RESPIRATORY AND MENINGEAL PATHOGENS RESEARCH UNIT

STATISTICAL ANALYSIS PLAN

Study Title	An adaptive phase I/II randomized placebo-controlled trial to determine safety, immunogenicity and efficacy of non-replicating ChAdOx1 SARS-CoV-2 vaccine in South African adults living without HIV; and safety and immunogenicity in adults living with HIV.
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Statistical Analysis Plan

An adaptive phase I/II randomized placebo-controlled trial to determine safety, immunogenicity and efficacy of non-replicating ChAdOx1 SARS-CoV-2 vaccine in South African adults living without HIV; and safety and immunogenicity in adults living with HIV.

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Contents

1 Introduction

In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China and were later confirmed to be infected with a novel coronavirus, known as 2019-nCoV. The virus was subsequently renamed to SARS-CoV-2 because it is similar to the coronavirus responsible for severe acute respiratory syndrome (SARS-CoV), a lineage B betacoronavirus. SARS-CoV-2 shares more than 79% of its sequence with SARS-CoV, and 50% with the coronavirus responsible for Middle East respiratory syndrome (MERS-CoV), a member of the lineage C betacoronavirus. COVID-19 is the infectious disease caused by SARS-CoV-2. By January 2020 there was increasing evidence of human to human transmission as the number of cases rapidly began to increase in China. Despite unprecedented containment measures adopted by the Chinese government, SARS-CoV-2 rapidly spread across the world.

ChAdOx1-nCoV-19 vaccine consists of the replication-deficient simian adenovirus vector ChAdOx1, containing the structural surface glycoprotein (Spike protein) antigen of the SARS CoV-2 (nCoV-19), with a leading tissue plasminogen activator (tPA) signal sequence. ChAdOx1-nCoV-19 expresses a codon-optimised coding sequence for the Spike protein from genome sequence accession GenBank: MN908947. The tPA leader sequence has been shown to be beneficial in enhancing immunogenicity of another ChAdOx1 vectored CoV vaccine (ChAdOx1 MERS) (CITE Alharbi, N.K., et al., ChAdOx1 and MVA based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. Vaccine, 2017. 35(30): p. 3780-3788.)

In April 2020 human trials of the experimental ChAdOx1-nCoV-19 vaccine against COVID-19 commenced in the UK and were followed by studies in Africa and South America with protocols that aligned with the UK study protocols. The South African trial is enrolling adults living with HIV and living without HIV to assess safety, immunogenicity and efficacy of two doses of ChAdOx-nCoV-19.

2 Objectives and endpoints

2.1 In adults living without HIV

Primary objective - safety To assess the safety, tolerability and reactogenecity profile of the candidate vaccine ChAdOx1 nCoV

The endpoint measures for this objective are the following Endpiont mea-

sures:

- occurrence of solicited local reactogenicity signs and symptoms for 7 days following vaccination
- occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following
- occurrence of unsolicited adverse events (AEs) for 28 days following vaccination
- change from baseline for safety laboratory measures
- occurrence of serious adverse events
- occurrence of disease enhancement episodes

Primary Objective - efficacy To assess the efficacy of the candidate ChAdOx1 nCov-19 against all-severity COVID-19

The endpoint for this objective is **virologically-confirmed COVID-19 clinical disease which is defined as an acute respiratory illness that is clinically consistent with COVID-19 based on presence of criteria indicated in Table 1 and Table 2 and positive SARS-Cov-2 specific reverse transcriptase polymerase chain-reaction (RT-PCR)**. Events will be included if they occurred more than 14 days after the booster dose.

Participants meeting this endpoint should be COVID-19 naïve at randomization and received two doses of ChAdOx1 nCoV-19 or placebo. "COVID-19 naïve" will be defined as seronegative, and tested negative for SARS-COV-2 infection in the 96 hours preceding randomisation, based on a high sensitivity serology antibody test and molecular detection testing of nasal swab samples, respectively.

Secondary objective - efficacy To assess the efficacy of the candidate ChAdOx1 nCov-19 against COVID-19 of differing severity Vaccine efficacy (VE) for the following endpoints will be analysed for the overall population and stratified by COVID-19 serological status at randomisation:

1. Virologically-confirmed COVID-19 clinical disease including all cases occurring onward from 21 days after a single dose.
2. Virologically-confirmed COVID-19 clinical disease occurring more than 14 days after a second dose for the overall population including those that were sero-positive at baselinev.
3. PCR positive COVID-19 disease cases.

4. Virologically-confirmed severe COVID-19 disease.
5. Virologically-confirmed moderate-severe COVID-19 clinical disease.
6. Hospitalization due to virologically-confirmed COVID-19 disease
7. Death associated with virologically-confirmed COVID-19 clinical disease
8. All-cause LRTI (overall and stratified by hospitalization or not), irrespective of test result for SARS-COV-2.
9. Oxford Primary Outcome definition (PCR+ at least one symptom of fever $> 37.8°C$, cough, shortness of breath, anosmia, aguesia.)

Secondary objective To assess the cellular and humoral immunogenicity of ChAdOx1 nCoV-19 The following outcomes will be described for all participants

1. Enzyme-linked immunosorbent assay (ELISA) or fluorescence based micro-bead immunosorbent assay on luminex platform to quantify antibodies against SARS-CoV-2 spike protein (seroconversion rates)
2. Interferon-gamma (IFN-?) enzyme- linked immunospot (ELISpot) responses to SARS-CoV-2 spike protein
3. Virus neutralising antibody (NAb) assays against live and/or pseudotyped SARS-CoV-2 virus
4. Th1 and Th2 cytokine response profile at 3-4 days after vaccination.

Exploratory objective To assess B cell responses to SARS-CoV-2 spike trimer and/or the receptor binding domain The following outcomes will be described for all participants

1. Cellular Fc effector functionality assays to measure the ability of vaccine elicited antibodies to mediate cellular cytotoxicity, complement deposition, and phagocytosis.
2. Flow cytometric sorting of plasmablasts and memory B cells to using spike and receptor binding domain "baits" to isolate SARS-CoV-2 specific B cells, sequence their immunoglobulin genes and define their epitope specificity.

2.2 In adults living with HIV

Primary objective - safety To assess the safety, tolerability and reactogenecity profile of the candidate vaccine ChAdOx1 nCoV

The endpoint measures for this objective are the following Endpiont measures:

- occurrence of solicited local reactogenicity signs and symptoms for 7 days following vaccination
- occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following
- occurrence of unsolicited adverse events (AEs) for 28 days following vaccination
- change from baseline for safety laboratory measures
- occurrence of serious adverse events
- occurrence of disease enhancement episodes

Co-primary objective To assess the cellular and humoral immunogenicity of ChAdOx1 nCoV-19 The following outcomes will be described for all participants

1. Enzyme-linked immunosorbent assay (ELISA) or fluorescence based micro-bead immunosorbent assay on luminex platform to quantify antibodies against SARS-CoV-2 spike protein (seroconversion rates)
2. Interferon-gamma (IFN-?) enzyme- linked immunospot (ELISpot) responses to SARS-CoV-2 spike protein
3. Virus neutralising antibody (NAb) assays against live and/or pseudotyped SARS-CoV-2 virus
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Exploratory objective To assess B cell responses to SARS-CoV-2 spike trimer and/or the receptor binding domain The following outcomes will be described for all participants

1. Cellular Fc effector functionality assays to measure the ability of vaccine elicited antibodies to mediate cellular cytotoxicity, complement deposition, and phagocytosis.
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Table 1: Symptoms of Suspected COVID-19

Respiratory	**Non-Respiratory**
New onset cough New onset rapid breathing New onset shortness of breath (or breathlessness or difficulty breathing) Sore throat Loss of smell (or smell disturbance) Nasal congestion Runny nose	Fever or feverishness (defined subjectively, or objective fever $\geq$37.8°C, regardless of use of anti-pyretic medication Myalgia (or muscle ache) Chills Loss of taste (or taste disturbance) Headache Diarrhea Tiredness (or fatigue or weakness) Nausea or vomitting Loss of appetite

Table 2: Efficacy Endpoint Definitions of COVID-19 Severity

COVID-19 Severity	Endpoint Definition
Mild	Any one of: • Fever (defined by subjective or objective measure, regardless of use of anti-pyretic medications) • New onset cough • ≥2 COVID-19 respiratory/non-respiratory symptoms in Table 1 AND • Does not meet criteria for moderate or severe
Moderate	≥1 of • Fever (≥ 37.8°C) + any 2 COVID-19 symptoms in table 4 for ≥3 days (need not be contiguous days) • High fever (≥ 38.4°C) for ≥ 3 days (need not be contiguous days) • Any evidence of significant LRTI: – Shortness of breath (or breathlessness or difficulty breathing) with or without exertion (beyond baseline) – Tachypnea: 20 to 29 breaths per minute at rest – SpO2: < 94% on room air – Abnormal chest x-ray/CT consistent with pneumonia or LRTI – Adventitious sounds on lung auscultation
Severe	≥1 of • Tachypnea: ≥ 30 breaths per minute at rest • SpO2: < 92% on room air or PAO2/FiO2 < 300 • High flow oxygen therapy, CPAP, or NIV (eg, CPAP/BiPAP) • Mechanical ventilation or ECMO • One or more major organ system failure[1](eg, cardiac/circulatory, pulmonary, renal, hepatic to be defined by diagnostic testing/clinical syndrome/interventions)

Abbreviations: BiPAP = bi-level positive airway pressure; CPAP = continuous positive air pressure; CT = computed tomography; ECMO = extracorporeal membrane oxygenation; FiO2 = fraction of inspired oxygen; LRTI = lower respiratory tract infection; NIV = non-invasive ventilation; PAO2 = partial pressure of oxygen in the alveolus; SpO2 = oxygen saturation

[1] Evidence of major organ dysfunction or failure includes but is not limited to any of acute respiratory distress syndrome (ARDS), acute renal failure, acute hepatic failure, acute right or left heart failure, septic or cardiogenic shock, or requirement for vasopressors, systemic corticosteroids, or hemodialysis

3 Study design

This is a Phase I/II, double-blinded, placebo-controlled, individually randomized study in adults aged 18-65 years living with and without HIV in South Africa. Two doses of ChAdOx1 nCoV-19 or placebo (normal saline - 0.9% NaCl) will be administered spaced 21-35 days apart via an intramuscular injection into the deltoid. Participants will be divided into three study groups. Group-1 (HIV-uninfected adults; n=70) and Group-3 (HIV-infected adults), a two dose schedule will be evaluated for safety and immunogenicity. In Group 2 (phase II; immunogenicity and efficacy cohort), we will target enrolling 1900 participants to accrue sufficient number of endpoints to analyze for efficacy of at least 60% (and a lower bound of >0%) and 80% power assuming an attack rate of 3.5% for COVID-19 in the placebo arm (see sample size section). Based on the endpoint case accrual and the trajectory of the epidemic in South Africa, the sample size may be adjusted in relation to number of endpoints being accrued. Participants already enrolled prior to implementation of Version 3.0 of the protocol, that tested positive for SARS-CoV-2 by PCR at randomization will continue on the study, including all further scheduled visits and procedures. However, an equal number of additional participants that test negative for SARS-CoV-2 on PCR testing will be enrolled into the study.

The three trial groups are detailed in Table 3, with an overall sample size of 2020. Randomisation will take place at an intervention to placebo ratio of 1:1 in blocks of 8 and all participants and clinical study staff will be blinded to IP or placebo. Site pharmacists and the person administering the allocated IP/placebo will be unblinded.

Participants will be followed over the duration of the study (through to 365 days post-randomization) to record adverse events and episodes of virologically confirmed symptomatic COVID-19. Participants will be tested for SARS-CoV-2 infection if they present with a new onset of symptoms suggestive of COVID-19 (Table 4) throughout the duration of their participation.

Detailed clinical parameters will be collected from medical records (or examination by study-staff) and aligned with the COVID-19 score; Table 2. These include measuring severity based on oxygen saturation, need for oxygen therapy, respiratory rate and other vital signs, need for ventilatory support, X-ray imaging and blood test results, amongst other clinically relevant parameters; Table 2.

The DSMC will periodically assess safety and efficacy data every 2-8 weeks and/or its discretion. All deaths and any serious adverse event considered to be study-related will be reviewed by the DSMC within 72 hours of site reporting of such cases to the DSMC (which will occur within 24 hours of site identification

on any such cases).

Table 3: Trial groups

Group	Group description	Objective
1 (n=70)	People living without HIV	Intensive safety and immunogenicity
2a (n=250)	People living without HIV	Safety, intensive immunogenicity and vaccine efficacy
2b (n=1650)	People living without HIV	Safety, immunogenicity and vaccine efficacy
3 (n=50)	People living with HIV	Intensive safety and immunogenicity

4 Study size and power

Sample size in Group 1 increased from 50 to 70, following higher than anticipated positivity for SARS-CoV-2 infection (six of initial 24 randomized into the study), to accommodate for non-evaluable (i.e. not COVID-19 naive) participants.

Sample size calculations for Group 2 were performed based on the total number of cases required to conclude with 80% power at least a 60% vaccine efficacy (lower bound of 95%CI >0) with an attack rate of 3.5% in the placebo arm. Calculations were adjusted for a 10% loss to follow-up. An estimated 42 cases are required in order to achieve the required power for the study.

A complete detail of the sample size calculations are provided in the study protocol.

5 Analysis datasets

We define the following study groups based on COVID-19 status at time of randomisation:

Overall population All participants

COVID naïve All participants that are sero-negative based on serology antibody test and a negative PCR result at time of randomisation.

COVID positive All participants that are sero-postive based on serology antibody test or have a positive PCR result at time of randomisation

We define the following terms based on investigational product received:

Per-protocol analysis Refers to when all participants who received two doses of planned product (ChAdOx1 nCOV-19 or placebo), where the second dose is ¿21 days after the first dose are included in the analysis.

Modified intention-to-treat Refers to when all participants who received two doses of ChAdOx1 nCOV-19 or placebo, where the second dose is ¿21 days after the first dose are included in the analysis.

6 Analysis plan

To determine which participants had a virologically confirmed COVID-19 episode, two independent reviewers will review study files of potential participants. Potential participants will be those which have a PCR positive result 14 days following the second vaccination and COVID-19 related symptoms.

6.1 Primary objective - safety

6.1.1 Solicited local reacotgenecity

All participants in Group 1 and 3 will be included in this analysis. Counts and proportions for the occurrence of each solicited local reactogenicity signs and symptoms for 7 days following vaccination will be presented along with the 95% confidence interval for proportions will be presented, stratified by group and vaccination visit (prime or booster).

6.1.2 Solicited systemic reactogenicity

All participants in Group 1 and 3 will be included in this analysis. Counts and proportions for the occurrence of each solicited systemic reactogenicity signs and symptoms for 7 days following vaccination will be presented along with the 95% confidence interval for proportions will be presented, stratified by group and vaccination visit (prime or booster).

6.1.3 Unsolicited adverse events

All participants in Group 1 and 3 will be included in this analysis. Counts and proportions for the occurrence of each unsolicited adverse events for 28 days following vaccination will be presented along with the 95% confidence interval for proportions will be presented, stratified by group and vaccination visit (prime or booster). MEDRA terms will be used to classify adverse events.

6.1.4 Safety laboratory measures

All participants in Group 1 and 3 will be included in this analysis.

6.1.5 Serious adverse events

All participants in Group 1 and 3 will be included in this analysis. Counts and proportions for the occurrence of serious adverse events will be presented along with the 95% confidence interval for proportions will be presented, stratified by group and vaccination visit (prime or booster).

6.1.6 Disease enhancement episodes

All participants in Group 1 and 3 will be included in this analysis. Counts and proportions for the occurrence of disease enhancement episodes will be presented along with the 95% confidence interval for proportions will be presented, stratified by group and vaccination visit (prime or booster).

6.2 Primary objective - efficacy

6.2.1 Main

Only per-protocol COVID-naïve participants will be included in this analysis. The endpoint is virologically-confirmed COVID-19 clinical disease where both the PCR positive result and the onset of the first symptom occurs 14 days after administration of the booster dose. Vaccine efficacy and 95% CI will be reported.

6.2.2 Sensitivity analysis

A sensitivity analysis will be conducted which includes modified intention-to-treat COVID-naïve participants irregardless of whether they received the planned investigational product or placebo. The endpoint is virologically-confirmed COVID-19 clinical disease where both the PCR positive result and the onset of the first symptom occurs 14 days after administration of the booster dose. Vaccine efficacy and 95% CI will be reported.

6.3 Secondary objective - efficacy

1. Virologically-confirmed COVID-19 clinical disease occurring more than 14 days after a second dose for the overall population including those that were COVID positive at baseline

   For this objective, the same endpoint for the primary efficacy objective is used. The analysis group includes all per-protocol participants. Vaccine

efficacy and 95% CI will be reported.

For the following objectives, a modified intention-to-treat cohort will be used. VE and 95% CI will be reported for the overall population and stratified by COVID-19 naïve status at baseline.

2. Virologically-confirmed COVID-19 clinical disease including all cases occurring onward from 21 days after a single dose
3. PCR positive COVID-19 disease cases
4. Virologically-confirmed severe COVID-19 disease
5. Virologically-confirmed moderate-severe COVID-19 clinical disease
6. Hospitalization with virologically-confirmed COVID-19 disease
7. Death with virologically-confirmed COVID-19 clinical disease
8. All-cause LRTI with and without hospitalisation

6.3.1 Oxford primary outcome definition

The endpoint of interest for this objective is a PCR positive result and at least one symptom of fever $> 37.8°C$, cough, shortness of breath, anosmia, aguesia occurring 14 days after administration of booster dose. VE and 95% CI will be reported for the overall population and stratified by COVID-19 serological status at baseline. Details of this analysis are including in the combined endpoints statistical analysis plan

6.4 Immunological secondary objective

1. Antibodies against SARS-CoV-2 spike protein All participants will be included in this analysis. ELISA results will be analysed using geometric mean concentrations (GMCs) and 95% CI for GMCs. Seroconversion results will be reported using counts, proportions and 95% CIs.

   ELISA results and the proportion of seroconversion, stratified by HIV status, will be reported at the following visit: prime vaccination, 14 days post prime vaccination, booster dose, 14 days post booster, 28 days post booster, six months post prime vaccination and one year post prime vaccination.

2. ELISpot responses to SARS-CoV-2 spike protein All participants in Group 1, 2a and 3 will be included in this analysis.

ELISpot results, stratified by HIV status, will be reported at the following visit: 14 days post prime vaccination and 14 days post booster.

3. Neutralising antibody assays All participants will be included in this analysis.

   Results, stratified by HIV status, will be reported at the following visit: 14 days post booster, 28 days post booster and one year post prime vaccination.

4. Th1 and Th2 cytokine response profile All participants in Group 1, 2a and 3 will be included in this analysis.

   Results, stratified by HIV status, will be reported seven days post prime vaccination. For Group 1 and 3 only, results will also be reported seven days post booster dose.

6.5 Exploratory objective

1. Cellular Fc effector functionality assays
2. Flow cytometric sorting

7 Analytical methods

7.1 Proportions

Proportions will be used to describe categorical data. The Agresti-Coull method will be used to calculate confidence intervals.

7.2 Geometric mean concentrations

Geometric mean concentrations (GMCs) will be used to describe immunological measurements. Immunological measurements will be log transformed. The mean and confidence interval will be calculated for the log values assuming a normal distribution. Confidence intervals for the GMC will be calculated by back transforming the confidence intervals for the log values.

7.3 Vaccine efficacy

Vaccine efficacy (VE) will be calculated as 1-RR, where the relative risk is the number of participants who reached the primary endpoint in vaccinated group divided by the number of participants who reached the primary endpoint in un-vaccinated group. Vaccinated and unvaccinated grouping is based on the

type of analysis: per-protocol or modified intention-to-treat. 95% confidence intervals will be estimated using the Clopper-Pearson exact method.

8 Analysis of safety and efficacy of ChAdOx1-nCoV-19

8.1 Global pooled analysis

The safety and efficacy of the ChAdOx1 nCoV-19 vaccine will be determined by a global pooled analysis that incorporates data from four ongoing phase 2 and 3 clinical studies of the vaccine in the UK (COV-001, COV-002, COV-003), Brazil, and South Africa (COV-005). The pooled analysis of data from these four studies will provide greater precision for both efficacy and safety outcomes than can be achieved in individual studies and provides a broader understanding of the use of the vaccine in different populations. The pooled statistical analysis plan has been developed in collaboration with AstraZeneca for the purposes of the marketing authorisation application (MAA) for the vaccine and is included as Appendix 1 of the Integrated Summary for Marketing Authorisation Application. The planned pooled analyses do not preclude additional analyses being conducted that are beyond those required for regulatory submissions. However, any additional analyses will follow all principles of the analysis detailed in the global MAA SAP to every extent possible. A declaration of efficacy from a global pooled analysis will not be considered a reason to stop the trial. All trial procedures will continue and further cases will accumulate depending on the progress of the epidemic in SA.

8.2 Study-specific analysis

Data from this study will contribute to the global pooled analysis for interim and primary efficacy analyses. In addition, at the end of this study (COV-005) a final study-specific analysis will be conducted to assess efficacy and safety of ChAdOx1 nCoV-19 in SA. The final analysis will incorporate only data from COV005 and will not be considered a formal assessment of efficacy as that is done at the global level but will be a supportive analysis. The timepoints for interim and final data locks for global analyses will be determined by the accumulation of cases that meet eligibility criteria for analysis across the four studies as detailed in Appendix 1 of the Integrated Summary for Marketing Authorisation Application. Alpha will be controlled globally at the 5Alpha for the study-specific analysis will be controlled separately at the 5The study-specific final analysis will incorporate both the data that previously contributed

to global pooled analysis, as well as additional data that has accumulated in the time after those analyses were conducted.

8.2.1 Handling of dropouts, missing Data, and outliers

All available data will be included in the analysis.