using the CELLECTRA® 2000 device will be administered following ID delivery of sterile saline sodium citrate (SSC) buffer (SSC-0001) on Day 0 and Day 28.

[0391] Primary Outcome Measure:

1. Phase 2: Change From Baseline in Antigen-specific Cellular Immune Response Measured by Interferon-gamma (IFN-γ) Enzyme-linked Immunospot (ELISpot) Assay [Time Frame: Baseline up to Day 393]

2. Phase 2: Change From Baseline in Neutralizing Antibody Response Measured by a Pseudovirus-based Neutralization Assay [Time Frame: Baseline up to Day 393]

3. Percentage of Participants With Virologically Confirmed COVID-19 Disease [Time Frame: From 14 days after completion of the 2-dose regimen up to 12 months post-dose 2 (i.e. Day 42 up to Day 393)]

[0392] Secondary Outcome Measures:

1. Phase 2 and 3: Percentage of Participants with Unsolicited and Solicited Injection Site Reactions [Time Frame: From time of consent up to 28 days post-dose 2 (up to Day 56)]

2. Phase 2 and 3: Percentage of Participants with Solicited and Unsolicited Systemic Adverse Events (AEs) [Time Frame: From time of consent up to 28 days post-dose 2 (up to Day 56)]

3. Phase 2 and 3: Percentage of Participants with Serious Adverse Events (SAEs) [Time Frame: Baseline up to Day 393]

4. Phase 2 and 3: Percentage of Participants with Adverse Events of Special Interest (AESIs) [Time Frame: Baseline up to Day 393]

5. Phase 3: Percentage of Participants With Death from All Causes [Time Frame: Baseline up to Day 393]
6. Phase 3: Percentage of Participants With Non-Severe COVID-19 Disease [Time Frame: From 14 days after completion of the 2-dose regimen up to 12 months post-dose 2 (i.e. Day 42 up to Day 393)]
7. Phase 3: Percentage of Participants With Severe COVID-19 Disease [Time Frame: From 14 days after completion of the 2-dose regimen up to 12 months post-dose 2 (i.e. Day 42 up to Day 393)]
8. Phase 3: Percentage of Participant With Death from COVID-19 Disease [Time Frame: From 14 days after completion of the 2-dose regimen up to 12 months post-dose 2 (i.e. Day 42 up to Day 393)]
9. Phase 3: Percentage of Participants With Virologically-Confirmed SARS-CoV-2 Infections [Time Frame: From 14 days after completion of the 2-dose regimen up to 12 months post-dose 2 (i.e. Day 42 up to Day 393)]
10. Phase 3: Days to Symptom Resolution in Participants With COVID-19 Disease [Time Frame: From 14 days after completion of the 2-dose regimen up to 12 months post-dose 2 (i.e. Day 42 up to Day 393)]
11. Phase 3: Change From Baseline in Antigen-specific Cellular Immune Response Measured by IFN-gamma ELISpot Assay [Time Frame: Baseline up to Day 393]
12. Phase 3: Change From Baseline in Neutralizing Antibody Response Measured by a Pseudovirus-based Neutralization Assay [Time Frame: Baseline up to Day 393]

[0393] Eligibility Criteria

Ages Eligible for Study:	18 Years and older
Sexes Eligible for Study:	All
Gender Based:	No
Accepts Healthy Volunteers:	Yes

[0394] Key Inclusion Criteria:

- Working or residing in an environment with high risk of exposure to SARS-CoV-2 for whom exposure may be relatively prolonged or for whom personal protective equipment (PPE) may be inconsistently used, especially in confined settings

- Screening laboratory results within normal limits for testing laboratory or are deemed not clinically significant by the Investigator.
- Be post-menopausal or be surgically sterile or have a partner who is sterile or use medically effective contraception with a failure rate of < 1% per year when used consistently and correctly from screening until 3 months following last dose.

[0395] Key Exclusion Criteria:

- Acute febrile illness with temperature > 100.4°F (38.0°C) or acute onset of upper or lower respiratory tract symptoms (e.g., cough, shortness of breath, sore throat).
- Positive serologic or molecular (Reverse transcription polymerase chain reaction [RT-PCR]) test for SARS-CoV-2 at Screening
- Pregnant or breastfeeding or intending to become pregnant or intending to father children within the projected duration of the trial starting from the screening visit until 3 months following the last dose.
- Known history of uncontrolled HIV based on a CD4 count less than 200 cells per cubic millimeter (/mm^3) or a detectable viral load within the past 3 months.
- Is currently participating or has participated in a study with an investigational product within 30 days preceding Day 0.
- Previous receipt of an investigational vaccine for prevention or treatment of COVID-19, middle east respiratory syndrome (MERS), or severe acute respiratory syndrome (SARS) (documented receipt of placebo in previous trial would be permissible for trial eligibility).
- Respiratory diseases (e.g., asthma, chronic obstructive pulmonary disease) requiring significant changes in therapy or hospitalization for worsening disease during the 6 weeks prior to enrollment.
- Immunosuppression as a result of underlying illness or treatment
- Lack of acceptable sites available for ID injection and EP
- Blood donation or transfusion within 1 month prior to Day 0.
- Reported alcohol or substance abuse or dependence, or illicit drug use (excluding marijuana use).
- Any illness or condition that in the opinion of the investigator may affect the safety of the participant or the evaluation of any study endpoint.

[0396] Example 8 One or two dose regimen of the SARS-CoV-2 DNA vaccine INO-4800 protects against respiratory tract disease burden in nonhuman primate (NHP) challenge model

[0397] The safety, immunogenicity and efficacy of the intradermal delivery of INO-4800, a synthetic DNA vaccine candidate encoding a SARS-CoV-2 spike antigen, was evaluated in the rhesus macaque model. Single and two dose vaccination regimens were evaluated. Vaccination induced both binding and neutralizing antibodies, along with IFN-γ-producing T cells against SARS-CoV-2. A high dose of SARS-CoV-2 Victoria01 strain (5 x 10^6 pfu) was used to specifically assess the impact of INO-4800 vaccination on lung disease burden to provide both vaccine safety and efficacy data. A broad range of lower respiratory tract disease parameters were measured by applying histopathology, lung disease scoring metric system, in situ hybridization, viral RNA RT-PCR and computed tomography (CT) scans to provide an understanding of the impact of vaccine induced immunity on protective efficacy and potential vaccine enhanced disease (VED).

[0398] This example describes the immunogenicity, efficacy and safety assessment of the SARS-CoV-2 DNA vaccine INO-4800 in a stringent high dose nonhuman primate challenge model. Intradermal delivery of 1 mg of INO-4800 to rhesus macaques induces humoral and T cell responses against the SARS-CoV-2 spike antigen in both a 2-dose regimen and a suboptimal 1 dose regimen. Throughout the study no overt clinical events were recorded in the animals. After a high dose SARS-CoV-2 challenge, a reduction in viral loads was observed and lung disease burden in both the 1 and 2 dose vaccine groups supporting the efficacy of INO-4800. Importantly, vaccine enhanced disease (VED) was not observed, even with the 1 dose group.

[0399] Methods:

[0400] Vaccine. The optimized DNA sequence encoding SARS-CoV-2 IgELS-spike was created using Inovio's proprietary in silico Gene Optimization Algorithm to enhance expression and immunogenicity. The optimized DNA sequence was synthesized, digested with BamHI and XhoI, and cloned into the expression vector pGX0001 under the control of the human cytomegalovirus immediate-early promoter and a bovine growth hormone polyadenylation signal.

[0401] Animals. Eighteen rhesus macaques of Indian origin (Macaca mulatta) were used in this study. Study groups comprised three males and three females of each species and all were adults aged between 2.5 and 3.5 years of age and weighing > 4 Kg at time of challenge.

Prior to the start of the experiment, socially compatible animals were randomly assigned to challenge groups, to minimize bias. Animals were housed in compatible social groups, in cages in accordance with the UK Home Office Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Procedures (2014) and National Committee for Refinement, Reduction and Replacement (NC3Rs) Guidelines on Primate Accommodation, Care and Use, August 2006. Housing prior and for the duration of challenge is described in [Salguero, F.J., et al., Comparison of Rhesus and Cynomolgus macaques as an authentic model for COVID-19. bioRxiv, 2020: p. 2020.09.17.301093.]. All experimental work was conducted under the authority of a UK Home Office approved project license (PDC57C033) that had been subject to local ethical review at PHE Porton Down by the Animal Welfare and Ethical Review Body (AWERB) and approved as required by the Home Office Animals (Scientific Procedures) Act 1986. Animals were sedated by intramuscular (IM) injection with ketamine hydrochloride (Ketaset, 100mg/ml, Fort Dodge Animal Health Ltd, Southampton, UK; 10mg/kg) for procedures requiring removal from their housing. None of the animals had been used previously for experimental procedures.

[0402] Vaccine administration. Animals received 1 mg of SARS-CoV-2 DNA vaccine, INO-4800, by intradermal injection at day 28 only (1 dose group) or 0 and 28 (2 dose group) followed by an EP treatment using the CELLECTRA 2000® Adaptive Constant Current Electroporation Device with a 3P array (Inovio Pharmaceuticals).

[0403] Serum and heparinised whole blood were collected whilst animals were sedated at bi-weekly intervals during the vaccination phase. Nasal and throat swabs were also collected on the day of challenge on D56. After challenge, nasal swabs, throat swabs and serum were collected at 1, 3, 5 dpc and at cull (6, 7 or 8 dpc – staggered due to the high level of labor involved in procedures), with heparinised whole blood collected at 3 dpc and at cull. Nasal and throat swabs were obtained as described [Salguero, F.J., et al., Comparison of Rhesus and Cynomolgus macaques as an authentic model for COVID-19. bioRxiv, 2020: p. 2020.09.17.301093.].

[0404] Clinical observations. Animals were monitored multiple times per day for behavioral and clinical changes. Behavior was evaluated for contra-indicators including depression, withdrawal from the group, aggression, changes in feeding patterns, breathing pattern, respiration rate and cough. Animals were observed and scored as follows for activity and health throughout the study. Key: Activity Level: A0 = Active & Alert; A1 = Only active when stimulated by operator; A2=Inactive even when stimulated/Immobile; H=Healthy; S=Sneeze, C=Cough, Nd=Nasal Discharge, Od=Ocular Discharge, Rn=Respiratory Noises,

Lb=Laboured breathing, L=Lethargy, Di=Diarrhoea, Ax = Loss of Appetite, Dx = Dehydration, RD=Respiratory Distress. Animal body weight, temperature and haemoglobin levels were measured and recorded throughout the study.

[0405] Viruses and Cells

[0406] SARS-CoV-2 Victoria/01/2020 [Caly, L., et al., Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. Med J Aust, 2020. 212(10): p. 459-462] was generously provided by The Doherty Institute, Melbourne, Australia at P1 after primary growth in Vero/hSLAM cells and subsequently passaged twice at PHE Porton Down in Vero/hSLAM cells [ECACC 04091501]. Infection of cells was with ~0.0005 MOI of virus and harvested at day 4 by dissociation of the remaining attached cells by gentle rocking with sterile 5 mm borosilicate beads followed by clarification by centrifugation at 1,000 x g for 10 mins. Whole genome sequencing was performed, on the P3 challenge stock, using both Nanopore and Illumina as described in Lewandowski, K., et al., Metagenomic Nanopore Sequencing of Influenza Virus Direct from Clinical Respiratory Samples. J Clin Microbiol, 2019. 58(1). Virus titer of the challenge stocks was determined by plaque assay on Vero/E6 cells [ECACC 85020206]. Cell lines were obtained from the European Collection of Authenticated Cell Cultures (ECACC) PHE, Porton Down, UK. Cell cultures were maintained at 37oC in Minimum essential medium (MEM) (Life Technologies, California, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, Dorset, UK) and 25 mM HEPES (Life Technologies, California, USA). In addition, Vero/hSLAM cultures were supplemented with 0.4 mg/ml of geneticin (Invitrogen) to maintain the expression plasmid. Challenge substance dilutions were conducted in phosphate buffer saline (PBS). Inoculum (5 x 10^6 PFU) was delivered by intratracheal route (2 ml) and intranasal instillation (1.0 ml total, 0.5 ml per nostril).

[0407] Clinical signs and in-life imaging by computerized tomography

[0408] CT scans were performed two weeks before and five days after challenge with SARS-CoV2. CT imaging was performed on sedated animals using a 16 slice Lightspeed CT scanner (General Electric Healthcare, Milwaukee, WI, USA) in both the prone and supine position and scans evaluated by a medical radiologist expert in respiratory diseases (as described previously [Salguero, F.J., et al., Comparison of Rhesus and Cynomolgus macaques as an authentic model for COVID-19. 2020: p. 2020.09.17.301093.]). To provide the power to discriminate differences between individual NHP's with low disease volume

(i.e. <25% lung involvement), a refined score system was designed in which scores were attributed for possession of abnormal features characteristic of COVID in human patients (COVID pattern score) and for the distribution of features through the lung (Zone score). The COVID pattern score was calculated as sum of scores assigned for the number of nodules identified, and the possession and extent of GGO and consolidation according to the following system: Nodule(s): Score 1 for 1, 2 for 2 or 3, 3 for 4 or more; GGO: each affected area was attributed with a score according to the following: Score 1 if area measured < 1 cm, 2 if 1 to 2 cm, 3 if 2 -3 cm, 4 if > 3 cm and scores for each area of GGO were summed to provide a total GGO score; Consolidation: each affected area was attributed with a score according to the following: 1 if area measured < 1 cm, 2 if 1 to 2 cm, 3 if 2 -3 cm, 4 if > 3 cm. Scores for each area of consolidation are summed to provide a total consolidation score. To account for estimated additional disease impact on the host of consolidation compared to GGO, the score system was weighted by doubling the score assigned for consolidation. To determine the zone score, the lung was divided into 12 zones and each side of the lung divided (from top to bottom) into three zones: the upper zone (above the carina), the middle zone (from the carina to the inferior pulmonary vein), and the lower zone (below the inferior pulmonary vein). Each zone was further divided into two areas: the anterior area (the area before the vertical line of the midpoint of the diaphragm in the sagittal position) and the posterior area (the area after the vertical line of the mid-point of the diaphragm in the sagittal position). This results in 12 zones in total where a score of one is attributed to each zone containing structural changes. The COVID pattern score and the zone are summed to provide the Total CT score.

[0409] Post-mortem examination and histopathology. Animals were euthanized at 3 different time-points, in groups of six (including one animal from each species and sex) at 6, 7 and 8 dpc. The bronchial alveolar lavage fluid (BAL) was collected at necropsy from the right lung. The left lung was dissected prior to BAL collection and used for subsequent histopathology and virology procedures. At necropsy nasal and throat swabs, heparinised whole blood and serum were taken alongside tissue samples for histopathology. Samples from the left cranial and left caudal lung lobe together with spleen, kidney, liver, mediastinal and axillary lymph nodes, small intestine (duodenum), large intestine (colon), trachea, larynx inoculation site and draining lymph node, were fixed by immersion in 10% neutral-buffered formalin and processed routinely into paraffin wax. Four µm sections were cut and stained with hematoxylin and eosin (H&E) and examined microscopically. A lung histopathology scoring system [Salguero, F.J., et al., Comparison of Rhesus and Cynomolgus macaques as

an authentic model for COVID-19. bioRxiv, 2020: p. 2020.09.17.301093] was used to evaluate lesions affecting the airways and the parenchyma. Three tissue sections from each left lung lobe were used to evaluate the lung histopathology. In addition, samples were stained using the RNAscope technique to identify the SARS-CoV-2 virus RNA in lung tissue sections. Briefly, tissues were pre-treated with hydrogen peroxide for 10 mins (RT), target retrieval for 15 mins (98-102°C) and protease plus for 30 mins (40°C) (Advanced Cell Diagnostics). A V-nCoV2019-S probe (SARS-CoV-2 Spike gene specific) was incubated on the tissues for two hours at 40°C. In addition, samples were stained using the RNAscope technique to identify the SARS-CoV-2 virus RNA. Amplification of the signal was carried out following the RNAscope protocol using the RNAscope 2.5 HD Detection kit – Red (Advanced Cell Diagnostics, Biotechne). All H&E and ISH stained slides were digitally scanned using a Panoramic 3D-Histech scanner and viewed using CaseViewer v2.4 software. The presence of viral RNA by ISH was evaluated using the whole lung tissue section slides. Digital image analysis was performed in RNAscope labelled slides to ascertain the percentage of stained cells within the lesions, by using the Nikon-NIS-Ar software package.

[0410] Viral load quantification by RT-qPCR. RNA was isolated from nasal swabs and throat swabs. Samples were inactivated in AVL (Qiagen) and ethanol. Downstream extraction was then performed using the BioSprint™96 One-For-All vet kit (Indical) and Kingfisher Flex platform as per manufacturer's instructions. Tissues were homogenized in Buffer RLT+ betamercaptoethanol (Qiagen). Tissue homogenate was then centrifuged through a QIAshredder homogenizer (Qiagen) and supplemented with ethanol as per manufacturer's instructions. Downstream extraction from tissue samples was then performed using the BioSprint™96 One-For-All vet kit (Indical) and Kingfisher Flex platform as per manufacturer's instructions.

[0411] Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) targeting a region of the SARS-CoV-2 nucleocapsid (N) gene was used to determine viral loads and was performed using TaqPath™ 1-Step RT-qPCR Master Mix, CG (Applied Biosystems™), 2019-nCoV CDC RUO Kit (Integrated DNA Technologies) and QuantStudio™ 7 Flex Real-Time PCR System. Sequences of the N1 primers and probe were: 2019-nCoV_N1-forward, 5' GACCCCAAAATCAGCGAAAT 3' (SEQ ID NO: 18) ; 2019-nCoV_N1-reverse, 5' TCTGGTTACTGCCAGTTGAATCTG 3'(SEQ ID NO: 19); 2019-nCoV_N1-probe, 5' FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1 3'(SEQ ID NO: 20). The cycling conditions were: 25°C for 2 minutes, 50°C for 15 minutes, 95°C for 2 minutes, followed by

45 cycles of 95°C for 3 seconds, 55°C for 30 seconds. The quantification standard was in vitro transcribed RNA of the SARS-CoV-2 N ORF (accession number NC_045512.2) with quantification between 1 and 6 log copies/µl. Positive swab and fluid samples detected below the limit of quantification (LoQ) of 4.11 log copies/ml, were assigned the value of 5 copies/µl, this equates to 3.81 log copies/ml, whilst undetected samples were assigned the value of < 2.3 copies/µl, equivalent to the assay's lower limit of detection (LoD) which equates to 3.47 log copies / ml. Positive tissue samples detected below the limit of quantification (LoQ) of 4.76 log copies/ml were assigned the value of 5 copies/µl, this equates to 4.46 log copies/g, whilst undetected samples were assigned the value of < 2.3 copies/µl, equivalent to the assay's lower limit of detection (LoD) which equates to 4.76 log copies/g.

[0412] Subgenomic RT-qPCR was performed on the QuantStudio™ 7 Flex Real-Time PCR System using TaqMan™ Fast Virus 1-Step Master Mix (Thermo Fisher Scientific) and oligonucleotides as specified by Wölfel, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 581, 465–469 (2020), with forward primer, probe and reverse primer at a final concentration of 250 nM, 125 nM and 500 nM respectively. Sequences of the sgE primers and probe were:

2019-nCoV_sgE-forward, 5' CGATCTCTTGTAGATCTGTTCTC 3' (SEQ ID NO: 21);
2019-nCoV_sgE-reverse, 5' ATATTGCAGCAGTACGCACACA 3' (SEQ ID NO: 22);
2019-nCoV_sgE-probe, 5' FAM- ACACTAGCCATCCTTACTGCGCTTCG-BHQ1 3' (SEQ ID NO: 23).

[0413] Cycling conditions were 50°C for 10 minutes, 95°C for 2 minutes, followed by 45 cycles of 95°C for 10 seconds and 60°C for 30 seconds. RT-qPCR amplicons were quantified against an in vitro transcribed RNA standard of the full length SARS-CoV-2 E ORF (accession number NC_045512.2) preceded by the UTR leader sequence and putative E gene transcription regulatory sequence described by Wolfel et al [Wölfel, R., Corman, V.M., Guggemos, W. et al. Virological assessment of hospitalized patients with COVID-2019. Nature 581, 465–469 (2020).]. Positive samples detected below the lower limit of quantification (LLOQ) were assigned the value of 5 copies/µl, whilst undetected samples were assigned the value of ≤0.9 copies/µl, equivalent to the assays lower limit of detection (LLOD). For nasal swab, throat swab and BAL samples extracted samples this equates to an LLOQ of 4.11 log copies/mL and LLOD of 3.06 log copies/mL. For tissue samples this equates to an LLOQ of 4.76 log copies/g and LLOD of 3.71 log copies/g.

[0414] Plaque reduction neutralization test. Neutralizing virus titers were measured in heat-inactivated (56°C for 30 minutes) serum samples. SARS-CoV-2 was diluted to a concentration of 1.4 x 10^3 pfu/ml (70 pfu/50 µl) and mixed 50:50 in 1% FCS/MEM with doubling serum dilutions from 1:10 to 1:320 in a 96-well V-bottomed plate. The plate was incubated at 37°C in a humidified box for one hour to allow the antibody in the serum samples to neutralize the virus. The neutralized virus was transferred into the wells of a washed plaque assay 24-well plate (see plaque assay method), allowed to adsorb at 37°C for a further hour, and overlaid with plaque assay overlay media. After five days incubation at 37°C in a humified box, the plates were fixed, stained and plaques counted.

[0415] Antigen Binding ELISA. Recombinant SARS-CoV-2 Spike- and RBD-specific IgG responses were determined by ELISA. A full-length trimeric and stabilized version of the SARS-CoV-2 Spike protein was supplied by Lake Pharma (#46328). Recombinant SARS-CoV-2 Receptor-Binding-Domain (319-541) Myc-His was developed and kindly provided by MassBiologics. High-binding 96-well plates (Nunc Maxisorp, 442404) were coated with 50 µl per well of 2 µg/ml Spike trimer (S1+S2) or RBD in 1x PBS (Gibco) and incubated overnight at 4°C. The ELISA plates were washed and blocked with 5% Fetal Bovine Serum (FBS, Sigma, F9665) in 1x PBS/0.1% Tween 20 for 1 hour at room temperature. Serum collected from animals after vaccination had a starting dilution of 1/50 followed by 8 two-fold serial dilutions. Post-challenge samples were inactivated in 0.5% triton and had a starting dilution of 1/100 followed by 8 three-fold serial dilutions. Serial dilutions were performed in 10% FBS in 1x PBS/0.1% Tween 20. After washing the plates, 50 µl/well of each serum dilution was added to the antigen-coated plate in duplicate and incubated for 2 hours at room temperature. Following washing, anti-monkey IgG conjugated to HRP (Invitrogen, PA1-84631) was diluted (1: 10,000) in 10% FBS in 1X PBS/0.1% Tween 20 and 100 µl/well was added to the plate. Plates were then incubated for 1 hour at room temperature. After washing, 1 mg/ml O-Phenylenediamine dihydrochloride solution (Sigma P9187) was prepared and 100 µl per well were added. The development was stopped with 50 µl per well 1M Hydrochloric acid (Fisher Chemical, J/4320/15) and the absorbance at 490 nm was read on a Molecular Devices versamax plate reader using Softmax (version 7.0). Titers were determined using the endpoint titer determination method. For each sample, an endpoint titer is defined as the reciprocal of the highest sample dilution that gives a reading (OD) above the cut-off. The cut-off was determined for each experimental group as the mean OD + 3SD of naïve samples.

[0416] Peripheral blood mononuclear cell isolation and resuscitation. PBMCs were isolated from whole blood anticoagulated with heparin (132 Units per 8 720 ml blood) (BD

Biosciences, Oxford, UK) using standard methods. PBMCs isolated from tissues were stored at −180 °C. For resuscitation PBMCs were thawed, washed in R10 medium (consisting of RPMI 1640 supplemented with 2 mM L-glutamine, 50 U/ml penicillin- 50 μg/ml streptomycin, and 10% heat-inactivated FBS) with 1 U/ml of DNase (Sigma), and resuspended in R10 medium and incubated at 37°C 5% CO_2 overnight.

[0417] ELISpot. An IFNγ ELISpot assay was used to estimate the frequency and IFNγ production capacity of SARS-CoV-2-specific T cells in PBMCs using a human/simian IFNγ kit (MabTech, Nacka. Sweden), as described previously [Sibley, L.S., et al., ELISPOT Refinement Using Spot Morphology for Assessing Host Responses to Tuberculosis. Cells, 2012. 1(1): p. 5-14.]. The cells were assayed at 2 x 10^5 cells per well. Cells were stimulated overnight with SARS-CoV-2 peptide pools spanning the ECD spike protein. Five peptide pools were 748 used, comprising of 15mer peptides, overlapping by 9 amino acids. Phorbol 12-myristate (Sigma) (100 ng/ml) and ionomycin (CN Biosciences, 753 Nottingham, UK) (1 mg/ml) were used as a positive control. Results were calculated and reported as spot forming units (SFU) per million cells. All SARS-CoV-2 peptides were assayed in duplicate and media only wells subtracted to give the antigen-specific SFU. ELISPOT plates were analyzed using a CTL scanner and software (CTL, Germany) and further analysis carried out using GraphPad Prism (GraphPad Software, USA).

[0418] Statistics. All statistical analyses were performed using GraphPad Prism 7 or 8 software (La Jolla, CA). These data were considered significant if $p < 0.05$. The type of statistical analysis performed is detailed in the figure legend. No samples or animals were excluded from the analysis.

[0419] Results:

[0420] Immunogenicity of one and two dose regimens of INO-4800. Twelve (6 male and 6 female) rhesus macaques were vaccinated with 1 dose (6 animals) or 2 doses (6 animals) of INO-4800 on day 28 or 0 and 28, respectively (**Figure 22A**). For each treatment 1 mg INO-4800 was administered intradermally followed by CELLECTRA-ID EP. A further six age- and sex- matched animals were not vaccinated and provided the control group. Animals were observed and scored as alert and healthy for the duration of the study, and no adverse events or clinical anomalies were recorded in the animals (**Figure 23**). The serum titers of SARS-CoV-2 spike antigen reactive IgG antibodies in all animals were measured biweekly between days 0 and 56. In the single dose group (INO-4800 X1) a mean endpoint titer of 467 against the SARS-CoV-2 spike antigen trimeric S1 + S2 ECD form and 442 against the RBD

antigen, and a live virus (Victoria/01/2020 matched to the challenge strain) neutralization titer of 239 14 days after vaccination (**Figures 22B-D**). In the 2 dose group (INO-4800 X2) a mean endpoint titer of 2,142 against the S1 + S2 ECD and 1,538 against the RBD antigen, and a live virus neutralization titer of 2,199 was measured 14 days after the 2nd vaccination (**Figures 22B-D**). Vaccination with INO-4800 induced SARS-CoV-2 spike antigen-specific Th1 T cell responses in the PBMC population as measured by an IFN-γ ELISpot (**Figure 22E**). In summary, intradermal delivery of INO-4800 induced a functional humoral and T cell response against SARS-CoV-2 spike protein which was boosted after a second dose. At the day of viral challenge (Day 56) the level of SARS-CoV-2 neutralizing antibodies in the serum was significantly higher in the vaccinated groups compared to the control group ($p = 0.015$). Following viral challenge there was a slight increase in SARS-CoV-2 spike binding and neutralizing antibody titers in all the groups between days 56 and 62-64 (**Figures 22B-D**). In the control group there was an increase in the cellular immune response to peptides spanning the SARS-CoV-2 spike antigen after viral challenge, but little change in the vaccinated groups, likely due to control of viral infection by the humoral arm of the host immune system (**Figure 22F**).

[0421] Viral loads in the upper and lower respiratory tracts after SARS-CoV-2 challenge

[0422] On day 56 all animals were challenged with a total of 5x10^6 pfu SARS-CoV-2 delivered to both the upper and lower respiratory tract. No overt clinical symptoms were observed throughout the duration (6-8 days) of the challenge in any of the animals (**Figures 23A-23C**). At indicated timepoints nasal and throat swabs were collected from the animals. SARS-CoV-2 viral genomic (viral RNA) and subgenomic (sgmRNA), which represents replicating virus were measured by RT-qPCR (**Figures 24A and 25A**). Analysis of viral RNA area under the curve (AUC) levels in the throat revealed significantly reduced levels in the vaccinated groups (**Figure 24B**). Additionally, the peak viral load level measured in the INO-4800 X2 group was significantly reduced compared to the control group (**Figure 24C**). Analysis revealed a significant negative correlation between throat viral loads and neutralizing and anti-RBD IgG titers (**Figures 15A-15D**). SARS-CoV-2 sgmRNA data revealed a similar trend to reduction of viral load in the vaccinated groups compared to controls (**Figures 25A-C**). Analysis in the nasal compartment revealed a trend for reduction and accelerated clearance of viral RNA and sgmRNA in the vaccinated groups compared to control, but did not reach a level of significance (**Figures 24D-F and 25D-F**). Analysis

revealed a significant negative correlation between nasal viral loads and neutralizing and anti-RBD IgG titers on day 3, but not day 1 (**Figures 15E-15H**).

[0423] At the time of necropsy (6-8 days post challenge), BAL fluid was collected from each animal. Measurement of the levels of SARS-CoV-2 viral RNA and sgmRNA revealed a reduction of the average virus in vaccinated groups, even though the levels were variable within each group dependent on the day of necropsy (**Figures 26A, 26B**). RT-qPCR was also performed on tissues collected at necropsy. At these timepoints post challenge the SARS-CoV-2 viral RNA levels detected were below limit of quantification in most tissues except the lungs (**Figure 27**). Measurements of the level of SARS-CoV-2 viral mRNA and sgmRNA detected in the lung tissue samples indicated reduced average viral load in the vaccinated animals (**Figures 26C and 26D**).

[0424] In summary data showed a significant reduction of viral load in the throat, and a trend for a reduction of viral loads in the lungs of the vaccinated groups. The collection of BAL and lung tissue samples at different timepoints (days 6, 7 or 8) after challenge likely added to the intragroup variability observed impacting statistical analysis. RT-qPCR viral load data indicate INO-4800 vaccination has a positive effect in reducing viral loads in rhesus macaques challenged with high dose SARS-CoV-2, in general, lower viral levels were measured in the 2 dose vaccine group compared to one dose vaccine group.

[0425] Disease burden in the lungs after SARS-CoV-2 challenge.

[0426] The pulmonary disease burden was assessed on harvested lung tissues collected at necropsy 6 to 8 days after challenge. Analysis was performed on all animals in the study in a double blinded manner. Histopathological analysis of lung tissue was performed on multiple organ tissues, but only the lungs showed remarkable lesions, compatible with SARS-CoV-2 infection. Pulmonary lesions consistent with infection with SARS-CoV-2 were observed in the lungs of animals from the unvaccinated control and at a reduced level in vaccinated groups. Representative histopathology images are provided in **Figure 28**. Briefly, the lung parenchyma was comprised of multifocal to coalescing areas of pneumonia surrounded by unaffected parenchyma. Alveolar damage, with necrosis of pneumocytes was a prominent feature in the affected areas. Alveolar spaces and interalveolar septa contained mixed inflammatory cells (including macrophages, lymphocytes, viable and degenerated neutrophils, and occasional eosinophils), and edema. Type II pneumocyte hyperplasia was also observed in distal bronchioles and bronchiolo-alveolar junctions. In the larger airways occasional, focal, epithelial degeneration and sloughing was observed in the respiratory

epithelium. Low numbers of mixed inflammatory cells, comprising neutrophils, lymphoid cells, and occasional eosinophils, infiltrated bronchial and bronchiolar walls. In the lumen of some airways, mucus admixed with degenerative cells, mainly neutrophils and epithelial cells, was seen. Within the parenchyma, perivascular and peribronchiolar cuffing was also observed, being mostly lymphoid cells comprising the infiltrates.

[0427] The histopathology score and percent tissue area of SARS-CoV-2 RNA positivity were applied to quantify the disease burden. The unvaccinated group showed the highest histopathological scores in the lung when compared with the vaccinated groups (**Figures 29A and 29C**). Animals from vaccinated groups showed reduced pathology when compared with unvaccinated animals, except for animal #10A from INO-4800X1 group, which showed histopathological scores similar to the unvaccinated animals. To detect the presence of SARS-CoV-2 RNA in the lung tissue, in situ hybridization (ISH) was performed. Viral RNA was observed in pneumocytes and inflammatory cells within the histopathological lesions with reduced frequency in the vaccinated animals (**Figure 29B**).

[0428] CT scans were performed to provide an in-life, unbiased, and quantifiable metric of lung disease. Results from lung CT imaging performed 5 days after challenge with SARS-CoV-2 were evaluated for the presence of COVID-19 disease features: ground glass opacity (GGO), consolidation, crazy paving, nodules, peri-lobular consolidation; distribution - upper, middle, lower, central 2/3, peripheral, bronchocentric, and for pulmonary embolus. The medical radiologist was blinded to the animal's treatment and clinical status. The extent of lung involvement was evaluated and quantified using a scoring system developed for COVID disease. The score system parameters are provided in materials and methods section. Pulmonary abnormalities characteristic of COVID-19 disease where observed in 3 out of 6 and 2 out of 6 animals in the INO-4800 one dose or two dose groups, respectively, and in 5 out of 6 unvaccinated animals in the control group (representative CT scan images are provided in **Figure 30**). The extent of lung involvement in the animals with disease involvement was less than 25% and considered low level disease (**Figure 29D**). There was a trend for disease scores to be highest in the unvaccinated control group with a reduction in the INO-4800 one and two dose groups (**Figures 29E-29G**). The comparison of scores between groups did not reach statistical difference ($p = 0.0584$ between INO-4800 two dose group and no vaccine group, Mann Whitney test). One outlier animal (10A) in the INO-4800 X1 group scored higher than other animals. However, the level of disease was still considered low and comparable disease burden had been observed in other NHP SARS-CoV-2 challenge studies performed under the same conditions. In summary, CT scanning provides a useful

measure of SARS-CoV-2-induced disease in rhesus macaques. Day 5 post SARS-CoV-2 infection, abnormalities where present were reported at low levels (<25% of lung involved). Evidence from CT scans suggested trends for differences in pulmonary disease burden between groups, with disease burden highest in the nonvaccinated control group.

[0429] In summary, after high dose SARS-CoV-2 challenge of nonhuman primates the disease burden was reduced in the animals receiving a single of two dose regimen of INO-4800 vaccine. There was no indication of vaccine enhanced disease, even in animals receiving a suboptimal one dose vaccination regimen.

[0430] Discussion

[0431] This example describes the safety, immunogenicity, and efficacy assessments of the SARS-CoV-2 DNA vaccine INO-4800 in a stringent high dose nonhuman primate challenge model. Intradermal delivery of 1 mg of INO-4800 to rhesus macaques induces both humoral and T cell responses against the SARS-CoV-2 spike antigen in both a 2-dose regimen and a 1 dose regimen. Throughout the study no overt clinical events were recorded in the animals. After a high dose SARS-CoV-2 challenge, a reduction in viral loads was observed and lung disease burden in both the 1 and 2 dose vaccine groups supporting the efficacy of INO-4800. Importantly, vaccine enhanced disease (VED) was not observed, even with the 1 dose group.

[0432] The rhesus macaque model has become a widely employed model for assessing medical countermeasures against SARS-CoV-2. Importantly, wildtype non-adapted SARS-CoV-2 replicates in the respiratory tract of rhesus macaques, and the animal presents with some of the characteristics observed in humans with mild COVID-19 symptoms [Salguero, F.J., et al., Comparison of Rhesus and Cynomolgus macaques as an authentic model for COVID-19. 2020: p. 2020.09.17.301093.; Muñoz-Fontela, C., et al., Animal models for COVID-19. Nature, 2020. 586(7830): p. 509-515]. Here, focus was placed on the lung disease burden in SARS-CoV-2 challenged rhesus macaques which had been vaccinated with INO-4800. While the level of lung disease burden measured in the animals was mild, a significant reduction in of histopathology and viral detection scores in the lungs of vaccinated animals was observed (**Figure 29**). This suggests the potential for a positive impact on the LRT disease which is observed in COVID-19 patients which progress to severe disease. Interestingly, a significant reduction in viral loads in the throat compartment in the upper respiratory tract was also observed, but only a trend for reduction in the nasal compartment. It may be that differential induction of mucosal immunity exists between the throat and nasal compartment. Interestingly, a significant negative correlation between the RBD targeting and

neutralizing antibodies in the serum with throat, but not nasal, viral loads was observed at day 1 post challenge (**Figure 15**). However, the levels of these antibodies in either of these URT compartments were not assayed to provide further evidence of the presence of increased levels of functional antibodies in the throat compared to nasal passage. Another possibility could be that viral control in the nasal compartment where the extensive ($5x10^6$ pfu) SARS-CoV-2 challenge dose was directly instilled may be a higher bar than in other mucosal compartments. In support of this, data in the control animals showed nasal swabs yielded higher viral titers than throat swabs, with similar observations being reported in COVID-19 subjects [Mohammadi, A., et al., SARS-CoV-2 detection in different respiratory sites: A systematic review and meta-analysis. EBioMedicine, 2020. 59: p. 102903.]

[0433] Importantly, the data indicated that enhanced respiratory disease (ERD) was not associated with INO-4800 immunization in either the 1 dose or 2 dose regimen. In the INO-4800 X1 dose group, one animal (10A) did present with the highest lung histopathology score and CT scan score. However, the multifocal lesions in animal 10A showed a similar histopathological pattern as those observed in the animals from the nonvaccinated group, with no apparent influx of different inflammatory cell subpopulations in the infiltrates. A potential hallmark of vaccine enhanced disease is the increased influx of inflammatory cells such as eosinophils [Bolles, M., et al., A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol, 2011. 85(23): p. 12201-15; Yasui, F., et al., Prior Immunization with Severe Acute Respiratory Syndrome (SARS)-Associated Coronavirus (SARS-CoV) Nucleocapsid Protein Causes Severe Pneumonia in Mice Infected with SARS-CoV. The Journal of Immunology, 2008. 181(9): p. 6337-6348.]. The CT scan and histopathology data for animal 10A are believed not to be associated with ERD, but rather a disease score and pattern similar to that of nonvaccinated animals. Similar lung histopathology inflammation scores ranging from minimal-mild to mild-moderate were reported in samples analyzed 7 or 8 days after challenge in rhesus macaques receiving other vaccine candidates [Corbett, K.S., et al., Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. New England Journal of Medicine, 2020. 383(16): p. 1544-1555]. Currently, VED remains a theoretical concern with SARS-CoV-2 vaccination and attempts to induce enhanced disease using a formalin inactivated whole virus preparation of SARS-CoV-2 have failed to repeat the lung pathology previously reported for other inactivated respiratory viral vaccines [Bewley, K.R., et al., Immunological and pathological outcomes of SARS-CoV-2 challenge after formalin-

inactivated vaccine immunization of ferrets and rhesus macaques. 2020: p. 2020.12.21.423746].

[0434] This data compliments the NHP SARS-CoV-2 challenge study which demonstrated reduction in LRT viral loads several months after INO-4800 immunization (Example 9). However, there are distinct differences between the studies, including different doses and variants used for the challenge stock, and the timing of the challenge. In the study described in this example, the animal was challenged four weeks after the last vaccination, at a timepoint when high levels of circulating neutralizing antibodies were present. In the other study, the level of serum SARS-CoV-2 neutralizing antibody was low at the time of challenge, protection appeared to be dependent on the recall of a memory response, with a strong humoral and cellular response against SARS-CoV-2 spike antigen detected in the animals. Here, an anamnestic response of a similar magnitude was not observed, suggesting protection may have been mediated by the antibodies present in circulation at time of challenge which is supported by the correlation between serum SARS-CoV-2 targeting antibody levels and reductions in viral loads (**Figure 15**).

[0435] In conclusion, the results here in a stringent preclinical SARS-CoV-2 animal model provide further support for the efficacy and safety of the DNA vaccine INO-4800 as a prophylactic countermeasure against COVID-19. Importantly, tested as a single dose immunization we observed a positive impact on the lung disease burden and no VED. Taken together with INO-4800 clinical data, INO-4800 has many attributes in terms of safety, efficacy and logistical feasibility due its high stability, negating the need for challenging cold chain distribution requirements for global access. Furthermore, synthetic DNA vaccine technology is amenable to highly accelerated developmental timelines, permitting rapid design and testing of candidates against new SARS-CoV-2 variants which display potential for immune escape [Wibmer, C.K., et al., SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. 2021: p. 2021.01.18.427166.; Moore, J.P. and P.A. Offit, SARS-CoV-2 Vaccines and the Growing Threat of Viral Variants. JAMA, 2021.].

[0436] EXAMPLE 9 SARS-COV-2 DNA vaccine induces humoral and cellular immunity resulting in memory responses which provide anamnestic protection in a rhesus macaque challenge

[0437] The immunogenicity of a synthetic DNA vaccine encoding the SARS-CoV-2 Spike protein was previously demonstrated in both mice and guinea pigs (Example 1). In this example, the durability of INO-4800-induced immune responses in rhesus macaques is

demonstrated. ID-EP administration in rhesus macaques induced cellular and humoral responses to SARS-Cov-2 S protein, with additional cross reactivity to the SARS-CoV-1 S protein. Protective efficacy is demonstrated more than 3 months post-final immunization, demonstrating establishment of amamnestic immune responses and reduced viral loads in vaccinated macaques. After viral challenge, a reduction in subgenomic messenger RNA (sgmRNA) BAL viral loads was observed compared to control animals with 1 mg (1/5th the DNA dose) administered via intradermal (ID) delivery. This was associated with induction of a rapid recall response in both cellular and humoral immune arms, supporting the potential for the INO-4800 candidate to moderate disease. No adverse events or evidence of vaccine enhanced disease (VED) were observed in animals in the vaccine group. Reduced viral subgenomic RNA loads in the lower lung and lower VL were observed. In the nose, a trend of lower VL was observed. These data support that immunization with this DNA vaccine candidate limits active viral replication and has the potential to reduce severity of disease, as well as reduced viral shedding in the nasal cavity.

[0438] It is important to note that the initial viral loads detected in control animals in this study were on average 1-2 logs higher (10^9 PFU/swab in 4/5 NHPs on day 1 post-challenge) than in similar published studies performed under identical conditions (~10^7 PFU/swab) (Yu et al., 2020, Science, eabc6284). Only two of the prior reported NHP studies included intranasal delivery as inoculation route for challenge (van Doremalen et al., 2020, bioRxiv 2020.05.13.093195; Yu et al., 2020, Science, eabc6284). High-dose challenge inoculums are frequently employed to ensure take of infection, however non-lethal systems such as this SARS-CoV-2 rhesus macaque model may artificially reduce the impact of potentially protective vaccines and interventions (Durudas et al., 2011, Curr HIV Res *9*, 276-288; Innis et al., 2019, Vaccine *37*, 4830-4834). Despite these limitations, this study demonstrated significant reduction in peak BAL sgmRNA and overall viral RNA, likely induced by rapid induction of immunological memory mediated by both B and T cell compartments. Wolfel et al reported nasal titers in patients average 6.5 x 10^5 copies/swab days 1-5 following onset of symptoms (Wolfel et al., 2020, Nature *581*, 465-469). These titers are significantly lower than the challenge dose and support potential for the vaccine candidate to control early during SARS-CoV-2 infection.

[0439] This study shows that DNA vaccination with a vaccine candidate targeting the full-length SARS-CoV-2 spike protein likely increases the availability T cell immunodominant epitopes leading to a broader and more potent immune response, compared to partial domains

and truncated immunogens. In this study, T cell cross-reactivity was observed to SARS-CoV-1.

[0440] In addition to T cells, INO-4800 induced durable antibody responses that rapidly increased following SARS-CoV-2 challenge. It is further demonstrated that INO-4800 induced robust neutralizing antibody responses against both D614 and G614 SARS-CoV-2 variants. While the D/G 614 site is outside the RBD, it has been suggested that this shift has the potential to impact vaccine-elicited antibodies (Korber B et al., 2020, Cell 182:1–16). Other studies report that the G614 variant exhibits increased SARS-CoV-2 infectivity (Hu et al., 2020, bioRxiv 2020.06.20.161323; Ozono S, 2020, bioRxiv 2020.06.15.151779). The data shows induction of comparable neutralization titers between D614 and G614 variants and that these responses are similarly recalled following SARS-CoV-2 challenge.

[0441] Materials & Methods

[0442] Non-human primate Immunizations, IFNγ ELISpot and ELISA

[0443] *DNA vaccine, INO-4800:* The highly optimized DNA sequence encoding SARS-CoV-2 IgE-spike was created using Inovio's proprietary in silico Gene Optimization Algorithm to enhance expression and immunogenicity (Smith et al., 2020, Nat Commun *11*, 2601). The optimized DNA sequence was synthesized, digested with BamHI and XhoI, and cloned into the expression vector pGX0001 under the control of the human cytomegalovirus immediate-early promoter and a bovine growth hormone polyadenylation signal.

[0444] Animals: All rhesus macaque experiments were approved by the Institutional Animal Care and Use Committee at Bioqual (Rockville, Maryland), an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility. Blood was collected for blood chemistry, PBMC isolation, serological analysis. BAL was collected on Week 8 to assay lung antibody levels and on Days 1, 2, 4, 7 post challenge to assay lung viral loads.

[0445] Immunizations, sample collection and viral challenge. Ten Chinese rhesus macaques (ranging from 4.55kg-5.55kg) were randomly assigned in study immunized (3 males and 2 females) or naïve (2 males and 3 females). Immunized macaques received two 1 mg injections of SARS-CoV-2 DNA vaccine, INO-4800 at week 0 and 4 by ID-EP administration using the CELLECTRA 2000® Adaptive Constant Current Electroporation Device with a 3P array (Inovio Pharmaceuticals). Blood was collected at indicated time points to analyse blood chemistry, peripheral blood mononuclear cells (PBMC) isolation, and serum was collected for serological analysis. Bronchoalveolar lavage was collected at Week

8 to assay lung antibody levels. BAL from naïve animals was run as control. At week 17, all animals were challenged with $1.2x10^8$ VP ($1.1x10^4$ PFU) SARS-CoV-2. Virus was administered as 1 ml by the intranasal (IN) route (0.5 ml in each nostril) and 1 ml by the intratracheal (IT) route.

[0446] Peripheral blood mononuclear cell isolation. Blood was collected from each macaque into sodium citrate cell preparation tubes (CPT, BD Biosciences). The tubes were centrifuged to separate plasma and lymphocytes, according to the manufacturer's protocol. Samples were transported by same-day shipment on cold-packs from Bioqual to The Wistar Institute for PBMC isolation. PBMCs were washed and residual red blood cells were removed using ammonium-chloride-potassium (ACK) lysis buffer. Cells were counted using a ViCell counter (Beckman Coulter) and resuspended in RPMI 1640 (Corning), supplemented with 10% fetal bovine serum (Atlas), and 1% penicillin/streptomycin (Gibco). Fresh cells were then plated for IFNγ ELISpot Assays and flow cytometry.

[0447] IFN-γ Enzyme-linked immunospot (ELISpot). Monkey interferon gamma (IFN-γ) ELISpot assay was performed to detect cellular responses. Monkey IFN-γ ELISpotPro (alkaline phosphatase) plates (Mabtech, Sweeden, Cat#3421M-2APW-10) were blocked for a minimum of 2 hours with RPMI 1640 (Corning), supplemented with 10% FBS and 1% penn/strep (R10). Following PBMC isolation, 200 000 cells from macaques were added to each well in the presence of 1) overlapping peptide pools (15-mers with 9-mer overlaps) corresponding to the SARS-CoV-1, SARS-CoV-2, or MERS-CoV Spike proteins (5µg/mL/well final concentration), 2) R10 with DMSO (negative control), or 3) anti-CD3 positive control (Mabtech, 1:1000 dilution). All samples were plated in triplicate. Plates were incubated overnight at 37°C, 5% CO_2. After 18-20 hours, the plates were washed in PBS and spots were developed according to the manufacturer's protocol. Spots were imaged using a CTL Immunospot plate reader and antigen-specific responses were determined by subtracting the number of spots in the R10+DMSO negative control well from the wells stimulated with peptide pools.

[0448] Antigen Binding ELISA. Serum and BAL was collected at each time point was evaluated for binding titers as indicated. Ninety-six well immunosorbent plates (NUNC) were coated with 1ug/mL recombinant SARS-CoV-2 S1+S2 ECD protein (Sino Biological 40589-V08B1), S1 protein (Sino Biological 40591-V08H), S2 protein (Sino Biological 40590-V08B), or receptor-binding domain (RBD) protein (Sino Biological 40595-V05H) in DPBS overnight at 4°C. ELISA plates were also coated with 1ug/mL recombinant SARS-CoV S1 protein (Sino Biological 40150-V08B1) and RBD protein (Sino Biological 40592-

V08B) or MERS-CoV Spike (Sino Biological 40069-V08B). Plates were washed four times with PBS + 0.05% Tween20 (PBS-T) and blocked with 5% skim milk in PBS-T (5% SM) for 90 minutes at 37°C. Sera or BAL from INO-4800 vaccinated macaques were serially diluted in 5% SM, added to the washed ELISA plates, and incubated for 1 hour at 37°C. Following incubation, plates were washed 4 times with PBS-T and an anti-monkey IgG conjugated to horseradish peroxidase (Southern Biotech 4700-5). Plates were washed 4 times with PBS-T and one-step TMB solution (Sigma) was added to the plates. The reaction was stopped with an equal volume of 2N sulfuric acid. Plates were read at 450nm and 570nm within 30 minutes of development using a Biotek Synergy2 plate reader.

[0449] ACE2 Competition ELISA-Non-human primates. 96-well half area plates (Corning) were coated at room temperature for 3 hours with 1 µg/mL PolyRab anti-His antibody (ThermoFisher, PA1-983B), followed by overnight blocking with blocking buffer containing 1x PBS, 5% skim milk, 1% FBS, and 0.2% Tween-20. The plates were then incubated with 10 µg/mL of His6x-tagged SARS-CoV-2 ("His6x" disclosed as SEQ ID NO: 25), S1+S2 ECD (Sinobiological, 40589-V08B1) at room temperature for 1-2 hours. NHP sera (Day 0 or Week 6) was serially diluted 3-fold with 1XPBS containing 1% FBS and 0.2% Tween and pre-mixed with huACE2-IgMu at constant concentration of 0.4ug/ml. The pre-mixture was then added to the plate and incubated at RT for 1-2 hours. The plates were further incubated at room temperature for 1 hour with goat anti-mouse IgG H+L HRP (A90-116P, Bethyl Laboratories) at 1:20,000 dilution followed by addition of one-step TMB ultra substrate (ThermoFisher) and then quenched with 1M H_2SO_4. Absorbance at 450nm and 570nm were recorded with BioTEK plate reader.

[0450] Flow cytometry-based ACE2 receptor binding inhibition assay. HEK-293T cells stably expressing ACE2-GFP were generated using retroviral transduction. Following transduction, the cells were flow sorted based on GFP expression to isolate GFP positive cells. Single cell cloning was done on these cells to generate cell lines with equivalent expression of ACE2-GFP. To detect inhibition of Spike binding to ACE2, S1+S2 ECD-his tagged (Sino Biological, Catalog #40589-V08B1) was incubated with serum collected from vaccinated animals at indicated time points and dilutions at concentration of 2.5 µg/ml on ice for 60 minutes. This mixture was then transferred to 150,000 293T-ACE2-GFP cells and incubated on ice for 90 minutes. Following this, the cells were washed 2x with PBS followed by staining for surelight® APC conjugated anti-his antibody (Abcam, ab72579) for 30 min on ice. As a positive control, Spike protein was pre-incubated with recombinant human

ACE2 before transferring to 293T-Ace2-GFP cells. Data was acquired using a BD LSRII and analyzed by FlowJo (version 10).

[0451] Pseudovirus Neutralization Assay. SARS-CoV-2 pseudovirus were produced using HEK293T cells transfected with GeneJammer (Agilent) using IgE-SARS-CoV-2 S plasmid (Genscript) and pNL4-3.Luc.R-E- plasmid (NIH AIDS reagent) at a 1:1 ratio. Forty-eight hours post transfection, transfection supernatant was collected, enriched with FBS to 12% final volume, steri-filtered (Millipore Sigma), and aliquoted for storage at -80°C. SARS-Cov-2 pseudovirus neutralization assay was set up using D10 media (DMEM supplemented with 10%FBS and 1X Penicillin-Streptomycin) in a 96 well format. CHO cells stably expressing Ace2 were used as target cells (Creative Biolabs, Catalog No. VCeL-Wyb019). SARS-Cov-2 pseudovirus were titered to yield greater than 20 times the cells only control relative luminescence units (RLU) after 72h of infection. For setting up neutralization assay, 10,000 CHO-ACE2 cells were plated in 96-well plates in 100ul D10 media and rested overnight at 37˚C and 5% CO2 for 24 hours. Following day, Monkey and Rabbit sera from INO-4800 vaccinated and control groups were heat inactivated and serially diluted as desired. Sera were incubated with a fixed amount of SARS-Cov-2 pseudovirus for 90 minutes at RT. 50ul media was removed from the plated CHO-Ace2 cell containing wells. Post 90 minutes, the mix was added to plated CHO-Ace2 cells and allowed to incubate in a standard incubator (37% humidity, 5% CO2) for 72h. Post 72h, cells were lysed using britelite plus luminescence reporter gene assay system (Perkin Elmer Catalog no. 6066769) and RLU were measured using the Biotek plate reader. Neutralization titers (ID50) were calculated using GraphPad Prism 8 and defined as the reciprocal serum dilution at which RLU were reduced by 50% compared to RLU in virus control wells after subtraction of background RLU in cell control wells.

[0452] Viral RNA assay. RT-PCR assays were utilized to monitor viral loads, essentially as previously described (Abnink P et al 2019 Science). Briefly, RNA was extracted using a QIAcube HT (Qiagen,Germany) and the Cador pathogen HT kit from bronchoalveolar lavage (BAL) supernatant and nasal swabs. RNA was reverse transcribed using superscript VILO (Invitrogen) and ran in duplicate using the QuantStudio 6 and 7 Flex Real-Time PCR System (Applied Biosystems) according to manufacturer's specifications. Viral loads were calculated of viral RNA copies per mL or per swab and the assay sensitivity was 50 copies. The target for amplification was the SARS-CoV2 N (nucleocapsid) gene. The primers and probes for the targets were: 2019-nCoV_N1-F :5'-GACCCCAAAATCAGCGAAAT-3' (SEQ ID NO:18);

2019-nCoV_N1-R: 5'-TCTGGTTACTGCCAGTTGAATCTG-3' (SEQ ID NO:19); 2019-nCoV_N1-P: 5'-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3' (SEQ ID NO:20).

[0453] Subgenomic mRNA assay. SARS-CoV-2 E gene subgenomic mRNA (sgmRNA) was assessed by RT-PCR using an approach similar to previously described (Wolfel R et al. 2020, Nature, 581, 465-469). To generate a standard curve, the SARS-CoV-2 E gene sgmRNA was cloned into a pcDNA3.1 expression plasmid; this insert was transcribed using an AmpliCap-Max T7 High Yield Message Maker Kit (Cellscript) to obtain RNA for standards. Prior to RT-PCR, samples collected from challenged animals or standards were reverse-transcribed using Superscript III VILO (Invitrogen) according to the manufacturer's instructions. A Taqman custom gene expression assay (ThermoFisher Scientific) was designed using the sequences targeting the E gene sgmRNA (18). Reactions were carried out on a QuantStudio 6 and 7 Flex Real-Time PCR System (Applied Biosystems) according to the manufacturer's specifications. Standard curves were used to calculate sgmRNA in copies per ml or per swab; the quantitative assay sensitivity was 50 copies per ml or per swab.

[0454] Results

[0455] Induction of memory humoral and cellular immune responses in INO-4800 immunized non-human primates. Non-human primates (NHP) are a valuable model in the development of COVID-19 vaccines and therapeutics as they can be infected with wild-type SARS-CoV-2, and present with similar pathology to humans (Chandrashekar et al., 2020, Science, eabc4776; Qin et al., 2005, J Pathol *206*, 251-259; Yao et al., 2014, J Infect Dis *209*, 236-242; Yu et al., 2020, Science, eabc6284). Rhesus macaques (n=5) received two immunizations of INO-4800 (1mg), at Week 0 and Week 4 (**Figure 33A**). Naïve control animals (n=5) did not receive vaccine. Humoral and cellular immune responses were monitored for 15 weeks (~4 months) following prime immunization for memory responses. All animals seroconverted following a single INO-4800 immunization, with serum IgG titers detected against the full-length S1+S2 extracellular domain (ECD), S1, S2, and RBD regions of the SARS-CoV-2 S protein (**Figure 33B and Figure 33C**). Cross-reactive antibodies were also detected against SARS-CoV S1 protein and RBD, but not MERS-CoV (**Figure 34**). SARS-CoV-2-reactive IgG against the ECD and RBD were detected in bronchoalveolar lavage (BAL) washes at Week 8 following immunization (**Figure 34**).

[0456] In serum samples of the animals SARS-CoV-2 pseudovirus neutralization activity was detected for >4 months following immunization (**Figure 33D**), demonstrating memory titers comparable to those observed in other reported acute protection studies in macaques

(Gao et al., 2020, Science 369, 77-81; Tian et al., 2020, Emerg Microbes Infect, 9:382-385; van Doremalen et al., 2020, bioRxiv 2020.05.13.093195; Yu et al., 2020, Science, eabc6284) and reported for convalescent humans (Ni et al., 2020, Immunity *52*, 971-977 e973; Robbiani et al., 2020, Nature, s41586-020-2456-9). During the course of the COVID-19 pandemic, a D614G SARS-CoV-2 spike variant has emerged that has potentially greater infectivity, and now accounts for >80% of new isolates (Korber B et al., 2020, Cell 182:1–16). There is concern that vaccines developed prior to this variant's appearance may not neutralize the D614G virus. Therefore, neutralization against this new variant was evaluated using a modified pseudovirus expressing the G614 Spike protein (**Figure 33E**). Similar neutralization ID50 titers were observed against both D614 and G614 spikes, supporting induction of functional antibody responses by INO-4800 against the now dominating SARS-CoV-2 variant.

[0457] To further investigate the neutralizing activities, the sera was also tested using an ACE2 competition ELISA, where sera from 80% of immunized NHPs inhibited the SARS-CoV-2 Spike-ACE2 interaction (**Figure 33F**). 100% of macaques responded in the flow cytometry ACE2-293T inhibition assay, with 53-96% inhibition of the Spike-ACE2 interaction at a 1:10 dilution and 24-53% inhibition at a 1:30 dilution (**Figure 33G**).

[0458] INO-4800 immunization also induced SARS-CoV-2 S antigen reactive T cell responses against all 5 peptide pools with T cells responses peaking at Week 6, two weeks following the second immunization (0-518 SFU/million cells) (**Figure 33H**). Distinct immunogenic epitope responses were detected against the RBD and S2 regions (**Figure 33B**). Cross-reactive T cells responses were also detected against the SARS-CoV Spike protein (**Figure 36A**). However, cross-reactivity was not observed to MERS-CoV Spike peptides, which supports the lower sequence homology between SARS-CoV-2 and MERS-CoV (**Figure 36B**).

[0459] Vaccine induced memory recall responses upon SARS-CoV-2 challenge in non-human primates. Vaccine immunized macaques along with unvaccinated controls were challenged with SARS-CoV-2 13 weeks (~3 months) post-final immunization (Study Week 17, **Figure 37A**). NHPs received a challenge dose of $1.1x10^4$ PFU of SARS-CoV-2 Isolate USA-WA1/2020 by intranasal and intratracheal inoculation as previously described (Chandrashekar et al., 2020; Yu et al., 2020). Upon viral challenge, 3/5 of INO-4800 vaccinated animals had an immediate increase in antibody titers against the SARS-CoV-2 full-length ECD. By day 7, 5/5 animals had an increase in antibody titers against both full length ECD and RBD (**Figure 37B**). Seven days post-challenge, robust geometric mean

endpoint titers ranging from 409,600 - 1,638,400 were observed in immunized animals, compared with the naïve group which displayed seroconversion of only 1/5 animals (GMT 100) (**Figure 37B**). A significant increase in pseudoneutralization titers was observed in all INO-4800 immunized animals against both D614 and G614 Spike variants by day 7 post-challenge (**Figure 37C**).

[0460] Cellular responses were evaluated before and after challenge. At week 15, IFN-γ ELISpot responses had contracted significantly since the peak responses observed at week 6. T cell responses increased in the vaccinated group following challenge (~218.36 SFU/million cells) implying recall of immunological T cell memory (**Figure 38 and Figure 39**).

[0461] Protective efficacy following SARS-CoV-2 challenge. At earlier time points post virus input at challenge, viral mRNA detection does not discriminate between input challenge inoculum and active infection, while sgmRNA levels are more likely representative of active cellular SARS-CoV-2 replication (Wolfel et al., 2020, Nature, 581, 465-469; Yu et al., 2020, Science, eabc6284). SARS-CoV-2 subgenomic mRNA (sgmRNA) was measured in nonvaccinated control and INO-4800 vaccinated macaques following challenge with $1.1x10^4$ PFU of SARS-CoV-2 Isolate USA-WA1/2020 (**Figure 40**). Peak viral sgmRNA loads in the BAL were significantly lower in the INO-4800 vaccinated group (Figure 40A and Figure 40B), along with significantly lower viral RNA loads at day 7 post-challenge (**Figure 40C**), indicating protection from lower respiratory disease. While sgmRNA was detected in the nasal swabs of both the control and INO-4800 vaccinated animals (**Figure 40D through Figure 40F**), viral RNA levels trended downwards in INO-4800 vaccinated animals by more than 2 logs (**Figure 40F**). Overall, the reduced viral loads afforded by INO-4800 vaccination are likely due to anamnestic B and T cell responses that are rapidly recalled immediately following exposure to SARS-CoV-2 infection.

[0462] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

[0463] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

[0464] ILLUSTRATIVE EMBODIMENTS

[0465] Embodiment 1. A nucleic acid molecule encoding a Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) spike antigen, the nucleic acid molecule comprising:

a nucleic acid sequence having at least about 90% identity over an entire length of the nucleic acid sequence set forth in nucleotides 55 to 3837 of SEQ ID NO: 2;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 2;

the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 2;

the nucleic acid sequence of SEQ ID NO: 2;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 3;

the nucleic acid sequence of SEQ ID NO: 3;

a nucleic acid sequence having at least about 90% identity over an entire length of nucleotides 55 to 3837 of SEQ ID NO: 5;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 5;

the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 5;

the nucleic acid sequence of SEQ ID NO: 5;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 6; or

the nucleic acid sequence of SEQ ID NO: 6.

[0466] Embodiment 2. A nucleic acid molecule encoding a SARS-CoV-2 spike antigen, wherein the SARS-CoV-2 spike antigen comprises:

an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1;

the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1;

an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 1;

the amino acid sequence of SEQ ID NO: 1;

an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4;

an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 4;

the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4; or

the amino acid sequence of SEQ ID NO: 4.

[0467] Embodiment 3. An expression vector comprising the nucleic acid molecule according to Embodiment 1 or Embodiment 2.

[0468] Embodiment 4. The expression vector according to Embodiment 3, wherein the nucleic acid molecule is operably linked to a regulatory element selected from a promoter and a poly-adenylation signal.

[0469] Embodiment 5. The expression vector according to Embodiment 3 or Embodiment 4, wherein the vector is a plasmid or viral vector.

[0470] Embodiment 6. An immunogenic composition comprising an effective amount of the expression vector according to any one of Embodiments 3-5.

[0471] Embodiment 7. The immunogenic composition according to Embodiment 6 further comprising a pharmaceutically acceptable excipient.

[0472] Embodiment 8. The immunogenic composition according to Embodiment 7 wherein the pharmaceutically acceptable excipient comprises a buffer, optionally saline-sodium citrate buffer.

[0473] Embodiment 9. The immunogenic composition of Embodiment 8, wherein the composition is formulated at a concentration of 10 mg per milliliter of a sodium salt citrate buffer.

[0474] Embodiment 10. The immunogenic composition according to any one of Embodiments 6-9, further comprising an adjuvant.

[0475] Embodiment 11. A SARS-CoV-2 spike antigen comprising:

an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1;

the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1;

an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 1;

the amino acid sequence of SEQ ID NO: 1;

an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4;

an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 4;

the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4; or

the amino acid sequence of SEQ ID NO: 4.

[0476] Embodiment 12. A vaccine for the prevention or treatment of Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) infection comprising an effective amount of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, or the antigen of Embodiment 11.

[0477] Embodiment 13. The vaccine according to Embodiment 12, further comprising a pharmaceutically acceptable excipient.

[0478] Embodiment 14. The vaccine according to Embodiment 13, wherein the pharmaceutically acceptable excipient comprises a buffer, optionally sodium salt citrate buffer.

[0479] Embodiment 15. The vaccine of Embodiment 14, formulated at a concentration of 10 mg of nucleic acid per milliliter of a sodium salt citrate buffer.

[0480] Embodiment 16. The vaccine according to any one of Embodiments 12 to 15, further comprising an adjuvant.

[0481] Embodiment 17. A method of inducing an immune response against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) in a subject in need thereof, the method comprising administering an effective amount of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 to the subject.

[0482] Embodiment 18. A method of protecting a subject in need thereof from infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), the method comprising administering an effective amount of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 to the subject.

[0483] Embodiment 19. A method of protecting a subject in need thereof from a disease or disorder associated with infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), the method comprising administering an effective amount of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 to the subject.

[0484] Embodiment 20. A method of treating a subject in need thereof against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) infection, the method comprising administering an effective amount of the nucleic acid molecule of Embodiment 1 or 2, the

vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 to the subject, wherein the subject is thereby resistant to one or more SARS-CoV-2 strains.

[0485] Embodiment 21. The method of any one of Embodiments 17 to 20, wherein administering comprises at least one of electroporation and injection.

[0486] Embodiment 22. The method of any one of Embodiments 17 to 20, wherein administering comprises parenteral administration followed by electroporation.

[0487] Embodiment 23. The method of any one of Embodiments 17 to 22, wherein an initial dose of about 0.5 mg to about 2.0 mg of nucleic acid is administered to the subject, optionally wherein the initial dose is 0.5 mg, 1.0 mg or 2.0 mg of nucleic acid.

[0488] Embodiment 24. The method of Embodiment 23, wherein a subsequent dose of about 0.5 mg to about 2.0 mg of nucleic acid is administered to the subject about four weeks after the initial dose, optionally wherein the subsequent dose is 0.5 mg,1.0 mg or 2.0 mg of nucleic acid.

[0489] Embodiment 25. The method of Embodiment 24, wherein one or more further subsequent doses of about 0.5 mg to about 2.0 mg of nucleic acid is administered to the subject at least twelve weeks after the initial dose, optionally wherein the further subsequent dose is 0.5 mg, 1.0 mg, or 2.0 mg of nucleic acid.

[0490] Embodiment 26. The method of any one of Embodiments 17 to 25, comprising administering INO-4800 or a biosimilar thereof to the subject.

[0491] Embodiment 27. The method of any one of Embodiments 17 to 26, further comprising administering to the subject at least one additional agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection.

[0492] Embodiment 28. The method of Embodiment 27 wherein the nucleic acid molecule, vector, the immunogenic composition, antigen, or vaccine is administered to the subject before, concurrently with, or after the additional agent.

[0493] Embodiment 29. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 in a method of inducing an immune response against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) in a subject in need thereof.

[0494] Embodiment 30. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 in a method of protecting a subject from infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2).

[0495] Embodiment 31. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 in a method of protecting a subject from a disease or disorder associated with infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2).

[0496] Embodiment 32. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, the immunogenic composition of any one of Embodiments 6-10, the antigen of Embodiment 11, or the vaccine of any one of Embodiments 12-16 in a method of treating a subject in need thereof against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) infection.

[0497] Embodiment 33. The use of any one of Embodiments 29 to 32 in combination with at least one additional agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection.

[0498] Embodiment 34. The use of any one of Embodiments 29 to 33, wherein the nucleic acid molecule, the vector, the immunogenic composition, the antigen, or the vaccine is administered to the subject by at least one of electroporation and injection.

[0499] Embodiment 35. The use of Embodiment 34, wherein the nucleic acid molecule, the vector, the immunogenic composition, the antigen, or the vaccine is parenterally administered to the subject followed by electroporation.

[0500] Embodiment 36. The use of any one of Embodiments 29 to 35, wherein an initial dose of about 0.5 mg to about 2.0 mg of nucleic acid is administered to the subject, optionally wherein the initial dose is 0.5 mg, 1.0 mg, or 2.0 mg of nucleic acid.

[0501] Embodiment 37. The use of Embodiment 36, wherein a subsequent dose of about 0.5 mg to about 2.0 mg of nucleic acid is administered to the subject about four weeks after the initial dose, optionally wherein the subsequent dose is 0.5 mg, 1.0 mg, or 2.0 mg of nucleic acid.

[0502] Embodiment 38. The use of Embodiment 37, wherein a further subsequent dose of about 0.5 mg to about 2.0 mg of nucleic acid is administered to the subject at least twelve

weeks after the initial dose, optionally wherein the further subsequent dose is 0.5 mg, 1.0 mg, or 2.0 mg of nucleic acid.

[0503] Embodiment 39. The use of any one of Embodiments 29 to 38, wherein the immunogenic composition is INO-4800 or a biosimilar thereof.

[0504] Embodiment 40. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, or the antigen of Embodiment 11 in the preparation of a medicament.

[0505] Embodiment 41. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, or the antigen of Embodiment 11 in the preparation of a medicament for treating or protecting against infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2).

[0506] Embodiment 42. Use of the nucleic acid molecule of Embodiment 1 or 2, the vector of any one of Embodiments 3-5, or the antigen of Embodiment 11 in the preparation of a medicament for protecting a subject in need thereof from a disease or disorder associated with infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2).

[0507] Embodiment 43. A method of detecting a persistent cellular immune response in a subject, the method comprising the steps of:

administering an immunogenic composition for inducing an immune response against a SARS-CoV-2 antigen to a subject in need thereof;

isolating peripheral mononuclear cells (PBMCs) from the subject;

stimulating the isolated PBMCs with a SARS-CoV-2 spike antigen comprising an amino acid sequence selected from the group consisting of an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 1, the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 1, an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 1, the amino acid sequence of SEQ ID NO: 1, an amino acid sequence having at least about 90% identity over an entire length of residues 19 to 1279 of SEQ ID NO: 4, an amino acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 4, the amino acid sequence set forth in residues 19 to 1279 of SEQ ID NO: 4, the amino acid sequence of SEQ ID NO: 4., and a fragment thereof comprising at least 20 amino acids; and

detecting at least one of the number of cytokine expressing cells and the level of cytokine expression.

[0508] Embodiment 44. The method of Embodiment 43, wherein the step of detecting at least one of the number of cytokine expressing cells and the level of cytokine expression is performed using an assay selected from the group consisting of Enzyme-linked immunospot (ELISpot) and Intracellular Cytokine Staining (ICS) analysis using flow cytometry.

[0509] Embodiment 45. The method of Embodiment 43, wherein the subject is administered an immunogenic composition comprising a nucleic acid molecule, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

a nucleic acid sequence having at least about 90% identity over an entire length of the nucleic acid sequence set forth in nucleotides 55 to 3837 of SEQ ID NO: 2;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 2;

the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 2;

the nucleic acid sequence of SEQ ID NO: 2;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 3;

the nucleic acid sequence of SEQ ID NO: 3;

a nucleic acid sequence having at least about 90% identity over an entire length of nucleotides 55 to 3837 of SEQ ID NO: 5;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 5;

the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 5;

the nucleic acid sequence of SEQ ID NO: 5;

a nucleic acid sequence having at least about 90% identity over an entire length of SEQ ID NO: 6; and

the nucleic acid sequence of SEQ ID NO: 6.

[0510] SEQUENCE LISTING

SARS-CoV-2 Consensus Spike Antigen amino acid insert sequence of pGX9501 (SEQ ID NO: 1) (IgE leader sequence underlined):

```
   1  MDWTWILFLV AAATRVHSSQ CVNLTTRTQL PPAYTNSFTR GVYYPDKVFR SSVLHSTQDL
  61  FLPFFSNVTW FHAIHVSGTN GTKRFDNPVL PFNDGVYFAS TEKSNIIRGW IFGTTLDSKT
 121  QSLLIVNNAT NVVIKVCEFQ FCNDPFLGVY YHKNNKSWME SEFRVYSSAN NCTFEYVSQP
 181  FLMDLEGKQG NFKNLREFVF KNIDGYFKIY SKHTPINLVR DLPQGFSALE PLVDLPIGIN
 241  ITRFQTLLAL HRSYLTPGDS SSGWTAGAAA YYVGYLQPRT FLLKYNENGT ITDAVDCALD
 301  PLSETKCTLK SFTVEKGIYQ TSNFRVQPTE SIVRFPNITN LCPFGEVFNA TRFASVYAWN
 361  RKRISNCVAD YSVLYNSASF STFKCYGVSP TKLNDLCFTN VYADSFVIRG DEVRQIAPGQ
 421  TGKIADYNYK LPDDFTGCVI AWNSNNLDSK VGGNYNYLYR LFRKSNLKPF ERDISTEIYQ
 481  AGSTPCNGVE GFNCYFPLQS YGFQPTNGVG YQPYRVVVLS FELLHAPATV CGPKKSTNLV
 541  KNKCVNFNFN GLTGTGVLTE SNKKFLPFQQ FGRDIADTTD AVRDPQTLEI LDITPCSFGG
 601  VSVITPGTNT SNQVAVLYQD VNCTEVPVAI HADQLTPTWR VYSTGSNVFQ TRAGCLIGAE
 661  HVNNSYECDI PIGAGICASY QTQTNSPRRA RSVASQSIIA YTMSLGAENS VAYSNNSIAI
 721  PTNFTISVTT EILPVSMTKT SVDCTMYICG DSTECSNLLL QYGSFCTQLN RALTGIAVEQ
 781  DKNTQEVFAQ VKQIYKTPPI KDFGGFNFSQ ILPDPSKPSK RSFIEDLLFN KVTLADAGFI
 841  KQYGDCLGDI AARDLICAQK FNGLTVLPPL LTDEMIAQYT SALLAGTITS GWTFGAGAAL
 901  QIPFAMQMAY RFNGIGVTQN VLYENQKLIA NQFNSAIGKI QDSLSSTASA LGKLQDVVNQ
 961  NAQALNTLVK QLSSNFGAIS SVLNDILSRL DKVEAEVQID RLITGRLQSL QTYVTQQLIR
1021  AAEIRASANL AATKMSECVL GQSKRVDFCG KGYHLMSFPQ SAPHGVVFLH VTYVPAQEKN
1081  FTTAPAICHD GKAHFPREGV FVSNGTHWFV TQRNFYEPQI ITTDNTFVSG NCDVVIGIVN
1141  NTVYDPLQPE LDSFKEELDK YFKNHTSPDV DLGDISGINA SVVNIQKEID RLNEVAKNLN
1201  ESLIDLQELG KYEQYIKWPW YIWLGFIAGL IAIVMVTIML CCMTSCCSCL KGCCSCGSCC
1261  KFDEDDSEPV LKGVKLHYT
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DNA insert sequence of pGX9501 (SEQ ID NO: 2) (IgE leader sequence underlined):

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   1  ATGGATTGGA CTTGGATTCT CTTTCTCGTT GCTGCAGCCA CACGCGTTCA TAGCAGCCAG
  61  TGTGTGAACC TGACCACCAG AACACAGCTG CCTCCTGCCT ACACCAACAG CTTCACCAGA
 121  GGAGTCTACT ACCCAGACAA AGTCTTCAGA AGCTCTGTGC TGCACAGCAC CCAGGACCTG
 181  TTCCTGCCTT TCTTCAGCAA CGTGACCTGG TTCCACGCCA TCCACGTGTC TGGCACCAAC
 241  GGCACCAAGA GATTTGACAA CCCTGTTCTT CCTTTCAATG ATGGCGTGTA CTTTGCCAGC
 301  ACAGAGAAGA GCAACATCAT CCGAGGCTGG ATCTTTGGCA CCACCCTGGA CAGCAAAACC
 361  CAGAGCCTGC TGATCGTGAA CAACGCCACC AACGTGGTCA TCAAGGTGTG TGAGTTCCAG
 421  TTCTGCAATG ACCCTTTCCT GGGCGTGTAC TACCACAAGA ACAACAAGTC CTGGATGGAG
 481  TCTGAGTTCA GAGTCTACAG CTCTGCCAAC AACTGCACAT TTGAATATGT GTCCCAGCCT
 541  TTCCTGATGG ACCTGGAGGG CAAGCAGGGC AACTTTAAGA ACCTGAGAGA ATTTGTGTTC
 601  AAGAACATCG ATGGCTACTT CAAGATCTAC AGCAAGCACA CACCCATCAA CCTGGTGAGA
 661  GACCTGCCTC AGGGCTTCTC TGCCCTGGAG CCTCTGGTGG ACCTGCCCAT CGGCATCAAC
 721  ATCACCAGAT TCCAGACCCT GCTGGCCCTG CACAGAAGCT ACCTGACCCC AGGAGACAGC
 781  AGCAGCGGCT GGACAGCTGG AGCTGCTGCC TACTACGTGG GCTACCTGCA GCCCAGGACC
 841  TTCCTGCTGA AGTACAACGA AAATGGCACC ATCACAGATG CTGTTGACTG TGCCCTGGAC
 901  CCTCTTAGCG AGACCAAGTG CACCCTGAAG TCCTTCACAG TGGAGAAAGG CATCTACCAG
 961  ACCAGCAACT TCCGAGTGCA GCCAACAGAG AGCATCGTGA GATTTCCAAA CATCACCAAC
1021  CTGTGCCCTT TTGGAGAAGT CTTCAATGCC ACCAGATTTG CTTCTGTGTA CGCCTGGAAC
1081  AGAAAAAGAA TCAGCAACTG TGTGGCTGAC TACTCTGTGC TGTACAACTC TGCCTCCTTC
1141  TCCACCTTCA AGTGCTATGG AGTCTCTCCA ACCAAGCTGA ATGACCTGTG CTTCACCAAC
1201  GTGTATGCTG ACAGCTTTGT GATCAGAGGA GATGAAGTGC GGCAGATTGC TCCTGGCCAG
1261  ACAGGCAAGA TTGCTGACTA CAACTACAAG CTGCCTGATG ACTTCACAGG CTGTGTCATC
1321  GCCTGGAACA GCAACAACCT GGACAGCAAG GTGGGCGGCA ACTACAACTA CCTGTACAGA
1381  CTTTTCAGGA AGAGCAACCT GAAGCCTTTT GAAAGAGACA TCTCCACAGA GATCTACCAG
1441  GCTGGCAGCA CACCCTGCAA TGGTGTGGAA GGCTTCAACT GCTACTTCCC TCTGCAGAGC
1501  TACGGCTTCC AGCCAACAAA TGGCGTGGGC TACCAGCCTT ACAGAGTGGT GGTGCTGTCC
1561  TTTGAGCTGC TGCACGCCCC TGCCACAGTG TGTGGCCCCA AGAAGAGCAC CAACCTGGTG
1621  AAGAACAAAT GTGTGAACTT CAATTTCAAT GGCCTGACAG GCACAGGAGT GCTGACAGAG
1681  AGCAACAAGA AGTTTCTTCC TTTCCAGCAG TTTGGAAGAG ACATTGCTGA CACCACAGAT
1741  GCTGTGAGAG ATCCTCAGAC CCTGGAGATC CTGGATATCA CACCCTGCTC CTTTGGAGGA
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3001 AGACTCATCA CAGGCCGCCT GCAGAGCCTG CAGACCTACG TGACCCAGCA GCTGATCAGA
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Single strand DNA sequence of pGX9501 (SEQ ID NO: 3):

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SARS-CoV-2 Outlier Spike Antigen amino acid insert sequence of pGX9503 (SEQ ID NO: 4) (IgE leader sequence underlined):

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 121 QSLLIVNNAT NVVIKVCEFQ FCNDPFLGVY YHKNNKSWME SEFRVYSSAN NCTFEYVSQP
 181 FLMDLEGKQG NFKNLREFVF KNIDGYFKIY SKHTPINLVR DLPQGFSALE PLVDLPIGIN
 241 ITRFQTLLAL HRSYLTPGDS SSGWTAGAAA YYVGYLQPRT FLLKYNENGT ITVAVACALD
 301 PLSETKCTLK SFTVEKGIYQ TSNFRVQPTE SIVRFPNITN LCPFGEVFNA TRFASVYAWN
 361 RKRISNCVAD YSVLYNSASF STFKCYGVSP TKLNDLCFTN VYADSFVIRG DEVRQIAPGQ
 421 TGKIADYNYK LPDDFTGCVI AWNSNNLDSK VGGNYNYLYR LFRKSNLKPF ERDISTEIYQ
 481 AGSTPCNGVE GFNCYFPLQS YGFQPTNGVG YQPYRVVVLS FELLHAPATV CGPKKSTNLV
 541 KNKCVNFNFN GLTGTGVLTE SNKKFLPFQQ FGRDIADTTD AVRDPQTLEI LDITPCSFGG
 601 VSVITPGANT SNQVTVLYQD VNCTEVPVAI HADQLTPTWR VYSTGSNVFK TRAGCLIGAE
 661 HVNNSYECDI PIGAGICASY QTQTNSPRRA RSTASQSIIA YTMSLGAENS VAYSNNSIVI
 721 PTNFTISVTT EILPVSMTKT SVDCTMYICS DSTECSNPLL QYGSFCTQLN RALTGIAVEQ
 781 DKNTQEVFAQ VKQIYKTPPI KDFGGFNFSQ ILPDPSKPSK RSFIEDLLFN KVTLADAGFI
 841 KQYGDCLGDI AARDLICAQK FNGLTVLPPL LTDEMIAQYT SALLAGTITS GWTFGAGAAL
 901 QIPFAMQMAY RFNGIRVTQN VLYENQKLIA NQFNSAIGKI QDSLSSTASA LGKLQDVVNQ
 961 NAQALNTLVK QLSSTFSTIS SVLNDILSRL DKVEAEVQID RLITGRLQSL QTYVTQQLIR
1021 AAEIRASANL KATKMSECVL GQSKRVDFCG KGYHLMSFPQ SAPHGVVFLH VTYVPAQEKN
1081 FTTAPATCHD GKAHFPREGV FVSNGTHWFV TQRNFDEPQI ITTDNTFVSG NCDVVIGIVN
1141 NTVYDPLQPE LDSFKEELDK YFKNHTSPDV DLGDISGINA SVVNIQKEID RLNEVAKNLN
1201 ESLIDLQELG KYEQYIKWPW YIWLGFIAGL IAIVMVTIML CCMTSCCSCL KGCCSCGSCC
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DNA insert sequence of pGX9503 (SEQ ID NO: 5) (IgE leader sequence underlined):

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 181 TTCCTGCCTT TCTTCAGCAA CGTGACCTGG TTCCACGCCA TCCACGTGTC TGGCACCAAC
 241 GGCACCAAGA GATTTGACAA CCCTGTGCTG CCTTTCAATG ATGGTGTGTA CTTTGCCAGC
 301 ACAGAGAAGA GCAACATCAT CCGAGGCTGG ATCTTTGGCA CCACCCTGGA CAGCAAAACA
 361 CAGAGCCTGC TGATCGTGAA TAATGCCACC AACGTGGTCA TCAAGGTGTG TGAGTTCCAG
 421 TTCTGCAATG ACCCTTTCCT GGGCGTGTAC TACCACAAGA ACAACAAGTC CTGGATGGAG
 481 TCTGAGTTCC GAGTGTACAG CTCTGCCAAC AACTGCACAT TTGAATATGT GTCCCAGCCT
 541 TTCCTGATGG ACCTGGAGGG CAAGCAGGGC AATTTCAAGA ACCTGAGAGA ATTTGTGTTC
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 661 GATCTTCCTC AGGGCTTCTC TGCCCTGGAG CCTCTGGTGG ACCTGCCCAT CGGCATCAAC
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3541 TCTGTGGTGA ACATCCAGAA GGAAATTGAC AGGCTGAACG AAGTGGCCAA GAACCTGAAC
3601 GAAAGCCTCA TCGACCTGCA GGAGCTGGGC AAGTACGAGC AGTACATCAA GTGGCCTTGG
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3661 TACATCTGGC TGGGCTTCAT TGCTGGCCTC ATCGCCATCG TGATGGTGAC CATCATGCTG
3721 TGCTGCATGA CCAGCTGCTG CTCTTGCCTG AAGGGCTGCT GCAGCTGTGG CAGCTGCTGC
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Single strand DNA sequence of pGX9503 (SEQ ID NO: 6):

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  61 atagtaatca attacggggt cattagttca tagcccatat atggagttcc gcgttacata
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 241 gtatttacgg taaactgccc acttggcagt acatcaagtg tatcatatgc caagtacgcc
 301 ccctattgac gtcaatgacg gtaaatggcc cgcctggcat tatgcccagt acatgacctt
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 421 gcggttttgg cagtacatca atgggcgtgg atagcggttt gactcacggg gatttccaag
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 541 aaaatgtcgt aacaactccg ccccattgac gcaaatgggc ggtaggcgtg tacggtggga
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6781 tttgctcaca tgttctt

SEQ ID NO: 7 pGX9501 Forward primer
CAGGACAAGAACACACAGGAA

SEQ ID NO: 8 pGX9501 Reverse primer
CAGGCAGGATTTGGGAGAAA

SEQ ID NO: 9 pGX9501 Probe
ACCCATCAAGGACTTTGGAGG

SEQ ID NO: 10 pGX9503 Forward primer
AGGACAAGAACACACAGGAAG;

SEQ ID NO: 11 pGX9503 Reverse primer
CAGGATCTGGGAGAAGTTGAAG

SEQ ID NO: 12 pGX9503 Probe
ACACCACCCATCAAGGACTTTGGA

SEQ ID NO: 13 β-actin Forward primer
GTGACGTGGACATCCGTAAA

SEQ ID NO: 14 β-actin Reverse primer
CAGGGCAGTAATCTCCTTCTG

SEQ ID NO: 15 β-actin Probe
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SEQ ID NO: 16
PHGVVFLHV

SEQ ID NO: 17
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SEQ ID NO:18: 2019-nCoV_N1-F
5′-GACCCCAAAATCAGCGAAAT-3′

SEQ ID NO:19: 2019-nCoV_N1-R
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SEQ ID NO:20: 2019-nCoV_N1-P
5′-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3′

SEQ ID NO:21: 2019-nCoV_sgE-forward
5′ CGATCTCTTGTAGATCTGTTCTC 3′

SEQ ID NO:22: 2019-nCoV_sgE-reverse
5′ ATATTGCAGCAGTACGCACACA 3′

SEQ ID NO:23: 2019-nCoV_sgE-probe
5′ FAM- ACACTAGCCATCCTTACTGCGCTTCG-BHQ1 3′

What is claimed:

1. A nucleic acid molecule encoding a Severe Acute Respiratory Syndrome coronavirus 2 (SARS- CoV-2) spike antigen, the nucleic acid molecule comprising:
a nucleic acid sequence having at least 99% identity over an entire length of the nucleic acid sequence set forth in nucleotides 55 to 3837 of SEQ ID NO: 2;
a nucleic acid sequence having at least 99% identity over an entire length of SEQ ID NO: 2;
the nucleic acid sequence of nucleotides 55 to 3837 of SEQ ID NO: 2; or
the nucleic acid sequence of SEQ ID NO: 2.

2. An expression vector comprising the nucleic acid molecule according to claim 1, said vector optionally comprising the nucleic acid sequence of SEQ ID NO: 3.

3. An immunogenic composition comprising an effective amount of the expression vector according claim 2 and a pharmaceutically acceptable excipient.

4. The immunogenic composition according to claim 3 wherein the pharmaceutically acceptable excipient comprises a buffer, optionally saline-sodium citrate buffer.

5. The immunogenic composition of claim 4, wherein the composition comprises 10 mg of the vector per milliliter of saline-sodium citrate buffer.

6. The immunogenic composition according to any one of claims 3-5, further comprising an adjuvant.

7. Use of the immunogenic composition of any one of claims 3-6 in the manufacture of a medicament for inducing an immune response against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) in a subject in need thereof.

8. Use of the immunogenic composition of any one of claims 3-6 in the manufacture of a medicament for protecting a subject from infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV- 2).

9. Use of the immunogenic composition of any one of claims 3-6 in the manufacture of a medicament for treating a subject in need thereof against Severe Acute Respiratory Syndrome coronavirus 2 (SARS- CoV-2) infection.

10. The use of any one of claims 7-9 in combination with at least one additional agent for the treatment of SARS-CoV-2 infection or the treatment or prevention of a disease or disorder associated with SARS-CoV-2 infection.

11. The use of any one of claims 7-10, wherein the immunogenic composition is formulated for administration by at least one of electroporation and injection.

12. The use of claim 11, wherein the immunogenic composition is formulated for parenteral administration followed by electroporation.

13. The use of any one of claims 7 to 12, wherein the immunogenic composition is formulated at an initial dose of 0.5 mg to 2.0 mg of the vector, optionally at a dose of 0.5 mg, 1.0 mg, or 2.0 mg of the vector.

14. The immunogenic composition of any one of claims 3-6 for use in therapy.

15. The vector of claim 2 for use as a medicament.

16. Use of the vector of claim 2 in the preparation of a medicament for treating or protecting against infection with Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2).