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# Supplementary appendix

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

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## Appendices

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## Clinical Trial Protocol

# Protocol Title: Phase 1 Dose Escalation Study to Evaluate the Safety and Biologically Effective Dose of COH04S1, a Synthetic MVA-based SARS-CoV-2 Vaccine, Administered as One or Two Injections to Healthy Adult Volunteers

| | |
|---|---|
| **Version Date:** | 03/29/2021 |
| **Protocol Version:** | 07 |
| **City of Hope #:** | 20447 |
| **Agents:** | **Synthetic MVA-Based SARS-CoV-2 Vaccine** |
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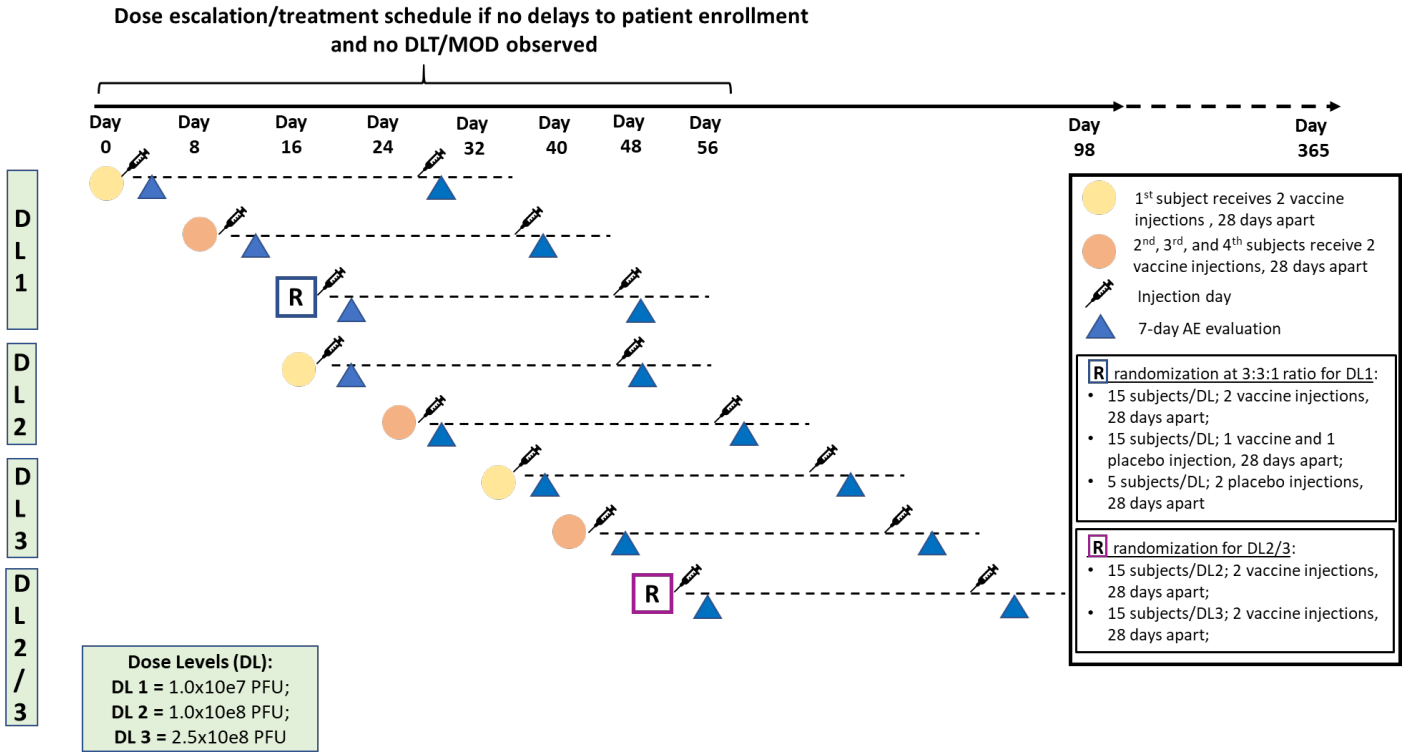
## STUDY SCHEMA




11.0. It is expected that enrollment during the randomization will be in 3:3:1 ratio for DL1. However, the PI may choose not to expand a DL or not enroll in the VP cohort, based on emerging immune response data from the vaccine (e.g., ≤1:20 neutralizing antibody titer at day 28 observed after only one injection on that DL). Because commercial vaccines may become available to the participants during this study (either through emergency use authorization or full approval), participants will be informed at the day 56 visit whether they have received vaccine or placebo. For DL1, after Day 56 unblinding, participants who have received VP will be offered a second dose of vaccine at that dose level, and participants who received PP will be offered the choice of an EUA vaccine or to be randomized to DL2 or DL3.

## PROTOCOL SYNOPSIS

**Protocol Title**

**Phase 1 Dose Escalation Study to Evaluate the Safety and Biologically Effective Dose of COH04S1, a Synthetic MVA-based SARS-CoV-2 Vaccine, Administered as One or Two Injections to Healthy Adult Volunteers**

**Study Detail**

| | |
|---|---|
| **Population/Indication(s):** | Participant must be older than 18 and younger than 55 years of age |
| **Phase:** | Phase 1 |
| **Sample Size:** | **Expected**: 81. Open-label safety study of approximately 4-6 subjects per dose level, plus 35 subjects in randomization portion of DL1 and 30 subjects randomized to DL2 vs DL3, 15 per dose level). **Maximum accrual**: 85 (81+4 potential replacement patients). |
| **Estimated Accrual Duration:** | 5 months |
| **Estimated Study Duration** | 17 months |
| **Participant Duration:** | ~13 – 15 months |
| **Participating Sites:** | City of Hope Duarte Campus and Upland |
| **Study Agents:** | Synthetic MVA-Based SARS-CoV-2 Vaccine: COH04S1 |
| **Sponsor:** | City of Hope |
| **Industry Partner:** | N/A |

**Rationale for this Study**

Background:

A novel coronavirus jumped from animal species to humans (zoonosis) in December 2019 in the Hubei province of China. The rapidly spreading virus, named SARS-CoV-2 after the samples were sequenced by Chinese investigators, was shown to be 96.2% identical to a bat coronavirus. Despite extended quarantine of individuals in China, the cases continued to mount with accompanying hospitalizations, need for ventilators and death in some cases [1]. The virus continued to spread to other regions of the world, including the United States (US) because of the interconnectedness of modern society. The spread is similar to that of the so-called “Spanish flu” in 1918. The penetrance of the virus worldwide suggested that therapeutics, while important, would never be as effective as prevention in stemming the outbreak, and two starkly different options for containment arose.

One option is that “herd immunity” will eventually lessen the impact of a new pathogen, but the unfortunate consequence of that strategy would be the 1% death rate estimated worldwide from COVID-19; in fact, the mortality rate reached ~10% in areas such as Northern Italy, Spain and France. In the US alone, ~3.3 million people would die as we establish herd immunity by natural means, even if done slowly to avoid overwhelming the medical system. The other option is a vaccine such as COH04S1, which will provide protective immunity to the recipient and with the hope for long-lived immunity, eliminating the need for repeated annual vaccination campaigns.

Rationale:

The properties of the Modified Vaccinia Ankara (MVA) are suitable for providing lifelong immunity as is the case for vaccination against smallpox infection and other infectious diseases [2, 3]. Despite significant experience using MVA with the prior SARS and MERS outbreaks, retreat of the infection into the background prevented launching of efficacy trials [4, 5]. Consequently, we do not know whether MVA will be protective against this pathogen nor for what period. Nonetheless, at City of Hope (COH) we demonstrated tolerability, marked and durable immunogenicity in healthy adults, and protective efficacy in stem cell transplant recipients of a recombinant MVA expressing three CMV antigens [6, 7]. The MVA vaccine platform has also been used to create a p53MVA vaccine at COH, which has been tested in clinical trials related to triple negative breast cancer, advanced solid tumors and late stage ovarian cancers [8-10]. Thus, we have currently developed COH04S1, an MVA-based vaccine that expresses the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins for evaluation in a first-in-humans trial.

**Objectives**

**Primary Objective:**

The primary objective is to evaluate the safety and tolerability of the COH04S1 vaccine in healthy volunteers at three different dose levels (DL): 1.0x10e7 PFU/dose, 1.0x10e8 PFU/dose, and 2.5x10e8 PFU/dose given by intramuscular (IM) injection in the upper arm.

**Secondary Objectives:**

Secondary objectives include the longitudinal evaluation of: SARS-CoV-2 S- and N-specific humoral immunity(IgG, IgM, and IgA in serum and saliva), with focus on quality and properties of antibodies elicited as a result of the vaccination; evaluation of SARS-CoV-2 S and N-specific Th1 vs Th2 polarization, T-cell levels and function; activated/cycling and memory phenotype markers, durability of immune responses, and maintenance of immunity that can be associated with protection over the study period. Additionally, we will explore the role of two injections (prime on Day 0 and boost on Day 28) versus one injection (prime), and enroll a placebo group in dose level 1 to help validate that the immune changes were not related to unexpected changes in the environment (e.g. circulating coronaviruses).

**Exploratory Objective:**

Surveillance for incidental COVID-19 infection after vaccination. This will include reporting on the severity of outcome to address concerns related to the potential for vaccine-induced disease enhancement. In addition, if infections are observed, correlative comparisons to uninfected cases will be conducted. A placebo group in DL1 will be enrolled to help provide information on a contemporary group of subjects for DL1.

**Study Design**

We propose to evaluate the safety/immunogenicity of the COH04S1 vaccine in adult healthy volunteers. These subjects will be screened based on eligibility criteria targeting adults with no significant illnesses, and subjects eligible for evaluation will show no sign of prior or current SARS-CoV-2 infection, as assessed by documented COVID-19 history and PCR diagnostic test.

We will evaluate the safety of the COH04S1 vaccine in research subjects treated at one of the 3 DLs: 1.0x10e7 PFU/dose, 1.0x10e8 PFU/dose, and 2.5x10e8 PFU/dose. DLs were chosen based on experiences with other MVA-based vaccines [11-13]. Following an initial open-label safety assessment (e.g. sentinel subjects) on each dose level, we initially planned to randomize subjects to three groups (VV; two IM vaccine injections in the upper non-dominant arm, 28 days apart, VP; one IM vaccine injection followed be a placebo injection, 28 days apart, or PP; two placebo injections, 28 days apart). Per version 7, this was changed for DL2 and DL3 (where randomization was modified to be between DL2 VV and DL3 VV). Any adverse event (AE any grade) will be evaluated from first vaccination to 7 days after the second injection (expected to be day 35) as shown in the Study Calendar. Long-term assessment on evaluations will continue through 365-days post-vaccination (first injection).

Each subject in the open-label safety evaluation is expected to receive 2 injections at the assigned DL on days 0 (prime) and 28 (boost; 2$^{nd}$ administration requires absence of DLT or MOD) and will be followed for 365 days post initial injection. DLT in a given subject is defined as any grade 3 or higher toxicity possibly, probably or definitely attributable to the research treatment, with the exception of expected local injection site AEs such as redness, pain, and swelling, and any fever, chills, malaise, headache, and flu-like symptoms such as myalgia and arthralgia of grade 3 that resolve to grade 1 or less in <7 days. A moderate toxicity (MOD) is a grade 2 possibly, probably or definitely attributable to the research treatment AE that persists for 7 days or more, or any grade 3 treatment related AE that was excluded from the DLT definition as noted above. Toxicity will be graded according to standard Division of Microbiology and Infectious Disease (DMID) adult toxicity tables. To be evaluable for dose escalation decisions, a subject must receive at least one vaccine injection. Dose escalation is primarily based on observations of MOD during the 7-day period after the initial injection, with observations of MOD or DLT later or after the second injection used as specified in the choice of Phase 2 dose decision discussion. All subjects in a cohort who do not experience a DLT or MOD must have received at least 1 injection and be followed for at least 7 days after the first injection or will be replaced during the open-label safety assessment. All subjects receiving any amount of vaccine (or placebo) will be followed for AEs and accounted for in the final data summary. Any DLT during the safety evaluation will qualify as a MOD event, but due to the increased severity, any DLT observed at any time during the study will also temporarily suspend all vaccine administrations at all dose levels pending review and approval of resumption of treatment by the PI, external DMC, IRB and, if necessary, in consultation with the FDA. Thus, dose escalation and accrual will depend on toxicity observed considering MOD, while DLTs will hold accrual. The design follows the Phase 1 queue (IQ) 3+3 design [14] adapted a) to decisions based on MOD (instead of DLT), and b) to require the first subject treated on each DL to be observed for at least 7 days before accruing further subjects. These rules stay within the risk constraints of a classic 3+3 design with a minimum of 1-week assessment time and adapted to lower the risk (moving from DLT to MOD) due to this being a study done in healthy subjects. In this design, 0/3 (or 0/4 assuming 3 are accrued immediately after the first subject on a dose level) with MOD would permit dose escalation, and

1/6 also permits dose escalation. Once a dose has passed the safety rules (represented by escalation or MTD determination), up to three cohorts of additional subjects will be enrolled at that dose level in a double-blind randomized expansion: 15 subjects at that dose level with prime and boost (VV), 15 subjects to receive a single injection (prime) cohort with placebo for boost (VP), and 5 subjects to receive two placebo injections (PP). Per version 7 of the protocol, the prime-only cohorts for dose levels 2 and 3 were removed. Thus, cohort at DL2 and DL3 will have 15 subjects receiving VV for both DL2 and DL3 (subjects will be randomized). Accrual to these cohorts will be randomized by a permuted block design (see statistical section), although expansion cohorts can be closed if accumulating data suggests insufficient immunological activity. The **Study Schema** (page 3) represents the expected subject flow, assuming no delays in accruing healthy subjects to open slots and no DLT/MOD toxicity.

**Evaluation Criteria and Endpoints**

**Primary endpoint:** The primary endpoint in this study is safety, which will be evaluated based on the DMID criteria (more details are in the Statistics section below).

**Secondary endpoints:**

1. Humoral immunity: SARS-CoV-2-specfic IgA, IgG, and IgM measured in serum and saliva by ELISA.
2. Neutralizing antibodies: measure and isolate the generation of neutralizing antibodies in participants, and test whether they prevent infection of a susceptible cell line with a pseudo-type of SARS-CoV-2 Wuhan isolate.
3. Th1 vs Th2 polarization: evaluation of SARS-CoV-2-S and -N specific IFN-gamma (Th1) and IL-4 (Th2) cytokine levels following stimulation with overlapping peptide libraries specific for SARS-CoV-2 S and N by ELISPOT
4. Evolution of activated/cycling and memory phenotype markers on the surface of antigen specific T cells: elicited as a result of the COH04S1 vaccination.

As an **exploratory endpoint**, this study will record any incidental COVID-19 infection occurring during the study follow-up period and will compare the biological correlatives of infected subjects with those uninfected. We will also report on the severity of outcome to address concerns related to the potential for vaccine-induced disease enhancement. Additionally, in a subgroup of volunteers we will evaluate in depth Th1 vs Th2 polarization by cytofluorimetry (FACS) using a panel of multiple Th1- (IFN-gamma, TNF-alpha, IL-2) and Th2- (IL-4, IL-6, IL-13) cytokines. Finally, we will measure neutralizing antibodies preventing infection of susceptible cells by relevant novel variants of concern (VOC) that may originate during the trial (e.g. UK variant, South African variant, Brazilian variant).

**Statistical Considerations**

**Primary Objective:**

The primary objective of this study is to evaluate the safety and tolerability of the COH04S1 vaccine in healthy volunteers at 3 different DL: 1.0x10e7 PFU/dose, 1.0x10e8 PFU/dose, and 2.5x10e8 PFU/dose, given by IM injection in the upper arm.

To explore these 3 doses safely, we will use the IQ 3+3 design [14] as noted above. These rules stay within the risk constraints of a classic 3+3 design (where 0/3 (or 0/4) with MOD permit dose escalation, and 1/6 also permits dose escalation), but reduce study duration by approximately 20% under a variety of scenarios, where accrual is staggered or subjects who are non-compliant are replaced. We have modified these risk-based rules with the additional rule that the first subject on each DL must be observed for 7 days after injection before any additional subjects can be accrued. In addition, once a dose has been cleared per these rules and escalation is permitted, additional subjects will be enrolled in a randomized expanded cohort on that DL. This will provide for more safety data to accumulate at that dose, and allow a comparison of single vs two injections, along with a placebo group (for DL1 only, per version 7 of protocol), for comparisons of adverse events and secondary objectives (15 expansion subjects assigned to VV, 15 to VP, and 5 to PP as noted above). Open-label slots will have priority over expansion slots. Per version 7, for DL2 and DL3 expansion slots, 30 subjects will be randomized between DL2 and DL3 (all receiving VV, prime plus boost). During the expansion cohorts, accrual may pause to be consistent with the safety constraints associated with the IQ 3+3. If multiple doses are in the randomization expansion portion simultaneously, the lower dose will enroll first. See detailed rules in "VaccineDecisionGrid.xlsx" at https://oneq.netlify.app/). During the expansion cohorts, if at any time ≥33% of subjects experience a MOD at any time in VV or VP on a dose level, that dose will hold accrual pending review by the DMC. If any DLT is observed (at any time), the study will hold accrual pending review by the DMC.

**SAMPLE SIZE RATIONALE (SAFETY):**

There is extensive experience with clinical delivery of MVA vaccines in which only mild reactogenicity has been observed [6-10, 15, 16]. The dose escalation is primarily designed to protect subjects against potential immunological reactions due to vaccine components, while allowing timely completion of the study. There is an open-label safety study of 4 subjects (maximum 8) per DL, plus a maximum of 35 subjects per DL in randomization portion. For placebo (PP), the total number of subjects was initially planned for 5 per dose level and 15 across all DLs. However, due to the recent wide availability of Emergency Use Authorization vaccines in California (all residents are eligible in April, 2021), the placebo was considered an unethical withholding/delay of available vaccines and was discontinued per version 7 of the protocol with a contemporary placebo comparison available for DL1 only (5 subjects). Initially 15 subjects were anticipated to be treated at each DL for single vaccine injection (VP) and the two vaccine injection cohort (VV) during the randomization portion. For safety evaluation, this will result in 19-23 subjects at any DL for two injections (VV). Per the amendment version 7, this remains unchanged. As a result, any AE with an incidence of 15% would be very likely to appear in at least one of the 19 subjects (>95%). For DL1, based on the first injection only (combining both single and double injections for the first 28 days), there would be 34-38 subjects on DL1, where any AE with an incidence of 9% would very likely to appear in at least one of the 34 subjects (>95% chance). As immunological data on DL1 and on sentinels on DL2 demonstrates a clear benefit for the boost without tolerability issues, for DL2 and DL3 21 subjects will be treated with VV (on each dose level, 15 during the expanded randomization portion), providing more than >96% probability of observing any AE with an incidence of 15%. Therefore, the trial will provide an adequate basis for judging the initial safety of the vaccine for future use in research subjects who are at risk for infection by COVID-19, while providing for an opportunity to evaluate immune response. Doses that are unacceptable due to toxicity will not be expanded. Other reasons (lack of immune response) may also close a cohort early, at the discretion of the PI, and similarly the PI can close a single injection cohort (VP) on a DL. As part of the safety assessment, we will evaluate the outcome of our immune correlate panel, including the potential of SARS-CoV-2-S and -N specific Th1 to Th2 polarization and any incidental infection of vaccinated subjects. The placebo group is not intended to test the hypothesis of no toxicity above the placebo, but does provide information on a contemporary group of subjects for DL1 from the same pool for a more thorough discussion of adverse events above normal variation. Data will be summarized both pooling the open-label and randomized portion, and with data restricted to the randomized expansion cohorts when comparing the adverse event profile of (VV), (VP) and (PP) groups in DL1, and for comparing DL2 to DL3 (VV) subjects (there are no (VP) or (PP) patients for DL2 or DL3.

**SAMPLE SIZE RATIONALE (HUMORAL IMMUNE RESPONSE):**

The primary immunogenicity outcome will be serum IgG against SARS-CoV-2. Enrollment requires a negative history for SARS-CoV-2 infection and a negative nasopharyngeal wash RT-PCR within 48 hours of vaccination. The determination of positivity by either test is based on the standards of the laboratory assay independent of this study. A “positive” IgG (immunogenicity) response, specific to any evaluation time, will be defined as a 4-fold raise from the baseline value (i.e. value prior to the first vaccination) during the 56-day period post-vaccination. Subjects with a positive immunogenicity result for IgG specific for SARS-CoV-2 S or N protein at any time after the first injection will be considered a success (with the exception of subjects who are diagnosed with SARS-CoV-2 prior to a “positive” immunogenicity result), and we will also evaluate the persistence of the positive IgG at 365 days. With 19-23 subjects at a DL on the two-vaccination plan (VV), the percent of success can be estimated with a standard error of 11%. While not initially randomized across dose levels (but randomized between DL2 and DL3), we will compare success rate of (V1,V1), (V2,V2) and (V3,V3), the planned two-injection cohorts from each of the expansion cohorts. If each of the three DLs accrue 19 subjects to (VV), and the success rate differs by 20% (e.g. 70% success for best dose, vs 50% success for two inferior DL), the probability of one inferior dose outperforming the superior dose is approximately 13%. If the success rate differs by 30% (e.g. 80% vs. 50% vs 50%) the probability of selection of the inferior dose by chance is <3%. With 15 subjects per cohort, the chance of selecting an inferior dose when it differs by 20% is <16%, and the chance of selecting an inferior dose when it differs by 30% is approximately 4%. For DL2 and DL3, when the expansion subjects are randomized across dose levels, if the success rate differs by 20%, there is less than a 10% chance of selecting the inferior dose with 15 subjects per dose level.

Comparison of immunogenicity within a dose of the single injection (VP) with the double injection (VV) and placebo (PP), is an exploratory endpoint as we consider IgG titers, persistence, adverse events and convenience. However, for the placebo comparison within a DL1, we will also compare the 5 placebo subjects to the 15 subject (VV) group on DL1, where we have 82% power to detect a statistically significant difference in the immune reaction success rate of 82% (VV) to 20% (PP) with a type I error (1-sided) of 10% (Exact test). If that test passes, comparison to the (VP) will be conducted with higher power(98% for the same effect size and type I error). We will not adjust for multiple comparisons. We note that the single injection recommended dose may exceed the recommended dose for the two-injection cohort and that

selection of dose and single vs double injection will depend on tolerability, compliance, and immunogenicity. The placebo group is primarily used to validate that the immune changes were not related to unexpected changes in the environment (e.g. circulating coronaviruses, subclinical exposure to SARS-CoV-2) on DL1.

Comparison across dose levels will include open-label safety subjects and will also include a comparison of the randomized 15 vs 15 subjects to DL2 and DL3.

**COMMUNITY ACQUIRED INFECTION**

Subjects will be followed for 365 days to document the incidence and severity of COVID-19 acquired infections. This is an exploratory endpoint as is the report on the severity of outcome to address concerns related to the potential for vaccine-induced disease enhancement. The placebo group may help provide related information on acquired COVID-19 infections on a contemporary group of subjects from the same population, although this will be notably underpowered based on the current infection rate. In addition, because commercial vaccines may become available to the participants during this study (either through emergency use authorization or full approval), participants will be informed on the day 56 visit whether they have received vaccine or placebo. As a result, early antibody responses and safety comparisons will focus on day 56 or before to avoid biases involved with the unblinding. For subjects on VP at DL1, subjects will be offered a second injection on DL1 of COH04S1 or can pursue an emergency use authorized vaccine. For PP subjects on DL1, on day 56 participants will be offered COH04S1 by random assignment to DL2 or DL3 VV groups or they can pursue an EUA vaccine. All subjects will continue on the trial for long-term follow-up, and retrospective analysis will take into consideration those who received the EUA vaccine.

**Abbreviated Eligibility Criteria**

Adult healthy volunteers will be screened based on eligibility criteria **targeting adults with no significant illnesses,** and eligible subjects will show no history of prior and no concurrent SARS-CoV-2 infection, as assessed by nasal wash followed by RT-PCR tests, and do not need to be naïve to smallpox vaccine, as CMV Triplex MVA studies have shown no difference in safety and immune recognition panels in subjects born before 1973 (during the compulsory smallpox campaign) or after [6].

Main Inclusion Criteria:

- Participant must be between 18 and <55 years of age at the time of screening;
- Absent history of SARS-CoV-2 infection and SARS-CoV-2 PCR test negative or pending at the time of vaccine injections
- Hepatitis B virus (HBV) surface antigen negative and hepatitis C virus (HCV) seronegative; HIV-1 seronegative

Main Exclusion Criteria:

- Any previous condition, or one that becomes known during the screening period, that would suggest that the individual could be immunologically impaired, or for which this study would pose a danger to him/herself or about which the P.I., in evaluating the subject for eligibility, determines that this exclusion is appropriate.
- No active infection for which the subject is receiving treatment;
- Any previous condition, or one that becomes known during the screening period, which would suggest that the technicians and health professionals involved in the study would be exposed to specific infectious risk;
- Any prior MVA vaccine, or treatment with whole or subunit SARS-CoV-2 or poxvirus vaccine in the last 12 months;
- Subjects who have had a live vaccine ≤30 days prior to administration of study vaccine or subjects who are ≤2 weeks within administration of inactivated vaccines (e.g. influenza vaccine).
- History of AE with a prior smallpox vaccination.
- Subjects at increased risk of exposure to SARS-CoV-2, such as patient-facing health care workers and emergency responders are excluded.

**Investigational Product Dosage and Administration**

The COH04S1 vaccine is a synthetic attenuated modified vaccinia Ankara (MVA) vector that expresses the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins. The COH cGMP laboratory will be responsible for conducting basic safety tests, while the release testing done at manufacture will be carried out by BioReliance CRO.

Volunteers will receive two* IM injections of 1.0 mL max. volume in the upper non-dominant arm over a 28-day period (**Table 1**). Subjects will be enrolled, treated and followed-up for a period of 365 days.

**Table 1: COH04S1 dose escalation schema**

| Dose schedules | Injection 1 (day 0) | Injection 2 (day 28))* |
|---|---|---|
| 1 (starting schedule) | $1x10^7$ PFU/dose (IM) | $1x10^7$ PFU/dose (IM) |
| 2 | $1x10^8$ PFU/dose (IM) | $1x10^8$ PFU/dose (IM) |
| 3 | $2.5x10^8$ PFU/dose (IM) | $2.5x10^8$ PFU/dose (IM) |

*All subjects in the open-label initial safety assessment will receive active vaccine. During the randomized portion of DL1, there will be subjects receiving the two vaccine injections (as shown in Table 1), or one vaccine injection followed by one placebo injection 28 days later, or two placebo injections 28 days apart. At the end of DL1 accrual, which will occur after the initial safety lead-in for DL2 and DL3, subjects will be randomized to DL2 or DL3 and will received two vaccine injections 28 days apart.

The COH Pharmacy will know the randomization status of participants and be able to conduct emergency unblinding of a subject if needed, while the clinical study team and participants will remain blinded to the randomization status to two vaccine and placebo injections.

**Clinical Observations and Tests to be Performed**

Study calendar for baseline, intervention and follow up procedures, SARS-CoV-2 screening, AE assessments, and blood and saliva collections are shown in Study Calendar.

At baseline, upon signing the informed consent, healthy volunteers will have a physical exam, evaluations of medical history and demographics, HIV, HCV, active HBV tests, pregnancy, lab and metabolic blood panels, and a baseline ECG with cardiac troponin test. To be evaluable, active infection with SARS-CoV-2 will be ruled out within 48 hours prior to each vaccine/placebo injection, but pending results are adequate for initial injection. Baseline SARS-CoV-2 (serological) tests will be conducted, but results are not required for eligibility. Saliva and/or blood (8-40 mL) for research laboratory testing will be collected at 10 of the 20 study visits, including baseline. Research subjects will receive the injections IM on days 0 and 28 and will be evaluated for AEs at 1-7 and 14 days after each injection, as well as Days 56, 90, 120, 180, 270, and 365. Finally, subjects will be monitored for SARS-CoV-2 infection serological (IgG) tests on Days 27, 56, 90, 120, 180, 270, and 365.MVA vector persistence will be evaluated on Days -2/-1, 42, 90, 180 and 365.

Laboratory testing: We will assess humoral immunity (IgA, IgG, and IgM) in serum and saliva by ELISA. Statistical power is based on positive serum IgG specific for the SARS-CoV-2 S protein, after the second vaccination. The neutralizing capability of the antibodies to prevent infection of a susceptible cell line will be evaluated using a pseudo-type of the SARS-CoV-2 virus carrying the original Wuhan Spike sequence. To evaluate the Th1 vs Th2 polarization of immune responses, which has been observed in convalescing COVID-19 cases [17], we will perform a SARS-CoV-2-specific ELISPOT to measure IFN-gamma and IL-4 cytokine levels, by using overlapping peptide libraries specific for SARS-CoV-2. Additionally, we will evaluate functional activated/cycling and memory phenotype marker evolution on the surface of antigen specific T cells elicited as a result of the vaccination.

Exploratory studies: All subjects with intercurrent infections will be tested for SARS-CoV-2 PCR assay, as a record any incidental COVID-19 infection during the study follow-up period, and we will compare the biological correlatives of infected subjects with those uninfected, along with recording the severity of disease to evaluate for the potential of vaccine-induced disease enhancement. Additionally, in depth analysis of Th1 and Th2 responses involving multiple cytokines will be evaluated in selected samples using intracellular cytokine staining (ICS). Finally, as new variants of concern (VOC) begin circulating in the population, we will measure in trial participants neutralizing antibodies capable of neutralizing new VOC using SARS-CoV-2 pseudoviruses carrying VOC Spike sequences.

## TABLE OF CONTENTS

## ABBREVIATIONS

| *Abbreviation* | *Meaning* |
|---|---|
| ACE2 | Angiotensin-converting enzyme 2 |
| ADE | Antibody-Dependent Enhancement of Infection |
| AE | Adverse Event |
| ALT | Alanine Transaminase |
| AP | Alkaline Phosphatase |
| ARDS | Acute respiratory distress syndrome |
| AST | Aspartate Transaminase |
| BAC | Bacterial artificial chromosome |
| BCCR | Briskin Center for Clinical Research |
| BHK-21 cells | Baby hamster kidney cell line (fibroblastic) |
| BMI | Body Mass Index |
| BUN | Blood Urea Nitrogen |
| CBC | Complete blood count |
| CBG | Center for Biomedicine and Genetics |
| CD137 | Cluster of differentiation 137, Marker for activated T cells |
| CD28 | Cluster of differentiation 28, Loss marks aging T cells |
| $CD3^+$ | Cluster of differentiation 3 positive, Marker for T cells |
| $CD4^+$ | Cluster of differentiation 4 positive, Marker for helper T cells |
| CD45RA | Cluster of differentiation 45 splice variant, Marker for TEMRA cells |
| $CD8^+$ | Marker for cytotoxic T cells |
| CEF cells | Chick embryo fibroblast cells |
| CFR | Code of Federal Regulations |
| cGMP | Current Good Manufacturing Practice |
| CMP | Comprehensive metabolic panel |
| CMV | Cytomegalovirus |
| COH | City of Hope |
| COH04S1 | COH's candidate vaccine against SARS-CoV-2 |
| COVID-19 | Coronavirus Disease 2019 |
| CR | Complete Response |
| CRO | Contract research organization |
| CRA | Clinical Research Coordinator |
| CRF | Case Report Form |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CVA | Chorioallantois Vaccinia Ankara |
| DCC | Data Coordinating Center |
| DL | Dose Level |
| DLT | Dose Limiting Toxicity |
| DMID | Division of Microbiology and Infectious Diseases |
| DMC | Data Monitoring Committee |
| ECG | Electrocardiogram |
| *E. coli* | *Escherichia. Coli* |
| ELISA | Enzyme-linked Immunosorbent Assay |
| ELISPOT | Enzyme-linked immune absorbent spot assay |
| EOT | End of Treatment |
| EVAL | Cleared first 7-day evaluation without a MOD toxicity event |
| FDA | Food and Drug Administration |
| FPV | Fowlpox Virus |
| GCP | Good Clinical Practice |
| HBV | Hepatitis B virus |

| | |
|---|---|
| HCV | Hepatitis C virus |
| HEK-293T cells | Homo sapiens embryonic kidney cells (epithelial) |
| HGB | Hemoglobin |
| HHS | Health and Human Services |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human Immunodeficiency Virus |
| IB | Investigator's Brochure |
| IDS | Investigational Drug Services |
| IFN-gamma | Interferon gamma |
| IL-2, IL-4, IL-6, IL-13 | Interleukins-2, -4, -6, -13 respectively |
| IgA, IgG, IgM | Immunoglobulin A, M, and G respectively |
| IM | Intramuscular |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| K | Potassium |
| LDH | Lactate dehydrogenase |
| MERS | Middle East respiratory syndrome |
| MVA | Modified Vaccinia Ankara |
| MOD | Moderate toxicity on DMID safety tables |
| MTD | Maximum tolerated dose |
| N | Coronavirus nucleocapsid protein |
| Na | Sodium |
| Nab | Neutralizing antibody |
| NCI | National Cancer Institute |
| NCT number | ClinicalTrials.gov identifier |
| OIDRA | Office of IND Development and Regulatory Affairs |
| PBMC | Peripheral blood mononuclear cells |
| PBS | Phosphate-buffered saline |
| PD | Progressive Disease |
| PFU | Plaque-forming Unit |
| PI | Principal Investigator |
| PMT | Protocol Management Team |
| PR | Partial Response |
| RBD | S1 receptor binding domain of Spike protein |
| S | Coronavirus spike protein |
| SAE | Serious Adverse Event |
| SARS | Severe Acute Respiratory Syndrome |
| SD | Stable Disease |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2 |
| sMVA | Synthetic MVA |
| TCM cells | Central memory T cells |
| TEM and TEMRAcells | Effector memory T cells |
| Th1, Th2 | Helper T cells, Type 1 and 2 respectively |
| THP-1 cells | Human monocyte-like cell line |
| TNF-alpha | Tumor necrosis factor alpha |
| TROVAC | Attenuated Fowlpox Virus strain FP-1 derived from the Duvette strain, plaque purified and amplified in CEF cells, designated at TROVAC and deposited to ATCC for purchase |
| ULN | Upper Limits of Normal |
| VOC | SARS-CoV-2 variant of concern |
| WB | Western blot |
| WBC | White Blood Cell |
| WHO | World Health Organization |

## 1.0 OBJECTIVES & ENDPOINTS

### 1.1 Primary Objectives

| Objectives | Endpoints/Measurements of Effect |
|---|---|
| ○ Safety and tolerability of the COH04S1 vaccine at three different dose levels (DL): 1.0x10e7 PFU/dose, 1.0x10e8 PFU/dose, and 2.5x10e8 PFU/dose | • Evaluated based on the Division of Microbiology and Infectious Diseases (DMID) criteria (APPENDIX B) |

### 1.2 Secondary Objectives

| Objectives | Endpoints/Measurements of Effect |
|---|---|
| ○ Longitudinal evaluation of: humoral immunity | • Humoral immunity: SARS-CoV-2-specfic IgA, IgG, and IgM measured in serum and saliva by ELISA during 1 year of observation. |
| ○ Quality and properties of cellular and humoral immunity elicited as a result of the vaccination | • SARS-CoV-2-specific neutralizing antibodies: measure the generation of neutralizing antibodies in participants, and test whether they prevent infection of a susceptible cell line with a pseudo-type of the SARS-CoV-2 virus.<br>• SARS-CoV-2-specific IFN-gamma, and IL-4 cytokine levels to assess via ELISPOT Th1 vs Th2 polarization using overlapping peptide libraries specific for SARS-CoV-2.<br>• Evolution of activated/cycling and memory phenotype markers on the surface of SARS-CoV-2- specific T cells elicited as a result of the COH04S1 vaccination. |
| ○ Explore the role of two injections versus one injection, and evaluate a placebo group. | • Comparison of immunogenicity and adverse events. The single injection recommended dose may exceed the recommended dose for the two-injection cohort. The placebo group is not intended to test the hypothesis of no toxicity above placebo, but instead to provide a contemporary group of subjects from the same pool for a more thorough discussion of both adverse events and immunogenicity above normal variation. |

### 1.3 Exploratory Objectives

| Objectives | Endpoints/Measurements of Effect |
|---|---|
| ○ Surveillance for incidental COVID-19 infection during follow-up (1 year) | • Record any incidental COVID-19 infection occurring during the study follow-up period and compare the SARS-CoV-2-specific immune correlates of infected subjects with those uninfected.<br>• Descriptive summary of the severity of COVID-19 and resolution to address concerns related to the potential for vaccine-induced disease enhancement<br>• Summarize the placebo group to provide initial data on acquired COVID-19 infections in the same time period and subject pool. Due to unblinding at day 56 visit and the related biases, this focuses on events up to the day 56 visit. |

| | |
|---|---|
| ○ Quality and properties of cellular and humoral immunity elicited as a result of the vaccination | • SARS-CoV-2-specific neutralizing antibodies: measure in participants the generation of neutralizing antibodies to new variants of concern (VOC) circulating in the population using a pseudo-type of SARS-CoV-2 VOC.<br>• In depth analysis of Th1 (IFN-gamma, TNF-alpha, IL-2)/Th2 (IL-4, IL-6, IL-13) cytokine expression via intracellular cytokine staining on selected samples following stimulation with overlapping peptide libraries specific for SARS-CoV-2. |

## 2.0 BACKGROUND

### 2.1 Disease Background

On February 4, 2020, the Secretary of Health and Human Services (HHS) determined that there is a public health emergency concerning the spread of a novel coronavirus. The outbreak of respiratory disease caused by this novel coronavirus, first detected in Wuhan City, Hubei Province, China, had continued to spread and had now been designated a pandemic by the World Health Organization (WHO). The virus was named "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption.

A considerable number of cases (10% or higher) of COVID-19 patients admitted to intensive care units develop an acute respiratory distress syndrome (ARDS), which is an acute inflammatory lung injury with hypoxemia and a high mortality rate [1]. In addition, there is currently no FDA-approved drug for treating COVID-19. The potential public health threat posed by COVID-19 has been high, both globally and to the United States, and the initial response to this crisis involved the development of rapid detection of cases and contacts, appropriate clinical management and infection control, and implementation of community mitigation efforts. Stay-at-home orders, intended to curtail the pandemic, have been disruptive to society and people are eager to return to normal activities despite the rising infection rate. In these circumstances, preventing the incidence of ARDS-associated pulmonary damage and mortality may be best accomplished by reducing SARS-CoV-2 infections through prophylactic vaccines. Without a vaccine, the pandemic will continue until herd immunity is established in the population, and an estimated ~3.3 million people in the United States are likely to die in the interim [3]. To hasten the end of the pandemic and protect the vulnerable, we have developed a preventative vaccine, COH04S1, against SARS-CoV-2, which we plan to test in this Phase 1 safety study.

Noteworthy, there are concerns associated with vaccination against SARS-CoV-2. One pertains to antibody-dependent enhancement of infection (ADE), which occurs when antibodies facilitate viral entry into host cells and enhance viral infection in these cells. In ADE, infection of immune cells via Fc receptors is thought to induce sustained inflammation and/or cytokine storm. It has been described in virus infections such as Dengue, Zika [18] and in SARS [19], and the cytokine storm precipitated by ADE can be reproduced using a SARS-CoV-1 pseudovirus [20]. The pathobiology of ADE was examined in SARS-CoV-1 in non-human primates and was shown to involve infected inflammatory cells such as macrophages, which results in an exacerbation of the inflammation leading to lung pathology [21]. Another concern is the report of Jaume et al. in which SARS-CoV-1 was associated with enhanced infection of B cells, despite the presence of neutralizing antibody [22]. Severe cases of COVID-19 illness have been linked to cytokine storm-like syndromes [20, 23, 24], and, in the lung, activation of innate immune cells, cascades of inflammatory activity, and tissue damage not unlike that seen in SARS-CoV-1 [19]. Thus, it is important to carefully evaluate the effect of vaccines for the potential of vaccine associated disease enhancement [25].

### 2.2 Study Agent Background

The candidate vaccine is based on a synthetic attenuated modified vaccinia Ankara (MVA) vector expressing spike (S) and nucleocapsid (N) antigens of SARS-CoV-2. We used MVA vectors because they are known for inducing humoral and cellular immune responses that provide long-term protection against a number of

infectious diseases, including smallpox and cytomegalovirus (CMV) [2, 7]. In fact, promising MVA vaccines for the related diseases SARS and MERS were in development in the last decade until their programs were put on hold before the launch of efficacy trials [26]. Although there is limited clinical evidence that MVA will be protective against SARS-CoV-2 and for how long, our team has demonstrated tolerability and protective efficacy in a recombinant multi-antigenic MVA against CMV in stem cell transplant recipients, a patient population that is especially vulnerable to infections [7]. Few adverse events (AE) of moderate or high severity have been observed in trials with adult and pediatric transplant recipients (NCT03354728, NCT03560752, and NCT04060277 studies performed at COH and NCT03383055 in Minnesota), and this demonstrates safety and tolerability of the MVA-based vaccine.

Although non-pathogenic and highly attenuated, MVA-based vaccines maintain high immunogenicity as demonstrated in various animal models and clinically in humans [2]. In the late phase of the smallpox eradication campaign, MVA was used as a priming vector for the replication competent vaccinia-based vaccine in over 120,000 individuals in Germany, and no AE were reported [2]. Since then, MVA has been used to develop a smallpox vaccine that is stored in the US Strategic National Stockpile in case of a smallpox outbreak [4].

### 2.2.1 Vaccine Design and Synthesis

We designed three unique synthetic sub-genomic sMVA fragments based on the MVA genome sequence published previously [27]. All three sMVA fragments were cloned in *Escherichia. coli* as bacterial artificial chromosome (BAC) clones. Using highly efficient BAC recombination techniques in *E. coli,* full-length SARS-CoV-2 S and N antigen sequences were inserted into commonly used MVA insertion sites located at different positions within the three sMVA fragments.

The sMVA SARS-CoV-2 virus was reconstituted with fowl pox virus (FPV) as a helper virus upon co-transfection of the DNA plasmids into BHK-21 cells, which are non-permissive for FPV (**Figure 1**) [28]. The virus was propagated on chicken embryo fibroblast (CEF) cells, which are commonly used for MVA vaccine production. The infected CEF cells were grown further and the virus media harvested, stored at -80ºC and subsequently titrated on CEF cells to grow ultrapurified virus. To simulate the process of transitioning vaccine candidates into clinical production, viruses were plaque purified and expanded.

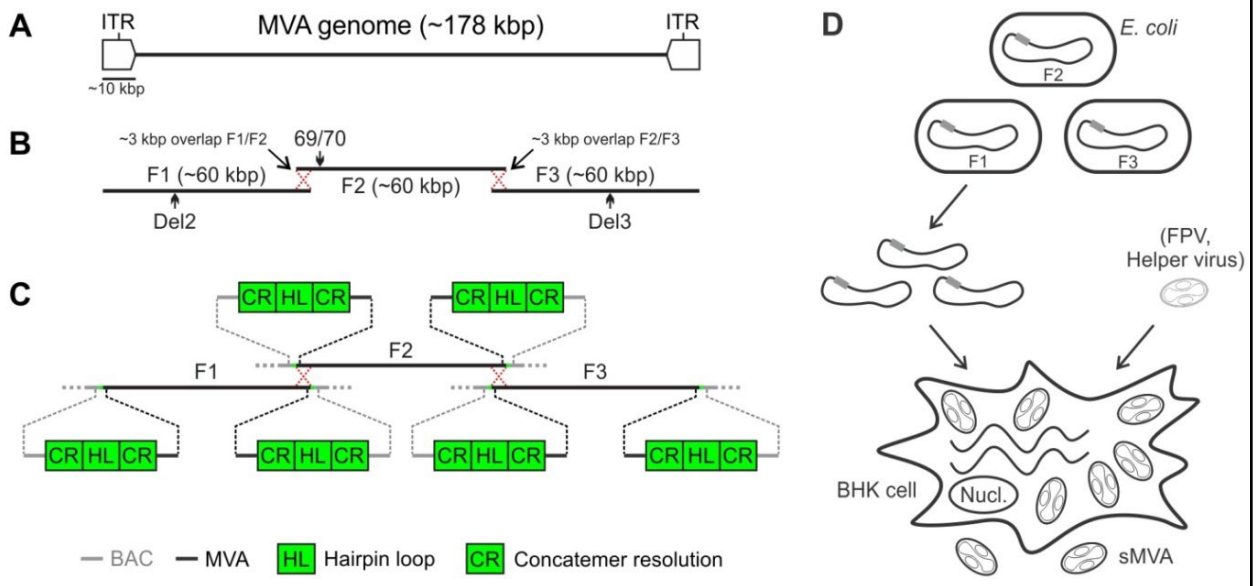



**Figure 1. sMVA construction. A)** MVA genome. The MVA genome is ~178 kbp in length and contains large ~9.6 kbp long inverted terminal repeat (ITR) sequences. **B)** sMVA fragments. Each of the three sMVA fragments (F1-F3) is ~60 kbp in length. sMVA F1 contains the left part of the MVA genome, including left ITR sequences; sMVA F2 contains the central part of the MVA genome; and sMVA F3 contains the right part of the MVA genome, including right ITR sequences. sMVA F1/F2 and F2/F3 share ~3 kbp overlapping homologous sequences for recombination (red dotted crossed lines). Indicated are the approximate genome positions of commonly used MVA insertion sites, including Del2 within sMVA F1, IGR69/70 (69/70) within sMVA F2, and Del3 within sMVA F3. **C)** Terminal CR/HL/CR sequence arrangements. Each of the three sMVA fragments contains at both ends a sequence composition comprising a duplex copy of the MVA terminal hairpin loop flanked by concatemeric resolution sequences (CR/HL/CR, green). The sMVA fragments are cloned in *E. coli* by a bacterial artificial chromosome vector (BAC, grey bars and dots). **D)** sMVA reconstitution procedure. The three sMVA fragments maintained as BACs in *E. coli* are isolated from the bacteria and co-transfected into BHK cells, which are subsequently infected with FPV as a helper virus to initiate the sMVA virus reconstitution process.

RT-PCR analysis confirmed the antigen sequences at the insertion sites. Expression of the SARS-CoV-2 S and N antigens by the constructs was confirmed by infecting BHK-21 cells and evaluating western blots (WB) with antibodies specific for the S1 and S2 domains of the S antigen, the N antigen, and anti-SARS-CoV-1 antiserum (**Figure 2**). Immunofluorescence analysis shows that the N antigen expression appears to be localized mostly in the cytoplasm and below the cell surface, and the S antigen is localized intracellularly and on the cell surface (data not shown,[29]). These observations are consistent with the expected cellular localization of the S and N antigens. No intracellular or surface SARS-CoV-2 antigen expression was observed from uninfected cells or control cells infected with the sMVA insert-free vector (data not shown, [29]). Overall, these results demonstrate that the constructs allow robust expression of both SARS-CoV-2 S and N proteins in infected cells.

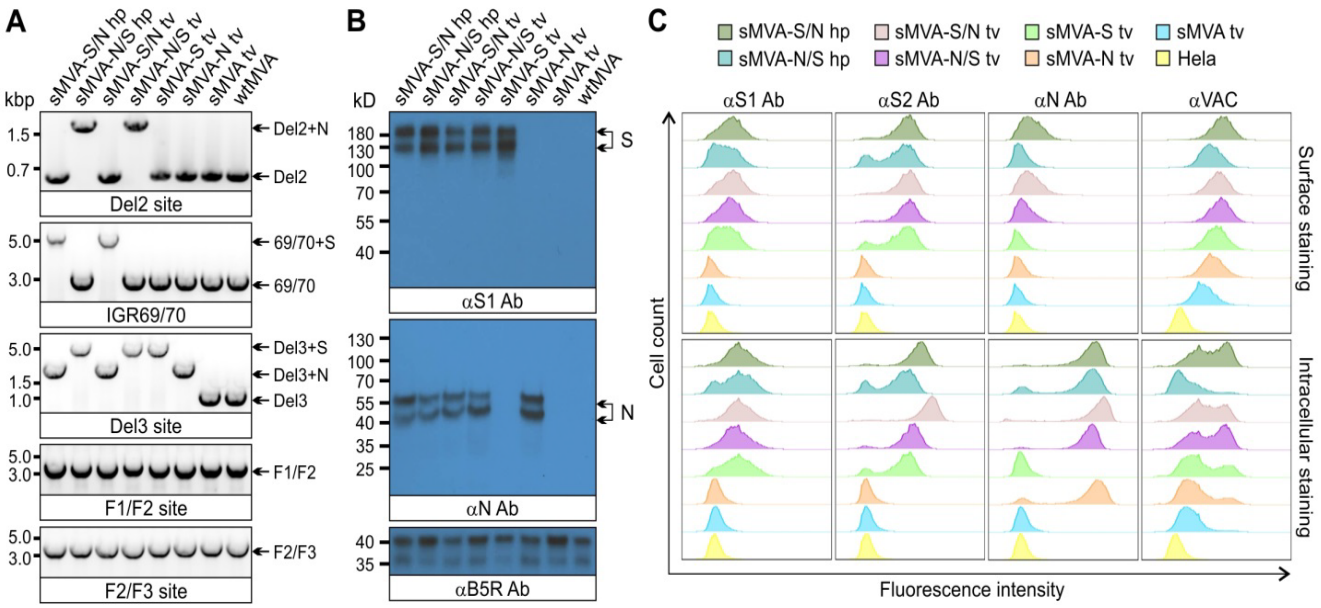



**Figure 2.** ***In vitro*** **characterization of sMVA-CoV2 vectors.** The single and double recombinant sMVA-CoV2 vectors derived with FPV HP1.441 (sMVA-S/N hp and sMVA-N/S hp) or fowlpox virus TROVAC (sMVA-S/N tv, sMVA-N/S tv, sMVA-S tv, and sMVA-N tv) were characterized by *in vitro* methods. **A)** PCR analysis. CEF infected with the vaccine vectors were evaluated by PCR to verify the S and N and antigen sequences inserted into Del2 (Del2+N), IGR69/70 (69/70+S), or Del3 (Del3+S, Del3+N. The F1/F2 and F2/F3 recombination sites were analyzed as controls. **B)** Western Blot analysis. BHK cells infected the vaccine vectors were evaluated by Western Blot using anti-S1 and N antibodies (αS1 and αN Ab) to verify the S and N antigen expression. The expression of the Vaccinia B5R protein was verified as control. The detected upper and lower molecular weight bands may represent mature and immature protein species of the S and N antigens. **C)** Flow cytometry staining. Hela cells infected with the vaccine vectors were evaluated by cell surface and intracellular flow staining using anti-S1, S2, and N antibodies (αS1, αS2, and αN Ab). Live cells (non-permeabilized) were used to evaluate cell surface antigen expression. Fixed and permeabilized cells were used to evaluate intracellular antigen expression. Anti-vaccinia virus antibody (αVAC) was used as staining control to verify MVA protein expression. Cells infected with sMVA or wtMVA or uninfected cells were used as controls for the experiments in A, B and C as indicated.

### 2.2.2 Preclinical Studies

In our preclinical studies with COH04S1, we confirmed that in mice the vaccine confers humoral and cellular immunity that protects against viral infection ( [29], and data not shown). To determine the immunogenicity of the sMVA-vectored S and N antigens, SARS-CoV-2-specific humoral and cellular immune responses were evaluated in Balb/c mice by two immunizations with COH04S1, 3 weeks apart.

#### *2.2.2.1 Humoral Immune Responses in Mice*

High-titer antigen-specific binding antibodies were detected after the first immunization, and an increase in these responses was observed after the second immunization. The vaccine induced binding antibodies against both the S and N antigens (**Figure 3**). Similar responses to those induced by the vaccine in Balb/c mice were elicited in C57BL/6 mice ( [29] and data not shown). Analysis of the IgG2a/IgG1 isotype ratio of the binding antibodies revealed $T_h1$-biased immune responses skewed toward IgG2a ([29] and data not shown) [30].

SARS-CoV-2-specific neutralizing antibody (NAb) responses, as assayed using pseudovirus, were detected after the first immunization, and these NAb titers increased after the second immunization. Similarly, NAb titers were also measured using infectious SARS-CoV-2 virus instead of pseudovirus (data not shown, [29]). These measurements were highly correlated with the pseudovirus assay ($r=0.7152$, $p<0.021$). Consequently, further

work will be exclusively conducted using safer pseudoviruses. Furthermore, the post-vaccination mouse serum did not promote infection of THP-1 monocytes (which do not express the ACE2 receptor, to which the S protein of SARS-CoV-2 binds) utilizing the Fc portion of antibodies that interact with Fc-gamma-R2 cell surface receptors on the antigen presenting and immune cells, even at low level antibody concentrations, suggesting low levels or the absence of conditions promoting antibody-associated disease enhancement (ADE) [22, 29, 31].

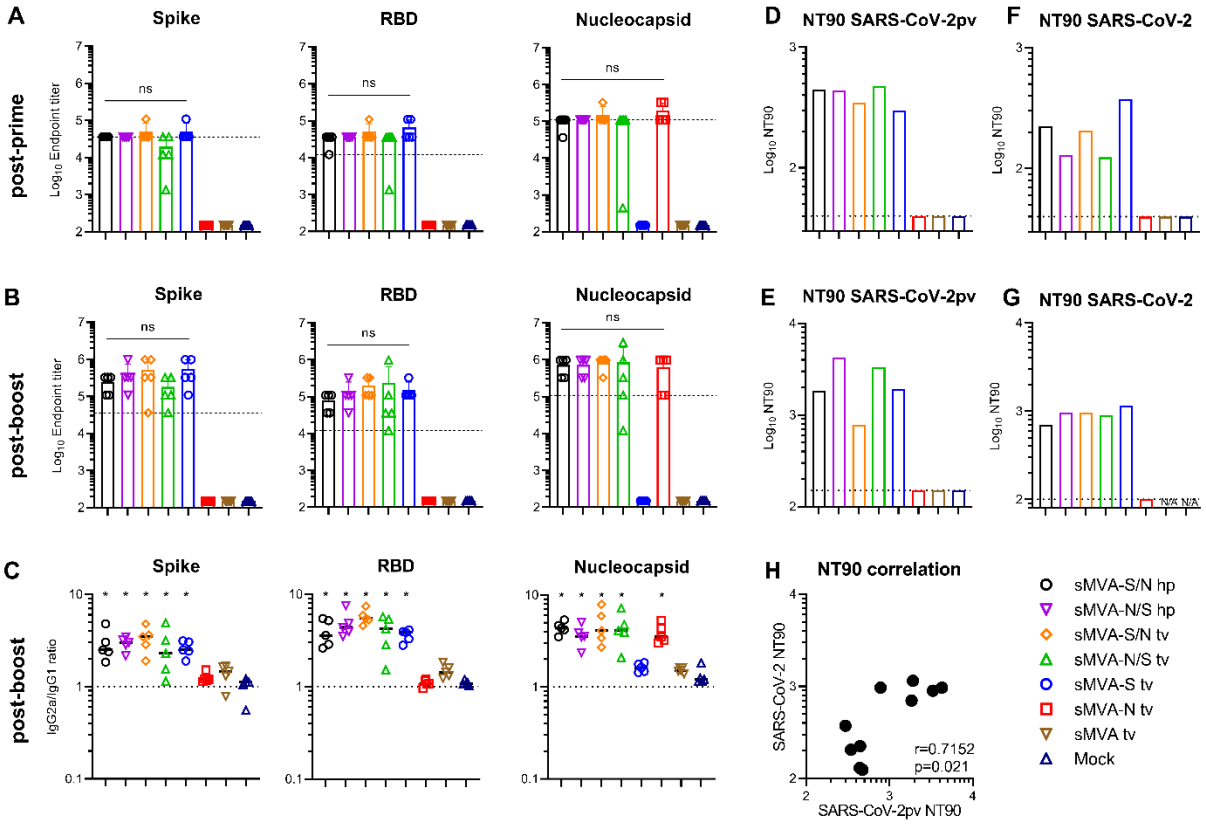



**Figure 3. Humoral immune responses stimulated by sMVA-CoV2 vectors.** Balb/c mice immunized twice in a three week interval with 5x10$^7$ PFU of the single and double recombinant sMVA-CoV2 vectors derived with FPV HP1.441 (sMVA-S/N hp and sMVA-N/S hp) or TROVAC (sMVA-S/N tv, sMVA-N/S tv, sMVA-S tv, sMVA-N tv) were evaluated for SARS-CoV-2-specific humoral immune responses **A-B)** Binding antibodies. S, RBD, and N-specific binding antibodies induced by the vaccine vectors were evaluated after the first (A) and second (B) immunization by ELISA. Dashed lines in A and B indicate median binding antibody endpoint titers measured in convalescent human sera [29]. One-way ANOVA with Tukey's multiple comparison test was used to evaluate differences between binding antibody end-point titers. **C)** IgG2a/IgG1 isotype ratio. S-, RBD-, and N-specific binding antibodies of the IgG2a and IgG1 isotype were measured after the second immunization using 1:10,000 serum dilution, and absorbance reading was used to calculate IgG2a/IgG1 antibody ratio. One-way ANOVA with Dunnett's multiple comparison test was used to compare each group mean IgG2a/IgG1 ratio to a ratio of 1 (balanced Th1/Th2 response). **D-G)** NAb responses. SARS-CoV-2-specific NAb (NT90 titer) induced by the vaccine vectors were measured after the first (D, F) and second (E, G) immunization against SARS-CoV-2 pseudovirus (pv) (D-E) or infectious SARS-CoV-2 virus (F-G) in pooled sera of immunized mice. Shown is the average NT90 measured in duplicate (D-E) or triplicate (F-G) infection. N/A=failed quality control of the samples. Dotted lines indicate lowest antibody dilution included in the analysis. **H)** SARS-CoV-2/SARS-CoV-2pv correlation analysis. Correlation analysis of NT90 measured in mouse sera after one and two immunizations using infectious SARS-CoV-2 virus and SARS-CoV-2pv. Pearson correlation coefficient (r) was calculated in H. *p<0.05. ns= not significant.

#### *2.2.2.2 Cellular Immune Responses in Mice*

Analysis of SARS-CoV-2 antigen-specific T-cells, as evaluated after the second (booster) immunization by *ex vivo* antigen stimulation, revealed S- and N-specific T-cell responses.

S-specific $CD8^+$ T-cells secreted high levels of the cytokines interferon gamma (IFN-gamma) and tumor necrosis factor alpha (TNF-alpha) with far lower levels of interleukin 4 and 10 (IL-4 and IL-10). S-specific $CD4^+$ T-cells mostly produced $T_h1$ cytokines IFN-gamma and TNF-alpha, while the production of $T_h2$ cytokines IL-4 and IL-10 was not increased following antigen stimulation. While activated N-specific $CD8^+$ T- cells were not present at significant levels, IFN-gamma and TNF-alpha-secreting $CD4^+$ T-cells were detected. The high levels of IFN-gamma and low levels of IL-4 and IL-10 indicate a $T_h1$-biased immune response for $CD8^+$ T cells (**Figure 4**).

## 2.3 **Correlative Studies**

Antiviral T and B cell-mediated adaptive immunity and memory are critical for SARS-CoV-2 viral clearance, and a vigorous virus specific T cell population is required for long term antiviral immunity [32]. In patients recovering from COVID 19, PBMC show evidence of clonal expansion, T cell activation and T cell memory formation, consistent with an effective adaptive immune response [32]. It has been reported that hospitalized patients who were recovering from COVID 19 mounted IgG and IgM responses to SARS-CoV-2-S and -N proteins, and that anti-SARS-CoV-2-S IgG may be predictive of serum neutralization capabilities in COVID 19 patients. Additionally, there was a significant correlation between the neutralizing antibody titers and the number of T cells specific for SARS-CoV-2-N, indicating that the development of neutralizing antibodies may be correlated with the activation of antiviral T cells [33]. Thus, effective clearance of the virus requires the concerted action of activated humoral and cellular adaptive responses.

Data from SARS-CoV-1 patients as well as recently infected SARS-CoV-2 patients documented relatively high levels of immune responses after infection, especially neutralizing antibody, $CD4^+$ and $CD8^+$ T cell responses to the surface S-protein that mediates entry into a wide range of host cells, through the angiotensin-converting enzyme 2 (ACE2) receptor [34], and cellular responses against the viral nucleocapsid (N) protein, that can activate antiviral B, and cytotoxic T cells [35]. To characterize the immune profile of the COH04S1 vaccine, we will use a comprehensive panel including antibody detection and neutralization protocols, evaluation of Th1 vs Th2 polarization [17], T cell activation and functional assays, established for previous immune-monitoring studies [6, 36-38] . In particular, we will assess humoral immunity (IgA, IgG, and IgM) in serum and saliva by ELISA. Statistical power will be based on positive serum IgG specific for the SARS-CoV-2 S protein, after the second vaccination. The neutralizing capability of the antibodies to prevent infection of a susceptible cell line will be evaluated using a pseudo-type of the SARS-CoV-2 virus. We will evaluate: a) antigen-specific T cell responses using overlapping peptide library specific for SARS-CoV-2; b) activated/cycling and memory phenotype marker evolution on the surface of antigen specific T cells elicited as a result of the vaccination; and c) Th1 vs Th2 polarization. The body of these correlative studies will provide longitudinal quantification and functional assessment of T and B-mediated response specific for SARS-CoV-2-S and -N antigenic proteins in the vaccinated volunteers.

Additionally, we will monitor the presence of the MVA vector, by measuring MVA DNAemia persistence in all vaccinated participants for up to one year (Day 365).

Since all subjects will undergo SARS-CoV-2-viral PCR and serological testing (see Study Calendar in Section 10.0), incidental COVID-19 infection occurring during the study follow-up period will be documented, and the SARS-CoV-2-specific immune correlatives outcomes of infected subjects will be compared with those uninfected. The severity of any incidental COVID-19 infection will be described in a detailed patient summary to consider the possibility of vaccine-induced disease enhancement.

The integrated panel of assays which will be implemented in this trial will provide comprehensive understanding of COH04S1 vaccine induced SARS-CoV-2-specific adaptive immunity.

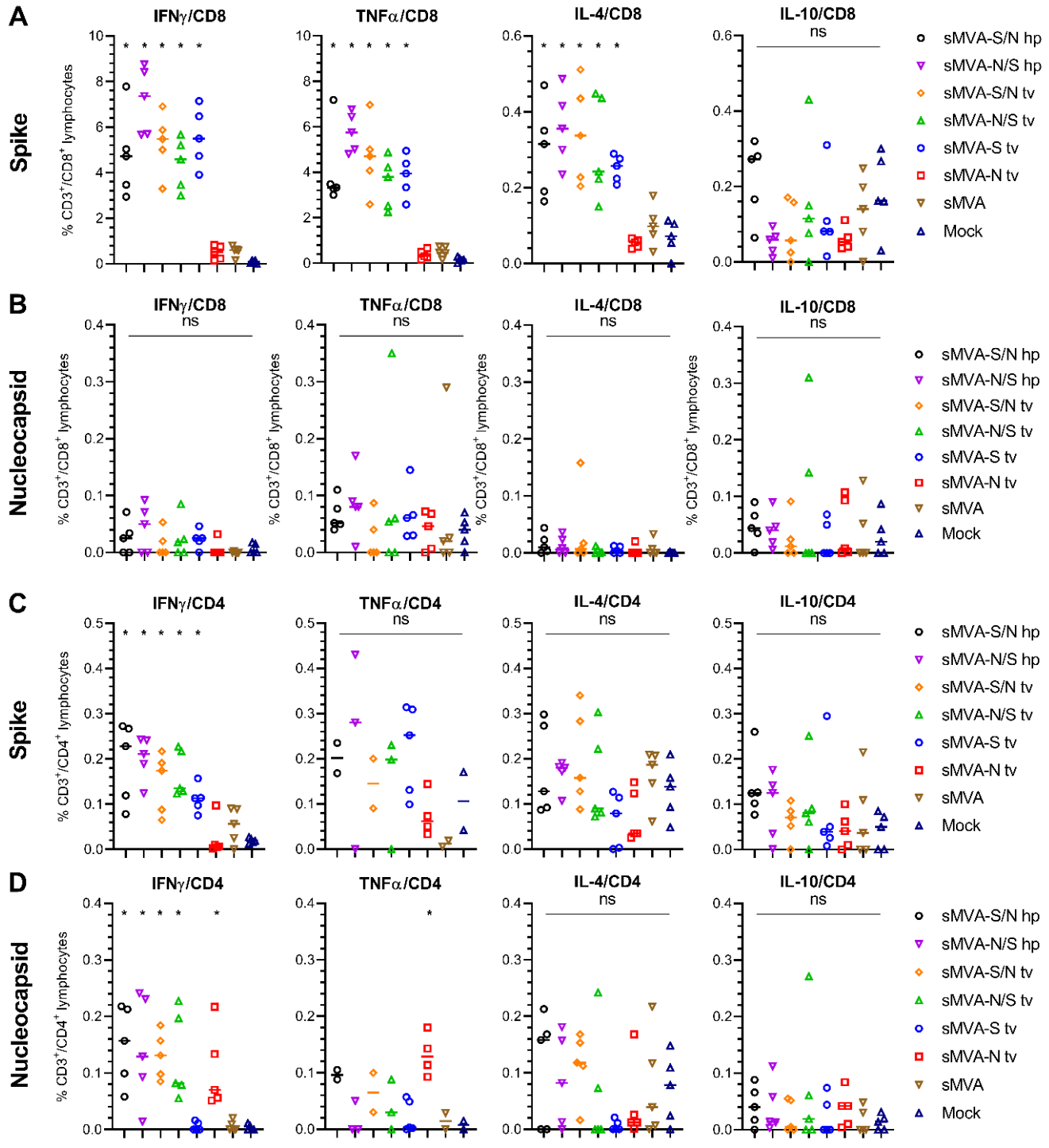



**Figure 4. Cellular immune responses stimulated by sMVA-CoV2 vectors.** Balb/c mice immunized 2 times in a 3-week interval with $5x10^7$ PFU of the single and double recombinant sMVA-CoV2 vectors derived with FPV HP1.441 (sMVA-S/N hp and sMVA-N/S hp) or TROVAC (sMVA-S/N tv, sMVA-N/S tv, sMVA-S tv, sMVA-N tv) were evaluated for SARS-CoV-2-specific cellular immune responses. Antigen-specific CD8+ and CD4+ T cell responses induced by the vaccine vectors were evaluated after the second immunization by IFN-gamma, TNF-alpha, IL-4 and IL-10 secreting flow cytometry staining following *ex vivo* antigen stimulation using SARS-CoV-2-specific S and N peptide libraries. Due to technical issues, for 1-3 animals/group % of CD4/TNFα cells stimulated with S and N libraries were not included. *indicates significance at the 0.05 level or below compared to controls.

## 2.4 Overview and Rationale of Study Design

We propose to evaluate the safety/immunogenicity of the COH04S1 vaccine in adult healthy volunteers. They will be screened based on targeting adults with no significant illnesses, and subjects eligible for evaluation will show no history of prior or current SARS-CoV-2 infection, as assessed by viral RT-PCR assay of nasopharyngeal wash.We will evaluate the safety of the COH04S1 vaccine in research subjects treated at one of the 3 dose levels (DL): 1.0x10e7 PFU/dose, 1.0x10e8 PFU/dose, and 2.5x10e8 PFU/dose. DL were chosen based on experiences with other MVA-based vaccines [11-13]. Following an initial open-label safety assessment (e.g. sentinel subjects) on each dose level, we initially planned to randomize subjects to three groups (VV: two IM vaccine injections in the upper non-dominant arm, 28 days apart, VP: one IM vaccine injection followed be a placebo injection, 28 days apart, or PP: two placebo injections, 28 days apart). Per version 7, this was changed for DL2 and DL3 (where VP was eliminated and randomization was modified to be between DL2 VV and DL3 VV). Any adverse event (AE) (any grade) will be evaluated from first vaccination to 7 days after the second injection (expected to be day 35) as per Study Calendar. Long-term assessment on evaluations will continue through 365-days post-vaccination (first injection).

Each subject in the open-label safety evaluation is expected to receive 2 injections at the assigned DL on days 0 and 28 ($2^{nd}$ administration requires absence of DLT or MOD) and will be followed for 365 days post initial injection. DLT in a given subject is defined as any grade 3 or higher toxicity possibly, probably or definitely attributable to the research treatment, with the exception of expected local injection site AEs such as redness, pain, and swelling, and any fever, chills, malaise, headache, and flu-like symptoms such as myalgia and arthralgia of ≤ grade 3 that resolve to grade 1 or less in <7 days. A moderate toxicity (MOD) is a grade 2 possibly, probably or definitely attributable to the research treatment AE that persists for 7 days or more, or any grade 3 treatment related AE that was excluded from the DLT definition as noted above. Toxicity will be graded according to standard Division of Microbiology and Infectious Disease (DMID) adult toxicity tables (APPENDIX B). To be evaluable for dose escalation decisions, a subject must receive at least one vaccine injection. Dose escalation is primarily based on observations of MOD during the 7-day after the initial injection, with observations of MOD later or after the second injection used as specified in the dose decision discussion. All subjects in a cohort who do not experience a DLT or MOD must have received at least 1 injection and be followed for at least 7 days after the first injection or will be replaced during the open-label safety assessment. All subjects receiving any amount of vaccine will be followed for AEs and accounted for in the final data summary. Any DLT during the safety evaluation will qualify as a MOD event, but due to the increased severity, any DLT observed at any time during the study will also temporarily suspend all vaccine administrations at all dose levels pending review and approval of resumption of treatment by the PI, external DMC, IRB and, if necessary, in consultation with the FDA. Thus, dose escalation and accrual will depend on toxicity observed considering MOD, while DLTs will hold accrual.

The design follows the Phase 1 queue (IQ) 3+3 design [14] adapted a) to decisions based on MOD (instead of DLT), and b) to require the first subject treated on each DL to be observed for at least 7 days before accruing further subjects. These rules stay within the risk constraints of a classic 3+3 design adapted to lower the risk (moving from DLT to MOD) due to this being a healthy-subjects study. In this design, 0/3 (or 0/4) with MOD would permit dose escalation, and 1/6 also permits dose escalation, with the additional staggered subject enrollment for the first subject on each DL for an added safety check. Once a dose has passed the safety rules (represented by escalation or MTD determination), up to three cohorts of additional subjects will be enrolled at that dose level in a double-blind randomized expansion: 15 subjects at that dose level with prime and boost (VV), 15 subjects to receive a single injection (prime) cohort with placebo for boost (VP), and 5 subjects to receive two placebo injections (PP). Per version 7 of the protocol, the VP cohorts were removed such that cohorts at per DL2 and DL3 have 15 subjects each receiving VV for both DL2 and DL3 by random assignment. Accrual to these cohorts will be randomized by a permuted block design (see statistical section), although expansion cohorts can be closed if accumulating data suggests insufficient immunological activity. The Study

Schema represents the expected subject flow, assuming no delays in accruing healthy subjects to open slots and no DLT/MOD toxicity.

## 3.0 ELIGIBILITY CRITERIA

| | |
|---|---|
| Subject MRN (COH Only) | Subject Initials (F, M, L): |
| Institution: | |

Participants must meet all of the following criteria on screening examination to be eligible to participate in the study, screening tests can only be repeated once:

### 3.1 Inclusion Criteria

*Informed Consent and Willingness to Participate*

__1. Documented informed consent of the participant.

*Age Criteria, Performance status, Language*

__2. Age: ≥ 18 years and <55 years

__3. Ability to read and understand English, Spanish, or Mandarin for consenting

*Clinical Laboratory and Organ Function Criteria (To be performed within 30 days prior to Day 0 of protocol therapy unless otherwise stated)*

| | | |
|---|---|---|
| __4. Platelets ≥ 100,000/$mm^3$ | Plts: | Date: |
| __5. WBCs 3,600-10,100/$mm^3$ | WBC: | |
| __6. Total bilirubin < 1.1 X ULN | ULN:<br>Bil: | Date: |
| __7. AST <1.5 x ULN | ULN:<br>AST: | Date: |
| __8. ALT <1.5x ULN | ULN:<br>ALT: | Date: |
| __9. AP < 1.1 x ULN | ULN:<br>AP: | Date: |
| __10. BUN < 1.25 x ULN | ULN:<br>BUN: | |
| __11. Creatinine less than or equal to the ULN | ULN:<br>Creatinine: | Date: |
| __12. Sodium 137-145 mEq/L | Na: | Date: |
| __13. Potassium 3.5-5.1 mEq/L | K: | Date: |
| __14. Carbon Dioxide 22-30 mmol/L | CO2: | Date: |
| __15. Glucose 80-128 mg/dL | Glucose: | Date: |
| __16. Albumin 3.5-5.0 g/dL | Albumin: | Date: |
| __17. HGB > 10.5 gm/dL | HGB: | Date: |
| __18. Hematocrit (Hct)<br>For females: 34.5-44.6 %<br>For males: 37.6-47.2 % | Hematocrit: | Date: |
| __19. Seronegative for HIV Ag/Ab combo, HCV*, active HBV (Surface Antigen Negative)<br>*If positive, Hepatitis C RNA quantitation must be performed. | HIV:<br>HCV:<br>HBV: | Date: |

| | | | |
|---|---|---|---|
| | | | |
| __20. History negative for COVID-19 and nasopharyngeal test results pending for SARS-CoV2 performed at COH on nasal wash samples using the Diasorin Simplexa™ test<br>*Baseline SARS-CoV2serologic test will be performed at TGen using the InBios assay; the result will not be required for eligibility. | PCR sent:<br>*Seroneg.: | | Date: |
| __21. A documented electrocardiogram (ECG) and cardiac troponin must be within normal institutional limits in the past 30 days; "normal ECG with sinus tachycardia" or "normal ECG with sinus bradycardia" is allowable based on a history of absent cardiac/exercise related symptoms as determined by the P.I. in consultation with a senior staff cardiologist. | ECG: | | Date: |
| __22. **Women of childbearing potential (WOCBP)**: negative urine or serum pregnancy test<br>If the urine pregnancy test is inconclusive a serum pregnancy test will be required | Urine: | Serum: | Date: |
| | | | |
| | | | |

*Contraception*

__23. Agreement by females **and** males of childbearing potential* to use an effective method of birth control or abstain from heterosexual activity for the course of the study through at least 6 weeks after the last dose of protocol therapy.

* Childbearing potential defined as not being surgically sterilized (men and women) or have not been free from menses for > 1 year (women only).

## 3.2 Exclusion Criteria

__1. Subjects at increased risk of exposure to SARS-CoV-2, such as patient-facing health care workers and emergency responders are excluded.

__2. Subjects who would be at higher risk for severe COVID-19 according to known risk factors are excluded e.g. type 2 diabetes, obesity (BMI >35), congestive heart failure (New York Heart Association Class ≥I, history of coronary artery disease, cardiomyopathies, sickle cell disease, smoking, chronic kidney disease, immunocompromised state from solid organ transplant, or chronic obstructive pulmonary disease.

*Prior and concomitant therapies*

__3. Subjects using investigational or licensed agents that may prevent or treat SARS-CoV-2 are excluded.

__4. Subjects are excluded, who have any history of allergic diatheses as defined by a history of asthma, anaphylaxis, or generalized urticaria, or by daily use of antihistamines, episodic (more than once in past 3 months) inhalational medications including steroidal agents, non-steroidal agents, or cromolyn sodium

__5. Any previous condition, or one that becomes known during the screening period, which would suggest that the technicians and health professionals involved in the study would be exposed to specific infectious risk;

__6. Surgery in past 6 months that required general anesthesia. Minor procedures, such as dental surgery and superficial diagnostic biopsies, are permitted;

__7. Taking daily medications for chronic or intercurrent illness. Medications excluded from this rule are: thyroid replacement, estrogen replacement, dietary vitamins and protein supplements, mild anti-depressant and anxiety medication, and any medication not known or likely to be immunosuppressive, as determined by the P.I.;

__8. Subjects who have had a live vaccine ≤30 days prior to administration of study vaccine or subjects who are ≤2 weeks within administration of inactivated vaccines (e.g. influenza vaccine). Flu shots are allowed >2 weeks before the first injection and >2 weeks post 2nd injection.

__9. Treatment with medication for high cholesterol or other lipid abnormality. Prophylactic medication is acceptable.

*Other illnesses or conditions*

__10. History of allergic reactions attributed to compounds of similar chemical or biologic composition to study agent

__11. History of adverse event with a prior smallpox vaccination

__12. Any previous condition, or one that becomes known during the screening period, that would suggest that the individual could be immunologically impaired, or for which this study would pose a danger to him/herself or about which the P.I., in evaluating the subject for eligibility, determines that this exclusion is appropriate.

__13. Subjects are excluded who have history of cancer other than basal cell skin cancer, or any condition, psychiatric or otherwise, that would preclude informed consent, consistent follow-up or compliance with any aspect of the study (e.g., untreated schizophrenia or other significant cognitive impairment, etc. as determined by the P.I.)

__14. Subjects with severe migraine headaches (more than one per month on average in the past 6 months or requiring preventive medication) are excluded but those on effective medication (less than one migraine per month) are allowed to enroll.

__15. History of heart disease, e.g. previous treated arrhythmia or myocardial infarction

__16. Horizontal positioning- induced or activities of normal living exercise-induced shortness of breath;

__17. History of stroke or claudication.

__18. Any of the following cardiac findings of ECG abnormality: 1) conduction disturbance (complete left or right bundle branch block, intraventricular conduction disturbance with QRS >120 ms, AV block of any degree, and QTc prolongation >450 msec for men and >460 msec for women; 2) repolarization (ST segment or T wave) abnormality; 3) significant atrial or ventricular arrhythmia, including frequent ectopy (e.g., 2 premature ventricular contractions in a row); and 4) evidence of past myocardial infarction.

__19. Poxvirus vaccine in the last 12 months

__20. Any MVA vaccine or poxvirus vaccine in the last 12 months;

__21. Any previous SARS-CoV-2 vaccine.

__22. History of or prior treatment for diabetes type 1 or diabetes type 2; BMI <18 or >35. BMI can be rounded to the nearest integer.

__23. Clinically significant uncontrolled illness

__24. Active infection requiring treatment

__25. Known history of immunodeficiency virus (HIV) or hepatitis B or hepatitis C infection

__26. Diagnosis which has been associated with immunodeficiency

__27. *Females only*: Pregnant or breastfeeding

__28. Men with partners of child-bearing potential and women of children-bearing potential who are not willing to use medically effective birth control methods, e.g. contraceptive pill, condom, or diaphragm, and continue this for 60 days after the second and last dose of vaccine;

__29. Subjects who are employed by or are a student at City of Hope and are in a chain of command that reports directly to persons listed on the protocol as Principal Investigator or Co-Investigators; or are relatives or partners of the investigators.

__30. Any other condition that would, in the Investigator's judgment, contraindicate the subject's participation in the clinical study due to safety concerns with clinical study procedures.

*Noncompliance*

__31. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

__32. Anyone considered to be in a vulnerable population as defined in 45 CFR §46.111 (a)(3) and 45 CFR §46, Subparts B-D

| **Eligibility Confirmed* by (Choose as applicable):** | **Print Name** | **Signature** | **Date** |
|---|---|---|---|
| ☐ Site PI | | | |
| ☐ Authorized study MD | | | |
| ☐ Study Nurse | | | |
| ☐ Study CRA/ CRC | | | |
| ☐ Other:_____ | | | |
| *Eligibility should be confirmed per institutional policies. | | | |

## 4.0 PARTICIPANT ENROLLMENT AND RANDOMIZATION

Subjects will be recruited from healthy volunteers by means of local advertisements. Subjects will be recruited from the COH campus. We have experience and have had great success recruiting participants, also healthy adult volunteers, to our previous study of the CMV-MVA vaccine [6]. Since the total number of volunteers required is a maximum of 129, it should not be difficult to complete the cohort from available persons at COH using the COH email system, newsletters, and publications for distribution of these advertisements. However, if we are unsuccessful at accruing the requisite number of volunteers by this means, then emphasis on reaching a larger pool of volunteers will be instituted through advertisements in local print media. The Principal Investigator, Co-Investigators, research staff and clinicians participating into the current study will be excluded from enrollment. All advertisements will first be submitted to the IRB for approval before using them to recruit study subjects.

### 4.1 Pre-Enrollment Informed Consent and Screening Procedures

**Informed Consent Procedure**. Candidates who respond to the advertisements will be invited to participate in a webinar conducted by the PI or designee approved by the IRB. A PowerPoint presentation will be used at this meeting that has been approved by the IRB for this purpose. The identity of the candidates involved in the webinar will be blinded from the other attendees. A Q&A session will be conducted at the conclusion of the presentation, and then those interested in continuing in the consenting process will be asked to indicate this by email (COVIDVACCINE@coh.org). Interested candidates will be send a copy of the informed consent to review and will be invited to a one-on-one video meeting with the PI or designee approved by the IRB. At that meeting, if/when there are no further questions about the trial, the candidate will have the opportunity to sign and return the consent (using DocuSign) or to indicate no further interest. When the PI or designee who participated in the consenting procedure countersigns the consent, the subject will be screened for eligibility.

Laboratory studies performed exclusively to determine eligibility will be done at a scheduled visit to the Briskin Center for Clinical Research (BCCR). Studies or procedures that are performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values and/or to determine pre-eligibility, even if the studies were done before informed consent was obtained.

The informed consent process is to be fully documented (see Section 16.4), and the prospective participant must receive a copy of the signed informed consent document. Screening procedures are listed in Section 10.0 (Study Calendar). Briefly, they include: physical exam, medical history and demographics, HIV, active HCV infection, active HBV infection, pregnancy test, a SARS-CoV-2 viral test by PCR, Chemistry/ Metabolic Panel (CMP), CBC with differential, ECG and cardiac troponin test.

### 4.2 Participant Enrollment

#### 4.2.1 COH DCC Availability and Contact Information

Eligible participants will be registered on the study centrally by the DCC at City of Hope.

DCC staff are available **between the hours of 8.00 am and 5.00 pm PST, Monday through Friday (except holidays)**.

- E-mail: DCC@coh.org

#### 4.2.2 Slot verification and reservation

A designated study team member will email the DCC and study biostatistician to verify current slot availability, and to reserve a slot for a specific prospective subject (provide DCC with subject initials), including a tentative treatment date. Slots can only be held for a limited time, at the discretion of the study PI.

The DCC should be notified of cancellations of prospective participants holding slots as soon as possible.

### 4.2.3 Registration Process

Allow up to 24 hours for the DCC to review to register a participant. The following procedure must be followed:

1. The study team should contact the DCC via email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window.
2. The study team will email a **Complete Registration Packet** to the DCC, which consists of a copy of the following documents:
    - Completed eligibility checklist (printed from Section 3.0 of the protocol) with required signature(s)
    - Signed Informed Consent
    - Signed HIPAA authorization form (if separate from informed consent)
    - Signed subject's bill of Rights (California only)
3. When all documents are received, the DCC will review and, work with the study team to resolve any missing elements. Any missing documents may delay registration. A participant failing to meet all requirements will not be registered and the study team will be immediately notified.
4. The DCC will send a Confirmation of Registration Form, including the Subject Study Number and cohort assignment to:
    - The COH Study PI and COH study team designees (including but not limited to study monitor(s) and statistician(s)).
5. Upon receipt of the Confirmation of Registration Form, COH study team will register the subject in OnCore.

### 4.2.4 Randomization

The biostatistician will be in charge of randomization and will assign participants to each dose level as described in the **Study Schema**.

The biostatistician and COH Pharmacy will know the randomization status of participants, along with the central Data Coordinating Center (DCC), while the clinical study team and participants will remain blinded to the randomization status. Once past the open-label initial safety assessment, subjects will be randomized in DL1 to two vaccine injections (prime and boost) 28 days apart, one vaccine injection (prime; followed by a placebo injection 28 days later), or control group (two placebo injections 28 days apart) and for DL2 and DL3 subjects will be randomized between these two dose levels (all subjects on DL2 or DL3 getting prime and boost 28 days apart).

### 4.2.5 Unblinding of the study

The following procedures should be followed for non-emergency unblinding:

- Because commercial vaccines may become available to the participants during this study (either through emergency use authorization or full approval), participants will be informed after or on the day 56 visit whether they have received vaccine or placebo (see section 11.3). The PI will ask the DCC or IDS Pharmacy for information to be able to notify the participant, confidentially, if they received "prime and boost vaccine" (VV) "prime vaccine only" (VP) or "placebo only" (PP),
- The respective placebo only DL1 participants who meet the public health rules will be offered an EUA vaccine at City of Hope if vaccine is available or offered the prime and boost of COH04S1 by continuing in this study with randomization to DL2/3; if choosing to take the EUA vaccine, subjects will be asked to inform the study team about this and continue follow-up. If choosing

to receive COH04S1 vaccine, they will need to again meet the original screening criteria for study eligibility.

- Participants who received the prime vaccine only at DL1 will be offered the booster injection of COH04S1 at DL1 and asked to continue in the study following the Study Calendar post injection 2. They would continue to be followed according to the visit schedule and complete the 12-month trial schedule. If they chose to receive an EUA vaccine, they will be informed that there is no information about the safety or effectiveness from vaccination with two different COVID-19 vaccines and they should continue to be followed up on this study.

#### 4.2.6 Emergency Unblinding

As authorized by Principal Investigator, the COH Pharmacy can break the blind in an emergency for an individual participant and inform the responsible party. Emergency un-blinding will occur if a subject on this study develops a life-threatening toxicity or SAE and the participant's physician feels that it is in the subject's best interest to know the randomization status of the subject.

In this very unlikely event, the PMT will determine if and how the unblinding should impact the subject's continued participation in the study or analysis of collection points post de-blinding. This plan will be provided to the COH IRB and external, independent DMC, as per COH institutional requirements. The date and reason for unblinding must be noted in the medical record and captured in the electronic Case Report Form (eCRF).

### 4.3 Screen Failures and Registered Participants Who Do Not Begin Study Treatment

Within 48 hours prior to vaccine/placebo injection, the participant will have a secondary eligibility screening to rule out active SARS-CoV-2 infection by RT-PCR assay and repeat CBC/CMP tests to rule out a > Grade 1 AE. Notify the DCC immediately if the participant secondary screen fails after registration or if the participant does not start treatment.

Issues that would cause treatment delays should be discussed with the Study Principal Investigator.

### 4.4 Dose Level Assignment

Eligible Phase 1 participants will be assigned a dose level (Section 11.1) and cohort (e.g., (V,V), (V,P), (P,P). If there are open-label slots available on a higher dose level and randomization slots available on a lower dose level, the open-label slots will be filled first. If there are two dose levels with randomization slots available, the lower dose will be filled first. Randomization slots will be supplied to central registration based on a permutated block design.

## 5.0 TREATMENT PROGRAM

### 5.1 Treatment Program Overview

The safety of the COH04S1 vaccine will be evaluated in healthy adult volunteers treated at one of the 3 DL: 1.0x10e7 PFU/dose, 1.0x10e8 PFU/dose, and 2.5x10e8 PFU/dose. DL were chosen based on experiences with other MVA-based vaccines [11-13]. Subjects will receive two IM injections in the upper arm in the outpatient setting, 28-days apart, on Days 0 and 28 of the study. Following an initial open-label safety assessment (e.g. sentinel subjects) on each dose level, we plan to randomize subjects to three groups in DL1 (VV: two IM vaccine injections in the upper non-dominant arm, 28 days apart, VP: one IM vaccine injection followed by a placebo injection, 28 days apart, or PP: two placebo injections, 28 days apart)and per amendment version 7, the VP cohorts were removed and two groups at DL2/DL3 remain (VV: two IM vaccine injections in the upper non-dominant arm, 28 days apart at DL2 or DL3). The placebo consists of a buffered solution (PBS) containing 7.5% lactose. Any adverse event (AE) of any grade will be evaluated from the first vaccination to 7 days after the

second injection (expected to be day 35) as per Study Calendar. Long-term assessment will continue through 365-days post-vaccination (first injection). Toxicity will be graded according to standard Division of Microbiology and Infectious Disease (DMID) adult toxicity tables.

## 5.2 **Treatment Plan**

The intervention consists of two intramuscular (IM) injections in the non-dominant upper arm, 28-days apart (delivered on days 0 and 28). Research subjects will have follow-up visits on Days 7, 14, 35, 42, 56, 90, 120, 180, 270 and 365. Blood (8-40 mL) and saliva will be collected for research testing at the visits indicated in the Study Calendar. Subjects enrolled on the single vaccine dose without booster injection and those receiving two placebo injections will have the same follow-up schedule as those receiving two vaccine injections. Any adverse event (AE any grade) will be evaluated from first vaccination to 7 days after the second injection (expected to be day 35); and long-term assessment on evaluations will continue through 365-days post-vaccination (first injection).
Each subject is expected to receive 2 injections at the assigned treatment/DL on days 0 and 28. The 2nd administration requires **absence of** DLT, serious adverse events that are considered to be at least possibly related to vaccination, no persistent AEs [MOD], positive pregnancy test, acute illness or new medical condition (see Study Calendar for more details). The schema below shows all participant visits and the main research procedures performed. Routine clinical procedures include:

- Physical exam at screening and Days 0, 7, 14, 28, 35, 42, 56, and 365
- CBC/differential at screening and Days -2/-1, 7, 26/27, 35, 56, 90, 120, 180 and 365; if a Grade 2 AE is reported for the CBC/diff at Days 7 and/or Day 35, the CBC/diff are repeated at Days 14 and/or 42, respectively
- Chem Metabolic Panel (CMP) at screening and Days -2/-1, 7, 26/27, 42, 56, 90, 120, 180 and 365; if a Grade 2 AE occurs at Day 7 and/or Day 35, CMP is repeated at Day 14 and/or 42
- Pregnancy testing at screening, Days -2/-1 and 26/27 (prior to injection days 0 and 28, respectively), and Day 365

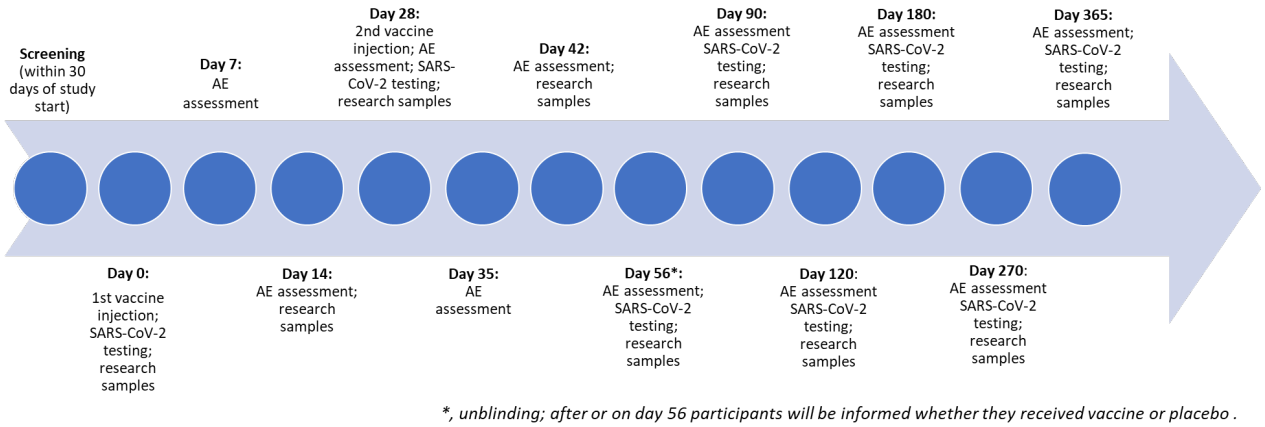



**Figure 5**. Main study visits, including the main procedures performed at each visit.

## 5.3 **Agent Administration**

Eligible healthy volunteers in the dose escalation evaluation will receive two IM injections of 1.0 mL max. volume in the upper non-dominant arm over a 28-day period or one injection (first injection on Day 0) (**Table 5.3-1**). Subjects will be enrolled, treated and followed-up for a period of 365 days.

**Table 5.3-1: COH04S1 dose escalation schema**

| Dose schedules | Injection 1 (day 0) | Injection 2 (day 28)* |
|---|---|---|
| 1 (starting schedule) | $1x10^7$ PFU/dose (IM) | $1x10^7$ PFU/dose (IM) |
| 2 | $1x10^8$ PFU/dose (IM) | $1x10^8$ PFU/dose (IM) |
| 3 | $2.5x10^8$ PFU/dose (IM) | $2.5x10^8$ PFU/dose (IM) |

* All subjects in the open-label initial safety assessment will receive vaccine. During the randomized portion of DL1, there will be subjects receiving two vaccine injections 28 days apart, or one vaccine injection and one placebo injection 28 days apart, or two placebo injections 28 days apart. At the end of DL1 accrual, which will occur after the initial safety lead-in for DL2 and DL3, subjects will be randomized to DL2 or DL3 and will received two vaccine injections 28 days apart.

The clinical study team and participants will remain blinded to the randomization status, and all participants will receive two physically identical injections.

The vaccine administration details are shown in **Table 5.3-2**.

**Table 5.3-2: Agent Administration**

| | |
|---|---|
| **Vaccine Dosage Form** | Sterile, preservative-free solution |
| **Placebo Dosage Form** | Sterile, preservative-free solution |
| **Dosing Schedule** | *Two injections administered on Days 0 and 28. |
| **Vaccine Formulation** | Sterile, preservative solution in PBS containing 7.5% lactose |
| **Placebo Formulation** | Sterile, preservative-free PBS containing 7.5% lactose |
| **Route of Administration** | Intramuscular (IM) injection |

*All subjects will receive two injections, whether enrolled in (VV), (VP) or (PP) groups (two vaccinations 28 days apart, one vaccination, one placebo 28 days apart, or two placebo injections 28 days apart).

Healthy eligible male and female volunteers during the open-label safety evaluation will receive the COH04S1 vaccine at the assigned open dose level; subjects enrolled to the randomized expanded DL1 cohort will be assigned (VV), (VP), or (PP) with 15 subjects each in VV and VP and 5 subjects in PP; subjects enrolled to the randomized expanded DL2/DL3 cohort will be assigned VV at DL2 (n=15) or VV at DL3 (n=15). The volume of injection will be ≤1.0 ml and will be given IM into the upper left arm (right arm if the subject is left-handed). The second injection will be given at 28 days (± 7 days) after the initial injection. The second injection will usually be given in the same anatomical location as the primary inoculation, but it can be given in the alternative arm if requested by the research subject. For subject convenience, if necessary, visits and vaccinations can occur within 7 days of the date. Injected subjects will be observed for at least 30 min following each injection. Subjects will be contacted by phone four times, at time periods days 1-3, days 4-6, days 29-31, and days 32-34 post-vaccination, and additionally they will be followed for a total of 365 days for safety observations and immunologic evaluations (see calendar in Section 10.0). The phone contact form is included in Appendix C: Subject Telephone contact record and dialog.

## 5.4 Assessments and Special Monitoring

For a detailed list of all study procedures including timing and windows, see Section 10.0 Study Calendar.

**Note:** Initiate a new cycle after all procedures/safety assessments have been completed.

## 5.5 **Duration of Therapy and Criteria for Removal from Protocol Therapy**

### 5.5.1 Duration of Therapy

This safety and dose finding study for COH04S1 vaccine will take ~17 months to complete. We anticipated accruing all subjects within the first ~60 days of the study. However, due to the availability of the EUA vaccines, enrollment has been lower than expected. Each volunteer will be actively involved in the study for a period of 365 days, plus the maximum of a 30-day screening period prior to immunization.

After enrollment, subjects will be required to make approximately 12 clinic visits over ~1-year period, and there are instances when a visit will require the subject to stay for more than a short outpatient clinic visit. These are as follows: the screening evaluation (1-2 hours, split in Screening visit 1 [~Day -10 to -3] and Screening Visit 2 or Baseline [Day -2/-1]) blood draw, ECG & cardiac troponin, and the primary immunization and booster injection visits (1.5 hour). Otherwise, visits for interval follow-up and blood draws require a very short stay in the outpatient area (15-30 min). The total time commitment by the volunteers for the duration of their involvement is <12 hours with an equal amount of time estimated for travel to and from the clinic.

In addition, baseline blood tests for safety and/or for immune status will be obtained on the days of immunization, 7 and 14 days post-vaccination, and at approximately days 56, 90, 120, 180, 270, and 365 post-vaccination. Pregnancy tests will be done at screening, before each immunization and at study end (Day 365, see Study Calendar in **Section 10.0**). Research subjects will have MVA antibody titer conducted for research purposes at the time of immunization, and then at Days 14, 42, 56, 90, 180 and 365.

All clinical laboratory tests will be performed in the Department of Clinical Pathology and Diagnostic Cardiology at COH. Immunologic laboratory studies listed below will be conducted under the direction of Don J. Diamond, Ph.D. in the Fox South laboratories, 1st Floor.

Participants will receive protocol therapy until one of the below criteria are met:

- Completed protocol therapy
- Participant is deemed intolerant to protocol therapy because of toxicity
- General or specific changes in the participant's health after the first vaccine injection that render the participant unacceptable for the second injection in the judgment of the investigator
- Withdrawal of consent for further protocol therapy (See Section 17.5)

Once participants meet criteria for removal from protocol therapy, the participant should then proceed to End of Treatment assessments, and then to follow-up (Refer to the Follow-Up section below).

Documentation of the reason for discontinuing protocol therapy and the date effective should be made in the Electronic Health Record/medical record and appropriate eCRF.

## 5.6 **Follow-Up**

### 5.6.1 Follow up post vaccine administration

All participants will enter follow-up after completing End of Treatment assessments 7 days after the last research injection. This is comprised of:

- **Safety Follow-up**- 7 days post-last dose of protocol therapy.
  - **Note** the period for safety follow-up will be extended until stabilization or resolution for all reportable AEs (per the agreement of the Study PI) and accompanying follow-up safety report.
- **Response Follow-up**- subjects will be followed to day 365 as indicated in the Study Calendar Section 10.0

Assessment time points and windows are detailed in Section 10.0.

### 5.6.2 Follow-up post potential SARS-CoV-2 exposure

All participants will be advised to contact us at any time if they think they have an intercurrent illness or when they might have been exposed to SARS-C0V-2. The nature of the illness or of the exposure will be evaluated upon contacting us. For further evaluation subjects will be seen at the clinic at the time of exposure, and 7, and 14 days later for assessment of SARS-CoV-2 infection by PCR and serology and to document severity and outcome of disease (follow-up will continue until resolution of SARS-CoV-2 infection related complications). The presence of any asymptomatic SARS-CoV-2 infection may be identified as well as any vaccine-induced disease enhancement.

## 5.7 Duration of Study Participation

Study participation may conclude when any of the following occur:

- Completion of study activities (treatment and 1 year of follow-up after protocol treatment)
- Withdrawal of consent (See Section 16.5)
- Participant is lost to follow-up. All attempts to contact the participant must be documented.
- At the discretion of the investigator for safety, behavioral, study termination or administrative reasons

Documentation of the reason for discontinuing study participation and the date effective should be made in the Electronic Health Record/medical record and appropriate eCRF.

## 5.8 Prohibited and Concomitant Therapies/Medications

### 5.8.1 Allowed concomitant medications

If concomitant therapy must be added or changed, including over-the-counter medications or alternative therapies, the reason and name of the agent/therapy should be recorded in the eCRF and documented in the Electronic Health Record/medical record.

### 5.8.2 Prohibited medications and therapies

Daily use of antihistamines, episodic (more than once in past 3 months) inhalational medications, including steroidal agents, non-steroidal agents, or cromolyn sodium are not allowed in this study. In addition, any immunosuppressive medication, any history of allergic diatheses treated chronically with immunosuppressive therapy may interfere with the vaccine response and will be prohibited. Any antipsychotic or mood stabilizing medications will be prohibited.

Chronic medications are excluded with the following exceptions: thyroid replacement, estrogen replacement, dietary vitamins and protein supplements, mild anti-depressant and anxiety medication, and any medication not known or likely to be immunosuppressive; all as determined by the P.I.

### 5.8.3 Management of adverse events

It is anticipated that the usual vaccine-related adverse events (AEs) will occur and these can be managed by over-the-counter medications. The subjects will be advised to take acetaminophen (500 mg oral) for symptoms of headache, muscle ache, and fever. For nausea or vomiting, the subject will take a clear liquid diet and choose whether to take Dramamine. If there is a more severe reaction, e.g. hives, blisters, the subject will take Benadryl (25 mg) and be seen in the clinic to determine if more aggressive management is necessary. If an acute anaphylactic attach occurs, the subject will be instructed to call 911 or go to the nearest emergency room.

## 6.0 ANTICIPATED ADVERSE EVENTS

### 6.1 COH04S1 vaccine

This is the first evaluation of the COH04S1 experimental vaccine in humans. Published experiences with MVA indicated minimal human toxicity in either short term or long term studies [6, 15, 39]. Recent published data from a randomized, placebo-controlled, double-blind study (Clinical Trials Registration: NCT00565929) have shown that MVA was safe, well tolerated and immunogenic when used as a vaccine in HCT recipients no less than two years post-hematopoietic cell transplant (HCT) [40]. The MVA Triplex vaccine containing 3 CMV proteins has been shown to be safe in phase 1 [6] and in phase 2 trials [7].

IM injections can cause local pain, redness and swelling, lasting for 1 or 2 days and possibly requiring analgesic agents (Tylenol, 650 mg p.o.) and/or antihistamine (Benadryl, 25-50 mg p.o.). If severe, this could require additional medication, and rarely could result in a sterile abscess that would need to be drained surgically, although this is very unlikely with IM injection. This has not been reported in the trial in healthy adults, or in the adult allogeneic-HCT recipients of the CMV-MVA vaccine to date.

Other common side effects of vaccination with CMV-MVA Triplex, and which may be associated with COH04S1, included injection pain, which has resolved within 7 days, rash, myalgia, headache, nausea, and fatigue. Should serious adverse reactions occur, treatment would be made available.

Although there is no current evidence with MVA-based vaccines, based on prior knowledge with coronaviruses, there is a remote possibility that there may be risk of antibody-dependent enhancement of disease.

## 7.0 DOSE DELAY / MODIFICATION GUIDELINES AND DLT DEFINITION

### 7.1 Dose Delays

The injection at Day 28 may be delayed by maximum 7 days. This visit must occur at Day 28 +/- 7 working days.

### 7.2 Dose Modifications

There will be no dose modifications in this study.

### 7.3 DLT and MOD Definition

DLT in a given subject is defined as any grade 3 or higher toxicity possibly, probably or definitely attributable to the research treatment, with the exception of expected local injection site AEs such as redness, pain, and swelling, and any fever, chills, malaise, headache, and flu-like symptoms such as myalgia and arthralgia of grade 3 that resolve to grade 1 or less in <7 days.

A MOD is a persistent moderate toxicity of grade 2 possibly, probably or definitely attributable to the research treatment AE that persists for 7 days or more, or any grade 3 treatment related AE that was excluded from the DLT definition as noted above. Toxicity will be graded according to standard Division of Microbiology and Infectious Disease (DMID) adult toxicity tables.

## 8.0 AGENT INFORMATION

### 8.1 Agent COH04S1

#### 8.1.1 Description

COH04S1 is a multi-antigen recombinant MVA based on a synthetic platform and encoding for SARS-CoV-2 (NCBI Accession# NC_045512) full-length Spike (S) and Nucleocapsid (N). We are pursuing the strategy of producing a double recombinant vectored vaccine to stimulate potent humoral and cellular immune responses against SARS-

CoV-2 S and N antigens. COH04S1 is a synthetic form of the highly attenuated, safe, and non-replicating MVA vaccine vector, designed to elicit humoral and cellular immune responses against SARS-CoV-2. Upon immunization, the vaccine vector infects cells at the local injection site, leading to the expression of the SARS-CoV-2 antigens that are visible to the immune system. The intrinsic adjuvant properties of the MVA vector backbone promote the stimulation of antigen-specific immune responses against SARS-CoV-2 infection.

All clinical batches of the COH04S1 vaccine will be manufactured at COH CBG, a California Food and Drug Branch (CFDB) licensed manufacturing facility which operates under the principles of cGMP for the manufacture of Phase 1/2 biologics.

### 8.1.2 Pharmacokinetics and Metabolism

There are no pharmaceutical or pharmacokinetic data for the vaccine molecule(s) proposed in this study. There is extensive data on the decay of MVA in rhesus macaques (RM) under immuno-suppression. MVA viral DNA is undetectable 6-8 weeks after administration of MVA viruses to RM [41].

### 8.1.3 Toxicology

This is the first evaluation of this experimental vaccine in humans. Published experiences with MVA indicated minimal human toxicity in either short term or long term studies [15, 39, 42, 43]. Nonetheless, published data from a randomized, placebo-controlled, double-blind study (Clinical Trials Registration: NCT00565929) have shown that MVA was safe, well tolerated and immunogenic when used as a vaccine in HCT recipients no less than two years post-HCT [40]. The MVA Triplex vaccine containing 3 CMV proteins has been shown to be safe in phase 1 [6] and in phase 2 trials [7].

#### *8.1.3.1 Expected human toxicities*

See Section 6.1 for detailed list of anticipated AEs.

#### *8.1.3.2 Potential effects on pregnancy and lactation*

This is the first evaluation of this experimental vaccine in humans. Published experiences with MVA indicated minimal human toxicity in either short term or long term studies [15, 39, 42, 43]. Nevertheless, pregnant or breast-feeding participants are excluded from this study.

### 8.1.4 Handling, storage, dispensing and disposal

MVA can be stored indefinitely at -70° C or lower without loss of activity (http://www.atcc.org/products/all/VR-1508.aspx; [44, 45]). For the purposes of this Phase 1 trial, COH04S1-MVA vaccine will be presented in sterile vials in PBS containing 7.5% lactose. For long-term storage, the vaccine will be placed in a monitored freezer between -60 to -90°C. This is a 24/7 centrally and electronically monitored freezer with excursions not exceeding -60 °C (max temperature)/-79 °C (minimum temperature), located at COH Pharmacy-Investigational Drug Service (**IDS**). The stability of the cGMP COH04S1-MVA vaccine will be tested at the COH CBG within 6 months from the first volunteer injection, and every 12 months thereafter. The stability of other recombinant MVA vaccines produced at the TVR have a half-life longer than the duration of the planned study. Of importance, cGMP MVA-p53 vaccine (produced at COH CBG) formulation, (IRB #10105), is still stable after 24 months. Prior to each injection, the thawed cGMP COH04S1 -MVA vaccine will be diluted with sterile diluent (PBS with 7.5% lactose) as appropriate for dose level, and then stored at 4-8°C prior to injection within 4 hours of thawing.

## 8.2 **Placebo: PBS containing 7.5% lactose solution for the placebo**

### 8.2.1 Description

PBS containing 7.5% lactose is a sterile, non-pyrogenic solution which is the diluent used in the formulation of the COH04S1 vaccine. This solution will be used for the placebo injection.

### 8.2.1 Toxicology

There are no known warnings. Lactose is a reducing sugar commonly used in multiple drugs as an excipient or bulking agent. It is a natural disaccharide consisting of galactose and glucose [46]. It can be administered by different routes including IM. There are no restrictions for diabetes and lactose intolerant subjects to take lactose containing medicines, since the amount of lactose delivered in drugs is minimal[46].

The possible risks of the placebo injection include: pain, swelling, hardness, redness, and itching at the injection site. Since this is a routine solution, no major reactions are expected.

### 8.2.2 Formulation

PBS containing 7.5% lactose will be supplied in a 1.2 mL polypropylene cryovial with a fill volume of approximately 1 mL/vial. The solution contains no bacteriostatic, antimicrobial agent or added buffer.

### 8.2.3 Supplier

PBS containing 7.5% lactose is produced and will be supplied by COH CBG.

### 8.2.4 Storage and Stability

PBS containing 7.5% lactose should be maintained between -60°C and -90°C in a temperature-monitored freezer. The release testing for PBS containing 7.5% lactose fill includes sterility, bacteriostasis and fungistasis, endotoxin, pH and particulate testing. The testing will be performed until the end of the study.

## 9.0 CORRELATIVE/ SPECIAL STUDIES

Minimum risk research blood samples (40 mL per time point, for a total of 9 blood draws) and saliva (2-3 ml per time point, for a total of 9 collections) will be obtained at time of research specimen collection in the outpatient setting.

### 9.1 **Correlative blood and saliva**

### 9.1.1 Overview and Time points

Peripheral blood and saliva will be collected at the time points indicated in Table 9.1-1, either at BCCR or at COH Upland, and promptly deliver by biospecimen couriers to the Diamond lab.

**Table 9.1-1 Overview of research specimens: collection and evaluation**

| Time points of collection | Total volume collected | Tube type | Receiving laboratory | Type of analysis |
|---|---|---|---|---|
| Days -2/-1**, 14, 26/27, 42, 56, 90, 120, 180, 270, and 365 | ~8 mL blood | 1x10 mL red-top (no anticoagulant) | Diamond Lab | Serum derivation; humoral immunity assays* |
| Days -2/-1**, 14, 26/27, 42, 56, 90, 120, 180, and 365 | ~30 mL blood | 3x10 mL green-top (containing heparin anticoagulant) | Diamond Lab | PBMC separation; cellular immunity assays, including cytokine analysis |
| Days -2/-1**, 42, 90, 180, and 365 | ~2 mL blood | 1x5 mL yellow top (containing citrate anticoagulant) | Diamond Lab | DNA separation MVA DNA detection assays |
| Days -2/-1**, 26/27, 42, 56, 90, 180, and 365 | ~ 2 mL saliva | 1x5 mL cryovials (freestanding polypropylene) | Diamond Lab | Saliva mucosal immunity assays |

*, Neutralization assay with live SARS-CoV-2 will be performed only on Day 42 serum.

**, Day -2/-1 visit, aka, baseline, can be combined with the Day 0 visit.

### 9.1.2 Labeling of blood and saliva samples

Label tubes with COH protocol #, subject ID (issued by DCC), institution, date and actual time point of collection (e.g. D for Day).

### 9.1.3 Collection and post-collection guidelines

Refer to Table 9.1-2 for collection and post-collection instructions.

**Table 9.1-2 Specimen collection and post-collection instructions.**

| Tube Type | Collection details | Site of collection | Post-collection instructions |
|---|---|---|---|
| Nasal wash specimen cups | 1- Specimens will be collected at BCCR<br>2- **Leave specimen cup at room temperature (r.t.)** | COH Duarte Campus | Deliver specimens at r.t. to COH Clinical Pathology Department. |
| | | COH Upland | |
| Green-top | 1- Blood samples will be collected by venipuncture.<br>2- Invert tubes eight times after collection.<br>3- **Leave tubes at r.t.** | COH Duarte Campus | **Promptly** deliver the blood samples at r.t. to the Diamond lab for processing (see 9.2 for details). |
| | | COH Upland | |
| Yellow-top | 1- Blood samples will be collected by venipuncture.<br>2- Invert tubes eight times after collection.<br>3- **Leave tubes at r.t.** | COH Duarte Campus | **Promptly** deliver the blood samples at r.t. to the Diamond lab for processing (see 9.2 for details). |
| | | COH Upland | |
| Red-top | 1- Blood samples will be collected by venipuncture.<br>2- Invert tubes eight times after collection.<br>3- **Leave tubes at r.t.** | COH Duarte Campus | **Promptly** deliver the blood samples r.t. to the Diamond lab for processing (see Section 9.2 for details). |
| | | COH Upland | |
| Cryovials (polypropylene) | 1- Saliva is allowed to accumulate in the floor of the mouth and the subject spits it out into the graduated test tube every 60 seconds, until ~2mL are collected and subsequently tubes will be closed and left at r.t. | COH Duarte Campus | **Promptly** deliver the saliva samples on ice to the Diamond lab for processing (see Section 9.2 for details). |
| | | COH Upland | |

## 9.2 **Research Laboratory Studies to be Performed**

We designed a comprehensive and innovative panel of immune assays combined with safe and versatile virological tools to characterize the COH04S1 vaccine induced SARS-CoV-2-specific adaptive immunity in this

clinical trial. Our integrated platform of immune- and pseudovirus-based methods includes state-of-the-art analytical multiparameter flow cytometry; qualitative in house developed ELISA, and neutralization assays based on a SARS-CoV-2 lentiviral-pseudovirus system, expressing the Spike antigen and infecting cell lines engineered to express ACE2 [34]. The assay system with Spike antigen "pseudotyped" onto non-replicative lentiviral particles alleviates the biosafety-level-3 (BSL3) hazard associated with working directly with SARS-CoV-2 and allows a safer approach to assess sera neutralizing activity to SARS-CoV-2. These methodologies are described below.

A thorough analysis of the DNA persistence of the COH04S1 vaccine viral vector will be performed to confirm vaccine safety.

### 9.2.1 SARS-CoV-2-specfic IgA, IgG, and IgM measured in serum and saliva by ELISA

To evaluate humoral immunity with the COH04S1 vaccine, we will measure SARS-CoV-2 specific antibodies, including IgA, IgG, and IgM, in serum and saliva by ELISA at the timepoints shown in the Study Calendar (see Section 10.0). The ELISA test has been developed by and will be conducted in the Diamond Laboratory at COH, Dept. of Hematology & Hematopoietic Cell Transplantation. The assay will identify SARS-CoV-2 antibodies specific for the S receptor-binding domain (RBD) that interacts with ACE2 on the surface of the cells, and the N protein that is one of the first B cell targets, during the initial phase of the SARS-CoV-2 infection [35]. The qualitative assays, based on previously established protocols [47], will be developed to investigate Spike subunit 1 (S1)- and N-specific antibodies of the IgG, IgM and IgA subclasses in serum and saliva. Pools of SARS-CoV-2 convalescent serum or SARS-CoV-2 negative serum will be used as a positive- and negative-controls (University of California at San Diego), respectively. End-point binding antibody titers will be expressed as the reciprocal of the last sample dilution to give an OD value above the cut-off [47]. Antibody levels in recipients will be graphed on a time plot and compared to baseline level in donors.

### 9.2.2 SARS-CoV-2-specific neutralizing antibodies

Evaluation of SARS-CoV-2 neutralizing antibody titers in serum samples of COH04S1 vaccinated volunteers will be performed at the timepoints listed in the Study Calendar (Section 10.0). We will use SARS-CoV-2 lentiviral-pseudovirus expressing the Spike antigen from the original Wuhan strain and infecting 293T cell lines engineered to express ACE2 [21]. Spike incorporation into the pseudovirus will be verified and quantified by Western blot using Spike-specific antibodies and by ELISA [34]. Serum samples from Day 42 will also be tested for neutralization of live SARS-CoV-2, and this test will be performed at the University of Louisville. As an exploratory endpoint we will test participants' serum samples for their ability to neutralize new variants of concern (VOC) as they appear in the population. Examples include the UK variant (VOC 202012/01) and the South African variant (VOC 501Y.V2). Pseudoviruses carrying the VOC Spike sequences will be used in a neutralization assay to measure neutralizing antibody titers to the VOC.

### 9.2.3 Th1 vs Th2 polarization

To evaluate the Th1 vs Th2 polarization of immune responses, we will perform dual fluorescence ELISPOT assay to detect and quantify cells secreting IFN-gamma and IL-4. Briefly, isolated PBMC will be stimulated with Spike and Nucleocapsid peptide libraries (15-mers with 11aa overlap) using fluorospot plates coated with IFN-gamma and IL-4 capture antibodies. Following 48h co-incubation, plates will be washed, and IFN-gamma and IL-4 detection antibodies followed by fluorophore conjugates will be added. Plates will be read and analyzed with a fluorescent ELISPOT reader and number of spots after stimulation expressed following subtraction of background from unstimulated samples. As an exploratory endpoint, in selected samples, a cytokine-based cytofluorimetric analysis (ICS) will be performed to analyzed multiple Th1 and Th2 cytokines. PBMC ($1\text{-}2x10^6$) will be stimulated for 16 hours with SARS-CoV-2-S or SARS-CoV-2-N overlapping peptide libraries (15-mers with 11aa overlap). Lymphocytes will be stained with viability dye and surface stained with antibodies to CD3, CD8 and CD4. After fixing and permeabilization, cells will be stained intracellularly with antibodies against IFN-gamma, TNF-alpha, IL-2, IL-4, IL6, IL-13. After washing, cells will be acquired using BD FACS Celesta Cell Analyzer and

analyzed with FlowJo software.

### 9.2.4 SARS-CoV-2-specific T-cell responses and evolution of activated/cycling and memory phenotype markers on the surface of antigens-specific T cells

Cellular immunity to SARS-CoV-2-S and -N, major domains of antiviral T cell immunity will be investigated in PBMC of COH04S1 vaccinated subjects, using multiparameter flow cytometry as previously described [6]. We will longitudinally monitor frequencies of T lymphocyte precursors responsive to SARS-CoV-2-S or SARS-CoV-2-N overlapping peptide libraries. In vaccine responders, SARS-CoV-2 specific T cells will be further evaluated by measuring levels [6] of CD137 surface marker expressed on $CD3^+$ $CD8^+$ and $CD3^+$ $CD4^+$ T cells stimulated for 24 hours with either SARS-CoV-2-S or SARS-CoV-2-N overlapping peptide libraries. CD137 is expressed only on recently activated T cells, and its expression correlates with functional activation of T cells [48]. Measurements of CD137 levels will be combined with immunophenotyping studies, by using antibodies to CD28 and CD45RA cell surface markers to assess and identify memory phenotype profiles percentage of effector memory (TEM and TEMRA), central memory (TCM) and naïve SARS-CoV-2-S or SARS-CoV-2-N specific T cells [7]. Additionally, we will assess the activated/cycling phenotype by using the CD38, HLA-DR, Ki67 and PD1 surface markers [38]. Approximately 300,000 events per sample will be acquired on a Gallios flow cytometer and analyzed by Kaluza software.
Taken together these studies will identify whether 2 immunizations with COH04S1 vaccine, given within 28 days, is an effective method to elicit SARS-CoV-2-specific adaptive immunity

### 9.2.5 Detection of persistence of COH04S1 MVA vector DNA

MVA vaccine vector DNAemia persistence will be monitored in all vaccinated participants for up to one year [41, 49], at Days -2/-1, 42, 90, 180 and 365. Real-time PCR will be performed using primers targeting the MVA Thymidine Kinase (TK) gene [50]. The assay will be performed in triplicate (0.5 µg cellular DNA/well) with one additional sample spiked with 51 copies of plasmid DNA with the identical CMV antigen cassette as contained in Triplex. The assay is sensitive to 2000 genomic units (GU)/ml (=20 copies MVA DNA/µg cellular DNA).

## 10.0 STUDY CALENDAR

All assessments may increase in frequency as clinically indicated.

| | Screening* | Day -2/-1** | Day 0 | Day 1-3 | Day 4-6 | Day 7 | Day 14 | Day 26/27 | Day 28 | Day 29-31[i] | Day 32-34[i] | Day 35[i] | Day 42[i] | Day 56 | Day 90 | Day 120 | Day 180 | Day 270 | Day 365 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Informed Consent | X | | | | | | | | | | | | | | | | | | |
| Physical Exam (PE) | X | | X | | | X | X | | X | | | X | X | X | | | | | X |
| Medical History and Demographics | X | | | | | | | | | | | | | | | | | | |
| HIV, HCV, active HBV Test | X | | | | | | | | | | | | | | | | | | X |
| Pregnancy Test[a] | X | X | | | | | | X | | | | | | | | | | | X |
| SARS-Cov-2 Viral PCR (Diasorin Simplexa™)[b] | | X | | | | | | X | | | | | | | | | | | |
| SARS-CoV-2 serological test | X[c] | X | | | | | | X | | | | | | X | X | X | X | X | X |
| Chemistry/Metabolic Panel (CMP) | X | X | | | | X | X[d] | X | | | | X | X[d] | X | X | X | X | | X |
| CBC with Differential | X | X | | | | X | X[d] | X | | | | X | X[d] | X | X | X | X | | X |
| ECG (single) and Cardiac Troponin Test | X | | | | | | X | | | | | | | | | | | | |
| Research Injection | | | X | | | | | | X | | | | | | | | | | |
| AE Assessment | | | | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X |
| Blood Draw (mL)[e] | | 40 | | | | | 40 | 40 | | | | | 40 | 40 | 40 | 40 | 40 | 8 | 40 |
| Phone call follow-up[f] | | | | X | X | | | | | X | X | | | | | | | | |
| Saliva Collection[g] | | X | | | | | | X | | | | | X | X | X | | X | | X |
| Unblinding[h] | | | | | | | | | | | | | | X | | | | | |

* Up to 30 days before vaccination. Window for all visits is the assigned Day +/- 7 days.

**, Day -2/-1 visit, aka, baseline, can be combined with the Day 0 visit, with SARS-CoV-2 virologic test, pregnancy test, labs, and research blood and saliva draw, and PE being performed very early in the morning, and injection in late afternoon.

[a] To be performed for women of child bearing potential.

[b] This test is performed at COH, in the Pathology Department, on nasal wash samples. Additional viral PCR tests will be performed on symptomatic individuals between Days 42 and 365 at the COH drive-through; COVID participants can be seen in the Febrile Research Clinic (FRC).

[c] At screening, the TGen will perform the InBios test, which has been authorized by FDA under an Emergency Use Authorization (EUA). For collection and shipping details see Appendix D; results are not required for eligibility but only for retrospective analysis to document a pre-vaccination subclinical SARS-CoV-2 infection. The SARS-CoV-2 serological tests done at the other visits will be performed in Dr. Don Diamond's Lab.

[d] If Grade 2 AEs with CBC/diff and/or CMP occur at Day 7, these tests are repeated on Day 14; similarly, if Grade 2 AEs with CBC/diff and/or CMP occur at Day 35, these tests are repeated on Day 42.

[e] A total of ~40 mL blood draw will include: ~8 mL of blood in tubes with no anticoagulant (red top) for serum derivation; ~30 mL of blood in tubes containing heparin (green top) for PBMC separation; ~2 mL of blood in tubes containing citrate (yellow top) for detecting MVA DNA (performed at the following time points: Day -2/-1, 42, 90, 180, 365) [6]. Blood samples will be used for the laboratory/correlative studies listed in Section 9.2, which will be done at Day -2/-1, 14, 26/27, 42, 56, 90, 120, 180 and 365, except for the live SARS-CoV-2 neutralization assay, which will be performed only at Day 42. At Day 270 only the ~8 mL of blood in tubes with no anticoagulant (red top) for serum derivation will be drawn for the correlative SARS-CoV-2 serological test.

[f] Subjects will be called twice in the first 7 days after both injections (day 0, day 28) for collection of AEs and follow up on any day between days 1-3 and days 4-6 and day 29-31 and 32-34.

[g] Whole oral fluid (saliva) will be collected by allowing the saliva to accumulate in the floor of the mouth and the subject spits it out into the graduated test tube every 60 seconds, until ~2mL are collected.

[h], participants are informed if they have received vaccine prime and boost, vaccine prime only, or placebo only; no information is disclosed about dose level of vaccine injections (see Appendix E: Routine Unblinding of IRB 20447). Participants in VP will be allowed to receive the booster injection at DL1 and follow the study calendar for injection #2 (day 26/27). Participants in PP will be offered EUA vaccine or to proceed to randomization of DL2/DL3 if all eligibility criteria are met and follow the study calendar for injection #1 (day -2/-1); if proceeding to randomization, eligibility will need to be reconfirmed (start visits with screening).

[i] Participants who opted to withdraw from the study or became unevaluable and never received their second injection, will not participate in the Day 35 visit and will not receive AE phone call follow up on days post second injection.

## 11.0 STATISTICAL CONSIDERATIONS

### 11.1 Study Design

Each subject during the initial open-label safety evaluation on each dose is expected to receive 2 injections at the assigned DL on days 0 and 28 (2nd administration requires absence of DLT or MOD) and will be followed for 365 days post initial injection. Subjects will be assessed for DLT, MOD, and biological correlatives as described above. To be evaluable for dose escalation decisions, a subject must receive at least one vaccine injection. Dose escalation is primarily based on observations of MOD during the 7-day after the initial injection, with observations of MOD or DLT later or after the second injection also used as specified in the dose decision discussion below. All subjects in a cohort who do not experience a DLT or MOD must have received at least 1 injection and be followed for at least 7 days after the first injection or will be replaced during the open-label safety assessment. All subjects receiving any injection will be followed for AEs and accounted for in the final data summary. Any DLT will qualify as a MOD event, but due to the increased severity, any DLT observed at any time during the study will also temporarily suspend all vaccine administrations at all doses pending review and approval of resumption of treatment by the PI, external DMC, IRB and in consultation with the FDA. Thus, dose escalation and accrual will depend on toxicity observed considered MOD, while DLTs will hold accrual. The design follows the Phase 1 queue (IQ) 3+3 design [14] adapted a) to decisions based on MOD (instead of DLT), and b) to require the first subject treated on each DL to be observed for at least 7 days before accruing further subjects. These rules stay within the risk constraints of a classic 3+3 design with a minimum of 1-week assessment time and adapted to lower the risk (moving from DLT to MOD) due to this being a healthy subjects study and requiring the first subject on a dose level be followed for at least 7 days before additional accrual is permitted on that dose level. In this design, 0/3 (or 0/4) with MOD would permit dose escalation, and 1/6 also permits dose escalation. Once a dose has passed the safety rules (represented by escalation or MTD determination), up to three cohorts of additional subjects will be enrolled at that dose level in a double-blind randomized expansion: 15 subjects at that dose level with prime and boost (VV), 15 subjects to receive a single injection (prime) cohort with placebo for boost (VP), and 5 subjects to receive two placebo injections (PP). Accrual to these cohorts will be randomized by a permuted block design, although expansion cohorts can be closed if accumulating data suggests insufficient immunological activity. Per version 7 of the protocol, the expansion on DL1 will include randomization of VV, VP and PP groups as above, but DL2 and DL3 expansion cohorts will only include VV and those two dose levels will be randomized (15 vs 15 subjects beyond the safety lead-in subjects). The permuted block design will be used for all randomization procedures. The permuted block design will also have an embedded “pause” rule to limit the number of subjects at risk consistent with table 11.1 below under the assumption that the higher dose is closed. The “Pause” rule will no longer apply once past the MTD call per 11.1 (this condition has been met per version 7 of the protocol).

The schema presented earlier represents the expected subject flow, assuming no delays in accruing healthy subjects to open slots and no DLT/MOD toxicity.

See Table 11.1-1 below for the dose level decision rules (detailed rules for all possibilities are provided in “VaccineDecisionGrid.xlsx” at https://oneq.netlify.app/)

In **Table 11.1-1**, “pause accrual per protocol” means pausing until there is a change in the disposition of the pending subjects (which would change the row and possibly the decision). Note that in the detailed decision grid online this called “suspend”. Implementation of these rules is based on PI and statistician sign-off for any new decisions. As an example of the rules, if the first subject clears the 7-day follow-up

(EVAL without a MOD), the rules permit accruing 3 additional subjects, as can be seen in rows 3-4 (1-3 total, 1 EVAL, 0 MOD) where a 4th subject is allowed and row 5 (4 total, 1 EVAL, 0 MOD), where a 5th subject is not accrued at that dose level or above until there is a change in the disposition of pending subjects. **The bolded decisions** represent differences between the traditional 3+3 and the IQ 3+3 modified for this vaccine study.

**Table 11.1-1. IQ 3+3 Design Decisions. Note: The bolded decisions in the right most column represent differences between the traditional 3+3 and the IQ 3+3 modified for the vaccine rules above.**

| Row | # on Current Level | | | IQ 3+3[b,c]: Dose Level (DL) Next Subject |
|---|---|---|---|---|
| | Total[a] | EVAL | MOD | |
| 0 | 0 | 0 | 0 | Same DL |
| 1 | 1 | 0 | 0 | **Pause accrual per protocol** |
| 2 | 2-3 | 0 | 0 | **Not possible** |
| 3 | 1-2 | 1 | 0 | Same DL |
| 4 | 3 | 1 | 0 | ***Same DL*** |
| 5 | 4 | 1 | 0 | ***Pause accrual per protocol*** |
| 6 | 2 | 2 | 0 | Same DL |
| 7 | 3 | 2 | 0 | ***Same DL*** |
| 8 | 4-5 | 2 | 0 | ***Same DL*** |
| 9 | 6 | 2 | 0 | ***Pause accrual per protocol*** |
| 10 | 3 | 3 | 0 | Escalate[d] |
| 11 | 4-6 | 3-5 | 0 | Escalate[d] |
| 12 | 6 | 6 | 0 | Escalate (or MTD)[d] |
| 13 | 1-2 | 1 | 1 | Same DL |
| 14 | 3 | 1 | 1 | Pause accrual per protocol |
| 15 | 2 | 2 | 1 | Same DL |
| 16 | 3 | 2 | 1 | ***Same DL*** |
| 17 | 4 | 2 | 1 | ***Pause accrual per protocol*** |
| 18 | 3-5 | 3-5 | 1 | Same DL |
| 19 | 6 | 3 | 1 | Pause accrual per protocol |
| 20 | 6 | 4 | 1 | ***Same DL*** |
| 21 | 6 | 5 | 1 | ***Same DL*** |
| 22 | 7 | 4 | 1 | ***Pause accrual per protocol*** |
| 23 | 7 | 5 | 1 | ***Same DL*** |
| 24 | 6-8 | 6-8 | 1 | Escalate[d] |
| 25 | 2-7 | 2-6 | 2 | De-escalate[e] |
| 26 | 7 | 7 | 2 | ***MTD*** |
| 27 | 8 | 7 | 2 | ***Pause accrual per protocol*** |
| 28 | 8 | 8 | 2 | ***MTD*** |
| 29 | <=8 | any | 3 | De-escalate[e] |

[a]Total number of subjects consented, excluding proven screen failures or subjects who are inevaluable for MOD.

[b]PI can choose to pause accrual per protocol at any time for pending subjects to complete evaluation.

[c]If a subject pending evaluation or during second injection on a lower dose experiences a MOD: if the rules of the lower dose define the MTD or do not permit escalation, subjects at higher doses will not proceed with second injections at higher dose, and further accrual at the higher doses will cease pending review by the external DMC.

[d]If the next higher DL is not available (there is no higher dose or the higher dose was already tested and too toxic), treat at current dose and declare the maximum-tolerated dose (MTD) with 0 or 1 MOD out of 6 (or 0 out of 5).

These rules stay within the risk constraints of a classic 3+3 design, where 0/3 (or 0/4) with MOD permit dose escalation, and 1/6 also permits dose escalation, but reduce study duration by approximately 20% under a variety of scenarios where accrual is staggered, and subjects are non-compliant and need to be replaced. We have added to these risk-based rules the additional rule that the first subject on each DL must be observed for 7 days after injection before any additional subjects can be accrued.

If all subjects are accrued as soon as slot is available, with no screen failures and all subjects are compliant with the evaluation process, this design reduces to a standard 3+3 design with the additional constraint of waiting until the first subject on a dose level is observed for the 7-day window. That scenario, with no concerning toxicity signal is pictured in the schema and completes in ~60 days. However, variations from the ideal create queuing issues better addressed by the IQ 3+3 design. For example, with a screening time of up to 14 days (uniform, reflecting subjecting testing), a 10% screen failure rate, a 5% inevaluability rate, a 3 day mean inter-arrival time, and a MOD rate of 5.9%, 6.6% and 7.6% at the three dose levels, the dose exploration portion with the IQ 3+3 takes an expected duration of 129 days, whereas the standard 3+3 (also with the 7-day rule for first subject per dose level) would have an expected duration of 156 days, a 21% increase. With regard to operating characteristics, given the MOD rates provided, the 3+3 declares the top dose safe in 86% of the simulations, and the IQ 3+3 declared the top dose safe in 84% of the simulations (based on 1000 simulations).

Once a dose has been cleared per these rules and escalation is permitted or the MTD is declared, additional subjects will be enrolled in a double-blind randomized expansion study. This will provide for more safety data to accumulate at that dose, and allow a comparison of single vs two injections, along with a placebo group, for comparisons of adverse events and secondary objectives (15 expansion subjects assigned to VV, 15 to VP, and 5 to PP as noted above) for DL1. DL2 and DL3 expansion cohorts will provide 15 subjects per dose level for comparison. Open-label slots will have priority over expansion slots. During the expansion cohorts, holds may be placed to be consistent with the safety constraints associated with the IQ 3+3 as noted above. If multiple doses are in the randomization expansion portion simultaneously, the lower dose will enroll first. See detailed rules in "VaccineDecisionGrid.xlsx" at https://oneq.netlify.app/). During expansion cohorts, if at any time ≥33% of subjects experience a MOD at any time in VV or VP that dose will hold accrual pending review by the DMC. If any DLT is observed the study will hold accrual pending review by the DMC.

**Accrual Rate/sample-size**

Expected (original): Open-label safety study of 4 subjects per dose level, plus 35 subjects per dose level in randomization portion. 39 x 3 dose levels = 117 subjects. Per version 7, the open label group for DL1 was 4 patients, with 35 expansion subjects on DL1, and for DL2 and DL3, there are 6 safety lead-in subjects each, and 15 additional expansion patients planned for a total of 39+21+21=81 subjects. For placebo, total number of subjects is now 5 subjects concurrent with DL1. Accrual is planned to take 5 months, including screening and assuming no delays in accruing healthy subjects to open slots and no DLT/MOD toxicity; entire study duration, ~17 months.

**Sample Size Rationale for Evaluation of Primary Endpoint: Safety**

There is extensive experience with clinical delivery of MVA vaccines in which only mild reactogenicity has been observed [6-10, 15, 16]. The dose escalation is primarily designed to protect subjects against potential immunological reactions due to vaccine components, while allowing timely completion of the study. There is an open-label safety study of 4 subjects (maximum 8) per DL, plus a maximum of 35 subjects per DL in randomization portion. For placebo (PP), the total number of subjects was initially

planned for 5 per dose level and 15 across all DLs. However, due to the recent wide availability of Emergency Use Authorization vaccines in California (all residents are eligible in April, 2021), the placebo was considered an unethical withholding/delay of available vaccines and was discontinued per version 7 of the protocol with a contemporary placebo comparison available for DL1 only (5 subjects). Initially 15 subjects were anticipated to be treated at each DL for single vaccine injection (VP) and for the two vaccine injection cohorts (VV) during the randomization portion. For safety evaluation, this will result in 19-23 subjects at any recommended DL for two injections (VV). Per the amendment version 7, this remains unchanged. As a result, any AE with an incidence of 15% would be very likely to appear in at least one of the 19 subjects (>95%). For DL1, based on the first injection only (combining both single and double injections for the first 28 days), there would be 34-38 subjects on DL1, where any AE with an incidence of 9% would very likely to appear in at least one of the 34 subjects (>95% chance). As immunological data on DL1 and sentinels on DL2 demonstrates a clear benefit for the boost without tolerability issues, for DL2 and DL3 21 subjects will be treated with VV (on each dose level, 15 during the expanded randomization portion), providing more than >96% probability of observing any AE with an incidence of 15%. Therefore, the trial will provide an adequate basis for judging the initial safety of the vaccine for future use in research subjects who are at risk for infection by COVID-19, while providing for an opportunity to evaluate immune response. Doses that are unacceptable due to toxicity will not be expanded. Other reasons (lack of immune response) may also close a cohort early, at the discretion of the PI, and similarly the PI can close a single injection cohort (VP) on a DL. As part of the safety assessment, we will evaluate the outcome of our immune correlate panel, including the potential of SARS-CoV-2-S and -N specific Th1 to Th2 polarization and any incidental infection of vaccinated subjects. The placebo group is not intended to test the hypothesis of no toxicity above the placebo, but does provide information on a contemporary group of subjects for DL1 from the same pool for a more thorough discussion of adverse events above normal variation. Data will be summarized both pooling the open-label and randomized portion, and with data restricted to the randomized expansion cohorts when comparing the adverse event profile of (VV), (VP) and (PP) groups in DL1, and for comparing DL2 to DL3 (VV) subjects (there are no (VP) or (PP) patients for DL2 or DL3.

**Sample Size Rationale for Evaluation of Humoral Immune Response**

The primary immunogenicity outcome will be serum IgG against SARS-CoV-2. Enrollment requires a negative history for SARS-CoV-2 infection and a pending nasopharyngeal wash. The determination of positivity by either test is based on the standards of the laboratory assay independent of this study. A “positive” IgG (immunogenicity) response, specific to any evaluation time, will be defined as a 4-fold raise from the baseline value (i.e. value prior to the first vaccination) during the 56-day period post-vaccination. Subjects with a positive immunogenicity result for IgG specific for SARS-CoV-2 S or N protein at any time after the first injection will be considered a success (with the exception of subjects who are diagnosed with SARS-CoV-2 prior to a “positive” immunogenicity result), and we will also evaluate the persistence of the positive IgG at 365 days. With 19-23 subjects at a DL on the two-vaccination plan (VV), the percent of success can be estimated with a standard error of 11%. While not initially randomized across dose levels (but randomized between DL2 and DL3), we will compare success rate of (V1,V1), (V2,V2) and (V3,V3), the planned two-injection cohorts from each of the expansion cohorts. If each of the three DLs accrue 19 subjects to (VV), and the success rate differs by 20% (e.g. 70% success for best dose, vs 50% success for two inferior DL), the probability of one inferior dose outperforming the superior dose is approximately 13%. If the success rate differs by 30% (e.g. 80% vs. 50% vs 50%) the probability of selection of the inferior dose by chance is <3%. With 15 subjects per cohort, the chance of selecting an inferior dose when it differs by 20% is <16%, and the chance of

selecting an inferior dose when it differs by 30% is approximately 4%. For DL2 and DL3, when the expansion subjects are randomized across dose levels, if the success rate differs by 20%, there is less 10% chance of selecting the inferior dose with 15 subjects per dose level.

Comparison of immunogenicity within a dose of the single injection (VP) with the double injection (VV) and placebo (PP), is an exploratory endpoint as we consider IgG titers, persistence, adverse events and convenience. However, for the placebo comparison within a DL1, we will also compare the 5 placebo subjects to the 15 subject (VV) group on DL1, where we have 82% power to detect a statistically significant difference in the immune reaction success rate of 82% (VV) to 20% (PP) with a type I error (1-sided) of 10% (Exact test). If that test passes, comparison to the (VP) will be conducted with higher power (98% for the same effect size and type I error). We will not adjust for multiple comparisons. We note that the single injection recommended dose may exceed the recommended dose for the two-injection cohort and that selection of dose and single vs double injection will depend on tolerability, compliance, and immunogenicity. The placebo group is primarily used to validate that the immune changes were not related to unexpected changes in the environment (e.g. circulating coronaviruses, subclinical exposure to SARS-CoV-2) on DL1.

Comparison across dose levels will include open-label safety subjects and will also include a comparison of the randomized 15 vs 15 subjects to DL2 and DL3.

**Community Acquired Infection:**

Subjects will be followed for 365 days to document the incidence and severity of COVID-19 acquired infections. This is an exploratory endpoint as is the report on the severity of outcome to address concerns related to the potential for vaccine-induced disease enhancement. The placebo group may help provide related information on acquired COVID-19 infections on a contemporary group of subjects from the same population, although this will be notably underpowered based on the current infection rate. In addition, because commercial vaccines may become available to the participants during this study (either through emergency use authorization or full approval), participants will be informed at the day 56 visit whether they have received vaccine or placebo. As a result, early antibody responses and safety comparisons will focus on day 56 or before to avoid biases involved with the unblinding. For subjects on VP on DL1, subjects will be offered a second injection on DL1 of COH04S1 or can pursue an emergency use authorized vaccine. For PP subjects on DL1, on day 56 patients will be allowed to re-enroll to the randomized DL2 or DL3 VV groups or can pursue an EUA vaccine. All subjects will continue on the trial for long-term follow-up, and retrospective analysis will take into consideration those who received the EUA vaccine.

### 11.2 Interim Analyses/Stopping Rules

The formal interim analysis is based on MOD and DLT observations as described above. However, correlative studies during the conduct of the study may be used to stop accrual to a given dose level (or the single-injection cohort, e.g. (VP) ) based on the judgement of the PI/sponsor. Additionally, during these expansion cohorts, if at any time ≥33% of subjects experience a MOD at any time on a dose level, that dose will hold accrual pending review by the DMC. If any DLT is observed (at any time, on any dose), the study will pause enrollment pending review by the independent DMC. The study will be paused for a safety review if any subject dies or requires ICU admission due to SARS-CoV-2 infection.

### 11.3 Partial Unblinding of Trial

For ethical reasons, which have been discussed at FDA public meetings for the EUA-related COVID-19 vaccines, provision of vaccine to the placebo group in the phase 3 trials was important. Thus, the

concept of loss of control groups prior to the end of a trial is potentially important. In the case of a phase 1 trial, which is recruiting subjects in the face of pending/variable EUA vaccine availability, there is a practical reason for breaking the blind, namely to assure participants that they can have access to an EUA vaccine or COH04S1 vaccine without the need to wait for one year and without the incentive to drop out of the study. This plan is to limit the blinding to 56 days post vaccination, during which the key safety data and immunological data are collected. Participants will be informed at the day 56 visit whether they have received vaccine (1 or 2 injections) or placebo (see Appendix E).

The subject will be asked to continue to remain in the study for documentation of immune response to the EUA and for any side effects from the EUA vaccine or additional COH04S1 vaccines post-unblinding.

## 12.0 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

### 12.1 Source Documents

Source documents are original documents, data, and records (e.g., medical records, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. The Investigator or their designee will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each subject enrolled in this clinical trial. Source documents must be adequate to reconstruct all data transcribed onto the case report forms.

### 12.2 Data Capture Methods and Management

Data for this trial will be collected using City of Hope's electronic capture system (EDC) that is compliant with 21 CFR Part 11.

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF).

### 12.3 Case Report Forms/Data Submission Schedule

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. All case report forms must be completed by designated study personnel. The completed case report forms must be reviewed, signed and dated by the Investigator or designee in a timely fashion.

All data will be collected using electronic data collection and will be submitted according to the timelines indicated in Table 12.3-1.

**Table 12.3-1 Data Submission Schedule**

| Form | Submission Timeline |
|---|---|
| Eligibility Checklist | Complete prior to registration |
| On Study Forms | Within 14 calendar days of registration |
| Baseline Assessment Forms | Within 14 calendar days of registration |
| Treatment Forms | Within 10 calendar days of treatment administration |
| Adverse Event Report Forms | **Injection1**: Within 7 calendar days of AE assessment/notification<br>**Injection 2**: Within 10 calendar days of AE assessment/notification |

| Form | Submission Timeline |
|---|---|
| Response Assessment Forms | Within 10 calendar days of the response assessment |
| Other Assessment Forms (concomitant medications) | Within 10 calendar days of the assessment |
| Off Treatment/Off Study Forms | Within 10 calendar days of end of treatment/study |
| Follow up/Survival Forms | Within 14 calendar days of the follow up activity |

### 12.4 Regulatory Records

The Investigator will maintain regulatory records, including updating records in accordance with Good Clinical Practice guidelines and FDA regulations.

## 13.0 REPORTING OF ADVERSE EVENTS, UNANTICIPATED PROBLEMS & OTHER EVENTS OF INTEREST

### 13.1 Adverse Event Definitions

**Adverse Event (AE)** - [Modified from 21 CFR 312.32 (a)] An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

**Serious Adverse Event (SAE)** - [Modified from 21 CFR 312.32] A serious adverse event is any expected or unexpected adverse event that results in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

### 13.2 Assessment of Adverse Events

The Study PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

#### 13.2.1 Assessment of Adverse Event Name and Grade:

Adverse events will be characterized using the descriptions and grading scales found in the most recent version of the standard DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) adult toxicity tables (APPENDIX B).

The determination of severity for all other events not listed in the DMID tables should be made by the investigator based on medical judgment and the severity categories of Grade 1 to 5 as defined below:

- Grade 1 (mild) - An event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Grade 2 (moderate) - An event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- Grade 3 (severe) - An event that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects the clinical status of the subject.
- Grade 4 (life threatening) - An event, and/or its immediate sequelae, that is associated with an imminent risk of death or with physical or mental disabilities that affect or limit the ability of the subject to perform activities of daily living (eating, ambulation, toileting, etc).
- Grade 5 (fatal) - Death (loss of life) as a result of an event.

13.2.2 Assessment of Attribution:

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

- **Definite** - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.
- **Probable** - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).
- **Possible** - The AE may be related to the investigational agent or study procedure and may be related to another cause(s).
- **Unlikely** - The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).
- **Unrelated** - The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

13.2.3 Assessment of expectedness:

The following definitions will be used to determine the expectedness of the event:

**Unexpected Adverse Event** - [Modified from 21 CFR 312.32 (a)] An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

**Expected Adverse Event** - An adverse event is expected if it does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

### 13.3 Unanticipated Problems

**Unanticipated Problem (UP)** - An unanticipated problem is any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

## 13.4 **Adverse Events of Special Interest (AESI)**

### 13.4.1 Immune-related AEs

An immune-related adverse event (irAE) is defined as an adverse event that is associated with drug exposure and is consistent with an immune-mediated mechanism of action and where there is no clear alternate etiology. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an irAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the irAE.

#### *13.4.1.1 Antibody dependent enhancement of infection (ADE)*

ADE is a process in which there is increased viral delivery to cells and tissues that are the direct targets of the pathogen and has been described in virus infections such as Dengue and Zika [18] and, in SARS *[19]*.

## 13.5 **Pregnancies**

### 13.5.1 Female participants:

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female participant occurring after the participant receives the first dose of protocol therapy up to 60 days post-last dose of vaccine are considered immediately reportable events. **Protocol therapy is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Study PI and the DCC immediately within 24 hours of awareness**. The female subject may be referred to an obstetrician-gynecologist (preferably one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The Investigator should make every effort to follow the female participant until completion of the pregnancy per institutional policies and should notify the Study PI.

Abnormal pregnancy outcomes and neonatal deaths that occur within 28 days of birth should be reported as an SAE per expedited reporting guidelines.

Any infant death after 28 days that the Investigator suspects is related to the *in utero* exposure to protocol therapy should also be reported as an SAE per expedited reporting guidelines.

### 13.5.2 Male participants:

If a female partner of a male participant becomes pregnant, the male participant should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

The Investigator should make every effort to follow the outcome of the pregnancy per institutional policies and should notify the Study PI.

### 13.6 Routine AE Collection and Reporting Guidelines

AEs will be collected from the signing of informed consent until ending study participation. Routine AE reporting will occur via data entry into the study eCRF. AEs will be monitored by the Protocol Management Team (PMT). AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.

AEs recorded in the eCRF include:

- Any Grade 1-5
- All SAEs

### 13.7 Expedited Reporting

**Table 13.6-1** indicates what events must be reported expeditiously. The coversheet for expedited reporting is included in APPENDIX A.

Serious Adverse Events that require expedited reporting and unanticipated problems will be reported according to the approved City of Hope's Institutional policy via electronic submission in iRIS at http://iris.coh.org.

**Table 13.6-1 Criteria for Expedited Reporting**

| Time point | What to report |
|---|---|
| From signing of the consent to study completion | • All UPs |
| For the time period beginning at treatment through one year post second vaccine injection | • All SAEs regardless of relationship to protocol therapy<br>• All UPs and AEs that meet the definition of a UP<br>• Pregnancies and lactation |
| NOTE: All events reported expeditiously require follow-up reporting until the event is resolved, stabilized, or determined to be irreversible by the investigator.<br>The Data Coordinating Center (DCC) will be consulted prior to ending the follow-up of events that have stabilized. | |

### 13.8 Reporting to the FDA

The study PI (or designee) will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy.

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the MedWatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA **no later than 7 calendar days** after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted **no later than 15 calendar days** after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported **as soon as** the relevant information becomes available. [21 CFR 312.32(d)(3)]

**In addition**, on behalf of the study PI, OIDRA will submit annually within 60 days (via COH OIDRA) of the anniversary of the date the IND went into effect, an annual report to the FDA which is to include a narrative summary and analysis of the information of all FDA reports within the reporting interval, a summary report adverse drug experiences, and history of actions taken since the last report because of adverse drug experiences.

## 14.0 ADHERENCE TO THE PROTOCOL & REPORTING OF PROTOCOL DEVIATIONS

A deviation is a divergence from a specific element of a protocol. It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. Protocol deviations may be on the part of the subject, the investigator, or study staff.

### 14.1 **Definitions**

#### 14.1.1 Unplanned Deviations:

- **Emergency modifications -** Investigators may implement a deviation from the protocol to eliminate an immediate hazard for the protection, safety, and well-being of the study subject to trial participants without prior COH IRB or Sponsor approval.
- **Deviations Discovered After They Have Occurred.**

Unplanned deviations from the protocol must be documented in study subject source documents.

#### 14.1.2 Planned Non-Emergency Deviations (Single Subject Exception)

A **planned deviation** involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific subject. It is a deviation that is anticipated and receives prior approval by the Study PI and the COH IRB.

### 14.2 **Reporting of Deviations**

#### 14.2.1 Reporting Unplanned Deviations

For any such deviation, the Study PI will notify the independent DMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy.

A list of these deviations, will be submitted along with the Protocol Management Team (PMT) reports to the independent DMC.

### 14.2.2 Reporting Planned Non-Emergency Deviations/ Single Subject Exceptions

Any planned deviation must be submitted as a "planned protocol deviation" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy. An IRB approved planned deviation does not need to be submitted as a deviation to the DMC.

## 15.0 STUDY OVERSIGHT, QUALITY ASSURANCE, & DATA AND SAFETY MONITORING

### 15.1 Study PI Responsibilities

The Study PI is responsible for the conduct of the clinical trial, including overseeing that sponsor responsibilities are fulfilled as defined in § 21 CFR 312.

### 15.2 All Investigator Responsibilities

All investigators agree to:

- Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when necessary to protect the safety, rights or welfare of subjects.
- Personally conduct or supervise the study (or investigation).
- Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
- Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
- Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
- Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
- Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
- Promptly report to the IRB and the Sponsor all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
- Seek IRB and Sponsor approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
- Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

### 15.3 Protocol Management Team (PMT)

The PMT, minimally consisting of the Study Principal Investigator, collaborating investigators, the research nurse, the clinical research associate/coordinator, and the study biostatistician, is responsible for ongoing monitoring of the data and safety of this study, including implementation of stopping rules for safety/toxicity.

The PMT will meet (in person or via teleconference) monthly to review study status. This review will include, but not be limited to, reportable adverse events (AEs) and unanticipated problems (UPs) involving risks to subjects or others, and an update of the ongoing study summary that describes study progress in terms of the study schema. The meeting will be a forum to discuss study related issues

including accrual, SAE/AEs experienced, study progress, deviations/violations and study management issues. The appropriateness of further subject enrollment and the specific intervention for subsequent subject enrollment are addressed, including the implementation of stopping rules. The minutes of these discussions be taken to document the date of these meetings, attendees and the issues that were discussed.

The Study PI is required to submit periodic status reports (the PMT Progress Report) according to the guidelines outlined in the City of Hope Institutional Data and Safety Monitoring Plan. The quarterly PMT Progress Reports is submitted quarterly to the independent DMC for its review.

### 15.4 **Clinical Trial Monitoring**

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected; the reported clinical trial data are accurate, complete and verifiable from original source documents; the clinical trial complies with the currently approved protocol, applicable regulatory requirements and Good Clinical Practice (GCP). All monitoring for this study will be performed by the Office of Clinical Trial Monitoring (OCTM), within City of Hope's Safety & Data Quality Office.

The Investigator/Institution will permit the study monitors and appropriate regulatory authorities direct access to the study data and corresponding source data to allow verification of data accuracy, quality and integrity. The Investigator will ensure that OCTM or other quality assurance reviewers are provided access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space for such monitoring activities.

Monitoring visits conducted by the Office of Clinical Trial Monitoring (OCTM) observe and report compliance of FDA rules & regulations, ICH Guidelines, GCP and overall study conduct. The document, *Interim Monitoring Visit Standard Operating Procedure for City of Hope Investigator Initiated Clinical Trials* defines policies and processes utilized to conduct interim monitoring visits for clinical investigations. In accordance with the standard operating procedure, the following activities will be specified for and/or occur: Establish frequency of monitoring visits and confirm parameter of data review (percentage of subject data); number of subjects to be reviewed; and monitoring responsibilities to include (not limited to) verifying informed consent process, verifying study subject eligibility, verifying prompt reporting of Serious Adverse Events and Adverse Events, verifying study drug accountability, and verifying source data against case report forms. At the monitoring visit conclusion, the Monitor will document their findings and observations in an Interim Monitoring Visit report. The Monitor will provide the report to the Investigator, Study Team and independent Data Monitoring Committee (DMC). The DMC is responsible for reviewing the report and determining if any findings/observations noted by the Monitor are to be classified as Major or Minor deviations. If applicable, the DMC is also responsible for requesting the study team to implement and submit a corrective and preventive action plan (CAPA) if not previously submitted. A review of the CAPA will be conducted by the DMC to determine whether CAPA fully addresses issues identified and meets required preventative measures. The CAPA is to be submitted as a protocol deviation report for additional review by the IRB.

### 15.5 **Independent Data Monitoring Committee (DMC)**

Because this is an interventional first in human clinical trial that also involves an institutional conflict of interest, an independent DMC will be used. The independent DMC will be a committee comprised of clinical specialists with experience in vaccines and who have no direct relationship with the study or with City of Hope. The DMC will oversee the monitoring of safety of participants in the clinical trial, and the conduct, progress, validity, and integrity of the data.

The independent DMC will consist of 3 experts in vaccinology who will approve the DMC Charter at or prior to the first meeting. The charter will reflect the DMC specific functions, the proposed frequency of meetings, data items to be submitted for review, and acknowledgment that conflict of interest review will be utilized in the selection of the members to ensure independence.

A DMC chairperson will be appointed who will be responsible for conducting the meeting and for summarizing the minutes of the closed portions of the meeting. All meetings will be held by teleconference at prescheduled times related to safety study progress, data analysis at study conclusion, and at any time when there is an unexpected adverse event or DLTs that require a DMC review as determined by the Protocol Management Team (PMT) or the DMC Chairperson. In addition, the DMC will review all unexpected problems and all serious adverse events possibly, probably, or definitely attributed to the COH04S1 vaccine product. However, because of the defined rules of dose escalation, the DMC will not be assembled to authorize escalation from one dose to another dose cohort. However, the DMC will be notified that dose escalation has occurred under the protocol rules.

Data and safety will be reported to the DMC using the standard COH PMT Progress Report. The DMC will review and monitor toxicity and accrual data from this trial. This study will utilize a Phase 1 Tracking Log to monitor data and safety for dose escalation. The tracking log will be submitted along with the PMT Progress Report to the DMC. The DMC will review up-to-date participant accrual; summary of all adverse events captured via routine and expedited reporting; a summary of deviations; any response information; monitoring reports, and summary comments provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request. A review of outcome results (response, toxicity and adverse events) and factors external to the study (such as scientific or therapeutic developments) is discussed, and the DMC votes on the issue relating to the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator, statistician and study team.

Unblinding for participants may be performed at the Day 56 visit, as noted previously. In case of emergency un-blinding, the plan will be provided to the COH IRB and independent DMC as per COH institutional requirements (refer to **Section 4.2.6** for more details for all unblinding).

## 16.0 ETHICAL AND REGULATORY CONSIDERATIONS

### 16.1 Ethical Standard

This study will be conducted in conformance with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979) and the Declaration of Helsinki.

### 16.2 Regulatory Compliance

This study is to be conducted in compliance with the IRB approved protocol and according to the following considerations:

- US Code of Federal Regulations (CFR) governing clinical study conduct
  - Title 21 Part 11 – Electronic Records; Electronic Signatures
  - Title 21 Part 50 – Protection of Human Subjects
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators

- Title 21 Part 56 – Institutional Review Boards
- Title 21 Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies
- Title 21 Part 312 – Investigational New Drug Application
- Title 45 Part 46 – Protection of Human Subjects

- US Federal legislation, including but not limited to
  - Health Insurance Portability and Accountability Act of 1996
  - Section 801 of the Food and Drug Administration Amendments Act
- Applicable state and local laws. For research occurring in California, this includes but is not limited to State of California Health and Safety Code, Title 17

- Applicable institutional research policies and procedures

### 16.3 **External Institutional Review Board**

An external Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, will be used to review and approve this protocol, informed consent form and any additional documents that the IRB may need to fulfill its responsibilities (Investigator's Brochure, information concerning subject recruitment, payment or compensation procedures, management of Institutional Conflict of Interest, or other pertinent information) prior to initiation of the study. Revisions to approved documents will require review and approval by the IRB before the changes are implemented in the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

The IRB's written unconditional approval of the study protocol and the informed consent document must be in the possession of the investigator, before the study is initiated.

The IRB will be informed of serious unexpected, unanticipated adverse experiences, and unanticipated problems occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the subjects of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

### 16.4 **Informed Consent**

The Principal Investigator or IRB approved named designee will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. This will be done in a group webinar meeting as a preliminary review of the study. In addition, the meeting will review the experimental subject's bill of rights, and the HIPAA research authorization form. Prospective participants will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope. Prospective participants will be afforded sufficient time to consider whether or not to participate in the research.

The webinar will utilize a PowerPoint presentation approved by the IRB. After the study has been fully explained, and at a time not less than 24 hours since the group meeting to allow discussion with family and other advisors, each interested prospective participant will meet privately by video with the PI/IRB-approved designee. At this time, they will review the consent in detail, answer any additional questions, and, if satisfied that the subject in fully informed, the participant will be asked to sign the consent using DocuSign. The electronic copy of the informed consent will then be emailed to COVIDVACCINE@coh.org and signed by the PI/IRB-approved designee who participated in the consenting procedure. Legally authorized representatives will not be allowed to provide consent. The method of obtaining and

documenting the informed consent and the contents of the consent will comply with the ICH-GCP and all applicable regulatory requirements. The consent will be available in English, Spanish, and Mandarin and if the Spanish or Mandarin version is used, an IRB approved translator will be present during the consenting procedure.

A copy of the signed informed consent will be given to the participant and will be uploaded into the EPIC COH medical record of the participant. The original signed consent must be maintained by the investigator and available for inspection by sponsor designated representatives, or regulatory authority at any time.

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Subjects will be notified of significant new findings or information that may relate to the subject's willingness to continue participation, such as new risk information. In such cases, subjects may be asked to sign a revised informed consent document that has been reviewed and approved by the IRB.

### 16.5 **Participant Withdrawal**

Participants may withdraw from the study at any time and for any reason without prejudice. The withdrawal must be documented per institutional policies. The COH DCC should be promptly notified of the change in participant status.

Participant withdrawal may consist of any of the following with regard to study procedures and data collection:

- Withdrawal from study treatment, but agreement to continue with active study procedures and chart review and survival follow-up.
- Withdrawal from study treatment and all active procedures, but agreement for chart review and survival follow-up.
- Withdrawal from study treatment, all active procedures, and any future data collection.

Participants who agreed to the collection of research blood samples may withdraw consent to use their specimens, if they are not yet processed as detailed in the consent form. Once the PI and site PI is notified of this withdrawal of informed consent, the research specimens will not be used in any research. At that time, any of the existing specimens will be destroyed.

### 16.6 **Special and Vulnerable Populations**

#### 16.6.1 Women and Minorities

The study is open to anyone, regardless of gender, but this first-in-human study is open only to those who can speak English, Spanish, or Mandarin to eliminate any misinformation during the consenting procedure. Efforts will be made to extend the trial to others without regard to race or ethnicity in the Phase 2 and 3 portions of this vaccine evaluations. The vaccine development is sensitive to the need to observe for differences in outcome that correlate to gender, racial, or ethnic identity, and if such is noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

Pregnant women are excluded because the effects of the study vaccine on embryogenesis and reproduction are unknown.

16.6.2 Pediatric Population

Pediatric participants (< 18 years of age) are excluded from this study because safety and effectiveness of protocol therapy has not yet been defined for the study population. Additional studies may be performed in the pediatric population once safety and effectiveness of protocol therapy is defined in the adult study population.

16.6.3 HIV Positive Individuals

Participants with HIV are included based on specifications outlined in inclusion criteria. However, persons living with HIV will be included in the phase 3 portion of this vaccine development.

16.6.4 Vulnerable Populations

Per 45 CFR §46.111 (a)(3) and 45 CFR §46, Subparts B-D identifies children, prisoners, pregnant women, mentally incapacitated persons, and economically or educationally disadvantaged persons as vulnerable populations.

Economically/educationally disadvantaged persons are not actively targeted for participation in this trial and are excluded from participation at this phase of the vaccine development. This study does not pose additional risks for economically/educationally disadvantaged persons than for the general population and future trials of this vaccine may include these persons.

16.7 **Use of Unused (Leftover) Specimens Collected for this Trial**

Unused samples in existence at study completion (i.e. completion of all research activities under this study) will either be: (a) placed in a COH IRB approved biorepository (COH IRB 20447) with some clinical information and potentially PHI attached.

16.8 **Conflict of Interest**

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study Sponsor (City of Hope) prior to participation in this study. All City of Hope investigators will follow the City of Hope conflict of interest policy.

16.9 **Financial Obligations, Compensation, and Reimbursement of Participants**

Anti SARS-CoV-2 Vaccine (COH04S1) will be provided free of charge to participants.

Neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

Standard of care drugs or procedures provided during the course of study participation will be the responsibility of the research participant and/or the insurance carrier. The participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the participant were not in a research study.

In the event of physical injury to a participant resulting from research procedures, appropriate medical treatment will be available at City of Hope to the injured participant. There are no plans for City of Hope to provide financial compensation in the event of physical injury to a participant.

Subjects will receive financial compensation for study participation. There are 14-16 visits to the clinic, and $50 will be provided per visit for a total compensation of up to approximately $800 per subject. Any subject who is compensated $600 or more within one calendar year will be provided with a 1099 form for purposes of preparing income taxes for that year.

### 16.10 **Publication/ Data Sharing**

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by City of Hope for the purposes of performing the study, will be published or passed on to any third party without the written approval of the Study PI. Any investigator involved with this study is obligated to provide City of Hope with complete test results and all data derived from the study.

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

In accordance with the U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801, this trial will be registered onto ClinicalTrials.gov. Results will be reported on ClinicalTrials.gov generally within 12 months after the completion date unless criteria to delay submission are met per the final rule.

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## APPENDIX A: EXPEDITED REPORTING COVERSHEET

### NOTIFICATION OF UNANTICIPATED PROBLEM/SERIOUS ADVERSE EVENT

### For Use by Participating Institutions Only

THIS FORM ALONG WITH A COPY OF THE MEDWATCH 3500 OR IRB REPORTING FORM MUST BE **EMAILED** TO DCC@COH.ORG WITHIN 24 HOURS OF KNOWLEDGE OF ONSET OF SERIOUS ADVERSE EVENT OR UNANCTICIPATED PROBLEM

**COH IRB #_____- Participating Site IRB # _____**

| | |
|---|---|
| From: | Date: |
| Phone No.: | Email: |
| Reporting Investigator: | |
| Event: | |
| Participant ID: | Institution: |
| Date Event Met Reporting Criteria (as defined in protocol): | |

| | |
|---|---|
| Type of Report: | ☐ Initial ☐ Follow-up |
| DMID Grade: | ☐ G1/mild ☐ G2/moderate ☐ G3/severe ☐ G4/life threatening ☐ G5 |
| Attribution to **Agent xx:** | ☐ Not Applicable* ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite |
| Attribution to **Agent xxy**: | ☐ Not Applicable* ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite |
| Historical/Known Correlation to **Agent xx**: | ☐ Expected ☐ Unexpected |
| Historical/Known Correlation to **Agent xxy**: | ☐ Expected ☐ Unexpected |
| Meets Definition of Serious AE: | ☐ Serious ☐ Non-serious |
| Meets Definition of Unanticipated Problem: | ☐ UP ☐ Not a UP |
| Has the event been reported to the following institution's IRB? | ☐ No ☐ Yes; Date:_____/____/_____ |

*** Not Applicable should only be used if subject has not received this agent.**

| | |
|---|---|
| Authorized Investigator Signature: | Date:____/____/____ |

## APPENDIX B: DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE

**ABBREVIATIONS:**

Abbreviations utilized in the Table:

ULN = Upper Limit of Normal

LLN = Lower Limit of Normal

Rx = Therapy

Req = Required

Mod = Moderate

IV = Intravenous

ADL = Activities of Daily Living

Dec = Decreased

**ESTIMATING SEVERITY GRADE**

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

**GRADE 1**

**Mild** Transient or mild discomfort (< 48 hours); no medical intervention/therapy required

**GRADE 2**

**Moderate** Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required

**GRADE 3**

**Severe** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible

**GRADE 4**

**Life-threatening** Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

**SERIOUS OR LIFE-THREATENING AEs**

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

**COMMENTS REGARDING THE USE OF THESE TABLES**

Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.

For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.

Criteria are generally grouped by body system.

Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria

| **HEMATOLOGY** | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Hemoglobin | 9.5 - 10.5 gm/dL | 8.0 - 9.4gm/dL | 6.5 - 7.9 gm/dL | < 6.5 gm/dL |
| Absolute Neutrophil Count | 1000-1500/$mm^3$ | 750-999/$mm^3$ | 500-749/$mm^3$ | <500/$mm^3$ |
| Platelets | 75,000-99,999/$mm^3$ | 50,000-74,999/$mm^3$ | 20,000-49,999/$mm^3$ | <20,000/$mm^3$ |
| WBCs | 11,000-13,000/$mm^3$ | 13,000-15,000/$mm^3$ | 15,000-30,000/$mm^3$ | >30,000 or <1,000/$mm^3$ |
| % Polymorphonuclear Leucocytes + Band Cells | > 80% | 90 – 95% | >95% | --------- |
| Abnormal Fibrinogen | Low: 100-200 mg/dL<br>High: 400-600 mg/dL | Low: <100 mg/dL<br>High: >600 mg/dL | Low: < 50 mg/dL<br>--------- | Fibrinogen associated with gross bleeding or with disseminated coagulation |
| Fibrin Split Product | 20-40 mcg/ml | 41-50 mcg/ml | 51-60 mcg/ml | > 60 mcg/ml |
| Prothrombin Time (PT) | 1.01 - 1.25 x ULN | 1.26-1.5 x ULN | 1.51 -3.0 x ULN | >3 x ULN |
| Activated Partial Thromboplastin (APPT) | 1.01 -1.66 x ULN | 1.67 - 2.33 x ULN | 2.34 - 3 x ULN | > 3 x ULN |
| Methemoglobin | 5.0 - 9.9 % | 10.0 - 14.9 % | 15.0 - 19.9% | > 20.0 % |

| **CHEMISTRIES** | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Hyponatremia | 130-135 mEq/L | 123-129 mEq/L | 116-122 mEq/L | < 116 mEq/L or abnormal sodium *with* mental status changes or seizures |
| Hypernatremia | 146-150 mEq/L | 151-157 mEq/L | 158-165 mEq/L | > 165 mEq/L or abnormal sodium *with* mental status changes or seizures |
| Hypokalemia | 3.0 - 3.4 mEq/L | 2.5 - 2.9 mEq/L | 2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required | < 2.0 mEq/L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia |
| Hyperkalemia | 5.6 - 6.0 mEq/L | 6.1 - 6.5 mEq/L | 6.6 - 7.0 mEq/l | > 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia |
| Hypoglycemia | 55-64 mg/dL | 40-54 mg/dL | 30-39 mg/dL | <30 mg/dL or abnormal glucose *with* mental status changes or coma |
| Hyperglycemia (nonfasting and no prior diabetes) | 116 - 160 mg/dL | 161- 250 mg/dL | 251 - 500 mg/dL | > 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures |
| Hypocalcemia (corrected for albumin) | 8.4 - 7.8 mg/dL | 7.7 - 7.0 mg/dL | 6.9 - 6.1 mg/dL | < 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany |

| **CHEMISTRIES (continued)** | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Hypercalcemia (correct for albumin) | 10.6 - 11.5 mg/dL | 11.6 - 12.5 mg/dL | 12.6 - 13.5 mg/dL | > 13.5 mg/dL or abnormal calcium *with* life threatening arrhythmia |
| Hypomagnesemia | 1.4 - 1.2 mEq/L | 1.1 - 0.9 mEq/L | 0.8 - 0.6 mEq/L | < 0.6 mEq/L or abnormal magnesium *with* life-threatening arrhythmia |
| Hypophosphatemia | 2.0 - 2.4 mg/dL | 1.5 -1.9 mg/dL or replacement Rx required | 1.0 -1.4 mg/dL intensive therapy or hospitalization required | < 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia |
| Hyperbilirubinemia (when accompanied by any increase in other liver function test) | 1.1 - <1.25 x ULN | 1.25 - <1.5 x ULN | 1.5 – 1.75 x ULN | > 1.75 x ULN |
| Hyperbilirubinemia (when other liver function are in the normal range) | 1.1 - <1.5 x ULN | 1.5 - <2.0 x ULN | 2.0 – 3.0 x ULN | > 3.0 x ULN |
| BUN | 1.25 - 2.5 x ULN | 2.6 - 5 x ULN | 5.1 - 10 x ULN | > 10 x ULN |
| Hyperuricemia (uric acid) | 7.5 – 10.0 mg/dL | 10.1 – 12.0 mg/dL | 12.1 – 15.0 mg/dL | >15.0 mg/dL |
| Creatinine | 1.1 - 1.5 x ULN | 1.6 - 3.0 x ULN | 3.1 - 6 x ULN | > 6 x ULN or dialysis required |

| ENZYMES | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| AST (SGOT) | 1.1 - <2.0 x ULN | 2.0 – <3.0 x ULN | 3.0 – 8.0 x ULN | > 8 x ULN |
| ALT (SGPT) | 1.1 - <2.0 x ULN | 2.0 – <3.0 x ULN | 3.0 – 8.0 x ULN | > 8 x ULN |
| GGT | 1.1 - <2.0 x ULN | 2.0 – <3.0 x ULN | 3.0 – 8.0 x ULN | > 8 x ULN |
| Alkaline Phosphatase | 1.1 - <2.0 x ULN | 2.0 – <3.0 x ULN | 3.0 – 8.0 x ULN | > 8 x ULN |
| Amylase | 1.1 - 1.5 x ULN | 1.6 - 2.0 x ULN | 2.1 - 5.0 x ULN | > 5.1 x ULN |
| Lipase | 1.1 - 1.5 x ULN | 1.6 - 2.0 x ULN | 2.1 - 5.0 x ULN | > 5.1 x ULN |

| URINALYSIS | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Proteinuria | 1+<br>or<br>200 mg - 1 g m loss/day | 2-3+<br>or<br>1- 2 g m loss/day | 4+<br>or<br>2-3.5 g m loss/day | nephrotic syndrome<br>or<br>> 3.5 g m loss/day |
| Hematuria | microscopic only<br><10 rbc/hpf | gross, no clots<br>>10 rbc/hpf | gross, with or without clots, OR red blood cell casts | obstructive or required transfusion |

| CARDIOVASCULAR | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Cardiac Rhythm | | asymptomatic, transient signs, no Rx required | recurrent/persistent; symptomatic Rx required | unstable dysrythmia; hospitalization and treatment required |
| Hypertension | transient increase > 20 mm/Hg; no treatment | recurrent, chronic increase > 20mm/Hg. /treatment required | acute treatment required; outpatient treatment or hospitalization possible | end organ damage or hospitalization required |
| Hypotension | transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required | symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment | requires IV fluids; no hospitalization required | mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment |
| Pericarditis | minimal effusion | mild/moderate asymptomatic effusion, no treatment | symptomatic effusion; pain; EKG changes | tamponade; pericardiocentesis or surgery required |
| Hemorrhage, Blood Loss | microscopic/occult | mild, no transfusion | gross blood loss; 1-2 units transfused | massive blood loss; > 3 units transfused |

| RESPIRATORY | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Cough | transient- no treatment | persistent cough; treatment responsive | Paroxysmal cough; uncontrolled with treatment | ---------- |
| Bronchospasm, Acute | transient; no treatment; 70% - 80% $FEV_1$ of peak flow | requires treatment; normalizes with bronchodilator; $FEV_1$ 50% - 70% (of peak flow) | no normalization with bronchodilator; $FEV_1$ 25% - 50% of peak flow; or retractions present | cyanosis: $FEV_1$ < 25% of peak flow or intubation necessary |
| Dyspnea | dyspnea on exertion | dyspnea with normal activity | dyspnea at rest | dyspnea requiring Oxygen therapy |

| GASTROINTESTINAL | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Nausea | mild or transient; maintains reasonable intake | moderate discomfort; intake decreased significantly; some activity limited | no significant intake; requires IV fluids | hospitalization required; |
| Vomiting | 1 episode in 24 hours | 2-5 episodes in 24 hours | >6 episodes in 24 hours or needing IV fluids | physiologic consequences requiring hospitalization or requiring parenteral nutrition |
| Constipation | requiring stool softener or dietary modification | requiring laxatives | obstipation requiring manual evacuation or enema | obstruction or toxic megacolon |
| Diarrhea | mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week | moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week | >7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required | hypotensive shock or physiologic consequences requiring hospitalization |
| Oral Discomfort/Dysphagia | mild discomfort; no difficulty swallowing | some limits on eating/drinking | eating/talking very limited; unable to swallow solid foods | unable to drink fluids; requires IV fluids |

| NEUROLOGICAL | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Neuro-Cerebellar | slight incoordination dysdiadochokinesis | intention tremor, dysmetria, slurred speech; nystagmus | locomotor ataxia | incapacitated |
| Psychiatric | mild anxiety or depression | moderate anxiety or depression; therapy required; change in normal routine | severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation | acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations |
| Muscle Strength | subjective weakness no objective symptoms/signs | mild objective signs/symptoms no decrease in function | objective weakness function limited | paralysis |
| Paresthesia (burning, tingling, etc.) | mild discomfort; no treatment required | moderate discomfort; non-narcotic analgesia required | severe discomfort; or narcotic analgesia required with symptomatic improvement | incapacitating; or not responsive to narcotic analgesia |
| Neuro-sensory | mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing | moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical | severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities) | sensory loss involves limbs and trunk; paralysis; or seizures |

| MUSCULOSKELATEL | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Arthralgia (joint pain) | mild pain not interfering with function | moderate pain, analgesics and/or pain interfering with function but not with activities of daily living | severe pain; pain and/or analgesics interfering with activities of daily living | disabling pain |
| Arthritis | mild pain with inflammation, erythema or joint swelling – but not interfering with function | moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living | severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living | permanent and/or disabling joint distruction |
| Myalgia | myalgia with no limitation of activity | muscle tenderness (at other than injection site) or with moderate impairment of activity | severe muscle tenderness with marked impairment of activity | frank myonecrosis |

| SKIN | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Mucocutaneous | erythema; pruritus | diffuse, maculo papular rash, dry desquamation | vesiculation or moist desquamation or ulceration | exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery |
| Induration | < 15mm | 15-30 mm | >30mm | |
| Erythema | < 15mm | 15-30 mm | >30mm | |
| Edema | < 15mm | 15-30 mm | >30mm | |
| Rash at Injection Site | < 15mm | 15-30 mm | >30mm | |
| Pruritus | slight itching at injection site | moderate itching at injection extremity | itching over entire body | |

| SYSTEMIC | | | | |
|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Allergic Reaction | pruritus without rash | localized urticaria | generalized urticaria; angioedema | anaphylaxis |
| Headache | mild, no treatment required | transient, moderate; treatment required | severe; responds to initial narcotic therapy | intractable; requires repeated narcotic therapy |
| Fever: oral | 37.7 - 38.5 C or 100.0 - 101.5 F | 38.6 - 39.5 C or 101.6 - 102.9 F | 39.6 - 40.5 C or 103 - 105 F | > 40 C or > 105 F |
| Fatigue | normal activity reduced < 48 hours | normal activity decreased 25-50% > 48 hours | normal activity decreased > 50% can't work | unable to care for self |

## APPENDIX C: SUBJECT TELEPHONE CONTACT RECORD AND DIALOG

Subject Telephone Contact Record

| | |
|---|---|
| Subject ID: _____ | Date of Call: _____/_____/_____ |
| Protocol # | Time of Call: _____:_____ |

**Day 1-3 Post Vaccination #_____**

**Have you observed changes at injection sites?**

| | | | | | |
|---|---|---|---|---|---|
| injection-site pain | ☐ Yes | ☐ No | swelling | ☐ Yes | ☐ No |
| rash | ☐ Yes | ☐ No | redness | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**Any Adverse Events since vaccination injection?**

| | | | | | |
|---|---|---|---|---|---|
| any chest pain | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| shortness of breath | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| fainting spell | ☐ Yes | ☐ No | near fainting spell | ☐ Yes | ☐ No |
| dry cough | ☐ Yes | ☐ No | fever | ☐ Yes | ☐ No |
| myalgia | ☐ Yes | ☐ No | headache | ☐ Yes | ☐ No |
| nausea | ☐ Yes | ☐ No | fatigue | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**If Yes, notify the P.I. and describe symptoms below:**

_____

_____

_____

Phone call made by: _____

Signature and Date: _____ _____/_____/_____

**Day 4-6 Post Vaccination #_____**

**Have you observed changes at injection sites?**

| | | | | | |
|---|---|---|---|---|---|
| injection-site pain | ☐ Yes | ☐ No | swelling | ☐ Yes | ☐ No |
| rash | ☐ Yes | ☐ No | redness | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**Any Adverse Events since vaccination injection?**

| | | | | | |
|---|---|---|---|---|---|
| any chest pain | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| shortness of breath | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| fainting spell | ☐ Yes | ☐ No | near fainting spell | ☐ Yes | ☐ No |
| dry cough | ☐ Yes | ☐ No | fever | ☐ Yes | ☐ No |
| myalgia | ☐ Yes | ☐ No | headache | ☐ Yes | ☐ No |
| nausea | ☐ Yes | ☐ No | fatigue | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**If Yes, notify the P.I. and describe symptoms below:**

_____

_____

_____

Phone call made by: _____

Signature and Date: _____ _____/_____/_____

| | |
|---|---|
| Subject ID: _____ | Date of Call: _____/_____/_____ |
| Protocol # | Time of Call: _____:_____ |

**Day 29-31 Post Vaccination #_____**

**Have you observed changes at injection sites?**

| | | | | | |
|---|---|---|---|---|---|
| injection-site pain | ☐ Yes | ☐ No | swelling | ☐ Yes | ☐ No |
| rash | ☐ Yes | ☐ No | redness | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**Any Adverse Events since vaccination injection?**

| | | | | | |
|---|---|---|---|---|---|
| any chest pain | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| shortness of breath | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| fainting spell | ☐ Yes | ☐ No | near fainting spell | ☐ Yes | ☐ No |
| dry cough | ☐ Yes | ☐ No | fever | ☐ Yes | ☐ No |
| myalgia | ☐ Yes | ☐ No | headache | ☐ Yes | ☐ No |
| nausea | ☐ Yes | ☐ No | fatigue | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**If Yes, notify the P.I. and describe symptoms below:**

_____

_____

_____

Phone call made by: _____

Signature and Date: _____ _____/_____/_____

**Day 32-34 Post Vaccination #_____**

**Have you observed changes at injection sites?**

| | | | | | |
|---|---|---|---|---|---|
| injection-site pain | ☐ Yes | ☐ No | swelling | ☐ Yes | ☐ No |
| rash | ☐ Yes | ☐ No | redness | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**Any Adverse Events since vaccination injection?**

| | | | | | |
|---|---|---|---|---|---|
| any chest pain | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| shortness of breath | ☐ Yes | ☐ No | awareness of heart beats | ☐ Yes | ☐ No |
| fainting spell | ☐ Yes | ☐ No | near fainting spell | ☐ Yes | ☐ No |
| dry cough | ☐ Yes | ☐ No | fever | ☐ Yes | ☐ No |
| myalgia | ☐ Yes | ☐ No | headache | ☐ Yes | ☐ No |
| nausea | ☐ Yes | ☐ No | fatigue | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**If Yes, notify the P.I. and describe symptoms below:**

_____

_____

_____

Phone call made by: _____

Signature and Date: _____ _____/_____/_____

| | |
|---|---|
| Subject ID: _____<br><br>Protocol # | Date of Call: _____/_____/_____<br>Time of Call: _____:_____<br>Date of Injection: _____ |

**After Day 35 Post Vaccination #_____**

**Have you observed changes at injection sites?**

| | | | | | |
|---|---|---|---|---|---|
| injection-site pain | ☐ Yes | ☐ No | swelling | ☐ Yes | ☐ No |
| rash | ☐ Yes | ☐ No | redness | ☐ Yes | ☐ No |
| bruising | ☐ Yes | ☐ No | other | ☐ Yes | ☐ No |

**Any Adverse Events since vaccination injection?**

| | | | | | |
|---|---|---|---|---|---|
| insomnia | ☐ Yes | ☐ No | diarrhea | ☐ Yes | ☐ No |
| bone pain | ☐ Yes | ☐ No | fever | ☐ Yes | ☐ No |
| vomiting | ☐ Yes | ☐ No | headache | ☐ Yes | ☐ No |
| nausea | ☐ Yes | ☐ No | If yes, rate headache on scale 1-10 with 10 | | |
| fatigue | ☐ Yes | ☐ No | being the worst): _____ | | |
| muscle ache | ☐ Yes | ☐ No | arthralgias:(joint pain) | ☐ Yes | ☐ No |
| skin rash (new/worse) | ☐ Yes | ☐ No | need for Tylenol | ☐ Yes | ☐ No |
| other | ☐ Yes | ☐ No | | | |

**If Yes, notify the P.I. and describe symptoms below:**

_____

_____

_____

Phone call made by: _____

Signature and Date: _____ _____/_____/_____

## APPENDIX D: SCREENING SPECIMEN COLLECTION AND SHIPPING GUIDELINES TO TGEN

| Tube Type | Collection details | Site of collection | Post-collection instructions |
|---|---|---|---|
| Red/gray top SST tube | 1. Label tube with the COH protocol number, donor ID number, donor's initials, DOB, and time/date of blood draw.<br>2. This is a single collection at screening.<br>3. Collect **1 x 8.5 mL in red/gray top SST tube.**<br>4. Invert the tube **5 times** immediately after collection.<br>5. Clot for **30 minutes** in a vertical position in a tube rack at room temperature.<br>6. Centrifuge the tube within 2 hours of collection to separate **serum** from cells, or refrigerate at 2-8ºC until centrifugation.<br>7. Spin the tube at room temperature at a speed of 1000 to 1300 RCF for 10 minutes in a swinging bucket centrifuge and 15 minutes in a fixed-angle centrifuge. | **COH** | **Ship at 2-8ºC on ice packs** by overnight carrier<br>To:<br>Erin Kelley<br>TGen North<br>3051 W Shamrell Blvd #106,<br>Flagstaff, AZ 86005 |

**Shipping guidelines**

All biological material must be shipped according to applicable government and International Air Transport Association (IATA) regulations.

Shipping guidelines can also be found on the FedEx website.

- Aim to ship samples on a **Monday through Wednesday**. If this is not feasible, advance arrangements should be made with Dr. Altin (jaltin@tgen.org) and Erin Kelley (ekelley@tgen.org) at TGen.
- **Blood and plasma samples will be sent overnight at 2-8°C on ice packs** in an appropriate container via FedEx.
- **On the day of shipment**, email Dr. Altin (jaltin@tgen.org) and Erin Kelley (ekelley@tgen.org) at TGen the FedEx shipment #.

Dr. John Altin/ Erin Kelley
TGen North
3051 W Shamrell Blvd #106,
Flagstaff, AZ 86005

## APPENDIX E: ROUTINE UNBLINDING OF IRB 20447

At the Day 56 visit, all subjects will be informed whether they had received the COH04S1 investigational vaccine prime and boost, prime only or the placebo only. No information about the dose level will be shared.

**Request for unblinding information:**

**FROM**: Principal Investigator (PI) for IRB 20447

**TO**: Investigational Drug Services (IDS) Pharmacy OR Data Coordinating Center (DCC)

This is a request to unblind treatment information for research participant (enter UPN), as follows:

☐ Vaccine prime and boost ☐ Vaccine prime only ☐ Placebo only

This information is requested in order to inform the participant at the Day 56 visit.

**PI**:

PRINT NAME:……. DATE: ……..

SIGNATURE: ……..

**IDS Pharmacy OR DCC Representative**:

PRINT NAME:……. DATE: ……..

SIGNATURE: ……..

## Supplementary methods

### COH04S1 generation

We designed three unique synthetic sub-genomic sMVA fragments based on the MVA genome sequence published previously (1). The entire sMVA was cloned as three fragments in Escherichia coli as bacterial artificial chromosome (BAC) clones using highly efficient BAC recombination techniques. In brief, unmodified full-length S and N antigen sequences based on the Wuhan-Hu-1 reference strain were inserted into commonly used MVA insertion sites located at different positions within the three sMVA fragments. The sMVA SARS-CoV-2 virus was reconstituted with fowl pox virus (FPV) as a helper virus upon co-transfection of the DNA plasmids into BHK-21 cells, which are non-permissive for FPV (2). The virus stocks were propagated on chicken embryo fibroblast (CEF) cells, which are commonly used for MVA vaccine production. The infected CEF cells were grown further, and the infected cells were harvested, freeze-thawed and stored at -80ºC, then titrated on CEF cells to grow expanded virus stocks. To transition vaccine candidates into clinical production, a double plaque-purified virus isolate derived from the previously constructed sMVA-N/S vaccine vector (3) was selected and named COH04S1. The clinical vaccine stock used in this trial was produced on CEF at the COH Center for Biomedicine and Genetics (CBG). The vaccine was manufactured as a liquid formulation containing PBS with 7·5% lactose.

### Dose-escalation rules

Dose-escalation was explored based on a queue-based design (IQ 3+3) adapted to healthy subjects' study using a biologic and designed to rapidly complete the phase 1 portion subject to specific traditional constraints on subject risk(4). This design uses the MOD as the event of concern and required the first participant at each dose level to be observed for 7 days after injection before any additional participants were permitted on that dose level. The detailed decision grid is available in "VaccineDecisionGrid.xlsx" (https://oneq.netlify.app/). There were two key safety signals used to limit risk: MOD which were used to limit subject risk per the dose escalation design, and toxicities exceeding MOD which would halt all accrual on all dose levels. Additionally, if a third or more of participants experienced a MOD at a dose level, that dose would hold accrual pending review by the DMC. By adapting to the subject queue, this design reduced the expected phase 1 study duration when compared to a non-queue-based 3+3 design by approximately 21%.
The expansion cohorts were intended to provide additional safety data and to help guide dose selection based on immunological correlatives. The design was expected to have between 19-23 subjects at a dose level receiving both prime and boost (including sentinel subjects). The conduct of the expansion cohorts was modified due to EUA vaccine availability.

### Enzyme-linked immunosorbent assay (ELISA) for IgG binding antibody detection

SARS-CoV-2-specific binding antibodies detected by indirect ELISA utilizing purified S, RBD, and N proteins (Sino Biological 40589-V08B1, 40592-V08H, 40588-V08B). Briefly, 96-well plates (Costar 3361) were coated with 100µl/well of S, RBD, or N proteins at a concentration of 1µg/ml in PBS pH 7·4 and incubated overnight at 4°C. Plates were washed 5X with wash buffer (0·1% Tween-20/PBS), then blocked with 250µl/well of assay buffer (0·5% casein/154mM NaCl/10mM Tris-HCl/0·1% Tween-20 [pH 7·6]/8% Normal goat serum) for 2 hours 37°C. After washing, 3-fold diluted heat-inactivated serum in blocking buffer was added to the plates starting from a dilution of 1:150. Plates were wrapped in foil and incubated 2 hours at 37°C. Plates were washed and 1:3,000 dilution of anti-human IgG HRP secondary antibody (BioRad 204005) in assay buffer was added for 1 hour at room temperature. Plates were washed and developed with 1 Step TMB-Ultra (Thermo Fisher 34029). After 2-4 minutes the reaction was stopped with 1M $H_2SO_4$ and 450nm absorbance was immediately quantified on FilterMax F3 (Molecular Devices). Positive and negative controls were included in each plate and consisted of serum pools of SARS-CoV-2 seropositive (S, RBD, and N endpoint titer 36450) and seronegative individuals (S, RBD, and N endpoint titer <150). Endpoint titers were calculated as the highest dilution to have an absorbance >0·100.

### Pseudovirus production

SARS-CoV-2 pseudovirus was produced using a plasmid lentiviral system based on pALD-gag-pol, pALD-rev, and pALD-GFP (Aldevron). Plasmid pALD-GFP was modified to express Firefly luciferase (pALD-Fluc). Plasmid

pCMV3-S (Sino Biological VG40589-UT) was utilized and modified to express SARS-CoV-2 Wuhan-Hu-1 S with D614G modification. Customized gene sequences cloned into pTwist-CMV-BetaGlobin (Twist Biosciences) were used to express SARS-CoV-2 VOC-specific S variants (5).
Mutations in the VOC-specific S variants compared to Wuhan-Hu-1 S included:

Alpha VOC: Δ69/70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H.
Beta VOC: L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G, A701V.
Gamma VOC: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F.
Delta VOC: T19R, G142D, Δ156-157, R158G, A222V, L452R, T478K, D614G, P681R, D950N.

All S antigens were expressed with C-terminal 19aa deletion. A transfection mixture was prepared 1ml OptiMEM that contained 30µl of TransIT-Lenti transfection reagent (Mirus MIR6600) and 6µg pALD-Fluc, 6µg pALD-gag-pol, 2·4µg pALD-rev, and 6·6µg S expression plasmid. The transfection mix was added to $5x10^6$ HEK293T/17 cells (ATCC CRL11268) seeded the day before in 10 cm dishes and the cells were incubated for 72h at 37°C. Supernatant containing pseudovirus was harvested and frozen in aliquots at -80°C. Lentivirus was titrated using the Lenti-XTM p24 Rapid Titer Kit (Takara) according to the manufacturer's instructions.

**Pseudovirus neutralization assay**

SARS-CoV-2 pseudoviruses were titrated in vitro to calculate the virus stock amount that equals 100,000-200,000 relative luciferase units. Flat-bottom 96-well plates were coated with 100µl poly-L-lysine (0·01%). Serial 2-fold serum dilutions starting from 1:20 were prepared in 50µl media and added to the plates in triplicates, followed by 50µl of pseudovirus. Plates were incubated overnight at 4°C. The following day, 10,000 HEK293T-ACE2 cells (6) were added to each well in the presence of 3µg/ml polybrene and plates were incubated at 37°C. After 48h of incubation, luciferase lysis buffer (Promega E1531) was added and luminescence was quantified using SpectraMax L (Molecular Devices) after adding Luciferase Assay Reagent (Promega E1483, 100µl/well). For each plate, positive (pseudovirus only) and negative (cells only) controls were added. The neutralization titer for each dilution was calculated as follows: NT = [1−(mean luminescence with immune sera/mean luminescence without immune sera)] × 100. The titers that gave 50% neutralization (NT50) were calculated by determining the linear slope of the graph plotting NT versus serum dilution by using the next higher and lower NT using Office Excel (v2019).

**WHO reference panel for anti-SARS-CoV-2 immunoglobulin**

WHO international reference panel 20/268 was obtained from the National Institute for Biological Standards and Control (NIBSC), reconstituted with distilled water, and analyzed by ELSA and PsV neutralization assay. The products were derived using pooled plasma samples from individuals recovered from COVID-19 and negative control plasma obtained from healthy blood donors before 2019. 20/268 included the following products ranked based on SARS-CoV-2 Ab titers: 20/150 (high), 20/148 (mid), 20/144 (low S, high N), 20/140 (low), and 20/142 (negative).

**IFNγ/IL-4-secreting T cells quantification by ELISPOT**

Peripheral blood mononuclear cells (PBMC) were isolated from fresh blood using Ficoll and counted using Luna-FL cell counter (Logos Biosystems). Frozen PBMCs were thawed and IFNγ/IL-4 secretion evaluated using human IFNγ/IL-4 FluoroSpot FLEX kit (Mabtech, X-01A16B) following manufacturer instructions. Briefly, 150,000 cells/well in CTL-test serum free media (Immunospot CTLT-010) were added to duplicate wells and stimulated with peptide pools (15-mers, 11 aa overlap, >70% purity). Spike peptide library (GenScript) consisted of 316 peptides and was divided into 4 sub-pools spanning the S1 and S2 domains (1S1=1-86; 1S2=87-168; 2S1=169-242; 2S2=243-316; peptides 173 and 304-309 were not successfully synthesized therefore excluded from the pools). Nucleocapsid (GenScript) and Membrane (in house synthesized) libraries consisted of 102 and 53 peptides, respectively. Each peptide pool (2µg/ml) and αCD28 (0·1µg/ml, Mabtech) were added to the cells and plates were incubated for 48h at 37°C. Control cells (50,000/well) were stimulated with PHA (10 µg/ml). After incubation, plates were washed with PBS and primary and secondary antibodies were added according to manufacturer's protocol. Fluorescent spots were acquired using CTL S6 Fluorocore (Immunospot). For each sample, spots in unstimulated DMSO-only control wells were subtracted from spots in stimulated wells. Zero spots were indicated as one. Total spike response was calculated as the sum of the response to each spike sub-pool. Fifty spots/$10^6$ cells were chosen as the arbitrary threshold discriminating negative from positive samples for the calculation of the fold-increase.

**SARS-CoV-2-specific CD137+ T cells and memory phenotype measurements**

SARS-CoV-2-specific T cells response was evaluated in sentinel subjects with available PBMCs samples (DL1: N=4; DL2: N=7, and DL3: N=4). Concentrations of T-cells expressing the 4-1BB (CD137) activation marker and the CD28, CD45RA memory phenotype profiles were measured following 24 hours stimulation with either S-15mer megapool (7) (overlapping 15-mers by 10aa) or N peptide library (Genscript), as previously detailed (8). PBMC for each time point were labeled and analyzed by fluorescence-activated cytometry (FC; Gallios™, Beckman Coulter with Kaluza analysis software, Brea, CA). Concentrations of S- or N-specific $CD3^+$ $CD4^+$ $CD137^+$ and $CD3^+$ $CD8^+$ $CD137^+$ T-cells were longitudinally measured using multiparameter (6 colors) FC. The lower limit of detection for $CD137^+$ T-cells was 0·02% or 0·1 cells/µl. When either S- or N- $CD3^+$ $CD8^+$ $CD137^+$ T-cell or $CD3^+$ $CD4^+$ $CD137^+$ T-cell populations were ≥0·2% a further analysis for CD28 and CD45RA memory membrane markers was performed. $CD45RA^+$ $CD28^+$ cells were classified as naïve/naïve-like, $CD45RA^-$ $CD28^+$ cells were classified as central memory (TCM), and $CD28^-$ cells were classified as effector T-cells. Within the effector T-cell group, two subpopulations were identified: $CD45RA^-$ $CD28^-$ cells (T effector memory, TEM) and $CD45RA^+$ $CD28^-$ effector "revertant" T-cells, re-expressing the RA isoform of the CD45 surface marker (TEMRA) (9, 10).

## Supplementary tables and figures

**Table S1. Local and systemic adverse reactions after one and two vaccinations in the open-label dose-escalation portion of the trial.**

| AE | DL1/DL1, n = 4 | | DL2/DL2, n = 7 | | | DL3/DL3, n = 6 | |
|---|---|---|---|---|---|---|---|
| | 1st shot, n = 4 | 2nd shot, n = 4 | 1st shot, n = 7 | | 2nd shot, n = 6 | 1st shot, n = 6 | 2nd shot, n = 6 |
| | Grade 1 | Grade 1 | Grade 1 | Grade 2 | Grade 1 | Grade 1 | Grade 1 |
| Injection site reaction | 3/4 (75%) | 3/4 (75%) | 6/7 (86%) | | 3/6 (50%) | 6/6 (100%) | 4/6 (67%) |
| Fatigue | 1/4 (25%) | | 4/7 (57%) | 1/7 (14%) | 4/6 (67%) | 2/6 (33%) | 2/6 (33%) |
| Headache | 1/4 (25%) | 1/4 (25%) | 5/7 (71%) | | 3/6 (50%) | 3/6 (50%) | |
| Myalgia | | | 2/7 (29%) | | 2/6 (33%) | 1/6 (17%) | 1/6 (17%) |
| Chills | | | | | 1/6 (17%) | 2/6 (33%) | |
| Diarrhea | | | 1/7 (14%) | | | 1/6 (17%) | 1/6 (17%) |
| Fever | | | | | | 3/6 (50%) | |
| Nausea | | | 2/7 (29%) | | | | |
| Increased alanine aminotransferase | | 1/4 (25%) | | | | | |
| Generalized muscle weakness | | | 1/7 (14%) | | | | |
| Hypertension | | | 1/7 (14%) | | | | |
| Hypoglycemia | | | | | | | 1/6 (17%) |
| Hyponatremia | 1/4 (25%) | | | | | | |
| Insomnia | | | | | | | 1/6 (17%) |
| Nasal congestion | 1/4 (25%) | | | | | | |
| Pruritus | | | | | 1/6 (17%) | | |
| Rash maculopapular | | | | | 1/6 (17%) | | |
| Sinus tachycardia | | 1/4 (25%) | | | | | |
| Urticaria | | | | | 1/6 (17%) | | |
| Anxiety | | | | 1/7 (14%) | | | |

Grade 2 notes: Anxiety and fatigue in same subject was classified a MOD toxicity. Both resolved within 2 weeks.
AE, adverse event; DL, dose level.

**Table S2. Local and systemic adverse reactions after one and two vaccinations in the DL2/DL3 RCT portion of the trial.**

| AE | DL2/DL2, n = 2 | | DL3/DL3, n = 4 | | | |
|---|---|---|---|---|---|---|
| | 1st shot, n = 2 | 2nd shot, n = 2 | 1st shot, n = 4 | | 2nd shot, n = 3 | |
| | Grade 1 | Grade 1 | Grade 1 | Grade 2 | Grade 1 | Grade 2 |
| Injection site reaction | 2/2 (100%) | 2/2 (100%) | 3/4 (75%) | | 2/3 (67%) | |
| Fatigue | 2/2 (100%) | 1/2 (50%) | 2/4 (50%) | | 1/3 (33%) | 1/3 (33%) |
| Headache | 2/2 (100%) | 1/2 (50%) | | | 1/3 (33%) | |
| Chills | | 1/2 (50%) | 1/4 (25%) | | 1/3 (33%) | |
| Fever | | 1/2 (50%) | 1/4 (25%) | | | 1/3 (33%) |
| Myalgia | 1/2 (50%) | 1/2 (50%) | | | 1/3 (33%) | |
| Bronchospasm | | | | 1/4 (25%) | | |
| Cough | | 1/2 (50%) | | | | |
| Dizziness | | | 1/4 (25%) | | | |
| Dry eye | | | 1/4 (25%) | | | |
| Generalized muscle weakness | 1/2 (50%) | | | | | |
| Insomnia | | | 1/4 (25%) | | | |
| Nausea | 1/2 (50%) | | | | | |
| Sore throat | | 1/2 (50%) | | | | |
| Vomiting | 1/2 (50%) | | | | | |
| Cornea tear | | | 1/4 (25%) | | | |

Grade 2 notes: Bronchospasm was attributed to seasonal allergies 2 weeks after first injection. No second injection was given. Grade 2 fever and fatigue were in the same subject on second injection and both AEs resolved within 2 days. AE, adverse event; DL, dose level.

**Table S3 Summary humoral responses (subjects with >4 fold increase in the parameters)**

| | Placebo/Placebo (N=5) | DL1/DL1 (N=17) | DL2/DL2 (N=8) | DL3/DL3 (N=9) | Total with Vaccine (N=34) |
|---|---|---|---|---|---|
| **S-IgG (endpoint)** | 0/5 | 17/17 | 8/8 | 9/9 | 34/34 |
| **N-IgG (endpoint)** | 0/5 | 15/17 | 8/8 | 9/9 | 32/34 |
| **RBD-IgG (endpoint)** | 0/5 | 17/17 | 8/8 | 9/9 | 34/34 |
| **NAb (NT50)** | 0/5 | 9/17 | 8/8 | 8/9 | 25/34 |

**Table S4 Spike IgG statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 150 vs. 1350 | 241·5 vs. 1265·5 | *0·0011* | ** |
| | Day 0 vs. Day 28 | 17 | 150 vs. 4050 | 241·5 vs. 2748·3 | *0·0003* | *** |
| | Day 0 vs. Day 42 | 17 | 150 vs. 12150 | 241·5 vs. 8244·8 | *0·0003* | *** |
| | Day 0 vs. Day 56 | 17 | 150 vs. 12150 | 241·5 vs. 9382·4 | *0·0003* | *** |
| | Day 0 vs. Day 90 | 15 | 150 vs. 4050 | 241·5 vs. 5841·1 | *0·0007* | *** |
| | Day 0 vs. Day 120 | 14 | 150 vs. 4050 | 241·5 vs. 4738·2 | *0·0011* | ** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 150 vs. 4050 | 171·7 vs. 2199·8 | *0·0090* | ** |
| | Day 0 vs. Day 28 | 9 | 150 vs. 4050 | 171·7 vs. 2808·1 | *0·0090* | ** |
| | Day 0 vs. Day 42 | 8 | 150 vs. 12150 | 171·7 vs. 9232 | *0·0141* | * |
| | Day 0 vs. Day 56 | 8 | 150 vs. 12150 | 171·7 vs. 9232 | *0·0140* | * |
| | Day 0 vs. Day 90 | 7 | 150 vs. 4050 | 171·7 vs. 4738·2 | *0·0223* | * |
| | Day 0 vs. Day 120 | 7 | 150 vs. 4050 | 171·7 vs. 3461·7 | *0·0223* | * |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 300 vs. 2700 | 361·2 vs. 2912·9 | *0·0090* | ** |
| | Day 0 vs. Day 28 | 10 | 300 vs. 4050 | 361·2 vs. 3251·1 | *0·0090* | ** |
| | Day 0 vs. Day 42 | 9 | 300 vs. 12150 | 361·2 vs. 8424·3 | *0·0088* | ** |
| | Day 0 vs. Day 56 | 9 | 300 vs. 12150 | 361·2 vs. 9518·1 | *0·0090* | ** |
| | Day 0 vs. Day 90 | 9 | 300 vs. 4050 | 361·2 vs. 6599·5 | *0·0091* | ** |
| | Day 0 vs. Day 120 | 9 | 300 vs. 4050 | 361·2 vs. 3172·7 | *0·0140* | * |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 150 vs. 1350 | 313·9 vs. 1350 | *0·0090* | ** |
| | Day 0 vs. Day 28 | 13 | 150 vs. 1350 | 313·9 vs. 2241·5 | *0·0025* | ** |
| | Day 0 vs. Day 42 | 13 | 150 vs. 1350 | 313·9 vs. 2439·2 | *0·0025* | ** |
| | Day 0 vs. Day 56 | 13 | 150 vs. 1350 | 313·9 vs. 2241·5 | *0·0038* | ** |
| | Day 0 vs. Day 70 | 10 | 150 vs. 12150 | 313·9 vs. 15135·6 | *0·0059* | ** |
| | Day 0 vs. Day 84 | 10 | 150 vs. 12150 | 313·9 vs. 16893·2 | *0·0059* | ** |
| | Day 0 vs. Day 118 | 10 | 150 vs. 12150 | 313·9 vs. 12150 | *0·0059* | ** |
| | Day 0 vs. Day 148 | 8 | 150 vs. 8100 | 313·9 vs. 6114·7 | *0·0223* | * |

Serum samples collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of S-specific IgG by ELISA and endpoint titers were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure 2A: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S5 Nucleocapsid IgG statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 75 vs. 150 | 150·6 vs. 201·7 | *0·0310* | * |
| | Day 0 vs. Day 28 | 17 | 75 vs. 150 | 150·6 vs. 314·9 | *0·0035* | ** |
| | Day 0 vs. Day 42 | 17 | 75 vs. 1350 | 150·6 vs. 1573·3 | *0·0005* | *** |
| | Day 0 vs. Day 56 | 17 | 75 vs. 1350 | 150·6 vs. 2318·6 | *0·0007* | *** |
| | Day 0 vs. Day 90 | 15 | 75 vs. 1350 | 150·6 vs. 1859·1 | *0·0016* | ** |
| | Day 0 vs. Day 120 | 14 | 75 vs. 1350 | 150·6 vs. 1758·5 | *0·0024* | ** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 75 vs. 150 | 81 vs. 159 | 0·0890 | |
| | Day 0 vs. Day 28 | 9 | 75 vs. 150 | 81 vs. 226·3 | *0·0104* | * |
| | Day 0 vs. Day 42 | 8 | 75 vs. 1350 | 81 vs. 1869·1 | *0·0220* | * |
| | Day 0 vs. Day 56 | 8 | 75 vs. 2700 | 81 vs. 4050 | *0·0130* | * |
| | Day 0 vs. Day 90 | 7 | 75 vs. 1350 | 81 vs. 1350 | *0·0215* | * |
| | Day 0 vs. Day 120 | 7 | 75 vs. 450 | 81 vs. 763·6 | *0·0335* | * |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 150 vs. 900 | 151·8 vs. 905·9 | *0·0355* | * |
| | Day 0 vs. Day 28 | 10 | 150 vs. 900 | 151·8 vs. 678·5 | *0·0355* | * |
| | Day 0 vs. Day 42 | 9 | 150 vs. 4050 | 151·8 vs. 3584·6 | *0·0090* | ** |
| | Day 0 vs. Day 56 | 9 | 150 vs. 4050 | 151·8 vs. 3584·6 | *0·0090* | ** |
| | Day 0 vs. Day 90 | 9 | 150 vs. 4050 | 151·8 vs. 2485·4 | *0·0088* | ** |
| | Day 0 vs. Day 120 | 9 | 150 vs. 1350 | 151·8 vs. 1525·3 | *0·0091* | ** |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 75 vs. 450 | 115·9 vs. 392·1 | *0·0057* | ** |
| | Day 0 vs. Day 28 | 13 | 75 vs. 450 | 115·9 vs. 838·8 | *0·0033* | ** |
| | Day 0 vs. Day 42 | 13 | 75 vs. 450 | 115·9 vs. 912·8 | *0·0035* | ** |
| | Day 0 vs. Day 56 | 13 | 75 vs. 450 | 115·9 vs. 730·8 | *0·0059* | ** |
| | Day 0 vs. Day 70 | 10 | 75 vs. 8100 | 115·9 vs. 6285 | *0·0058* | ** |
| | Day 0 vs. Day 84 | 10 | 75 vs. 4050 | 115·9 vs. 5631·1 | *0·0058* | ** |
| | Day 0 vs. Day 118 | 10 | 75 vs. 4050 | 115·9 vs. 5254 | *0·0091* | ** |
| | Day 0 vs. Day 148 | 8 | 75 vs. 4050 | 115·9 vs. 3237·3 | *0·0223* | * |

Serum samples collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of N-specific IgG by ELISA and endpoint titers were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure 2B: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S6 D614G neutralizing antibodies statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 10 vs. 10 | 13·8 vs. 17·7 | 0·0680 | |
| | Day 0 vs. Day 28 | 17 | 10 vs. 10 | 13·8 vs. 17·8 | 0·0684 | |
| | Day 0 vs. Day 42 | 17 | 10 vs. 62 | 13·.8 vs. 66·7 | *0·0003* | *** |
| | Day 0 vs. Day 56 | 17 | 10 vs. 40 | 13·8 vs. 43·4 | *0·0027* | ** |
| | Day 0 vs. Day 90 | 15 | 10 vs. 43 | 13·8 vs. 32·4 | *0·0249* | * |
| | Day 0 vs. Day 120 | 14 | 10 vs. 17 | 13·8 vs. 26·9 | *0·0300* | * |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 10 vs. 25 | 10·9 vs. 37·2 | *0·0225* | * |
| | Day 0 vs. Day 28 | 9 | 10 vs. 10 | 10·9 vs. 20·6 | 0·1775 | |
| | Day 0 vs. Day 42 | 8 | 10 vs. 131 | 10·9 vs. 161·7 | *0·0141* | * |
| | Day 0 vs. Day 56 | 8 | 10 vs. 181·50 | 10·9 vs. 166·9 | *0·0078* | ** |
| | Day 0 vs. Day 90 | 7 | 10 vs. 60 | 10·9 vs. 58·5 | *0·0360* | * |
| | Day 0 vs. Day 120 | 7 | 10 vs. 52 | 10·9 vs. 46·2 | 0·0591 | |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 10 vs. 32 | 12·1 vs. 40·7 | *0·0223* | * |
| | Day 0 vs. Day 28 | 10 | 10 vs. 25 | 12·1 vs. 23·7 | 0·0754 | |
| | Day 0 vs. Day 42 | 9 | 10 vs. 152 | 12·1 vs. 162·9 | *0·0039* | ** |
| | Day 0 vs. Day 56 | 9 | 10 vs. 113 | 12·1 vs. 136·6 | *0·0039* | ** |
| | Day 0 vs. Day 90 | 9 | 10 vs. 75 | 12·1 vs. 56·5 | *0·0143* | * |
| | Day 0 vs. Day 120 | 9 | 10 vs. 29 | 12·1 vs. 37·1 | *0·0360* | * |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 10 vs. 10 | 10·7 vs. 12·2 | 0·3711 | |
| | Day 0 vs. Day 28 | 13 | 10 vs. 10 | 10·7 vs. 12·4 | 0·3711 | |
| | Day 0 vs. Day 42 | 13 | 10 vs. 10 | 10·7 vs. 12·3 | 1·0000 | |
| | Day 0 vs. Day 56 | 13 | 10 vs. 10 | 10·7 vs. 12·3 | 1·0000 | |
| | Day 0 vs. Day 70 | 10 | 10 vs. 140·50 | 10·7 vs. 117 | *0·0092* | ** |
| | Day 0 vs. Day 84 | 10 | 10 vs. 59 | 10·7 vs. 110.5 | *0·0059* | ** |
| | Day 0 vs. Day 118 | 10 | 10 vs. 43 | 10·7 vs. 61·4 | *0·0143* | * |
| | Day 0 vs. Day 148 | 8 | 10 vs. 26·50 | 10·7 vs. 37·6 | *0·0360* | * |

Serum samples collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of SARS-CoV-2-specific neutralizing antibodies using a S PsV based on the original SARS-CoV-2 Wuhan strain with D614G substitution. NT50s at different timepoints were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure 2C: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S7 RBD IgG statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 75 vs. 150 | 99·8 vs. 136·9 | 0·0975 | |
| | Day 0 vs. Day 28 | 17 | 75 vs. 150 | 99·8 vs. 176 | *0·0084* | ** |
| | Day 0 vs. Day 42 | 17 | 75 vs. 12150 | 99·8 vs. 7728·8 | *0·0003* | *** |
| | Day 0 vs. Day 56 | 17 | 75 vs. 12150 | 99·8 vs. 6366·7 | *0·0003* | *** |
| | Day 0 vs. Day 90 | 15 | 75 vs. 4050 | 99·8 vs. 3021·5 | *0·0007* | *** |
| | Day 0 vs. Day 120 | 14 | 75 vs. 2700 | 99·8 vs. 1708·3 | *0·0016* | ** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 75 vs. 150 | 81 vs. 194 | *0·0335* | * |
| | Day 0 vs. Day 28 | 9 | 75 vs. 450 | 81 vs. 531·9 | *0·0130* | * |
| | Day 0 vs. Day 42 | 8 | 75 vs. 12150 | 81 vs. 7014·8 | *0·0131* | * |
| | Day 0 vs. Day 56 | 8 | 75 vs. 12150 | 81 vs. 12150 | *0·0120* | * |
| | Day 0 vs. Day 90 | 7 | 75 vs. 4050 | 81 vs. 4050 | *0·0201* | * |
| | Day 0 vs. Day 120 | 7 | 75 vs. 4050 | 81 vs. 2161·8 | *0·0213* | * |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 112·5 vs. 450 | 118·4 vs. 450 | *0·0136* | * |
| | Day 0 vs. Day 28 | 10 | 112·5 vs. 450 | 118·4 vs. 560·6 | *0·0137* | * |
| | Day 0 vs. Day 42 | 9 | 112·5 vs. 12150 | 118·4 vs. 9518·1 | *0·0088* | ** |
| | Day 0 vs. Day 56 | 9 | 112·5 vs. 4050 | 118·4 vs. 6599·5 | *0·0089* | ** |
| | Day 0 vs. Day 90 | 9 | 112·5 vs. 4050 | 118·4 vs. 3172·7 | *0·0091* | ** |
| | Day 0 vs. Day 120 | 9 | 112·5 vs. 1350 | 118·4 vs. 1723·3 | *0·0090* | ** |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 75 vs. 75 | 79·1 vs. 101 | 0·1736 | |
| | Day 0 vs. Day 28 | 13 | 75 vs. 150 | 79·1 vs. 195 | *0·0050* | ** |
| | Day 0 vs. Day 42 | 13 | 75 vs. 150 | 79·1 vs. 169·9 | *0·0077* | ** |
| | Day 0 vs. Day 56 | 13 | 75 vs. 150 | 79·1 vs. 143·5 | *0·0103* | * |
| | Day 0 vs. Day 70 | 10 | 75 vs. 12150 | 79·1 vs. 7829·4 | *0·0057* | ** |
| | Day 0 vs. Day 84 | 10 | 75 vs. 8100 | 79·1 vs. 6285 | *0·0057* | ** |
| | Day 0 vs. Day 118 | 10 | 75 vs. 4050 | 79·1 vs. 3251·1 | *0·0057* | ** |
| | Day 0 vs. Day 148 | 8 | 75 vs. 1350 | 79·1 vs. 2038·2 | *0·0130* | * |

Serum samples collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of RBD-specific IgG by ELISA and endpoint titers were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure S2: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S8 Spike-specific IFNγ-secreting T cells statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 10 vs. 462·50 | 9·2 vs. 373·2 | *<0·0001* | **** |
| | Day 0 vs. Day 28 | 17 | 10 vs. 216·65 | 9·2 vs. 256·3 | *0·0003* | *** |
| | Day 0 vs. Day 42 | 17 | 10 vs. 512·50 | 9·2 vs. 517·5 | *<0·0001* | **** |
| | Day 0 vs. Day 56 | 17 | 10 vs. 283·31 | 9·2 vs. 406·8 | *<0·0001* | **** |
| | Day 0 vs. Day 90 | 15 | 10 vs. 443 | 9·2 vs. 379·2 | *0·0001* | **** |
| | Day 0 vs. Day 120 | 14 | 10 vs. 653·50 | 9·2 vs. 467·6 | *0·0001* | *** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 57·50 vs. 835 | 32·3 vs. 909·5 | *0·0039* | ** |
| | Day 0 vs. Day 28 | 9 | 57·50 vs. 212.50 | 32·3 vs. 263·8 | *0·0117* | * |
| | Day 0 vs. Day 42 | 8 | 57.50 vs. 468 | 32·3 vs. 603·5 | *0·0078* | ** |
| | Day 0 vs. Day 56 | 8 | 57·50 vs. 535 | 32·3 vs. 604·1 | *0·0078* | ** |
| | Day 0 vs. Day 90 | 7 | 57·50 vs. 497 | 32·3 vs. 386·9 | *0·0313* | * |
| | Day 0 vs. Day 120 | 7 | 57·50 vs. 157 | 32·3 vs. 210 | 0·0781 | |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 30·08 vs. 711·50 | 25·3 vs. 633·5 | *0·0020* | ** |
| | Day 0 vs. Day 28 | 10 | 30·08 vs. 298·25 | 25·3 vs. 273·2 | *0·0020* | ** |
| | Day 0 vs. Day 42 | 9 | 30·08 vs. 310 | 25·3 vs. 324·9 | *0·0039* | ** |
| | Day 0 vs. Day 56 | 9 | 30·08 vs. 167 | 25·3 vs. 219·2 | *0·0273* | * |
| | Day 0 vs. Day 90 | 9 | 30·08 vs. 123 | 25·3 vs. 113·8 | 0·0742 | |
| | Day 0 vs. Day 120 | 9 | 30·08 vs. 277 | 25·3 vs. 271·6 | *0·0039* | ** |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 16·50 vs. 353 | 13·6 vs. 429·6 | *0·0002* | *** |
| | Day 0 vs. Day 28 | 13 | 16·50 vs. 306·64 | 13·6 vs. 295·1 | *0·0002* | *** |
| | Day 0 vs. Day 42 | 13 | 16·50 vs. 200 | 13·6 vs. 229 | *0·0002* | *** |
| | Day 0 vs. Day 56 | 13 | 16·50 vs. 196·50 | 13·6 vs. 179·1 | *0·0002* | *** |
| | Day 0 vs. Day 70 | 10 | 16·50 vs. 359·965 | 13·6 vs. 472·5 | *0·0020* | ** |
| | Day 0 vs. Day 84 | 10 | 16·50 vs. 432 | 13·6 vs. 454·2 | *0·0020* | ** |
| | Day 0 vs. Day 118 | 10 | 16·50 vs. 367 | 13·6 vs. 426·7 | *0·0020* | ** |
| | Day 0 vs. Day 148 | 8 | 16·50 vs. 308·25 | 13·6 vs. 382·3 | *0·0078* | ** |

PBMCs collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of S-specific T cells secreting IFNγ using ELISPOT. S-specific T cells/$10^6$ PBMCs at different timepoints were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure 3A: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S9 Nucleocapsid-specific IFNγ-secreting T cells statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 6·50 vs. 213 | 4·4 vs. 192·2 | *<0·0001* | **** |
| | Day 0 vs. Day 28 | 17 | 6·50 vs. 120 | 4·4 vs. 119·9 | *<0·0001* | **** |
| | Day 0 vs. Day 42 | 17 | 6·50 vs. 273 | 4·4 vs. 218·4 | *<0·0001* | **** |
| | Day 0 vs. Day 56 | 17 | 6·50 vs. 173·50 | 4·4 vs. 170·9 | *<0·0001* | **** |
| | Day 0 vs. Day 90 | 15 | 6·50 vs. 230 | 4·4 vs. 194·7 | *0·0001* | **** |
| | Day 0 vs. Day 120 | 14 | 6·50 vs. 149 | 4·4 vs. 220·3 | *0·0001* | *** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 7·50 vs. 390 | 6·5 vs. 487·2 | *0·0039* | ** |
| | Day 0 vs. Day 28 | 9 | 7·50 vs. 120 | 6·5 vs. 125·3 | *0·0128* | * |
| | Day 0 vs. Day 42 | 8 | 7·50 vs. 198·75 | 6·5 vs. 306·6 | *0·0078* | ** |
| | Day 0 vs. Day 56 | 8 | 7·50 vs. 246·50 | 6·5 vs. 314·7 | *0·0078* | ** |
| | Day 0 vs. Day 90 | 7 | 7·50 vs. 167 | 6·5 vs. 135·3 | *0·0313* | * |
| | Day 0 vs. Day 120 | 7 | 7·50 vs. 197 | 6·5 vs. 98·7 | 0·0781 | |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 8·25 vs. 515·06 | 8·6 vs. 418·4 | *0·0020* | ** |
| | Day 0 vs. Day 28 | 10 | 8·25 vs. 316·66 | 8·6 vs. 192·1 | *0·0092* | ** |
| | Day 0 vs. Day 42 | 9 | 8·25 vs. 363·30 | 8·6 vs. 268·5 | *0·0195* | * |
| | Day 0 vs. Day 56 | 9 | 8·25 vs. 240 | 8·6 vs. 133·7 | 0·0547 | |
| | Day 0 vs. Day 90 | 9 | 8·25 vs. 56 | 8·6 vs. 74·6 | 0·1289 | |
| | Day 0 vs. Day 120 | 9 | 8·25 vs. 150 | 8·6 vs. 149·3 | *0·0391* | * |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 10 vs. 183 | 7·5 vs. 252·7 | *0·0002* | *** |
| | Day 0 vs. Day 28 | 13 | 10 vs. 183·50 | 7·5 vs. 170·9 | *0·0002* | *** |
| | Day 0 vs. Day 42 | 13 | 10 vs. 116·66 | 7·5 vs. 109·6 | *0·0002* | *** |
| | Day 0 vs. Day 56 | 13 | 10 vs. 99·99 | 7·5 vs. 69·1 | *0·0033* | ** |
| | Day 0 vs. Day 70 | 10 | 10 vs. 228·32 | 7·5 vs. 263·7 | *0·0039* | ** |
| | Day 0 vs. Day 84 | 10 | 10 vs. 205 | 7·5 vs. 215·3 | *0·0020* | ** |
| | Day 0 vs. Day 118 | 10 | 10 vs. 166·65 | 7·5 vs. 194·4 | *0·0020* | ** |
| | Day 0 vs. Day 148 | 8 | 10 vs. 135·50 | 7·5 vs. 155·7 | *0·0156* | * |

PBMCs collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of N-specific T cells secreting IFNγ using ELISPOT. S-specific T cells/$10^6$ PBMCs at different timepoints were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure 3B: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S10 Spike-specific IL-4-secreting T cells statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 6·50 vs. 40 | 6 vs. 22·1 | *0·0131* | * |
| | Day 0 vs. Day 28 | 17 | 6·50 vs. 27·50 | 6 vs. 22·5 | *0·0245* | * |
| | Day 0 vs. Day 42 | 17 | 6·50 vs. 59·99 | 6 vs. 30·1 | *0·0066* | ** |
| | Day 0 vs. Day 56 | 17 | 6·50 vs. 15 | 6 vs. 18·9 | *0·0280* | * |
| | Day 0 vs. Day 90 | 15 | 6·50 vs. 30 | 6 vs. 34·1 | *0·0144* | * |
| | Day 0 vs. Day 120 | 14 | 6·50 vs. 26·50 | 6 vs. 31·2 | *0·0084* | ** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 25 vs. 96·50 | 25·4 vs. 138·5 | *0·0039* | ** |
| | Day 0 vs. Day 28 | 9 | 25 vs. 37·50 | 25·4 vs. 44·2 | 0·0578 | |
| | Day 0 vs. Day 42 | 8 | 25 vs. 94·25 | 25·4 vs. 72·6 | *0·0156* | * |
| | Day 0 vs. Day 56 | 8 | 25 vs. 44·75 | 25·4 vs. 69 | *0·0078* | ** |
| | Day 0 vs. Day 90 | 7 | 25 vs. 23 | 25·4 vs. 32·3 | 1·0000 | |
| | Day 0 vs. Day 120 | 7 | 25 vs. 16·50 | 25·4 vs. 18·1 | 0·9375 | |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 19·915 vs. 122·75 | 14·4 vs. 74·6 | *0·0092* | ** |
| | Day 0 vs. Day 28 | 10 | 19·915 vs. 53·33 | 14·4 vs. 33·6 | *0·0330* | * |
| | Day 0 vs. Day 42 | 9 | 19·915 vs. 66·50 | 14·4 vs. 80·4 | *0·0039* | ** |
| | Day 0 vs. Day 56 | 9 | 19·915 vs. 46·50 | 14·4 vs. 64 | *0·0117* | * |
| | Day 0 vs. Day 90 | 9 | 19·915 vs. 20 | 14·4 vs. 20·2 | 0·5933 | |
| | Day 0 vs. Day 120 | 9 | 19·915 vs. 20 | 14·4 vs. 28·5 | 0·6353 | |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 16·66 vs. 56.50 | 17·5 vs. 37·7 | 0·0574 | |
| | Day 0 vs. Day 28 | 13 | 16·66 vs. 43 | 17·5 vs. 38·7 | *0·0042* | ** |
| | Day 0 vs. Day 42 | 13 | 16·66 vs. 30 | 17·5 vs. 24·7 | 0·0942 | |
| | Day 0 vs. Day 56 | 13 | 16·66 vs. 30 | 17·5 vs. 17·6 | 0·2163 | |
| | Day 0 vs. Day 70 | 10 | 16·66 vs. 61·66 | 17·5 vs. 41·5 | *0·0488* | * |
| | Day 0 vs. Day 84 | 10 | 16·66 vs. 50 | 17·5 vs. 34·3 | 0·1055 | |
| | Day 0 vs. Day 118 | 10 | 16·66 vs. 42 | 17·5 vs. 42·7 | 0·0645 | |
| | Day 0 vs. Day 148 | 8 | 16·66 vs. 67 | 17·5 vs. 48·7 | 0·0781 | |

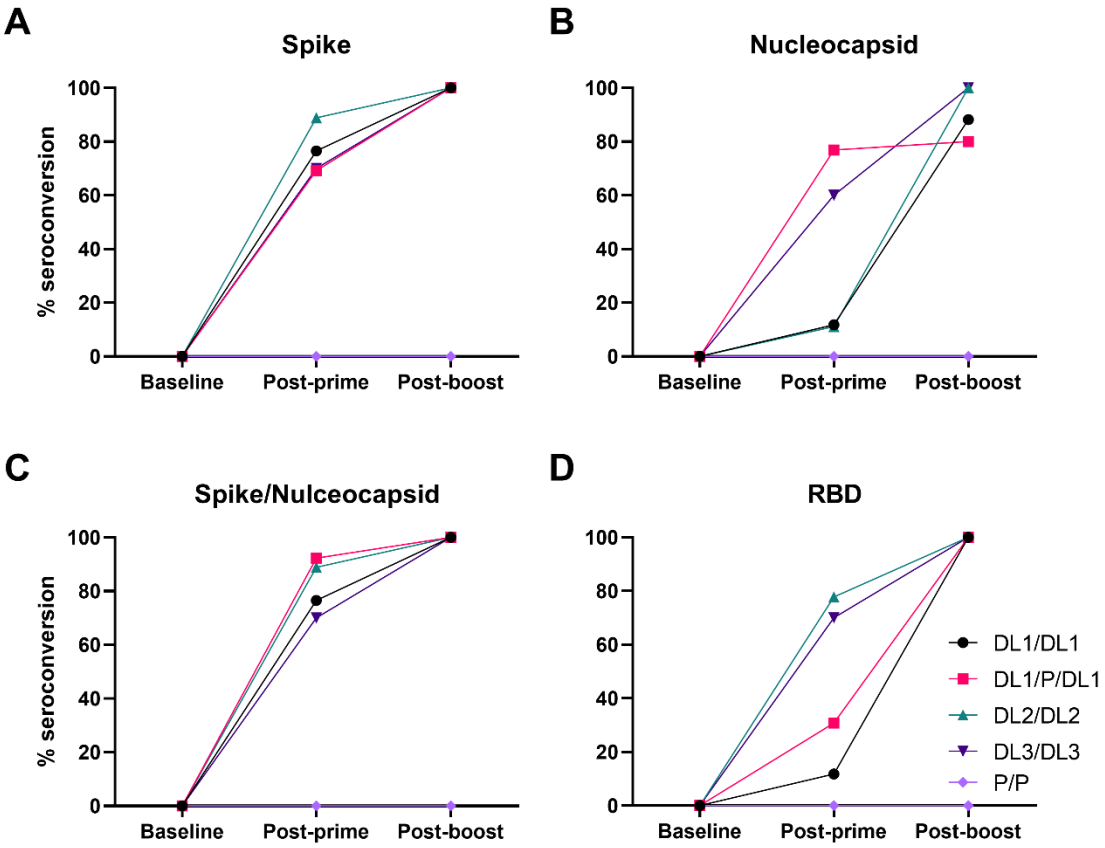
PBMCs collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of S-specific T cells secreting IL-4 using ELISPOT. S-specific T cells/$10^6$ PBMCs at different timepoints were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure S5A: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Table S11 Nucleocapsid-specific IL-4-secreting T cells statistical testing**

| group | comparison | n | median | geometric mean | p.value | sig |
|---|---|---|---|---|---|---|
| **DL1/DL1** | Day 0 vs. Day 14 | 17 | 1 vs. 10 | 2·4 vs. 9·6 | *0·0158* | * |
| | Day 0 vs. Day 28 | 17 | 1 vs. 16·66 | 2·4 vs. 9 | *0·0071* | ** |
| | Day 0 vs. Day 42 | 17 | 1 vs. 17 | 2·4 vs. 13 | *0·0049* | ** |
| | Day 0 vs. Day 56 | 17 | 1 vs. 5 | 2·4 vs. 7·6 | *0·0427* | * |
| | Day 0 vs. Day 90 | 15 | 1 vs. 13·33 | 2·4 vs. 10·9 | *0·0280* | * |
| | Day 0 vs. Day 120 | 14 | 1 vs. 6·835 | 2·4 vs. 8·5 | *0·0086* | ** |
| **DL2/DL2** | Day 0 vs. Day 14 | 9 | 5 vs. 50 | 5·3 vs. 36·6 | *0·0209* | * |
| | Day 0 vs. Day 28 | 9 | 5 vs. 12·50 | 5·3 vs. 9·9 | 0·0661 | |
| | Day 0 vs. Day 42 | 8 | 5 vs. 37·25 | 5·3 vs. 32 | *0·0225* | * |
| | Day 0 vs. Day 56 | 8 | 5 vs. 19·75 | 5·3 vs. 25·1 | *0·0141* | * |
| | Day 0 vs. Day 90 | 7 | 5 vs. 10 | 5·3 vs. 12·4 | 0·2188 | |
| | Day 0 vs. Day 120 | 7 | 5 vs. 4 | 5·3 vs. 4·4 | 1·0000 | |
| **DL3/DL3** | Day 0 vs. Day 14 | 10 | 1 vs. 51·50 | 2·3 vs. 44·5 | *0·0020* | ** |
| | Day 0 vs. Day 28 | 10 | 1 vs. 17·58 | 2·3 vs. 14·9 | *0·0195* | * |
| | Day 0 vs. Day 42 | 9 | 1 vs. 40 | 2·3 vs. 32·6 | *0·0143* | * |
| | Day 0 vs. Day 56 | 9 | 1 vs. 23·50 | 2·3 vs. 25·9 | *0·0039* | ** |
| | Day 0 vs. Day 90 | 9 | 1 vs. 13·50 | 2·3 vs. 7·3 | *0·0391* | * |
| | Day 0 vs. Day 120 | 9 | 1 vs. 10 | 2·3 vs. 8·4 | 0·0753 | |
| **DL1/placebo/DL1** | Day 0 vs. Day 14 | 13 | 1 vs. 10 | 2·6 vs. 8·7 | *0·0144* | * |
| | Day 0 vs. Day 28 | 13 | 1 vs. 7 | 2·6 vs. 10·6 | *0·0118* | * |
| | Day 0 vs. Day 42 | 13 | 1 vs. 10 | 2·6 vs. 8·2 | *0·0178* | * |
| | Day 0 vs. Day 56 | 13 | 1 vs. 3·50 | 2·6 vs. 3·4 | 0·9188 | |
| | Day 0 vs. Day 70 | 10 | 1 vs. 13·33 | 2·6 vs. 12·3 | 0·0580 | |
| | Day 0 vs. Day 84 | 10 | 1 vs. 25 | 2·6 vs. 15 | 0·0592 | |
| | Day 0 vs. Day 118 | 10 | 1 vs. 8·50 | 2·6 vs. 8·3 | 0·0960 | |
| | Day 0 vs. Day 148 | 8 | 1 vs. 15 | 2·6 vs. 8·1 | 0·0759 | |

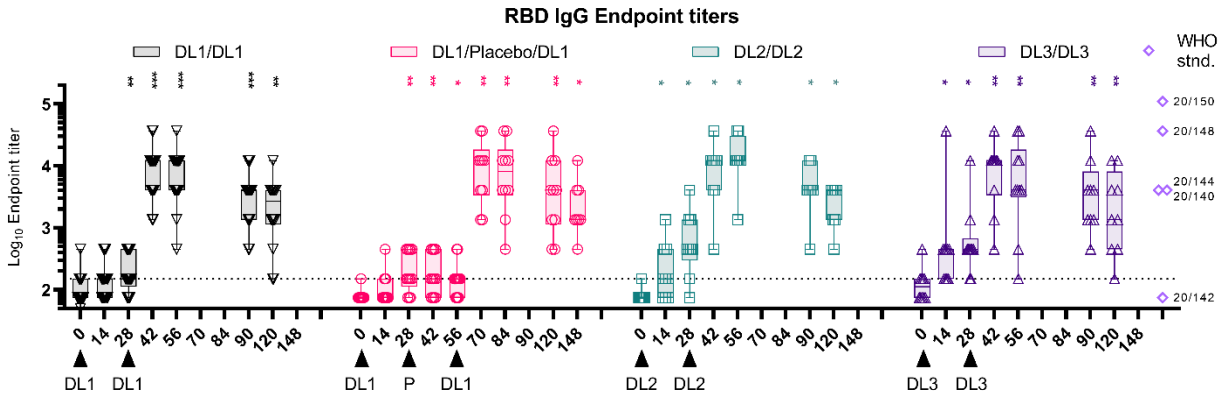
PBMCs collected from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of N-specific T cells secreting IL-4 using ELISPOT. S-specific T cells/$10^6$ PBMCs at different timepoints were statistically compared to baseline values using two-sided Wilcoxon paired signed-rank test. P-values less than 0·05 are color coded in red. Asterisk ranking as shown in Figure S5B: *=p<0·05, **=p<0·01, ***=p<0·001, ****=p<0·0001.

**Figure S1. Seroconversion rates in different COH04S1 vaccine groups and placebo controls**



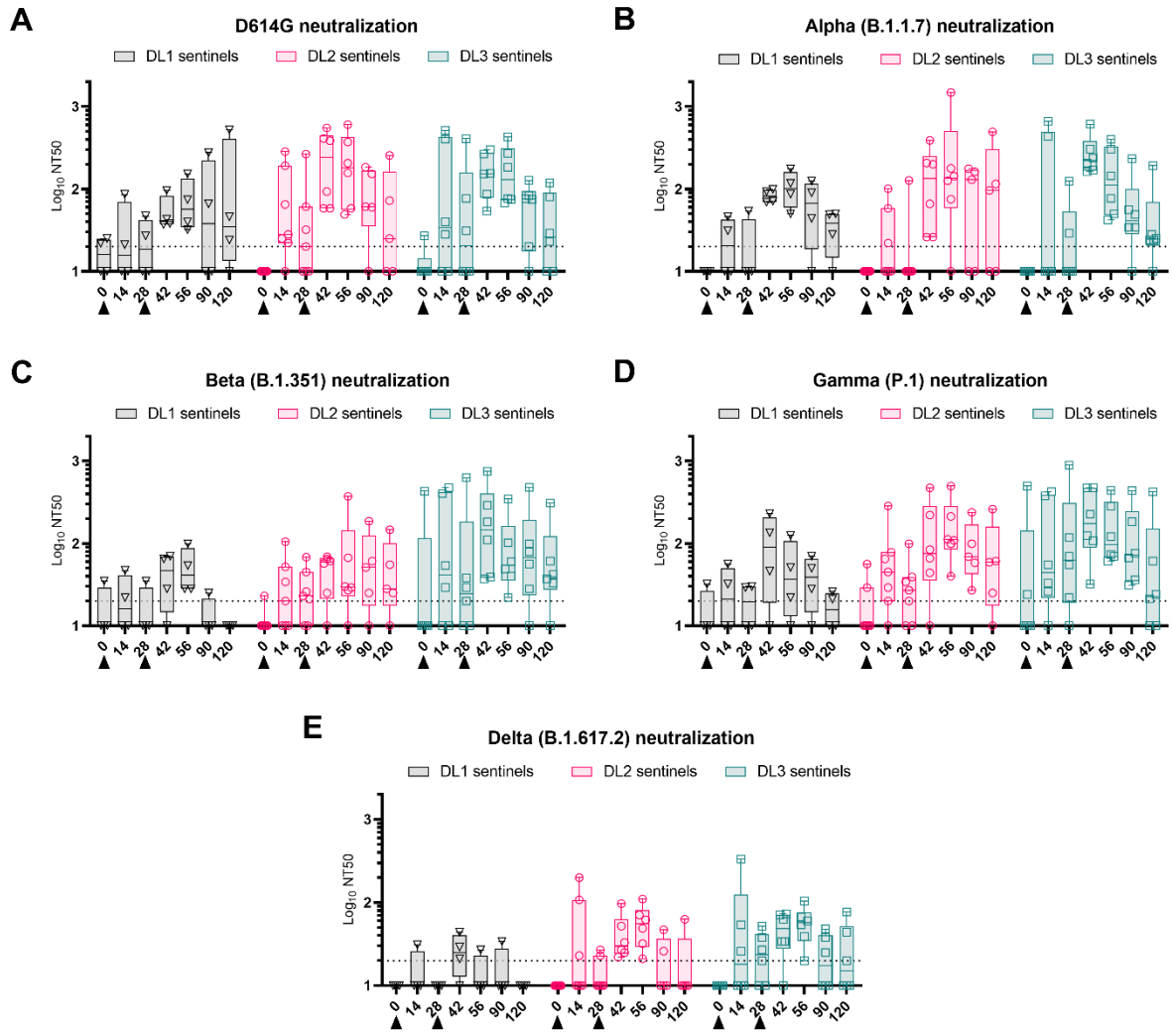

Serum samples from subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) were evaluated for the presence of S-, RBD, and N-specific IgG by ELISA and endpoint titers quantified. Seroconversion is shown as a four-fold increase in S (A), N (B), S or N (C), and RBD (D) IgG titers from day 0. Per-protocol seroconversion was defined as a post-boost four-fold increase in S or N IgG titers from day 0 (C). Post-prime is considered any time before booster vaccination, post-boost is considered the first month post-boost vaccination. P, placebo.

**Figure S2. Serum RBD-specific binding antibodies in COH04S1-vaccinated subjects**



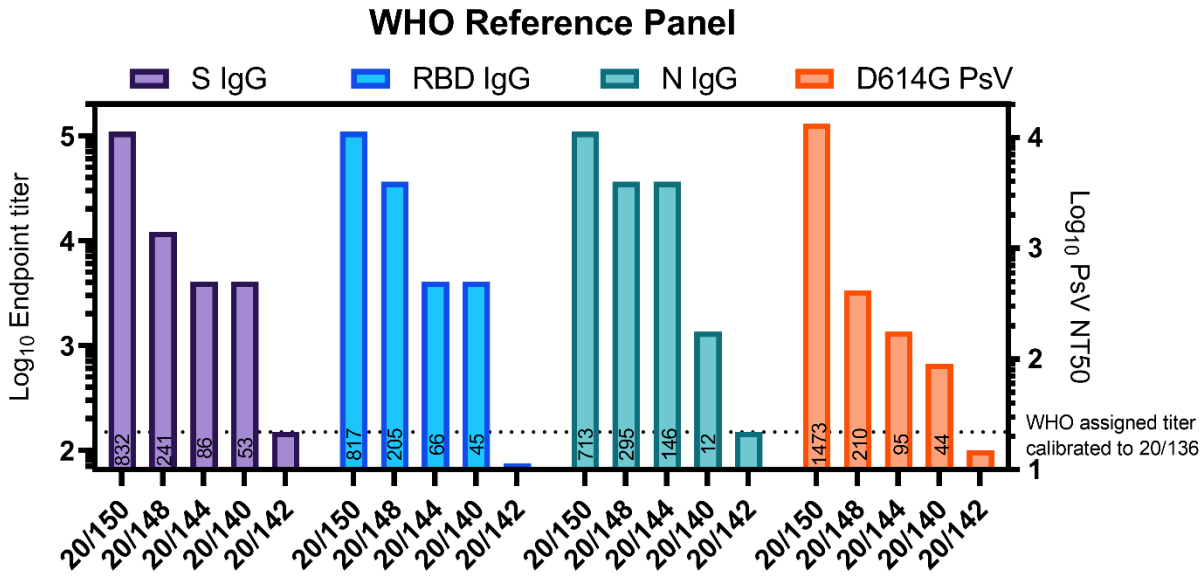

Receptor binding domain (RBD) IgG endpoint titers were quantified by ELISA at the indicated time points in subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3). Box plots extends from the 25th to the 75th percentiles, median values are shown as a line (key geometric means are discussed in the text), whiskers extend from minimum to maximum values. Individual values are superimposed. Reported are statistical testing results using Wilcoxon rank sum paired test and comparing each time point to baseline (day 0). Significance levels are indicated as follow: *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001. Exact p values are shown in Table S6. Dotted lines represent the lower limit of quantification. Arrowheads represent time of vaccination. P, placebo. Indicated are RBD endpoint titers measured in WHO reference panel 20/268 (ranked based on SARS-CoV-2 Ab titers: 20/150=high, 20/148=mid, 20/144=low S, high N, 20/140=low, and 20/142=negative. WHO assigned values are shown in Figure S4).

**Figure S3. NAb responses to SARS-CoV-2 VOCs in COH04S1-vaccinated DL1-3 sentinels**



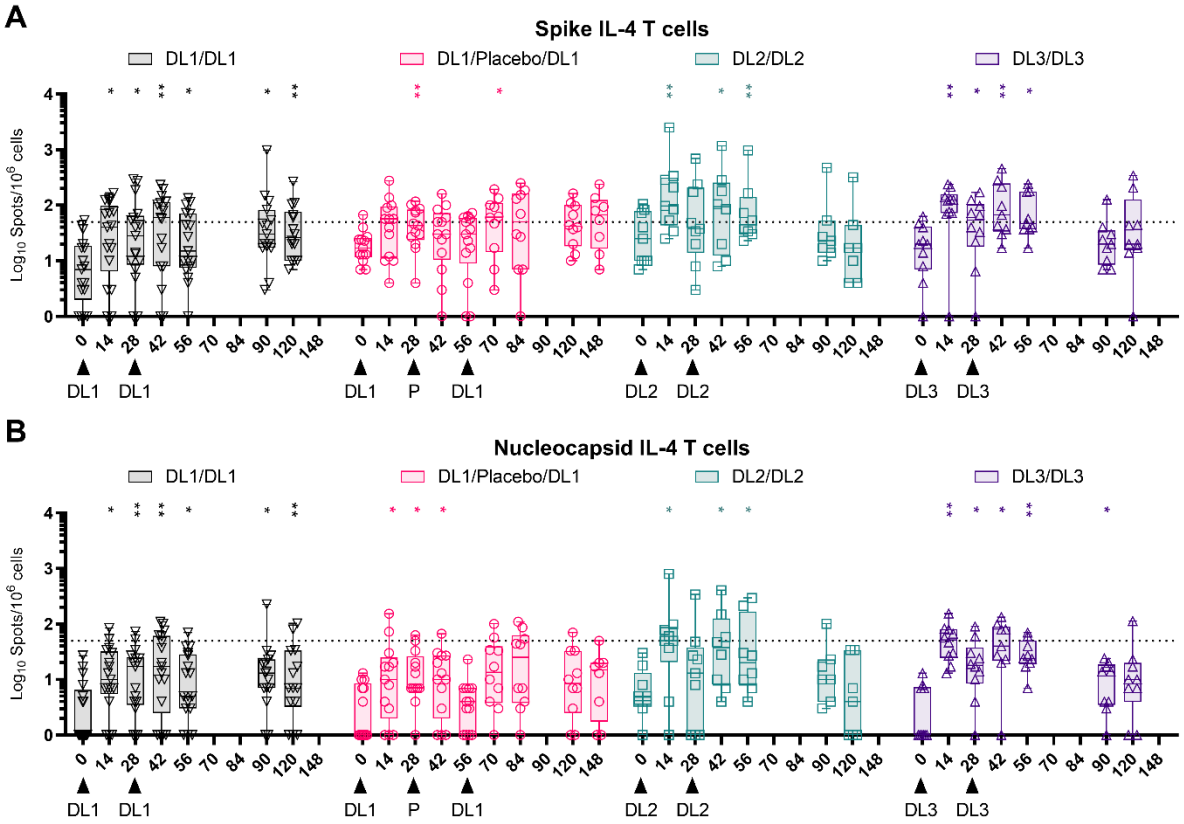

SARS-CoV-2-specific NAb titers (NT50) to VOC were evaluated at the indicated time points in serum samples from sentinel subjects vaccinated with COH04S1 dose level (DL) 1 (N=4), DL2 (N=7), and DL3 (N=6) using a SARS-CoV-2 PsV based on (A) Wuhan S sequence with D614G substitution, or several VOC, including (B) Alpha (B.1.1.7), (C) Beta (B.1.351), (D) Gamma (P.1), and (E) Delta. Box plots extends from the 25$^{th}$ to the 75$^{th}$ percentiles, median values are shown as a line, whiskers extend from minimum to maximum values. Individual values are superimposed. Arrowheads represent time of vaccination. Dotted lines represent the lower limit of quantification.

**Figure S4. WHO Reference Panel binding and neutralizing antibody titers**



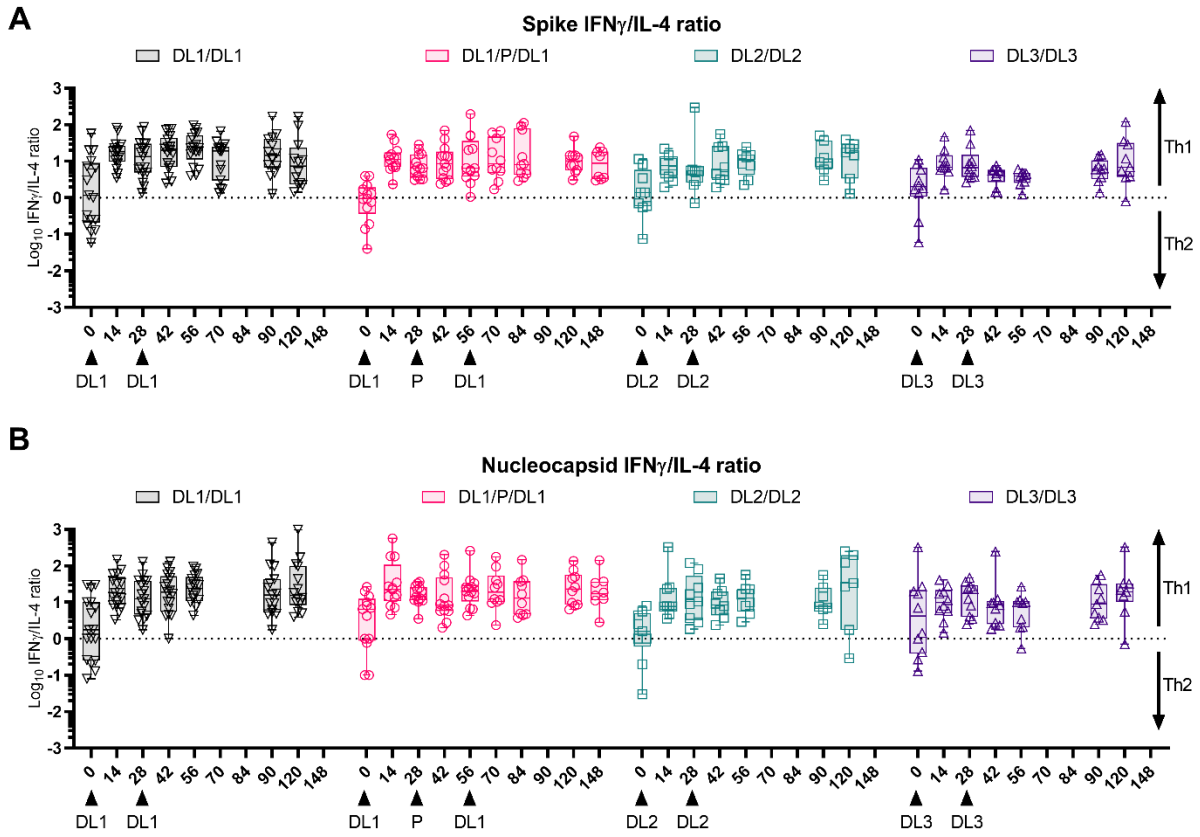

WHO reference panel 20/268 individual panel members were evaluated for IgG endpoint titers by ELISA and neutralizing titer (NT50) using SARS-CoV-2 PsV based on Wuhan S sequence with D614G substitution. Panel products 20/150 (high), 20/148 (mid), 20/144 (low S, high N), 20/140 (low), and 20/142 (negative) were reconstituted with water and analyzed. Dotted line indicates the lower limit of detection of the assays. Annotations inside the bars indicate the binding antibody titers (BAU/ml) and neutralizing titers (IU/ml) disclosed by NISBC for each product based on the calibration with the NIBSC international standard for anti-SARS-CoV-2 immunoglobulin (20/136).

**Figure S5. IL-4 T cell responses following COH04S1 vaccination with different DL and schedules.**



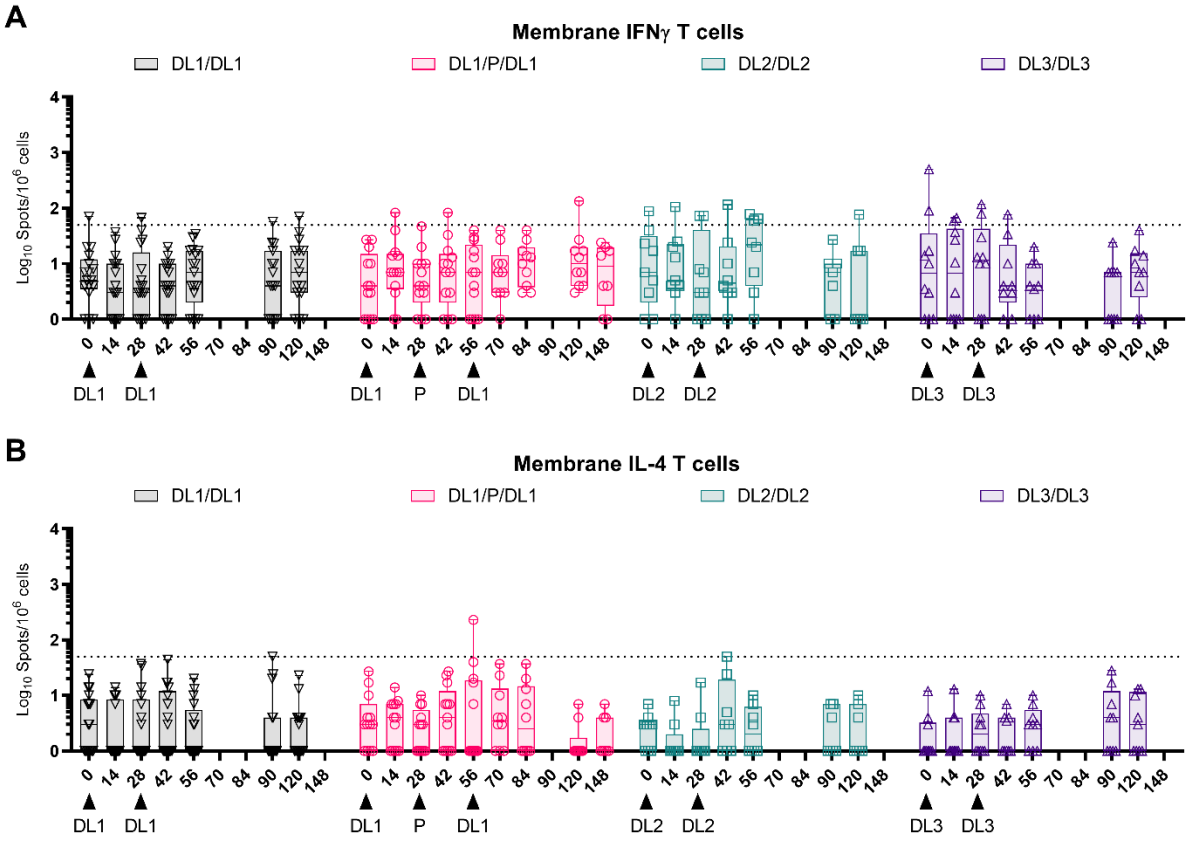

Spike- (A) and Nucleocapsid- (B) specific IL-4 T cell responses were quantified at the indicated time-points by IFNγ/IL-4 ELISPOT upon PBMCs stimulation with S and N peptide libraries in subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3). Shown are spot forming cells per $10^6$ PBMCs that were obtained after subtraction of spots in unstimulated controls from stimulated samples. Box plots extends from the 25th to the 75th percentiles, median values are shown as a line, whiskers extend from minimum to maximum values. Individual values are superimposed. Reported are statistical testing results using Wilcoxon rank sum paired test and comparing each time point to baseline (day 0). Significance levels are indicated as follow: *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001. Exact p values are shown in tables S10-S11. Dotted lines represent the arbitrary threshold for positive response (50 spots/$10^6$ PBMCs). Arrowheads represent time of vaccination. P, placebo.

**Figure S6. Spike and Nucleocapsid IFNγ/IL-4 T cells ratio in COH04S1-vaccinated subjects**



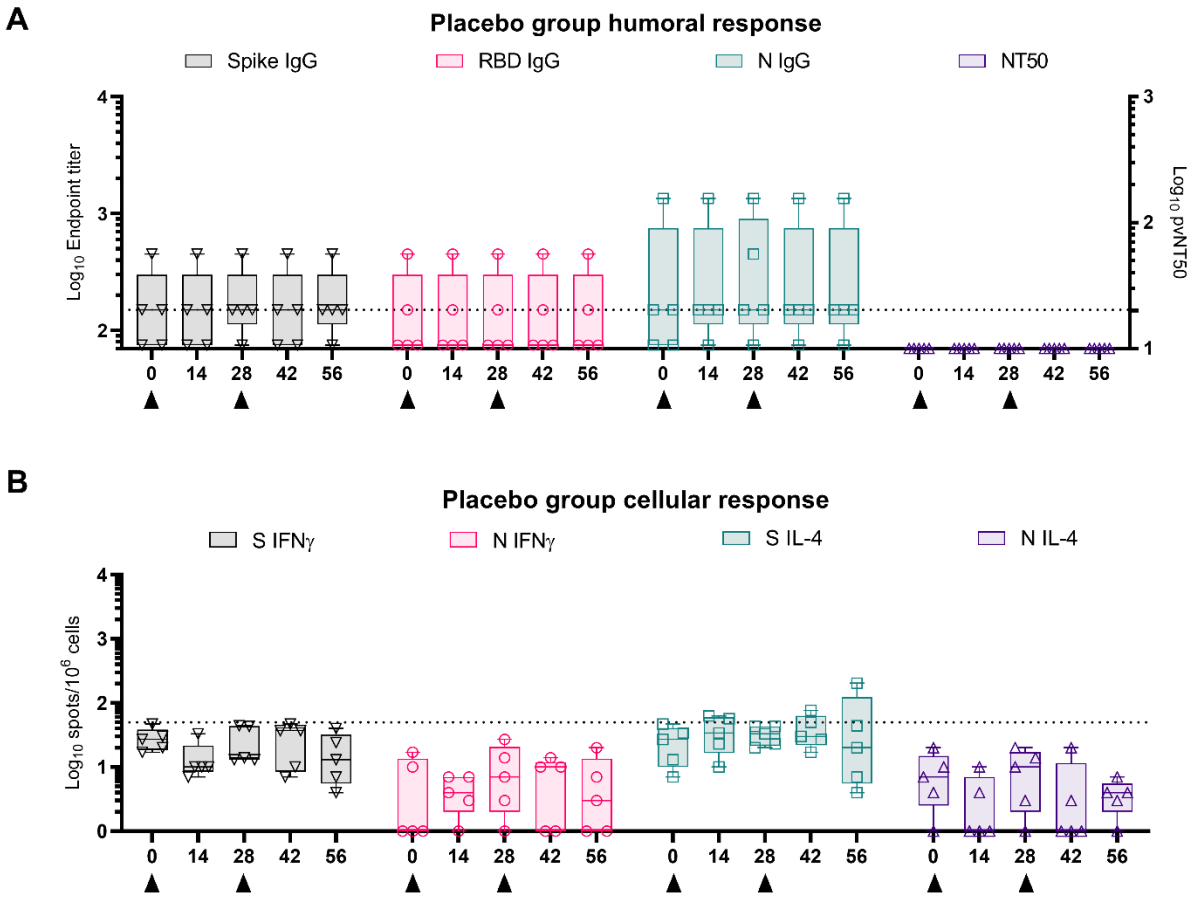

S- and N-specific IFNγ and IL-4 T cells were quantified by ELISPOT at the given time points in subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) and ratios of S- (A) and N (B)-specific cells secreting IFNγ and IL-4 were evaluated. Box plots extends from the 25$^{th}$ to the 75$^{th}$ percentiles, median values are shown as a line, whiskers extend from minimum to maximum values. Individual values are superimposed. Dotted lines indicate a ratio of 1. A Th1-biased response is >1, a Th2-biased response is <1. P, placebo.

**Figure S7. IFNγ/IL-4 T cell responses to Membrane peptide library in vaccinated subjects**



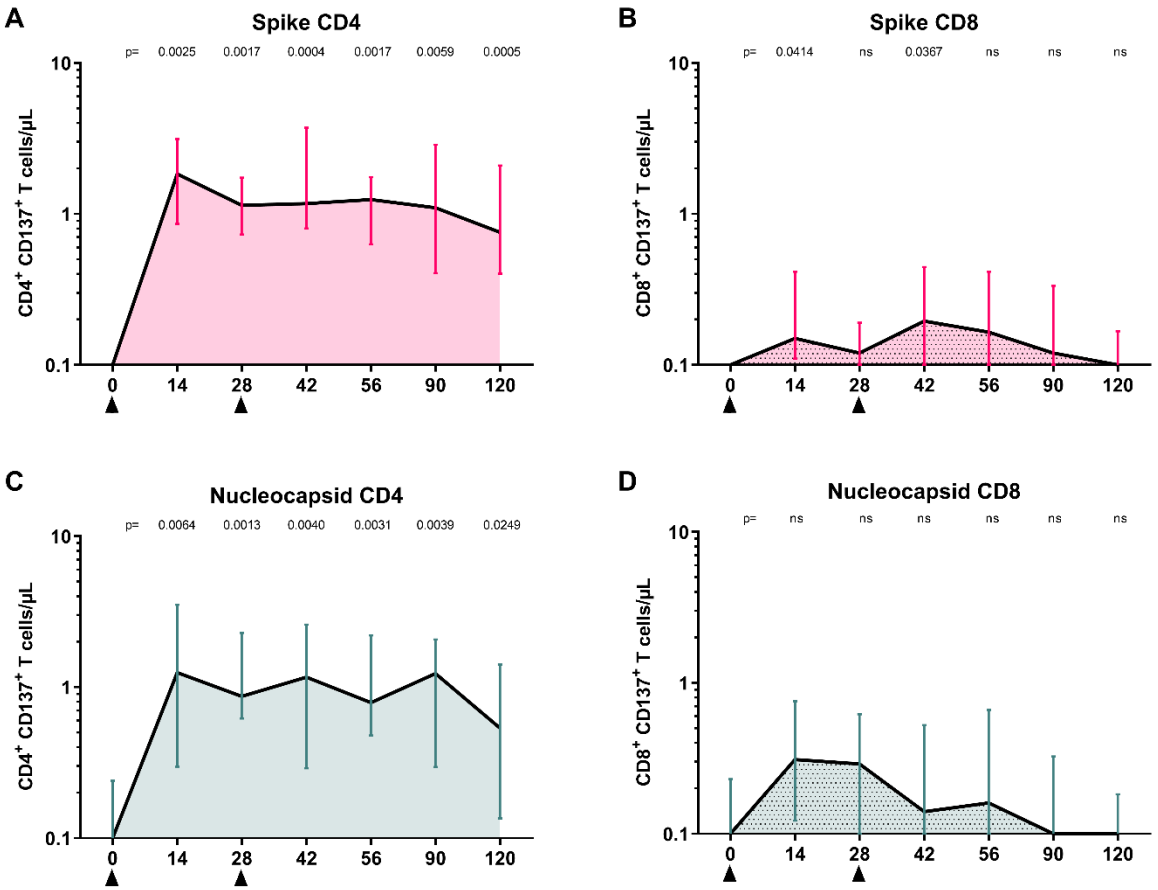

Membrane-specific T cells secreting IFNγ (A) and IL-4 (B) were quantified at the given days post vaccination in subjects vaccinated with COH04S1 at dose-level (DL) 1 (DL1/DL1 and DL1/placebo/DL1), DL2 (DL2/DL2), and DL3 (DL3/DL3) by ELISPOT upon PBMCs stimulation with a membrane peptide library. Shown are spot forming cells per $10^6$ PBMCs that were obtained after subtraction of spots in unstimulated controls from stimulated samples. Box plots extends from the 25$^{th}$ to the 75$^{th}$ percentiles, median values are shown as a line, whiskers extend from minimum to maximum values. Individual values are superimposed. Dotted lines represent the arbitrary threshold for positive response (50 spots/$10^6$ PBMCs). Arrowheads represent time of vaccination. P, placebo.

**Figure S8. Humoral and cellular responses in placebo recipients**



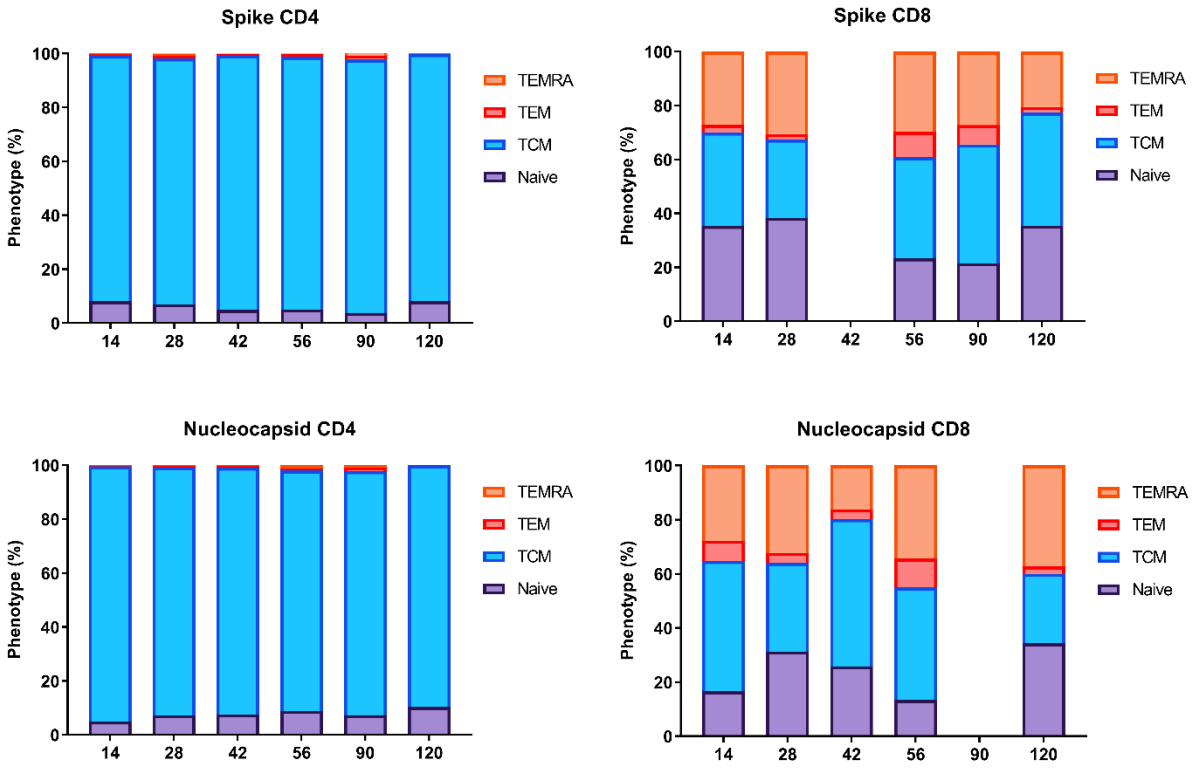

IgG binding antibody titers specific for S, RBD, and N, and neutralizing antibody titers were evaluated at the given time points in placebo recipients (N=5) up to day 56 post-prime immunization (A). Box plots extends from the 25$^{th}$ to the 75$^{th}$ percentiles, median values are shown as a line, whiskers extend from minimum to maximum values. Individual values are superimposed. Dotted line represents the lower limit of detection for the assays (ELISA=150, pvNT50=20). S- and N-specific T cells secreting IFNγ or IL-4 were quantified by ELISPOT (B). Dotted line represents the arbitrary threshold for positivity (50 spots/$10^6$ cells). Arrowheads indicate time of placebo vaccinations.

**Figure S9. Spike and Nucleocapsid CD137+ T cells in DL1-3 sentinels**



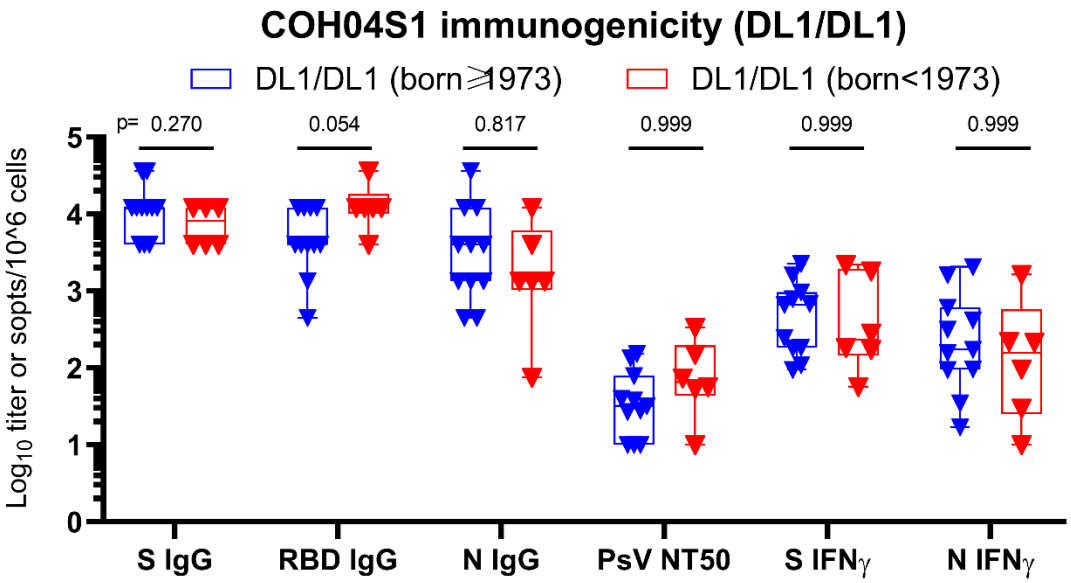

S- and N-specific $CD4^+CD137^+$ and $CD8^+$ $CD137^+$ T cells were measured in available samples from sentinel subjects vaccinated with COH04S1 DL1 (N=4), DL2 (N=7), and DL3(N=4). Shown are $CD4^+CD137^+$ and $CD8^+$ $CD137^+$ T cell counts per µl of blood. Black lines indicate median values, lines indicate interquartile ranges. Two-sided, Wilcoxon rank sum paired test was performed and each timepoint was compared to baseline. P values are indicated above each timepoint. ns=not significant ($p>0.05$). Arrowheads indicate time of vaccinations.

**Figure S10. Spike and Nucleocapsid CD137+ T cells activated/cycling phenotype in DL1-3 sentinels**




Samples from COH04S1-vaccinated DL1-3 sentinel subjects with S- and N-specific $CD4^{+}CD137^{+}$ and $CD8^{+}$ $CD137^{+}$ T cell % ≥ 0.2% (Figure S9) were analyzed for the presence of CD28 and CD45RA memory membrane markers. Shown are longitudinal memory phenotypes % classified as follow: naïve/naïve-like ($CD45RA^{+}$ $CD28^{+}$ cells), central memory (TCM; $CD45RA^{-}$ $CD28^{+}$ cells), effector memory T-cells (TEM; $CD45RA^{-}$ $CD28^{-}$ cells), and effector "revertant" T-cells, re-expressing the RA isoform of the CD45 surface marker (TEMRA; $CD45RA^{+}$ $CD28^{-}$).

**Figure S11. COH04S1 immunogenicity in subjects born before or after 1973**




Subjects in the DL1/DL1 cohort were divided based on date of birth between individuals born before (N=6) or after 1973 (N=11), the year of the end of the smallpox eradication campaign. Subjects' orthopoxvirus immune status was not disclosed and only the date of birth could be used to define possibility of a poxvirus pre-existing immunity. Shown are COH04S1-induced binding (S IgG, RBD IgG, and N IgG), and neutralizing antibody titers (PsV NT50), and T cell responses (S and N IFNγ). Two-way ANOVA followed by Sidak's multiple comparison test was used to define significance.

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