3. BACKGROUND AND RATIONALE

3.1. Background

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 [1]. 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 [2]. COVID-19 is the illness 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. The WHO declared the COVID-19 outbreak a public health emergency of international concern on 30th January 2020. As of 22nd April 2020, over 2.5 million cases have been reported with more than 177 000 deaths globally (Worldometers.info).

Coronaviruses (CoVs) are spherical, enveloped, large positive-sense single-stranded RNA genomes. One-fourth of their genome is responsible for coding structural proteins, such as the spike (S) glycoprotein, envelope (E), membrane (M) and nucleocapsid (N) proteins. E, M, and N are mainly responsible for virion assembly whilst the S protein is involved in receptor binding, mediating virus entry into host cells during CoVs infection via different receptors [3]. SARS-CoV-2 belongs to the phylogenetic lineage B of the genus *Betacoronavirus* and it uses the angiotensin-converting enzyme 2 (ACE2) as the entry receptor [4]. It is the seventh CoV known to cause human infections and the third known to cause severe disease after SARS-CoV and MERS-CoV.

The spike protein is a type I, trimeric, transmembrane glycoprotein located at the surface of the viral envelope of CoVs, which can be divided into two functional subunits: the N-terminal S1 and the C-terminal S2. S1 and S2 are responsible for cellular receptor binding via the receptor binding domain (RBD) and fusion of virus and cell membranes respectively, thereby mediating the entry of SARS-CoV-2 into target cells.[3] Neutralizing antibodies to SARS-CoV-2 are widely assumed to be correlated with recovery from infection, and the use of passively infused convalescent sera is being assessed for treatment of COVID-19. Such antibodies may protect from infection, as in vitro studies showed that cross-reactive SARS-CoV-1 antibodies prevented SARS-CoV-2 infection. The roles of S in receptor binding and membrane fusion, and the fact that it is the main target for neutralising antibodies, makes it an ideal target for vaccine and antiviral development. Furthermore, the potential of spike antibodies to mediate Fc effector functions has not been examined in SARS-CoV-2 vaccines,

nor extensively in any

related coronaviruses including SARS-CoV-1. Fc effector function is protective against Ebola and HIV as well as against respiratory diseases such as tuberculosis and Influenza (Saphire, et al., 2018; Lu, et al., 2016; Su, et al., 2019; Vanderven and Kent, 2020).

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) [5].

3.2. Pre-Clinical Studies

3.2.1. Immunogenicity (Jenner Institute, unpublished)

Mice (balb/c and CD-1) were immunised with ChAdOx1 expressing SARS-CoV-2 Spike protein or green fluorescent protein (GFP). Spleens were harvested for assessment of IFY ELISpot responses and serum samples were taken for assessments of S1 and S2 antibody responses on ELISA at 9 or 10 days post vaccination. The results of this study show that a single dose of ChAdOx1 nCoV was immunogenic in mice.

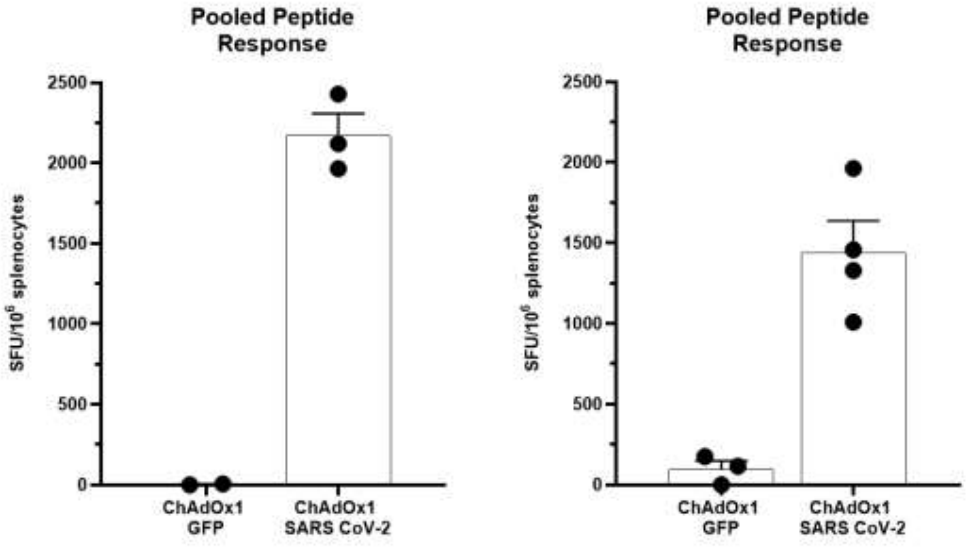

Figure 1. Summed splenic IFN-γ ELISpot responses of BALB/c (left panel) and CD-1 (right panel) mice, in response to peptides spanning the spike protein from SARS-CoV-2, nine or ten days post vaccination, with 1.7 × 10^{10} viral particles (vp) ChAdOx1 nCoV-19 or 8 × 10^{9} vp ChAdOx1 GFP.

Mean with SEM are depicted.

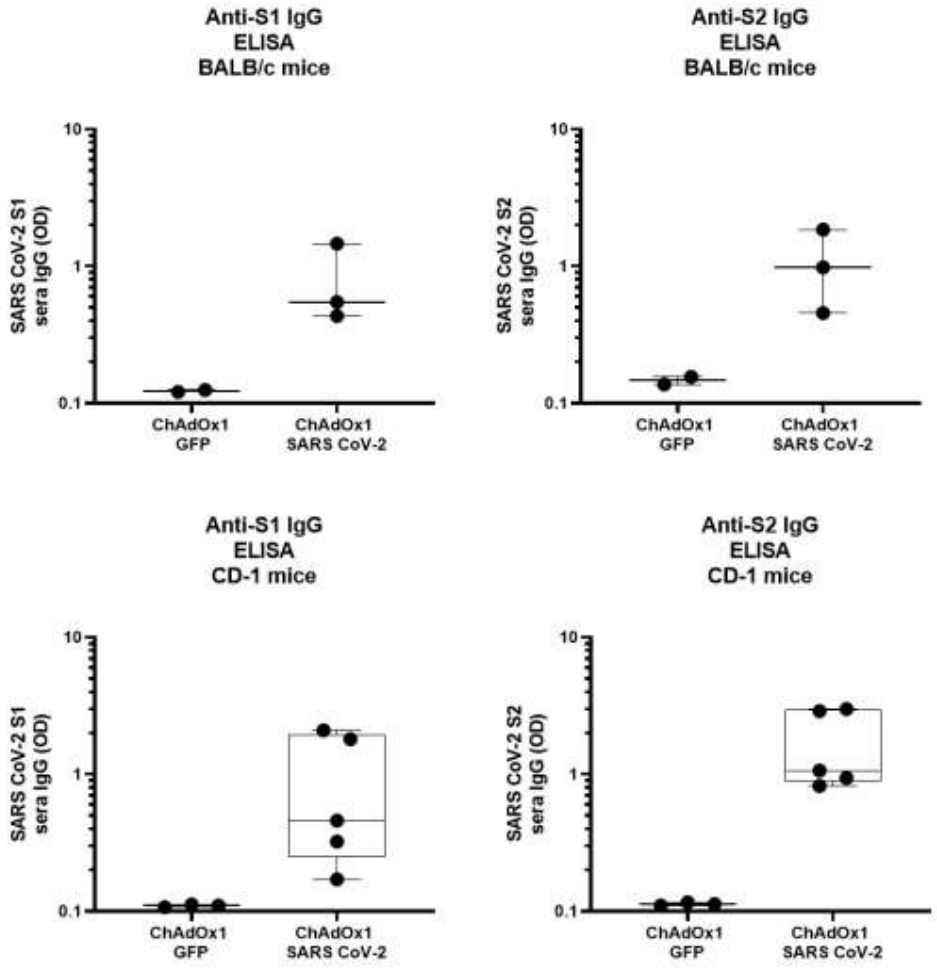

Figure 2. Box and whisker plot of the optical densities following ELISA analysis of BALB/C mouse sera (Top panel) incubated with purified protein spanning the S1 domain (left) or purified protein spanning the S2 domain (right) of the SARS-CoV-2 spike nine or ten days post vaccination, with 1.7×10^{10} vp ChAdOx1 nCoV-19 or 8×10^{9} vp ChAdOx1 GFP. Box and whisker plots of the optical densities following ELISA analysis of CD-1 mouse sera (Bottom panel) incubated with purified protein spanning the S1 domain (left) or purified protein spanning the S2 domain (right) of the SARS-CoV-2 spike.

3.2.2. Efficacy

Pre-clinical efficacy studies of ChAdOx1 nCoV-19 in ferrets and non-human primates are underway. Results will be included in an updated Investigator's Brochure when available.

3.3. Antibody Dependent Enhancement

Safety concerns around the use of full length coronavirus Spike glycoproteins and other viral antigens(nucleoprotein) as a vaccine antigen have been raised following historical and limited reports of immunopathology and antibody dependent enhancement (ADE) reported in vitro and post SARS-CoV challenge in mice, ferrets and non-human primates immunised with whole SARS-CoV inactivated or full-length S protein based vaccines, including a study using Modified Vaccinia Ankara as a vector [6-8]. To date, there has been one report of lung immunopathology following MERS-CoV challenge in mice immunised with an inactivated MERS-CoV candidate vaccine [9]. However, in preclinical studies of ChAdOx1 immunisation and MERS-CoV challenge, no ADE was observed in hDPP4 transgenic mice, dromedary camels or non-human primates (van Doremalen et al, manuscript submitted) [10, 11].

The risks of inducing lung immunopathology in the event of COVID-19 following ChAdOx1 nCoV-19 vaccination are unknown. Challenge studies on ferrets and NHPs are underway and these pre-clinical studies will report on presence or absence of lung pathology. Results will be reviewed as soon as they emerge and will inform discussions on risk/benefit to participants receiving the Investigational Medical Product (IMP). All pathology data arising from challenge studies of other SARS-CoV-2 vaccine candidates will also be taken into account.

3.4. Previous clinical experience

The phase I/II study in health adults in the UK, initiated in late April 2020 is the first-in-human study employing ChAdOx1 nCoV-19, and as of mid-June 2020 had enrolled more than 7000 participants. Furthermore, ChAdOx1 vectored vaccines expressing different inserts have previously been used in over 320 healthy participants taking part in clinical trials conducted by or in partnership with the University of Oxford in the UK, Switzerland Uganda and Saudi Arabia (Table 1, Table 2). Most importantly, a ChAdOx1 vectored vaccine expressing the full-length Spike protein from another Betacoronavirus, MERS-CoV, has been given to 31 participants to date as part of MERS001 and MERS002 trials. ChAdOx1 MERS was given at doses ranging from $5x10^9$ vp to $5x10^{10}$ vp (table 2) with no serious adverse reactions reported. Further safety and immunogenicity results on ChAdOx1 MERS can be found on the Investigator's Brochure for ChAdOx1 nCoV-19 for reference.

Clinical trials of ChAdOx1 vectored vaccines encoding antigens for Influenza (fusion protein NP+M1), Tuberculosis (85A), Prostate Cancer (5T4), Malaria (LS2), Chikungunya (structural polyprotein), Zika (prM and E), MERS-CoV (full-length Spike protein) and Meningitis B are listed below.

None of the below mentioned clinical trials reported serious adverse events associated with the administration of ChAdOx1, which was shown to have a good safety profile.

Table 1: Clinical experience with ChAdOx1 viral vector vaccines.

Country	Trial	Vaccine	Age	Route	Dose	Number of Participants (Received ChAdOx1)	Publication / Registration Number
UK	FLU004	ChAdOx1 NP+M1	18-50	IM	$5x10^8$ vp	3	Antrobus et al, 2014. Molecular Therapy. DOI: 10.1038/mt.2013.284 [12]
					$5x10^9$ vp	3	
					$2.5x10^{10}$ vp	3	
					$5x10^{10}$ vp	6	
UK	FLU005	ChAdOx1 NP+M1 MVA NP+M1 (week 8)	18-50	IM	$2.5x10^{10}$ vp	12	Coughlan et al, 2018. EBioMedicine DOI: 10.1016/j.ebiom.2018.02.011 DOI: 10.1016/j.ebiom.2018.05.001 [13]
		ChAdOx1 NP+M1 MVA NP+M1 (week 52)	18-50	IM	$2.5x10^{10}$ vp	12	
		MVA NP+M1 ChAdOx1 NP+M1 (week 8)	18-50	IM	$2.5x10^{10}$ vp	12	
		MVA NP+M1 ChAdOx1 NP+M1 (week 52)	18-50	IM	$2.5x10^{10}$ vp	9	
		ChAdOx1 NP+M1	>50	IM	$2.5x10^{10}$ vp	12	
		ChAdOx1 NP+M1 MVA NP+M1 (week 8)	>50	IM	$2.5x10^{10}$ vp	12	

Country	Trial	Vaccine	Age	Route	Dose	Number of Participants (Received ChAdOx1)	Publication / Registration Number
UK	TB034	ChAdOx1 85A	18-50	IM	$5x10^9$ vp	6	Wilkie et al, 2020 Vaccine DOI: 10.1016/j.vaccine.2019.10.102 [14]
					$2.5x10^{10}$ vp	12	
		ChAdOx1 85A MVA85A (week 8)	18-50	IM	$2.5x10^{10}$ vp	12	
		ChAdOx1 85A (x2, 4weeks apart) MVA85A (at 4 months)	18-50	IM	$2.5x10^{10}$ vp	12	
Switzerland	TB039 (ongoing)	ChAdOx1 85A	18-55	Aerosol	$1x10^9$ vp	3	Clinicaltrials.gov: NCT04121494
				Aerosol	$5x10^9$ vp	3	
				Aerosol	$1x10^{10}$ vp	11	
				Aerosol/IM	$1x10^{10}$ vp	15	
Uganda	TB042 (ongoing)	ChAdOx1 85A	18-49	IM	$5x10^9$ vp	6	Clinicaltrials.gov: NCT03681860
					$2.5 x10^{10}$	6	
UK	VANCE01	ChAdOx1.5T4 MVA.5T4	18 – 75	IM	$2.5x10^{10}$ vp	34	Clinicaltrials.gov: NCT02390063

Country	Trial	Vaccine	Age	Route	Dose	Number of Participants (Received ChAdOx1)	Publication / Registration Number
UK	ADVANCE (ongoing)	ChAdOx1.5T4 MVA.5T4	≥18	IM	$2.5x10^{10}$ vp	23 (as of Feb 20)	Clinicaltrials.gov: NCT03815942
UK	VAC067	ChAdOx1 LS2	18-45	IM	$5x10^{9}$ vp	3	Clinicaltrials.gov: NCT03203421
					$2.5x10^{10}$ vp	10	
UK	VAMBOX	ChAdOx1 MenB.1	18-50	IM	$2.5x10^{10}$ vp	3	ISRCTN46336916
					$5x10^{10}$ vp	26	
UK	CHIK001	ChAdOx1 Chik	18-50	IM	$5x10^{9}$ vp	6	Clinicaltrials.gov: NCT03590392 DOI: https://doi.org/10.4269/ajtmh.abstract2019 Abstract #59, page 19.
					$2.5x10^{10}$ vp	9	
					$5x10^{10}$ vp	9	
UK	ZIKA001 (ongoing)	ChAdOx1 Zika	18-50	IM	$5x10^{9}$ vp	6	Clinicaltrials.gov: NCT04015648
					$2.5x10^{10}$ vp	3 (as of Feb 20)	
					$5x10^{10}$ vp	-	

Table 2: Clinical experience with ChAdOx1 MERS vaccine

Country	Trial	Vaccine	Age	Route	Dose	Number of Participants (Received ChAdOx1)	Publication / Registration Number
UK	MERS001 (ongoing)	ChAdOx1 MERS	18-50	IM	$5x10^9$ vp	6	Clinicaltrials.gov: NCT03399578 DOI: https://doi.org/10.4269/ajtmh.abstract2018 Abstract#973, page 305. Folegatti et.al. 2020, Lancet Infect.Dis, *In press*.
					$2.5x10^{10}$ vp	9	
					$5x10^{10}$ vp	9	
					$2.5x10^{10}$ vp (homologous prime-boost)	3	
Saudi Arabia	MERS002 (ongoing)	ChAdOx1 MERS	18-50	IM	$5x10^9$ vp	4	Clinicaltrials.gov: NCT04170829
					$2.5x10^{10}$ vp	3	
					$5x10^{10}$ vp	-	

3.5. Rationale

The COVID-19 pandemic has caused major disruption to healthcare systems with significant socioeconomic impacts. Containment measures have failed to stop the global spread of virus. There are currently no specific treatments available against COVID-19 and accelerated vaccine development is urgently needed. South Africa is still at an early stage of its COVID-19 outbreak, which is expected to start peaking toward the end of July 2020, but has already documented 3,500 cases and 58 deaths as of 22 April 2020 (Wordometer.info). Recent modelling data indicates that globally there are likely to be 3-4 waves of COVID-19 outbreaks, possibly extending through to 2022.

Live attenuated viruses have historically been among the most immunogenic platforms available, as they have the capacity to present multiple antigens across the viral life cycle in their native conformations. However, manufacturing live-attenuated viruses requires complex containment and biosafety measures. Furthermore, live-attenuated viruses carry the risks of inadequate attenuation causing disseminated disease, particularly in immunocompromised hosts. Given that severe disease and fatal COVID-19 disproportionally affect older adults with co-morbidities, making a live-attenuated virus vaccine is a less viable option. Replication competent viral vectors could pose a similar threat for disseminated disease in the immuno-suppressed. Replication deficient vectors, however, avoid that risk while maintaining the advantages of native antigen presentation, elicitation of T cell immunity and the ability to express multiple antigens [15]. Subunit vaccines usually require the use of adjuvants and whilst DNA and RNA vaccines can offer manufacturing advantages, they are often poorly immunogenic requiring multiple doses, which is highly undesirable in the context of a pandemic.

Chimpanzee adenovirus vaccine vectors have been safely administered to thousands of people using a wide range of infectious disease targets. ChAdOx1 vectored vaccines have been given to over 320 participants with no safety concerns and have been shown to be highly immunogenic at single dose administration. Of relevance, a single dose of a ChAdOx1 vectored vaccine expressing full-length spike protein from another betacoronavirus (MERS-CoV) has shown to induce neutralising antibodies in recent clinical trials (Folegatti et. Al. 2020. Lancet Infect Dis, In press).

A Phase I single-blind, randomised controlled trial in the UK of ChAdOx1 nCoV-19 enrolled healthy adults aged 18–55 years with no history of laboratory confirmed SARS-CoV-2 infection or of COVID-19-like symptoms, who were randomly assigned (1:1) to receive ChAdOx1 nCoV-19 at a dose of 5×10^{10} viral particles or MenACWY as a single intramuscular injection. Local and systemic reactions were more common in the ChAdOx1 nCoV-19 group and many were reduced by use of prophylactic paracetamol, including pain, feeling feverish, chills, muscle ache, headache, and malaise. There were no serious adverse events related to ChAdOx1 nCoV-19. In the ChAdOx1 nCoV-19 group, spike-specific T-cell responses peaked on day 14 (median 856 spot-forming cells per million peripheral blood mononuclear

cells, IQR 493–1802; n=43). Anti-spike IgG responses rose by day 28 (median 157 ELISA units [EU], 96–317; n=127), and were boosted following a second dose (639 EU, 360–792; n=10). Neutralising antibody responses against SARS-CoV-2 were detected in 32 (91%) of 35 participants after a single dose when measured in MNA80 and in 35 (100%) participants when measured in PRNT50. After a booster dose, all participants had neutralising activity (nine of nine in MNA80 at day 42 and ten of ten in Marburg VN on day 56). Neutralising antibody responses correlated strongly with antibody levels measured by ELISA (R^2=0·67 by Marburg VN; p<0·001).[16] These data support the decision to pursue a two dose schedule for evaluation of efficacy of the vaccine candidate. Hence, this protocol has been adapted as such, with all Group now assigned to receive either two doses of ChAdOx1 nCoV-19 or placebo (Folegatti et al, Lancet 2020).

The trial to be conducted in South Africa will enroll adults living without and with HIV to assess safety, immunogenicity and efficacy two doses of ChAdOx1-nCoV-19. The South Africa study on ChAdOx1-nCoV-19 (Group 1 enrolment) was initiated following review by the Data and Safety Monitoring Committee (which oversees multiple ChAdOx1 nCoV-19 including the UK, South African and a planned study in Kenya) of the initial safety cohort (n=50) that will be enrolled in the UK. Enrolment into Group-1 of the study in South Africa occurred in tandem with opening of enrolment of the expanded immunogenicity and "efficacy-cohort" in the UK.

Recent guidelines from the Food and Drug Administration on conduct of COVID-19 vaccine trials recommend that "although establishing vaccine safety and efficacy in SARS-CoV-2 naïve individuals is critical, vaccine safety and COVID-19 outcomes in individuals with prior SARS-CoV-2 infection, which might have been asymptomatic, is also important to examine because re-vaccination screening for prior infection is unlikely to occur in practice with the deployment of licensed COVID-19 vaccines. Therefore, COVID-19 vaccine trials need not screen for or exclude participants with history or laboratory evidence of prior SARS-CoV-2 infection. However, individuals with acute COVID-19 (or other acute infectious illness) should be excluded from COVID-19 vaccine trials".[17]

4. OBJECTIVES AND ENDPOINTS

In adults without HIV (HIV-uninfected)

Primary objective:

To assess the safety of the candidate vaccine ChAdOx1 nCoV-19 in healthy HIV-uninfected adults.

Co-primary objective:

To assess efficacy of the candidate ChAdOx1 nCoV-19 against COVID-19, defined as virologically confirmed (PCR positive) COVID-19 disease, in participants that were COVID-19 naïve at the time of randomization and who received two doses of ChAdOx1 nCoV-19 or placebo. Events will be included if they occurred more than 14 days after the booster dose. "COVID-19 naïve" will be defined as sero-negative and tested negative for SARS-CoV-2 infection, based on a high sensitivity serology antibody

test and molecular detection testing of nasal swab, respectively.

Secondary objective

To assess the immunogenicity of ChAdOx1 nCoV-19 in healthy HIV-uninfected adults

Table 3: Details of objectives Groups 1 & 2 (HIV-uninfected):

Objective	Objective details	Endpoint measures
Primary Objective (Group 1 and Group 2 a and b)	To assess the safety, tolerability and reactogenicity profile of the candidate vaccine ChAdOx1 nCoV-19	a) occurrence of solicited local reactogenicity signs and symptoms for 7 days following vaccination; b) occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following vaccination; c) occurrence of unsolicited adverse events (AEs) for 28 days following vaccination; d) change from baseline for safety laboratory measures and; e) occurrence of serious adverse events e) occurrence of disease enhancement episodes
Co- Primary objective (Group 2a and 2b; efficacy cohort)	To assess efficacy of the candidate ChAdOx1 nCoV-19 against all-severity COVID-19	The **primary efficacy [objective] and endpoint** include PCR positive COVID-19 disease cases occurring in participants that were COVID-19 naïve at randomization and who received two doses of ChAdOx1 nCoV-19 or placebo. Events will be included if they occurred more than 14 days after the booster dose. Virologically-confirmed COVID-19 clinical disease will be defined as an acute respiratory illness that is clinically consistent with COVID-19 based on presence of criteria indicated in Table 4 and Table 5 **AND** a positive SARS-CoV-2 specific reverse transcriptase polymerase chain reaction (RT-PCR)

Secondary efficacy [objectives], endpoints	To assess efficacy of the candidate ChAdOx1 nCoV-19 against COVID-19 of differing severity	**Secondary efficacy [objectives], endpoints** in for the overall population and stratified by COVID-19 serological status at randomisation include: a. VE in preventing virologically-confirmed COVID-19 clinical disease including all cases occurring onward from 21 days after a single dose. b. VE in preventing virologically-confirmed COVID-19 clinical disease occurring more than 14 days after a second dose for the overall population and those that were sero-positive at baseline. c. VE in preventing PCR positive COVID-19 disease cases. d. VE in preventing severe confirmed COVID-19 disease. e. VE in preventing virologically-confirmed moderate-severe COVID-19 clinical disease. f. VE in preventing hospitalization due to virologically confirmed COVID-19 disease g. VE in preventing death associated with virologically-confirmed COVID-19 clinical disease h. VE in preventing] all-cause LRTI (overall and stratified by hospitalization or not, irrespective of test result for SARS-COV-2. i. VE using Oxford Primary Outcome definition (PCR+ at least one symptom of fever > 37.8oC, cough, shortness of breath, anosmia, aguesia.)
Secondary objective (Group 1 and Group 2)	To assess cellular and humoral immunogenicity of ChAdOx1 nCoV-19	a) 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) b) Interferon-gamma (IFN-γ) enzyme- linked immunospot (ELISpot) responses to SARS-CoV-2 spike protein c) Virus neutralising antibody (NAb) assays against live and/or pseudotyped SARS-CoV-2 virus d) Th1 and Th2 cytokine response profile at 3-4 days after vaccination.
Exploratory immunology:	To assess B cell responses to SARS-CoV-2 spike trimer and/or the receptor binding domain	a.Cellular Fc effector functionality assays to measure the ability of vaccine elicited antibodies to mediate cellular cytotoxicity, complement deposition, and phagocytosis. b. 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.

Table 4: Symptoms of Suspected COVID-19

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

Abbreviations: COVID-19 = coronavirus disease 2019.

Table 5: Efficacy Endpoint Definitions of COVID-19 Severity

COVID-19 Severity	Endpoint Definitions
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 4 **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 – SpO_2: < 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 • SpO_2: < 92% on room air or PAO_2/FiO_2 < 300 • High flow oxygen therapy, CPAP, or NIV (eg, CPAP/BiPAP) • Mechanical ventilation or ECMO • One or more major organ system failure[a] (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; FiO_2 = fraction of inspired oxygen; LRTI = lower respiratory tract infection; NIV = non-invasive ventilation; PAO_2 = partial pressure of oxygen in the alveolus; SpO_2 = oxygen saturation.

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.

In adults living with HIV (HIV-infected)

Primary co-objectives:

- To assess the safety of the candidate vaccine ChAdOx1 nCoV-19 in adults living with HIV.
- To evaluate the immunogenicity of ChAdOx1 nCoV-19 after first and second doses of vaccine in adults living with HIV.

Table 6: Details of objectives Groups 3 (HIV-infected):

Objective	Objective details	Endpoint measures
Primary objective	To assess the safety, tolerability and reactogenicity profile of the candidate vaccine ChAdOx1 nCoV-19 in people living with HIV	a) occurrence of solicited local reactogenicity signs and symptoms for 7 days following vaccination; b) occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following vaccination; c) occurrence of unsolicited adverse events (AEs) for 28 days following vaccination; d) change from baseline for safety laboratory measures and; e) occurrence of serious adverse events; f) occurrence of disease enhancement episodes
Co-primary objective	To assess cellular and humoral immunogenicity of ChAdOx1 nCoV-19 in people living with HIV after one and two doses of vaccine	a) 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) b) Interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot) responses to SARS-CoV-2 spike protein c) Virus neutralising antibody (NAb) assays against live and/or pseudotyped SARS-CoV-2 virus d) Th1 and Th2 cytokine response profile at 3-4 days after vaccination.
Secondary objective	To descriptively compare immune responses to ChAdOx1 nCoV-19 in people living with HIV to HIV-uninfected individuals, overall and stratified by COVID-19 sero-status at enrolment.	a) 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) b) Interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot) responses to SARS-CoV-2 spike protein c) Virus neutralising antibody (NAb)

		assays against live and/or pseudotyped SARS-CoV-2 virus d) Th1 and Th2 cytokine response profile at 3-4 days after vaccination.
Exploratory immunology:	To assess B cell responses to SARS-CoV-2 spike trimer and/or the receptor binding domain	a. Cellular Fc effector functionality assays to measure the ability of vaccine elicited antibodies to mediate cellular cytotoxicity, complement deposition, and phagocytosis. b. 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.

5. TRIAL 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. ChAdOx1 nCoV-19 or placebo will be administered via an intramuscular injection into the deltoid. The protocol has been adapted to confirm that the study will assess safety, immunogenicity and efficacy of two doses of ChAdOx1 nCoV-19 based on the phase I study results from the UK immunogenicity cohort.[16] For Group-1 (HIV-uninfected adults; n=70) and Group-3 (HIV-infected adults; n=100), a two dose schedule spaced 21-35 days apart will be evaluated for safety and immunogenicity. In Group II (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 7, with an overall sample size of ~2070. 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. Once group 1 is fully recruited, safety data will be reviewed by DSMC. Group 3 enrolment will follow on from group 1 enrolment. This decision will be guided by DSMC review of COV001 trial in the UK. Initiation of enrolment into Group 2 will be contingent upon review by the joint DSMC of the ongoing study in the UK, which will also ultimately inform whether to pursue a single or two-dose schedule for the efficacy-cohort in South Africa.

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 5. 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 5.

Safety will be assessed in real time and at least monthly interim reviews by the DSMC will be scheduled after Group 1 (70 HIV-uninfected) participants received the IP (dose 1 and dose 2 if given), after enrolment of 100 HIV-infected adults (Group-3) and once all Group-2 participants are enrolled. The DSMC will periodically assess safety and efficacy data every 4-8 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 7: Trial groups

Group #	Group description	Objective	Follow up	Treatment	Vaccination schedule
1 (n=70)	People without HIV (HIV-uninfected)	Intensive Safety and immunogenicity	Intensive	ChAdOx1 nCoV-19 5-7.5x1010 vp; OR Normal saline (0.9% NaCl)	2* doses, 4 weeks (21-35 days) apart
2a (n=250)[§]	People without HIV (HIV-uninfected)	Safety, intensive immunogenicity and vaccine efficacy	Extended	ChAdOx1 nCoV-19 5-7.5x10^{10} vp; OR Normal saline (0.9% NaCl)	2* doses, 4 weeks (21-35 days) apart
2b (n=1650)[§]	People without HIV (HIV-uninfected)	Safety, immunogenicity and vaccine efficacy	Extended	ChAdOx1 nCoV-19 5-7.5x10^{10} vp; OR Normal saline (0.9% NaCl)	2* doses, 4 weeks (21-35 days) apart
3 (n=100)	People living with HIV (HIV-infected),	Intensive Safety and immunogenicity	Intensive	ChAdOx1 nCoV-19 5-7.5x10^{10} vp; OR Normal saline (0.9% NaCl)	Prime-boost 2* doses, 4 weeks (21-35 days) apart

[¥]Sample size 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.

*Participants will receive 2 doses of the same injection (EITHER IP or placebo) as assigned at randomization.

[§]Numbers will be increased to supplement for corresponding number of individuals randomized prior to implementation of Version 3.0 of the protocol that tested positive for SARS-CoV-2 on PCR at time of randomization.

[#] Following a review of the initial safety/immunogenicity trial being conducted in the UK; COV0001 trial, and review of the initial safety/ immunogenicity trial COV0001 by the DSMC, it was decided that Group 2 in this trial will receive 2 doses of assigned study intervention. SAHPRA and WHREC have already been informed of Group 2 receiving a two-dose schedule based on the earlier version of the protocol.

Also, considering the unpredictability of the force of SARS-CoV-2 infection and the lower than anticipated attack rate for the primary-endpoint cases in the study being undertaken in the UK, the sample size for Group 2 (efficacy cohort) has been expanded. This will involve enrolling up to a total of 1900 people in Group-2, which will provide 80% power to detect at least a 60% vaccine efficacy (lower bound of 95%CI >0) with an attack rate of 3.5% in the placebo arm. Ongoing review of the number of COVID-19 endpoint cases accrued during the course of the study, may lend itself to enrolling smaller number of participants should the attack rate be higher than 3.5%. The sample size for Group 1 has been increased to 70 to accommodate for the higher than anticipated infection rate with SARS-

CoV-2 (6 of the initial 24 participants randomized in group 1). Similarly, in anticipation of approximately one-third of Group-3 participants possibly being already infected with SARS-CoV-2, the sample size will be increased to 100 to have approximately 30 sero-negative vaccinees and placebo recipients enrolled into the study.

5.1. Trial participants

Adult participants (healthy HIV-uninfected; and generally well people living with HIV [Group 3]) aged 18-65 years will be enrolled. Participants will be considered enrolled immediately following randomization to receive first vaccination.

5.2. Definition of End of Trial

The end of the trial is the date of the last assay conducted on the last sample collected.

5.3. Duration of study

The total duration of the study will be 12 months from the day of enrolment for all participants.

5.4. Potential Risks for participants

The potential risks are those associated with phlebotomy, vaccination and disease enhancement

5.4.1. Venipuncture

Localised bruising and discomfort can occur at the site of venipuncture. Infrequently fainting may occur. These will not be documented as AEs if they occur. The total volume of blood drawn over a six-month period will be 160- 315mL (blood volumes may vary slightly for participants at different investigator sites due to use of different volume vacutainers, following local SOPs). This should not compromise these participants, as they would donate 470mL during a single blood donation for the National Blood transfusion Service over a 3-4-month period. Participants will be asked to refrain from blood donation for the duration of their involvement in the trial.

5.4.2. Allergic reactions

Allergic reactions from mild to severe may occur in response to any constituent of a medicinal product's preparation. Anaphylaxis is extremely rare (about 1 in 1,000,000 vaccine doses) but can occur in response to any vaccine or medication.

5.4.3. Vaccination

Local reaction from IM vaccination

The typical local reaction as a result of IM injection is temporary pain, tenderness, redness, and swelling at the site of the injection.

Systemic reactions

Constitutional influenza-like symptoms such as fatigue, headache, malaise, feverishness, and muscle aches can occur with any vaccination and last for 2-3 days. Presyncopal and syncopal episodes may occur at the time of vaccination which rapidly resolve. As with any other vaccine, temporary ascending paralysis (Guillain-Barré syndrome, GBS) or immune mediated reactions that can lead to organ damage may occur, but this should be extremely rare (1 in 100,000-1,000,000 vaccine doses).

Control participants will receive a placebo injection containing sterile normal saline (0.9% NaCl). The volume of the IP and placebo injections will be equal.

5.4.4. Disease Enhancement

The risks of inducing disease enhancement and lung immunopathology in the event of COVID-19 disease following ChAdOx1 nCoV-19 vaccination are unknown. Challenge studies on ferrets and Non-human primates (NHPs) are underway and results will be reviewed as they emerge. All pre-clinical data from challenge studies using ChAdOx1 nCoV-19 and other vaccine candidates (when available) will inform decisions on risks and benefits to participants receiving the IP.

5.5. Known Potential Benefits

Recipients of ChAdOx1 nCoV-19 do not have any guaranteed benefit. However, the information gained from this study could contribute to the development of a safe and effective vaccine against COVID-19. IP recipients may benefit from receipt of the ChAdOx1 nCoV-19 vaccine if the vaccine is found to be effective against reducing COVID-19. Placebo recipients will not benefit from receipt of placebo, however, may benefit from regular follow-up during the SARS-CoV-2 pandemic as they will be tested for infection if they are symptomatic.

6. RECRUITMENT AND WITHDRAWAL OF TRIAL PARTICIPANTS

6.1. Identification of Trial Participants

Adults in South Africa will be recruited by the following methods:

- Research sites will utilize databases available in the research units which contain contact details of participants or parents of participants in previous (completed) vaccine trials.
- Adverts, approved by local ethics committee, may be utilized and places in health care clinics and other public places.
- Radio announcements
- Community engagement via the community action boards affiliated to the research sites

6.2. Informed consent

All participants will have the opportunity to read the information sheet prior to or during screening visit. An 'assessment of understanding' (AOU) will be completed by participant to assess their understanding of participant information sheet. Participants will have the opportunity to discuss the trial information with investigators and family members. Informed consent will be signed and dated before any study specific procedures are performed. The informed consent process will be undertaken in two stages. In the first instance, following brief introduction about the study at the screening visit, including inclusion and exclusion criteria, volunteers will be asked to consent to procedures to determine their eligibility for possible randomization (full-study participation). This will include collection of key demographic information, a brief clinical history, testing inter alia for HIV-1 infection (except for Group 3), Hepatitis B surface antigen (HBsAG) positivity, evidence for current (by molecular detection) infection with SARS-CoV-2, pregnancy test (for women of reproductive age group), HbA1C (glycosylated hemoglobin) as well as general physical well-being (including blood pressure check). This approach has been adopted to accommodate for the higher than anticipated number of participants (six of 24) that tested positive for SARS-CoV-2 infection by PCR after having started enrolment of Group-1 participants.

Following fulfilment of inclusion and exclusion criteria based on findings from the screening visit, those who remain eligible and agree to undergo randomization into the full study, will be consented further for study participation. Screened participants will be encouraged to take complete informed (enrolment) consent forms home from the screening visit, and read and discuss the trial and their possible involvement in the trial with family. At the randomization visit, the participant will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasized:

Participation in the study is entirely voluntary

Refusal to participate involves no penalty or loss of medical benefits

The participant may withdraw from the study at any time.

The participant is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved

The study involves research of an investigational vaccine

There is no direct benefit to the participant from participating

Participants will be asked to provide detailed medical and surgical history to investigator

verbally and if possibly, patient-held medical records (outpatient cards) will be reviewed.

Blood samples taken as part of the study may be sent to laboratories outside South Africa (e.g. University of Oxford and InNexus BioPharma Inc in Canada) for immunogenicity testing. These will be anonymised samples. Participants who agree to full study participation will be asked if they consent to storage of any leftover samples for use in other ethically approved research for up to 25-year period, which will be optional.

The aims of the study and all tests to be carried out will be explained. The participant will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, the participant and the investigator will sign and date the relevant screening consent form, and full-study participation consent form (if eligible). However, in the current crisis, there may be occasions when it is necessary for the consent form to be signed by an appropriately trained and delegated research nurse instead of the investigator. The participant would always have the opportunity to discuss the study with a medically qualified investigator if they wish. The participant will then be provided with a copy of the consent forms to take away and keep, with the original being stored in the case report form (CRF).

6.3. Inclusion and exclusion criteria

This trial will be conducted in generally healthy adults without HIV, except for Group-3 (i.e. safety and immunogenicity in people with HIV).

6.3.1. Inclusion Criteria for all participants

The participants must satisfy all the following criteria to be eligible for the trial:

- Healthy adults aged 18-65 years.
- Documented result of not being infected with HIV (including screening by a rapid HIV antibody test) within two weeks of randomization into the study for Group-1 and Group-2 participants only.
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Willing to allow investigators review available medical records, and review all medical and laboratory records if participant is admitted to hospital with respiratory tract infection suspected or confirmed to be COVID-19.
- For females only, willingness to practice continuous effective contraception (see below) during the study and a negative pregnancy test on the day(s) of screening (within 14 days of randomization) or vaccination.
- For Group-3 only (i.e. HIV-infected), need to have been on anti-retroviral treatment for at least three months and HIV-1 viral load is <1,000 copies/ml within two weeks of randomization.

- Agreement to refrain from blood donation during the course of the study.
- Provide written informed consent.

6.3.2. Exclusion Criteria

The participant may not enter the study if any of the following apply:

- Planned receipt of any vaccine other (licensed or investigational) than the study intervention within 30 days before and after each study vaccination.
- Use of any unproven registered and unregistered treatments for COVID-19.
- Evidence of current SARS-CoV-2 infection detected by molecular assay detection of SARS-CoV-2 done within 96 hours prior to randomization.
- Acute respiratory and/or non-respiratory illness consistent with potential COVID-19 (see Table 4 for list of symptoms) concurrent or within 14 days prior to first study vaccination (medical history and/or physical examination) or documented temperature of > 38°C during this period. NOTE: This is a temporary exclusion for which the subject may be re-evaluated if they remain free from acute respiratory and/or non-respiratory illness consistent with potential COVID-19 after 14 days. Should a subject have a SARS-CoV-2 positive test, they may NOT be randomized.
- Prior receipt of an investigational or licensed vaccine likely to impact on interpretation of the trial data (e.g. Adenovirus vectored vaccines, any coronavirus vaccines).
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.
- HbSAg positivity on the screening sample, or any sample obtained within three months of randomization.
- Grade 2 or higher level of abnormality for FBC, U&E or LFT based on DAIDS Grading Criteria (Version 2.1, July 2017)
- History of allergic disease or reactions likely to be exacerbated by any component of the ChAdOx1 nCoV-19 vaccine.
- Any history of hereditary angioedema or idiopathic angioedema.
- Any history of anaphylaxis in relation to vaccination.
- Pregnancy, lactation or willingness/intention to become pregnant during the study.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of serious psychiatric condition likely to affect participation in the study.
- Bleeding disorder (e.g. factor deficiency, coagulopathy or platelet disorder), or prior history of

significant bleeding or bruising following IM injections or venipuncture.

- Any other serious chronic illness requiring hospital specialist supervision.
- Chronic respiratory diseases, including poorly controlled/ unstable asthma
- Chronic disease inclusive of:

  a) hypertension if ≥Grade 2 based on DAIDS AE Grading Version 2.1-July 2017

  b) congestive heart failure;

  c) chronic obstructive pulmonary disease by Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of ≥ 2;

  d) evidence of coronary artery disease as manifested by cardiac interventions or cardiac medications for control of symptoms;

  e) chronic type 2 diabetes (adult onset) requiring insulin;

  f) chronic kidney disease/renal insufficiency;

  g) chronic gastrointestinal and hepatic diseases; or

  h) chronic neurological diseases.

- Seriously overweight (BMI ≥ 40 Kg/m^2)
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week (% alcohol x volume (ml)/1000= number of units; e.g. Normal beer= 2 units, Glass of wine =3 units).
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Any clinically significant abnormal finding on screening urinalysis.
- Any other significant disease, disorder or finding which may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to participate in the study or impair interpretation of the study data.
- History of laboratory confirmed COVID-19 illness or known close contact with a person that was infected with SARS-COV-2. Close contact refers to being in contact with someone in the same household, or for at least 15 minutes and in close proximity with an infected person in the absence of wearing of a face masks.
- New onset of fever or a cough or shortness of breath in the 30 days preceding screening and/or enrolment

- In addition to above, Group 1 & 2 participants need to fulfil the following exclusion criteria: Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent severe infections and chronic use (more than 14 days) immunosuppressant medication within the past 6 months (topical steroids are allowed).

- Any confirmed or suspected immunosuppressive or immunodeficient state (except HIV infection for Group-3), asplenia, recurrent severe infections and chronic use (more than 14 days) immunosuppressant medication within the past 6 months (topical steroids are allowed).

Note: Stable endocrine disorders that have a confirmed autoimmune etiology (eg, thyroid, pancreatic), including stable diabetes not requiring insulin are allowed.

Should participants develop COVID-19 prior to the second dose of vaccine, or test positive for SARS-CoV-2 infection and be asymptomatic, the participants will remain eligible to receive a second dose of assigned study-intervention. The second dose of assigned study-drug will however be delayed for at least: i. 14 days in individuals that had asymptomatic SARS-CoV-2 infection, ii. 14 days after symptom resolution if mild illness; iii. 28 days after illness onset following moderate or severe illness, and is clinically stable based on the discretion of the investigator; iv. For cases requiring hospitalization for COVID-19, the 2nd dose should be delayed for at least 14 days post-discharge and participant needs to be clinically stable.

6.3.3. Re-vaccination exclusion criteria

The following AEs associated with any vaccine, or identified on or before the day of vaccination constitute absolute contraindications to further administration of an IP to the participant in question. If any of these events occur during the study, the participant will continue follow-up in the study but will not receive any further study investigational vaccine:

- Anaphylactic reaction following administration of vaccine
- Pregnancy

6.3.4. Effective contraception for female participants

Female participants of childbearing potential (any woman or adolescent who has begun menstruation) are required to use an effective form of contraception during the course of the study (i.e. until their last

follow-up visit).

Acceptable forms of contraception for female participants include:

Established use of oral, injected or implanted hormonal methods of contraception.

Placement of an intrauterine device (IUD) or intrauterine system (IUS).

Total abdominal hysterectomy.

Bilateral tubal Occlusion

Barrier methods of contraception (condom or occlusive cap with spermicide).

Post-menopausal women, defined as a woman over the age of 45 who has not had a menstrual period for at least 12 months.

Female participants will be requested to continue obtaining their contraceptives from their nearest clinic, which is provided at no-cost in the public-sector. Should this not be feasible, the study will provide female participants with the contraceptives.

Female participants in a same-sex relationship and women that are post-menopausal will not be required to be on contraception.

6.3.5. Prevention of 'Over Participating'

Participants will be excluded from the study if they are concurrently involved in another trial where an IP has been administered within 30 days prior to enrolment, or will be administered during the trial period. They will not be enrolled they are actively registered on another investigational vaccine or medication trial.

6.3.6. Withdrawal of Participants

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a participant has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the participant at any time in the interests of the participant's health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

Administrative decision by the Investigator.

Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).

Significant protocol deviation.

Participant non-compliance with study requirements.

An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the AE has resolved, stabilized or a non-trial related causality has been assigned. The DSMC or DSMC chair may recommend withdrawal of participants.

Any participant who is withdrawn from the study may be replaced, if that is possible within the specified time frame.

If a participant withdraws from the study, data and blood samples collected before their withdrawal will still be used on the analysis. Storage of blood samples will continue unless the participant specifically requests otherwise.

In all cases of participant withdrawal, long-term safety data collection, including some procedures such as safety bloods, will continue as appropriate if participants have received one or more vaccine doses, unless they decline any further follow-up.

6.4. Pregnancy

Should a participant become pregnant during the trial, no further study IP will be administered. She will be followed up for clinical safety assessment with her ongoing consent and in addition will be followed until pregnancy outcome is determined. We would not routinely perform venipuncture in a pregnant participant unless there is clinical need. Women falling pregnant during the study will also continue in follow-up for COVID-19 and be retained in the efficacy analyses. A 'Pregnancy reporting' form must be completed within 7 days of site staff becoming aware of the pregnancy, and a pregnancy outcome form must be completed within 7 days of delivery, or as soon as possible (within 7 days of site awareness) if site is notified more than 7 days post-delivery.

7. TRIAL PROCEDURES

This section describes the trial procedures for evaluating study participants and follow-up after administration of study vaccine.

7.1. Schedule of Attendance

All participants in groups 1 and 3 will have the same schedule of clinic attendances and procedures as indicated in the schedules of attendance (Table 8). Participants will receive either the ChAdOx1 nCoV-19

vaccine or NaCl (0.9%) placebo injection, and undergo follow-up for a total of 12 months' post enrolment. The total volume of blood donated during the study will be 160-315mL depending on which group they are allocated to. Additional visits or procedures may be performed at the discretion of the investigators, e.g., further medical history and physical examination, urine microscopy in the event of positive urinalysis or additional blood tests if clinically relevant.

7.2. Observations

Pulse, respiratory rate, oxygen saturation, blood pressure and temperature will be measured at the time-points indicated in the schedule of procedures and may also be measured as part of a physical examination if indicated at other time-points.

7.3. Blood tests, Nasal swab/saliva and urinalysis

Blood will be drawn for the following laboratory tests and processed at an accredited Laboratory for:

Haematology; Full Blood Count and differential count (Groups 1 and 3)

Biochemistry; U&E (Sodium, Potassium, Urea, Creatinine), Liver Function Tests (Albumin, ALT, ALP, Bilirubin) (Groups 1 and 3)

Diagnostic serology; HBsAg, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses). HIV Elisa will be repeated on HIV-negative participants at trial conclusion visit (day 364). HbA1C will be done on all participants, and those with an abnormal result will be referred for further medical care, but remain eligible for study enrolment. (All Groups)

Genetics; Human Leukocyte Antigen (HLA) typing (All Groups)

COVID-19 PCR processing (nasal swab and/or saliva)

A nasal swab and/or saliva will be collected for testing of SARS-COV-2. Sample processing will be done at the RMPRU using molecular detection methods, with confirmatory testing at another accredited laboratory. In the case of discordant results between RMPRU and the second laboratory, a further aliquot of the sample will be submitted to a third accredited laboratory for testing, and the result from the third laboratory will be assumed to be the final result.

Additional safety blood tests may be performed if clinically relevant at the discretion of the medically qualified investigators, including potential prognostic indicators or markers of severe COVID-19 disease.

Urinalysis; Urine will be tested for protein, blood and glucose at screening. For female participants only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening and immediately prior to vaccination.

Immunology; Immunogenicity will be assessed by a variety of immunological assays.

Serum IgG and IgM: The serum samples will be analysed by ELISA or on other appropriate immuno-assays platforms such as Luminex) for titres of IgG and IgM to two different versions of the spike protein (full length spike protein and receptor binding domain (RBD). These proteins are the major targets for neutralizing antibodies for SARS-CoV-2. Plasmids for these proteins were procured from the laboratory of Prof Florian Krammer, Mount Sinai USA and proteins were successfully expressed and purified in the laboratory of Prof Penny Moore at NICD, South Africa. Fluorescence based micro-bead immunosorbent assay for IgG against SARS-CoV-2 whole length spike protein and the RBD domain on luminex platform has been developed at RMPRU and assay harmonization will be done in collaboration with Prof. Andrew Pollard lab, Oxford University, United Kingdom.

This will be a two-step analysis in which first step includes screening of serum samples against the RBD and second step in which positive samples from the first step undergo a confirmatory testing against the full length spike protein. COVID-19 IgG assay against RBD and Spike protein has been set up at RMPRU and checked for sensitivity (compared to PCR positivity) and specificity (using serum samples from pre COVID months, Sep, Oct, Nov, Dec 2019). Covid 19 IgM assay set up underway. In house reference serum for both IgG and IgM will be developed by pooling convalescent serum from COVID-19 positive participants, and will be calibrated against standard reference reagent from National Institute for Biological Standards and Control (NIBSC), which is providing references sera to laboratories as part of a WHO COVID-19 serology working groups. COVID-19 assay will be further harmonized using NIBSC serum panel which includes high, medium and negative control serum panel. Each luminex run will include in-house high, medium and negative serums for quality control. The assay will further expand to IgA for breast milk analysis.

In addition, samples may be sent to the Oxford collaborators group, and possibly another reference group for further testing.

Ex vIvo- Elispot Assay:

The *ex vivo* IFN-gamma ELISpot assay, will be used to quantify the frequency of antigen-specific effector T cells in response to vaccination. The assay will be performed on standardized procedure from Oxford University. Pools of peptides needed for the assay will be supplied from Oxford University. For Elispot assay, peptides are designed to cover the length of the SARS-CoV2 spike construct and comprise 15mer peptides overlapping by 10 aa, giving a total of 258 peptides.

Cytokine analysis: Serum samples will be analysed at RMPRU for a panel of 25 cytokines which includes

TH1 cytokines (IFN-γ and interleukin (IL)-2), TH2 Cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13) and other pro-inflammatory markers such as TNF-α on multiplex bead-based immunoassay using commercial kits as per manufacturer instructions (Novex, Human Cytokine Magnetic 25-Plex PanelCatalog #: LHC0009M).

Neutralisation measurements will use an assay adapted from well-validated existing HIV-based pseudovirus neutralization assays using the pNL4–3.luc.R-E HIV construct with a SARS-CoV-2 spike protein. This assay is being developed and validated in collaboration with Dr David Montefiori, Duke University.

Pseudotyped neutralization assay:

The SARS-CoV-2 neutralization assay (optimized in collaboration with Dr Nicole-Doria-Rose, VRC) is an adaptation of a highly validated HIV neutralization assay routinely in use at the NICD. The SARS-CoV-2 assay measures neutralization of pseudotyped virus in ACE2-over-expressing 293T target cells (developed by Dr Michael Farzan, The Scripps Research Institute) as a function of a reduction in Luc reporter gene expression. The pseudotyped virus consists of lentiviral particles that are deficient for lentiviral env, but have surface SARS-CoV-2 spike protein and package the gene for firefly luciferase. Infected cells express luciferase, and luciferase activity is quantified by relative light units (RLU) of luminescence. Virus is applied to cells with or without pre-incubation with antibodies; neutralizing antibodies reduce infection, resulting in lower RLUs. Serial dilution of antibodies can be used to produce a dose-response curve to quantify potency. The assay is performed in 96-well flat-bottom black culture plates for high throughput capacity and enhanced luciferase signal Use of a clonal cell line provides enhanced precision and uniformity. Controls will be neutralizing monoclonal antibodies expressed in-house as well as COVID-19 HIV positive and negative serum samples.

Live virus neutralization assays:

This assay, being developed at the NICD by Prof Janusz Paweska utilizes live SARS-CoV-2 coronavirus cultured for one week in Vero cells. After visualization of microscopic cytopathic effects by microscopy, cultures are confirmed positive by PCR and viral stocks cryopreserved. Microneutralization assays will be performed by incubation of SARS-CoV-2 virus with Vero cells with or without pre-incubation with antibodies. Neutralizing antibodies reduce infection, resulting in reduced cytopathic effect. Cross-validation of the live and pseudotyped neutralization assays will be performed using shared SARS-CoV-2 convalescent sera and neutralizing monoclonal antibodies expressed in-house.

Fc effector functionality, including antibody dependent cellular cytotoxicity, complement deposition, and phagocytosis will assess responses to spike trimer or the receptor binding domain. ADCC will use spike trimer or receptor binding domain (RBD)-coated Huh7 cells that express the SARS-CoV-2 receptor ACE-2. Targets for ADCD and ADCP will be neutravidin fluorescent beads coated with spike or RBD proteins.

Targets will be incubated with SARS-CoV-2 convalescent sera. Effector cells for ADCC will be PBMCS from uninfected donors, and will measure granzyme B. ADCD will be measured as the amount of C3b deposition on the surface of antigen-coated beads. ADCP will be measured as the percentage of antigen-coated beads taken up by THP-1 cells in an antibody dependent manner. In addition, samples may be sent to the Oxford collaborators group, and possibly another reference group for further testing. A detailed update of the specific immunology assays to be used in this study will be provided prior to enrolling the first subject in the study.

Other exploratory immunological assays including antibody subtype assays, DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity, monoclonal antibody isolation and gene expression studies amongst others may be performed at the discretion of the Investigators.

Collaboration with other specialist laboratories in South Africa, the UK, Europe, Canada and elsewhere for further exploratory tests may occur. This would involve the transfer of serum or plasma and, PBMC and/or other study samples to these laboratories, but these would remain anonymised. Informed consent for this will be gained from participants. Samples collected for the purposes of COVID-19 diagnosis will be sent to reference laboratories in South Africa for confirmatory testing.

All participants testing positive for SARS-COV2 will be notified through the Notifiable Medical Conditions, per regulatory requirements in South Africa, and includes providing personal data to implement isolation measures of infected individuals and tracing of their contacts. Participants will be informed the obligation on the part of the investigators to submit this level of information to the Notifiable Medical Conditions registry, and local authorities that are responsible for monitoring of infected cases and their contacts.

Participants will be informed that there may be leftover samples of their blood (after testing for this study is completed), and that such samples may be stored up to 25 years for possible future research (exploratory immunology), including genotypic testing of genetic polymorphisms potentially relevant to vaccine immunogenicity. Participants will be able to decide if they will permit such future use of any leftover samples. With the participants' informed consent, any leftover cells, urine and serum/plasma will be frozen for future analysis of COVID-19 and other coronaviruses related diseases or vaccine-related responses. If a participant elect not to permit this, all of that participant's leftover samples will be discarded after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

Samples that are to be stored for future research will be stored at RMPRU.

7.4. Study visits

The study visits and procedures will be undertaken by one of the clinical trials team. The procedures to be included in each visit are documented in the schedule of attendances. Each visit is assigned a time-point and a window period, within which the visit will be conducted.

7.4.1. Screening visit

Participants will be required to share past medical and past surgical history, and medication at screening visit as an initial confirmation of eligibility. All potential participants will have a screening visit, which may take place up to 14 days prior to vaccination, although some results such as molecular testing for SARS-CoV-2 need to be done within 96 hours of randomization. The screening informed consent will be taken before screening. If consent is obtained, the procedures indicated in the schedule of attendances will be undertaken including a medical history, physical examination, blood tests and height and weight. Individually each participant will have the opportunity to question an appropriately trained and delegated researcher before signing the full-study participation consent at enrollment visit.

Abnormal clinical findings from the urinalysis or blood tests at screening will be assessed by a medically qualified study member. Abnormal clinical and blood tests following screening will be assessed according to specific laboratory adverse event grading tables (DAIDS Laboratory Grading of Abnormal Results; Version 2.1, July 2017). Any abnormal test result deemed clinically significant may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant (Grade 2 or higher abnormality), the participant will be informed and appropriate medical care arranged with the permission of the participant.

The eligibility of the participant will be reviewed at the end of the screening visit and again when all results from the screening visit have been considered. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be based on fulfilling the inclusion and exclusion criteria, as well as at the discretion of the Investigator. If eligible, a day 0 visit will be scheduled for the participant to receive the vaccine and subsequent follow-up. Participants will be consented for full study participation prior to randomization at the day-0 visit.

If more than 14 days elapse between screening and an eligible and willing participant presents for enrolment, re-screening will be required. Informed consent should be verified and discussion recorded in source document. All applicable screening procedures, except for HIV if negative in the past three months should be repeated at re-screening visit. Also, if applicable, safety bloods should be repeated, to ensure that they are done ≤14 days prior to vaccination, and SARS-CoV-2 swab needs to be done in

96 hours prior to randomisation.

7.4.2. Day 0: Enrolment and vaccination visit

Participants will be considered enrolled into the trial at the point of written, signed consent for full-study participation, i.e. following confirmation of eligibly through the screening visit. The initiation of the consenting process for full-study participation may precede the date on which the consent form is signed, to allow adequate time for potential participants to consider their willingness to participate. Before randomization, the eligibility of the participant will be reviewed. Pulse, respiratory rate, oxygen saturation, blood pressure and temperature will be observed and if necessary, a medical history and physical examination may be undertaken. Vaccinations will be administered as described below.

7.4.3. Vaccination

All vaccines will be administered intramuscularly according to specific SOPs. The injection site will be covered with a sterile dressing and the participant will stay in the trial site for observation, in case of immediate adverse events. Observations will be taken 60 minutes after vaccination (+/- 30 minutes). Post-vaccination observations include pulse rate, respiratory rate, oxygen saturation, blood pressure, temperature and vaccination site review.

In all groups, participants will be given an oral thermometer, measurement device and diary card (paper or electronic), with instructions on use, along with the emergency 24-hour telephone number to contact the on-call study doctor if needed. Participants will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms. Diary cards will collect information on the timing and severity of the following solicited AEs:

Table 8: Solicited AEs as collected on post vaccination diary cards

Local solicited AEs	Systemic solicited AEs
Pain	Fever
Tenderness	Feverishness
Redness	Chills
Warmth	Joint pains
Itch	Muscle pains
Swelling	Fatigue
Induration	Headache
	Malaise
	Nausea

7.4.3.1. Sequence of Enrolment and Vaccination of Participants

Prior to initiation of the study, any newly available safety data will be reviewed from animal studies (including non-human primate studies being conducted in UK) and clinical trials of coronavirus vaccines (including data from first 50 participants being enrolled in similar trial in UK, COV001) being tested elsewhere, and discussed with the DSMC and/or regulatory and ethics committees as necessary. Participants in group 1 (HIV-uninfected adults, prime-boost 2-dose, intensive follow up) and Group-3 may be enrolled concurrently, contingent upon approval by the DSMC.

Based on the immunogenicity data from the initial safety/immunogenicity cohort enrolled in the UK study (COV001);[1] and following review by the DSMC it was decided that all participants will receive two doses of the assigned study-intervention. A notification on the dosing schedule for Group-2 was submitted the Ethics committee and SAPHRA.

7.4.4. Subsequent visits:

Follow-up visits will take place as per the schedule of attendances described in Table 8, Table 9 and Table 10 with their respective windows. Participants will be assessed for local and systemic adverse events, interim history, physical examination, review of diary cards (paper or electronic) and blood tests at these time points as detailed in the schedule of attendances. Blood will also be taken for immunology purposes.

If participants experience adverse events (laboratory or clinical), which the investigator (physician), CI and/or DSMC chair determine necessary for further close observation, the participant may be admitted to a hospital for observation and further medical management under the care of the attending-physicians.

7.4.5. Participants under quarantine

Given the evolving epidemiological situation both globally and in South Africa, should a participant be under isolation or quarantine and unable to attend any of the scheduled visits, a telephone consultation will be arranged in order to obtain core study data where possible. Any study samples from participants under quarantine or isolation will be collected at the place of residence at the time of the participant (or in hospital if hospitalized), by trained study staff with appropriate precautionary measures being implemented (including use of protective personal equipment).

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Table 8: Schedule of visits: Groups 1 & 3

Visit number	Screening	V1	V2	V3	V4	V5	V6*	V7*	V8*	V9*	V10	V11	COVID-19
Day #	-14 to -1	0 (Vax1)	3	7	14	28 (Vax2)	31	35	42	56	182	364	Illness
	Screening	D0	V1+ 3 days ±1; (day 2-4)	V1 +7 days ±2 (day 5-9)	V1+ 14 days ±3 (day 11-17)	Visit 1 + 28 days ±7 day 21-35)	V5+3 days ±1	V5+7 days ±2	V5+14 days ±3	V5 +28 (±7)	D182 (±14)	D364 (±14 days)	As required [$]
Eligibility	X	X											
Consenting	X[§]	X[¥]											
Inclusion/ exclusion	X	X				X							
Contraindications	X	X				X							
Vital signs [#]	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X												X
Physical examination	X (full)	X	X	X	X	X (full)	X	X	X	X	X	X	X (full)
Vaccination		X				X							
Post vaccination obs		X	X (deltoid)	X (deltoid)		X	X (deltoid)	X (deltoid)					
Diary cards provided		X				X							X (illness DC)
DC collected				X				X					
Safety bloods (FBC, U&E, LFT)	X		X	X		X		X		X			
Screening bloods (HBsAg, , HIV, HbA1C)	X											X (HIV Gr 1)	
HIV Viral load and CD4 (Grp 3 only)	VL and CD4												
Immunology bloods***		E, PAX (12.5-17.5ml)	Cyt, PAX (12.5 - 17.5ml)		E & CMI (20-25ml)	E, N, PAX (17.5-22.5ml)		Cyt (10-15mls)	E & N & CMI (25-30ml)	E (10-15ml)	E (10-15ml)	E & N (15-20ml)	E (10-20ml)
Urinalysis	X												

Urinalysis bHCG (women only)	X	(X)				X							
Nasal swab/ saliva	X (V1-96 hours)	X		X	X	X		X	X	X	X	X	X

* Visit 5 to Visit 9 are scheduled relative to when the 2nd dose of vaccine/placebo (Visit 4) has been administered.

§ Screening informed consent form (ICF).

¥Full study participation informed consent form, if remain eligible after completion of screening procedures.

Vital signs includes pulse, respiratory rate, oxygen saturation, blood pressure and temperature;

** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window

***Abbreviations for laboratory tests: E =Elisa; Cyt= Th1 and Th2 cytokine profile; N= neutralization and/or pseudo-neutralisation assay; CMI= cell-mediated immunity assay, PAX= PAXgenes.

Blood test summary:

- Screening: Safety bloods (Full Blood Count, FBC; Urea and Electrolytes, U&E; Liver Function tests, LFT); Screening bloods (HBsAg, HIV, Glycosylated hemoglobin; HbA1c), In group 3 only- CD4+ -lymphocyte count, CD4+ & VHIV-1 viral load, VL)
- Visit 1: Immunogenicity- Elisa
- Visit 2 Safety bloods (FBC, U&E, LFT), Immunogenicity- Th1 and Th2 cytokine profile
- Visit 3 Safety bloods (FBC, U&E, LFT)
- Visit 4 Immunogenicity- Elisa & cell-mediated immunity
- Visit 5 Safety bloods (FBC, U&E, LFT), Immunogenicity- Elisa & neutralization and/or pseudo-neutralisation assay
- Visit 6 NIL
- Visit 7 Safety bloods (FBC, U&E, LFT), Immunogenicity- Th1 and Th2 cytokine profile
- Visit 8 Immunogenicity- Elisa, neutralization and/or pseudo-neutralisation assay & cell-mediated immunity
- Visit 9 Safety bloods (FBC, U&E, LFT), Immunogenicity- Elisa
- Visit 10 Immunogenicity- Elisa
- Visit 11 Immunogenicity- Elisa & neutralization and/or pseudo-neutralisation assays
- Illness visit Immunogenicity- Elisa

$ Nasal swabs/ saliva and Elisa (illness) will be repeated at Days 5-8, 12-15 and 28-35 days.

Table 9: Visit schedule for group 2a

Visit number	Screening	V1	V2	V3	V4	V5*	V6*	V7*	V8	V9	COVID-19
Day #	-14 to -1	0 (Vax1)	7	14	28 (Vax2)	35	42	56	182	364	Illness
	Screening	D0	V1 +7 days ±2 (day 5-9)	V1+ 14 days ±3 (day 11-17)	Visit 1 + 28 days ±7	V4+7 days ±2	V4+14 days ±3	V4 +28 (±7)	D182 (±14)	D364 (±14 days)	As required [$]
Eligibility	X	X									
Consenting	X[§]	X[¥]									
Inclusion/ exclusion	X	X			X						
Contraindications	X	X			X						
Vital signs [#]	X	X	X	X	X	X	X	X	X	X	X
Medical history	X										X
Physical examination	X (full)	X	X	X	X (full)	X	X	X	X	X	X (full)
Vaccination		X			X						
Post vaccination obs		X	X (deltoid)		X	X (deltoid)					
Diary cards provided		X			X						X (illness DC)
DC collected			X			X					
Screening bloods (HBsAg, , HIV, HBA1C)	X									X (HIV)	
Immunology bloods***		E, PAX (12.5-17.5ml)	Cyt, PAX (12.5 -17.5ml)	E & CMI (20-25ml)	E, N, PAX (17.5-22.5ml)		E & N & CMI (25-30ml)	E (10-15ml)	E (10-15ml)	E & N (15-20ml)	E (10-20ml)
Urinalysis	X										
Urinalysis bHCG (women only)	X	(X)			X						
Nasal swab/ saliva	X (V1-96 hours)	X	X	X	X	X	X	X	X	X	X

- Visit 5 to Visit 7 are scheduled relative to when the 2nd dose of vaccine/placebo (Visit 4) has been administered.

[§] Screening informed consent form (ICF).
[¥] Full study participation informed consent form, if remain eligible after completion of screening procedures.

[#] Vital signs includes pulse, blood pressure and temperature;

** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window

***Abbreviations for laboratory tests: E=Elisa; Cyt=Th1 and Th2 cytokine profile; N=neutralization and/or pseudo-neutralisation assay; CMI= cell-mediated immunity assay, PAX= PAXgenes.

[#] Vital signs includes pulse, respiratory rate, oxygen saturation, blood pressure and temperature;

** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window

***Abbreviations for laboratory tests: E =Elisa; Cyt= Th1 and Th2 cytokine profile; N= neutralization and/or pseudo-neutralisation assay; CMI= cell-mediated immunity assay, PAX= PAXgenes.

Blood test summary:

- Screening: Safety bloods (Full Blood Count, FBC; Urea and Electrolytes, U&E; Liver Function tests, LFT); Screening bloods (HBsAg, HIV, Glycosylated hemoglobin; HbA1c), In group 3 only- CD4+ -lymphocyte count, CD4+ & VHIV-1 viral load, VL)
- Visit 1: Immunogenicity- Elisa
- Visit 2 Safety bloods (FBC, U&E, LFT), Immunogenicity- Th1 and Th2 cytokine profile
- Visit 3 Safety bloods (FBC, U&E, LFT)
- Visit 4 Immunogenicity- Elisa & cell-mediated immunity
- Visit 5 Safety bloods (FBC, U&E, LFT), Immunogenicity- Elisa & neutralization and/or pseudo-neutralisation assay
- Visit 6 NIL
- Visit 7 Safety bloods (FBC, U&E, LFT), Immunogenicity- Th1 and Th2 cytokine profile
- Visit 8 Immunogenicity- Elisa, neutralization and/or pseudo-neutralisation assay & cell-mediated immunity
- Visit 9 Safety bloods (FBC, U&E, LFT), Immunogenicity- Elisa
- Visit 10 Immunogenicity- Elisa
- Visit 11 Immunogenicity- Elisa & neutralization and/or pseudo-neutralisation assays
- Illness visit Immunogenicity- Elisa

[$] Nasal swabs/ saliva and Elisa (illness) will be repeated at Days 5-8, 12-15 and 28-35 days.

Table 10: Visit schedule, group 2b (extended efficacy cohort; remaining 1650 participants)

Visit number	Screening	V1	V2	V3	V4	V5	V6	COVID-19
Day #	-14 to -1	0 (Vax1)	28 (Vax2)	42	56	182	364	Illness
	Screening	D0	Visit 1 + 28 days ±7	V2+14 days ±3	V2 +28 (±7)	D182 (±14)	D364 (±14 days)	As required [$]
Eligibility	X	X						
Consenting	X[§]	X[¥]						
Inclusion/ exclusion	X	X	X					
Contraindications	X	X	X					
Vital signs [#]	X	X	X	X	X	X	X	X
Medical history	X							X
Physical examination	X (full)	X	X (full)	X	X	X	X	X (full)
Vaccination		X	X					
Post vaccination observation		X	X					
Diary cards provided		X	X					X (illness DC)
DC collected			X					
Screening bloods (HBsAg, HIV, HbBA1C)	X						X (HIV)	
Immunology bloods***		E (10-15ml)	E, N, HLA (17.5-22.5ml)	E & N (15-20ml)	E (10-15ml)	E (10-15ml)	E & N (15-20ml)	E (10-20ml)
Urinalysis	X							
Urinalysis bHCG (women only)	X	(X)	X					
Nasal swab/ saliva	X (V1-96 hours)	X	X	X	X	X	X	X

[§] Screening informed consent form (ICF).
[¥] Full study participation informed consent form, if remain eligible after completion of screening procedures.

* IF participants receive two doses of vaccine, then dose 2 will be administered at Visit 2, and follow up visits will be completed 14 days post dose 2 (6- visit schedule). IF participants only receive ONE dose of IP, then no vaccine will be administered at visit 2, and day 42 visit will not be included in visit schedule (5-visit schedule)
[#] Vital signs includes pulse, respiratory rate, oxygen saturation, blood pressure and temperature;

** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window

***Abbreviations for laboratory tests: E=Elisa; Cyt=Th1 and Th2 cytokine profile; N=neutralization and/or pseudo-neutralisation assay; CMI=cell-mediated immunity assay.

Blood test summary:

- Screening: Screening bloods (HBsAg, HIV, HBA1C)
- Visit 1: Immunogenicity- Elisa
- Visit 2 Immunogenicity- Elisa & neutralization and/or pseudo-neutralisation assay and HLA
- Visit 3 Immunogenicity- Elisa, neutralization and/or pseudo-neutralisation assay
- Visit 4 Immunogenicity- Elisa
- Visit 5 Immunogenicity- Elisa
- Visit 6 Immunogenicity- Elisa & neutralization and/or pseudo-neutralisation assay
- Illness visit Immunogenicity- Elisa

$ Nasal swabs/ saliva and Elisa (illness) will be repeated at Days 5-8, 12-15 and 28-35 days