

Clinical Assessment of ChAdOx1 Vectored Vaccines Against Emerging Pathogens

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Abstract

Traditional vaccine development strategies are costly and time-consuming which makes them unsuitable for clinical assessment of new vaccines in the context of infectious diseases outbreaks. Platform technologies provide substantial manufacturing advantages and have the potential to significantly reduce these limitations to clinical development in health emergencies.

Adenovirus vectors were initially developed as gene transfer vehicles for the treatment of rare genetic conditions, and over the past decade have been under investigation as a platform technology for vaccine development. A replication-deficient adenoviral vector based on the simian adenovirus type Y25 and named ChAdOx1 was first evaluated in clinical trials in 2012 as a universal influenza vaccine candidate. Since then, and before this work began, ChAdOx1 vectored vaccines had been administered to 160 healthy adult volunteers as part of clinical trials of candidate vaccines against influenza, tuberculosis, and malaria.

This project consists of an assessment of ChAdOx1 vectored vaccines against emerging pathogens and its suitability as a platform technology for accelerated R&D and response to epidemic threats. Safety, immunogenicity, and efficacy profiles for ChAdOx1 vectored vaccines against chikungunya, Zika, MERS-CoV and SARS-CoV-2 viruses are presented here. These data have substantially contributed to the approvals of the ChAdOx1 vectored COVID-19 vaccine, now in use in over 180 countries.

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Abbreviations

ADE	Antibody-dependent enhancement
AE	Adverse event
A&E	Accident and Emergency
AESI	Adverse event of special interest
Ad	Adenovirus
AdHu	Adenovirus Human
AdHu4	Adenovirus human serotype 4
AdHu5 or Ad5	Adenovirus human serotype 5
Ad26	Adenovirus human serotype 26
ANVISA	Agencia Nacional de Vigilancia Sanitaria (Brazilian regulatory agency)
BAC	Bacterial artificial chromosome
CBF	Clinical Biomanufacturing Facility
CCHF	Crimean-Congo Haemorrhagic Fever
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CDC	Center for Disease Control
CEPI	Coalition for epidemic preparedness innovations
ChAd63	Chimpanzee Adenovirus 63
ChAdOx1	Chimpanzee Adenovirus Ox1
CHIKV	Chikungunya virus
CI	Chief Investigator
CMA	Conditional Marketing Authorisation
COVID-19	Coronavirus disease 2019

CRF	Case Report Form or Clinical Research Facility
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
ECSA	East/Central/South Africa CHIKV lineage
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot assay
EMA	European Medicines Agency
EU	ELISA units
EUA	Emergency use authorisation
FCγ	Fragment crystallizable gamma
FDA	Food and Drug Administration
GBS	Guillain-Barré Syndrome
GCP	Good Clinical Practice
GMSC	Genetically Modified organisms Safety Committee
HAdV	Human adenovirus
HAdV-4	Human adenovirus serotype 4
HBV	Hepatitis B virus
HEK 293	Human embryonic kidney cells 293
HIV	Human Immunodeficiency virus
HPV	Human papilloma virus
ICU	Intensive Care Unit
IM	Intramuscular
IMP	Investigational Medicinal Product
LLD	Lower limit of detection
LSM	Local Safety Monitor

MenACWY	Meningococcal group A, C, W-135 and Y conjugate vaccine
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	Messenger RNA
MVA	Modified Vaccinia Virus Ankara
NAb	Neutralising antibody
NHP	Non-human primate
NHS	National Health Service
NIAID	National Institute of Allergy and Infectious Diseases
ORF	Open reading frame
OUH	Oxford University Hospitals
PBMC	Peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralization test
PTMF	Platform technology master file
qPCR	Quantitative PCR
R&D	Research and Development
REC	Research Ethics Committee
RNA	Ribonucleic acid
RR	Relative Risk
RT	Room temperature
SAdV	Simian adenovirus
SAE	Serious Adverse Event
SARS-CoV	Severe acute respiratory syndrome coronavirus

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SFC	Spot forming cells
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
tPA	Tissue plasminogen activator
UK	United Kingdom
UKVN	UK Vaccine Network
VLP	Virus-like particle
vp	Viral particles
UTMB	University of Texas Medical Branch
VMIC	Vaccine Manufacturing and Innovation Centre
VSV	Vesicular Stomatitis Virus
WA	West African CHIKV lineage
WHO	World Health Organisation
ZIKV	Zika virus

Main publications resulting from this work

Folegatti, PM., Jenkin, D., Morris, S., Gilbert, S., Kim, D., et al. Vaccines based on the replication-deficient simian adenoviral vector ChAdOx1: Standardized template with key considerations for a risk/benefit assessment. Vaccine. *Accepted for publication*.

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**contributed equally*

Chapter 1: Introduction

1.1 Priority pathogens for accelerated research & development

As the COVID-19 pandemic takes its toll worldwide, deadly outbreaks of currently unknown pathogens will inevitably happen in the future. In less than a century, the world has seen 4 influenza pandemics happening (1918 H1N1, 1957-1958 H2N2, 1968 H3N2, and 2009 H1N1), and 3 different deadly coronaviruses outbreaks struck in the past 20 years alone (severe acute respiratory syndrome coronavirus [SARS-CoV] in 2003, middle east respiratory syndrome coronavirus [MERS-CoV] in 2012 and severe acute respiratory syndrome 2 [SARS-CoV-2] in 2020). Despite therapeutic advances in recent decades, the continued emergence and resurgence of infectious pathogens causes significant disruption to individuals' lives, nationwide health systems, and economies across the globe. The increasing global interdependence, higher mobility of people, animals, and trade, and human destruction of many natural environments for animal species, facilitate the transmission of these agents with multiple opportunities for the spread of pathogens.

The catastrophic impacts of the Ebola epidemic in West Africa in 2014, and the Zika outbreak in Brazil in 2015, have highlighted the need for better-coordinated rapid response actions, and early engagement of the international community in outbreak situations, avoiding the collapse of fragile health systems and the development of pandemics. These actions involve encouraging technological development of new diagnostic and therapeutic strategies but most importantly, of methods of epidemiological surveillance and transmission control in outbreak

situations. In this context, new vaccination strategies are recognized as the most cost-effective way to protect communities and contain epidemics.

Pandemic preparedness and biodefense plans have been put in place for several years now by individual governments and international health agencies following past epidemics of infectious diseases of public health concern. Since 2015 and following the Ebola outbreak in West Africa, the National Institute of Allergy and Infectious Diseases (NIAID) in the United States of America (USA), the United Kingdom (UK) Vaccine Network, the World Health Organization (WHO), and the Coalition for Epidemic Preparedness Innovations (CEPI) amongst others have created lists of priority pathogens for accelerated research and development (R&D). These priority pathogens have been chosen based on the absence of effective control strategies, the potential socio-economic impact, risk of pandemics and threats to national security through bioterrorism activities.

Table 1. Timeline of significant events leading to pandemic preparedness actions

Year	Event
2001	Launch of the Global Health Security Initiative
2003	SARS-CoV Outbreak
2005	WHO International Health Regulations revision
2009	H1N1 pdm09' pandemic
2012	MERS-CoV Outbreak in Saudi Arabia
2014	Launch of Global Health Security Agenda
2014-2016	Ebola outbreak in West Africa
2015	Zika outbreak in Brazil
2015	First WHO consultation/workshop on Prioritizing Severe Emerging Diseases for R&D
2017	Launch of CEPI
2018	Disease X added to WHO R&D blueprint
2020	COVID-19 Pandemic

Diseases caused by haemorrhagic fever viruses (e.g., Ebola, Marburg, Lassa, Crimean-Congo, and Rift Valley fever), coronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2), Nipah, Zika and chikungunya viruses are examples featured on these priority lists. In 2018, the WHO R&D Blueprint included ‘Disease X’ as a placeholder to their priority disease list. Disease X represents a hypothetical, unknown pathogen that could cause a future epidemic. The WHO priority pathogens list remains largely unchanged since it was first proposed in 2015, except for the Disease X and COVID-19 additions. The NIAID has an extensive list of pathogens which are divided into different categories (A, B and C) where those in category A represent the highest risk to USA national security and public health threat. Only the NIAID and UKVN have bacterial pathogens added to their respective pathogen priority lists.

Table 2. List of priority pathogens from different agencies (excluding SARS-CoV-2)

Pathogen/Disease	WHO	CEPI	UKVN	NIAID Category
CCHF	✓		✓	A
Ebola	✓	✓	✓	A
Marburg	✓		✓	A
Lassa	✓	✓	✓	A
MERS-CoV	✓	✓	✓	C
Nipah	✓	✓	✓	C
Rift Valley fever	✓	✓	✓	A
Zika	✓		✓	B
Disease X	✓	✓		
Chikungunya		✓	✓	B
Hantavirus			✓	A
Plague			✓	A
Q Fever			✓	B
Anthrax				A
Botulism				A
Smallpox				A
Tularaemia				A
Dengue				A
Other Arenavirus (excluding Lassa)				A

Note: All NIAID category A included, but category B and C pathogens are only shown if common to at least one other agency. CCHF: Crimean-Congo haemorrhagic fever

For many of these agents, the initial preclinical development of new vaccines does not necessarily represent a barrier to technological progress, but the production and testing of such vaccines in clinical trials is a slow, risky, and costly process with limited market potential for outbreak pathogens vaccines. However, there are opportunities to apply lessons learned from one vaccine to reduce development time and costs for others by using a manufacturing and testing platform.

1.2 Platform technologies for vaccine development

Vaccine development against infectious diseases and bioterrorism has been traditionally based on a “one bug, one vaccine” approach. However, platform technologies have been developed over the years, making it possible for vaccine manufacturing processes to be streamlined by using a common established approach [1]. Vaccine platform technologies for rapid response broadly refer to systems that use the same backbone, often also described as carriers or vehicles, that can be adapted for use against different pathogens by inserting new genetic or protein sequences. They are also referred to as ‘plug-and-play’ physical frameworks that can be used for vaccine development against different emerging infectious diseases. In contrast, traditional mechanisms of vaccine production which are only suitable for a single disease application are not necessarily classed as platform technologies, as is the case for annual influenza vaccines [2]. Similarly, molecular “clamps” used to stabilize viral protein targets are better described as adjuvants that can be used in multiple vaccines, rather than true platforms [1]. The use of platform technologies represents a significant advantage compared to traditional whole inactivated or attenuated vaccines as it eliminates the need for manipulation and culture of infectious and hazardous pathogens in high-level biosafety containment laboratories.

The time from viral sequencing of the pathogen to phase 1 trials of the vaccines has decreased from 20 months in 2003 during the SARS-CoV outbreak, to less than 4 months during the 2016 Zika virus epidemic [3], and to a record-breaking 63 days for the current SARS-CoV-2 pandemic [4], thanks to platform technologies for vaccine development.

The spectrum of different platforms includes expression systems, virus-like particles (VLP), nucleic acid, and viral vectored vaccines.

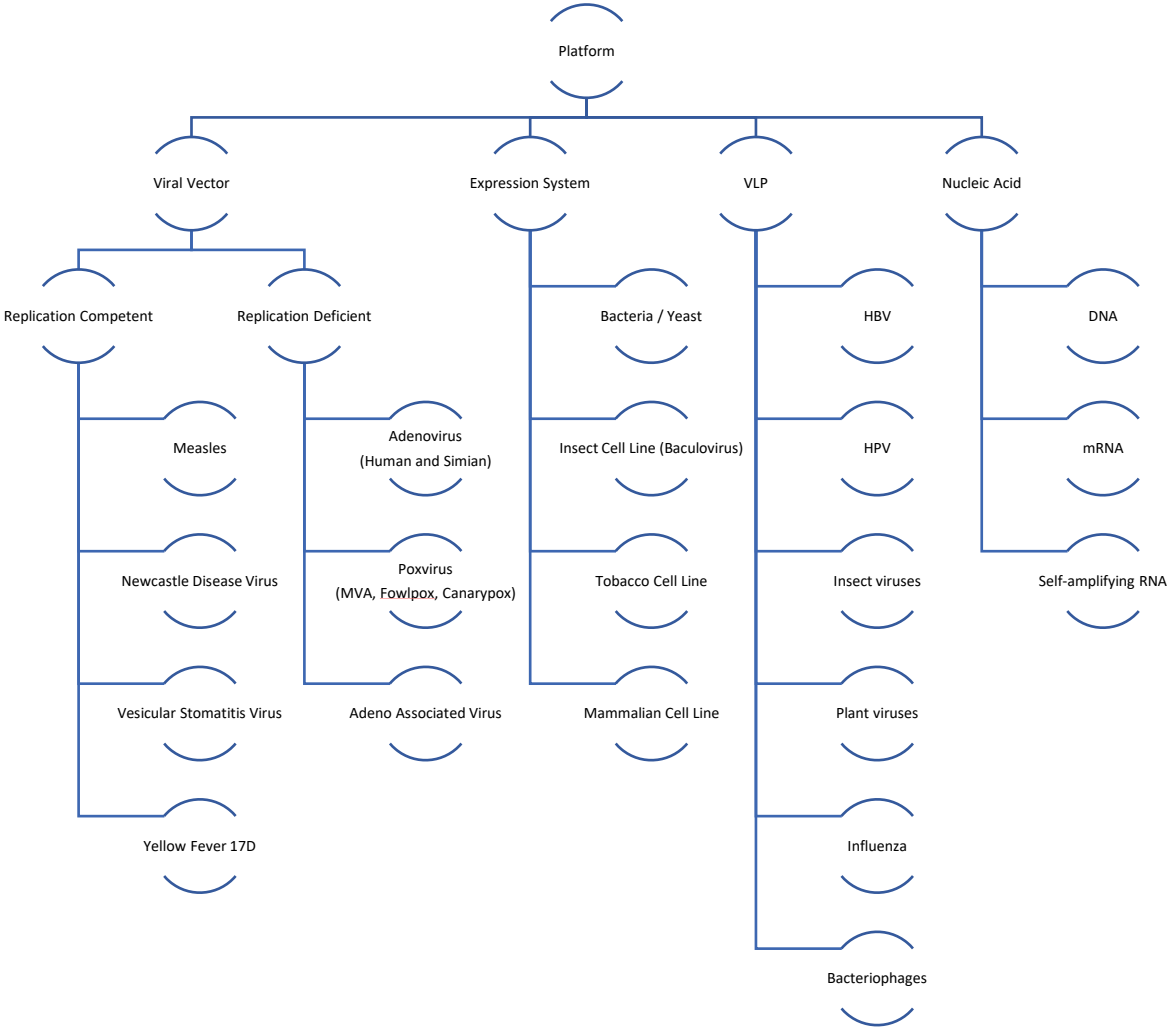


Figure 1. Examples of platform technologies for vaccine development

1.2.1 Recombinant protein expression systems

Expression systems are genetic constructs that are designed to produce a protein, either inside or outside a cell. They are commonly used in subunit protein vaccine development and are mostly derived from insect, mammalian and tobacco plant cell lines or bacterial and yeast hosts [5].

In general, a recombinant viral vector is created with an expression cassette which contains the elements required for the expression of the antigen of interest (promoter, coding sequence and transcription terminator). The recombinant virus infects the cells and the cell line is used to produce the antigen which can then be purified and used in a vaccine [5]. One example is the licensed recombinant influenza vaccine (Flublock®) which is manufactured using an insect cell line that is infected with a baculovirus containing the influenza hemagglutinin protein but can also be adapted to other antigens [6].

Drawbacks from expression systems technologies include a) suboptimal antigen expression that can differ from human cells, mostly through different post-translational modifications (notably glycosylation) which can impact binding functions and immunogenicity profiles; and b) the need for adjuvants in most subunit protein vaccines, as they often lack pathogen-associated molecular patterns resulting in poor activation of innate immune cells and weaker adaptive immune responses.

1.2.2 Virus-like particles

VLP platforms generally consist of self-assembling recombinant proteins that function as a scaffold to which any antigen can be linked. Well-characterized viral, bacterial and/or parasite proteins and polypeptides can be obtained by

recombinant techniques and linked to the virus-like particles to form the final vaccine antigen. Examples of VLPs used to express heterologous antigens used in clinical development include hepatitis B, human papillomavirus, influenza, bacteriophages, plant viruses and insect viruses VLPs [7, 8].

1.2.3 Nucleic acid

DNA or mRNA-based vaccines can be rapidly adapted when new viruses emerge with speed of vaccine manufacture being an important advantage of nucleic acid vaccine platforms.

DNA vaccines are made of a synthetic DNA construct encoding the vaccine antigen. To obtain an efficient uptake of the construct into cells, passage across the cell membrane and the nuclear membrane is required. This can be achieved by electroporation methods, which also represent significant delivery challenges and higher costs. After uptake into cells, the vaccine antigen is expressed from the DNA construct.

The same principles described for DNA vaccines apply to mRNA-based ones, except that nuclear translocation of the DNA construct and transcription into mRNA are bypassed. It was hoped that self-amplifying RNA vaccines were able to induce similar immune responses with lower doses, as more vaccine antigen is expressed per cell. However, immunogenicity results from a phase I COVID-19 self-amplifying RNA vaccine indicate that further optimisation of the technology is required to realise its potential as an effective RNA vaccine [9]. mRNA is very unstable, so these constructs include modified nucleosides to prevent degradation. A carrier molecule is necessary to enable the entry of the mRNA into cells and lipid nanoparticles are the most commonly used. Nucleic

acid-based vaccines can induce both humoral and cellular immune responses. They may provide intrinsic adjuvant activity to drive dendritic cell maturation and thus elicit robust T and B cell immune responses. However, the inclusion of traditional adjuvants as well as novel approaches based on the mRNA ability to encode immune-modulatory proteins have also been proposed to increase immune responses generated by nucleic acid-based strategies [10].

1.2.4 Viral vectors

Viral vector vaccines are usually made of a recombinant vector virus in which genes encoding antigens are cloned using recombinant DNA techniques. They can be replicating or non-replicating.

Replicating vector vaccines infect cells in which the vaccine antigen is produced as well as more infectious vectors which can infect new cells that will then also produce the vaccine antigen [11]. Examples of replication-competent vectors commonly used in vaccine clinical development include measles [12], Newcastle disease virus (NCT04764422) [13], yellow fever 17D [14] and the first licensed viral vectored vaccine following the Ebola outbreak in West Africa in 2014, the vesicular stomatitis virus (VSV) [15]. Non-replicating vector vaccines enter cells and produce the vaccine antigen, but no new vector virus particles are formed. Most used replication-deficient vectored vaccines include adenovirus, modified vaccinia Ankara (MVA) and adeno associated virus. As viral vector vaccines result in endogenous antigen production, both humoral and cellular immune responses are stimulated [11].

Replication competent vectors normally require doses to be administered that are 2-3 logs lower compared to replication-deficient viruses [16], which means more

vaccine can be available for clinical use after each manufacturing run. However, they are unsuitable for use in those who are immunosuppressed and may require additional biodistribution testing to look for viscerotropism or neurotropism. Testing for replication-competent viruses may be necessary for certain replication-deficient vectors to look for potential recombination between viral vector and the necessary complementing cell line during manufacture.

1.2.4.1 Adenovirus vectored vaccines

Adenovirus vectors were initially developed as gene transfer vehicles and their first use in clinical research dates from 1993 [17]. The double-stranded DNA genome can be manipulated by recombinant DNA techniques, so foreign genes can be inserted by deletion of adenovirus genes. They can also be grown in a large variety of cell types. Most of the currently developed adenovirus vectors are replication-incompetent through full deletion of the E1 early gene. Propagation of E1 deleted adenovirus is easily obtained with good yields by growing them *in vitro* using cell lines that constitutively produce E1 from adenovirus human serotype 5 - AdHu5 (e.g., human embryonic kidney cells 293 - HEK 293 cells), and therefore complement the replication-defective E1 deleted adenovirus vectors. As a result of E1 deletion, these vectors have lower cytotoxicity of the target cell during gene transfer.

Whilst adenovirus vectors were initially developed for use in gene therapy in patients with rare genetic diseases, it quickly became apparent they could also be used as vaccine vectors that deliver an antigen to induce immune responses [18]. Adenoviruses are attractive vectors for human vaccination. They possess a stable genome so that inserts of foreign genes are not deleted. They can infect

large numbers of cells and the transferred information remains extra-chromosomal, thus avoiding any potential for insertional mutagenesis.

The first adenovirus vectors investigated for gene transfer were based on common human serotypes Ad5 and Ad2. However, these vectors suffered from high pre-existing anti-adenovirus neutralising antibodies in human sera which potentially reduces their effectiveness both as therapeutic agents and as viral vaccine vectors. Despite this, several Ad5 vectored vaccines against HIV [19-22], Ebola [23] and COVID-19 [24] amongst others have been developed and tested in humans. To circumvent pre-existing immunity to Ad5, vectors based on other rare human adenoviruses (such as Ad26), and simian adenoviruses (such as ChAd68, the first E1-deleted simian adenovirus created, and ChAd63, the first to enter clinical trials) have been developed. Ad5 vectored Ebola vaccines developed in China and Russia were licensed for domestic use in their respective countries in the event of an Ebola outbreak in 2017, based on animal rule (see section 1.4.1 Animal Rule for concept details) and data from phase I/II studies [25]. The Janssen Ad26 Ebola vaccine, used in a prime-boost regimen with MVA [26] was the first adenoviral vectored vaccine licensed for human use by a major western regulator in 2020. Since then, Ad5 and Ad26 vectored COVID-19 vaccines have received emergency use authorisation in several countries during the COVID-19 pandemic [27, 28].

The ubiquity of human adenovirus infections generates high levels of host anti-vector immunity which may limit their utility as vectors. The prevalence of immunity to human adenoviruses prompted the consideration of simian adenoviruses as vectors. Simian adenoviruses (SAdV) and human adenoviruses (HAdV) are not phylogenetically distinguishable and fall into the same eight

species (A, B1, B2, and C to G). ChAd63, ChAdOx1 and ChAdOx2 are, like many SAdV isolated to date, members of species E, which contains only one human virus (HAdV-4). Chimpanzee adenoviruses exhibit hexon structures homologous to that of human adenoviruses. Indeed, ChAd63 hexons are most similar in sequence to the hexons of AdHu4, previously used by the US military in mass vaccination campaigns where over 2 million adults received tablets of serially passaged adenovirus with good safety and efficacy data [29]. Simian adenoviruses are not known to cause pathological illness in humans and the prevalence of antibodies to chimpanzee origin adenoviruses is less than 5% in humans residing in the US. In equatorial Africa (the natural habitat for chimpanzees), prevalence is higher but still below that of AdHu5 [30]. In a study in Kenya, 23% of children (aged 1-6 years) had high-titre neutralising antibodies to AdHu5, whilst only 4% had high-titre neutralising antibodies to ChAd63. Immunity to both vectors was age-dependent [31].

1.3 ChAdOx1 vector characteristics

ChAdOx1 (Chimpanzee Adenovirus Oxford 1) consists of the E1/E3 deleted chimpanzee adenovirus Y25 with the human adenovirus (AdHu) serotype 5 E4 open reading frame (ORF) 4, 6 and 6/7 genes. The wildtype chimpanzee adenovirus isolate Y25 was originally isolated from chimpanzees in Central Africa by William Hillis, John Hopkins University of Medicine in the 1960s. Y25 was then shared with research collaborators in Germany and Sweden before reaching the Jenner Institute. The virus was passaged in HEK293A cells and purified by CsCl gradient ultracentrifugation. Viral DNA was phenol extracted and cloned into a bacterial artificial chromosome (BAC) containing Y25 LFI/II and RFI by recombination in such a manner as to delete the E1 region. The E3 region was

deleted by recombineering. Recombineering is an *in vivo* method of genetic engineering applicable to chromosomal as well as episomal replicons in *Escherichia coli*. This method circumvents the need for most standard *in vitro* cloning techniques. Recombineering is based on homologous recombination which allows construction of DNA molecules with precise junctions without the constraints imposed by restriction enzyme site location. Bacteriophage homologous recombination proteins catalyse these recombineering reactions using targeting constructs introduced by electroporation. Gene knockouts, deletions and point mutations are readily made, gene tags can be inserted and regions of BAC genome can be subcloned by gene retrieval using recombineering [32]. The E4 region was modified by recombineering to replace the native E4 orf 4, orf 6 and orf 6/7 with those from Human Adenovirus serotype 5. The E1 region was modified to allow insertion of the antigen expression cassette. The E1 region is essential for viral replication, hence the ability to delete E1 renders the vector immediately replication incompetent. E1-deleted adenoviral vectors can be amplified in E1 complementing cell lines (e.g., HEK293, PER.C6). Deletion of E3, a non-essential adenovirus gene, increases the insert capacity of the new vector by approximately 5kb. It is known that the proteins encoded by the E4 region of adenoviruses interact with E1 during viral replication, and the imperfect interaction between the gene products of the AdHu5 E1 gene produced by HEK293 cells, and simian E4 gene products have been found to result in impaired viral replication in this cell line, and consequently lower virus yields. In ChAdOx1, Ad5 E4Orf4 E4Orf6 and E4 Orf6/7 have been inserted to replace the homologous simian virus coding sequence, resulting in improved viral replication during vaccine production. Since no replication of the virus takes

place after immunisation, this replacement has no effect on immunogenicity of the viral vector [33]. Insertion of the transgene occurs at the E1 locus for single valent vaccines or E1 and E4 for multivalent vaccines.

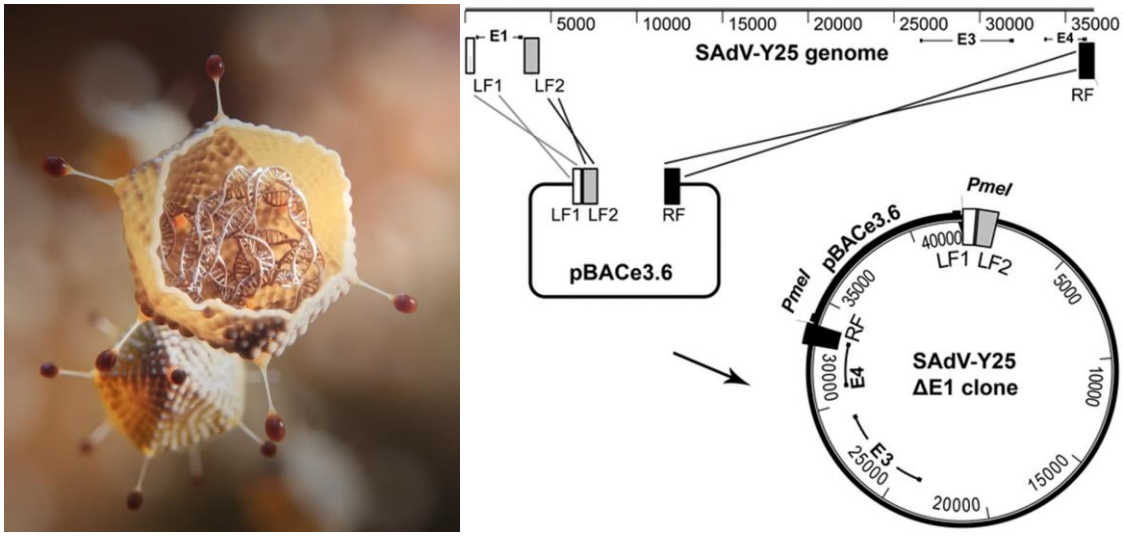


Figure 2. Adenovirus vector illustration and ChAdOx1 vector clone.

Reproduced from https://www.astrazeneca.com/media-centre/covid-19-media.html and Dicks et. al [33]

The transgene expression is controlled by the cytomegalovirus immediate early promoter with or without intron A sequence. Polyadenylation sequence is controlled from the bovine growth hormone gene or the simian virus 40 polyadenylation signal (SV40). The insertion or expression of the transgene does not affect the phenotype of the vector as it remains structurally the same. Any transgene expressed from a ChAdOx1 vectored vaccine would also not alter cell tropism. Unlike other vectors (e.g., VSV which replaces the surface glycoprotein with the vaccine antigen [34]), adenoviruses are comprised of a protein capsid and do not incorporate foreign proteins into their structure.

ChAdOx1 can accommodate multigenic inserts but with a limit on the transgene size up to 8kbp, including the required promoter and terminator sequences. All transgenes are synthesized and cloned into a shuttle vector containing the promoter and poly-A sequence. The expression cassette is inserted into the adenovirus BAC by recombination.

ChAdOx1 is derived from wild type Chimpanzee adenovirus Y25, belonging to the family *Adenoviridae*, genus *Mastadenovirus*, species *Human mastadenovirus E* (same as ChAd63, and AdHu4) [35, 36].

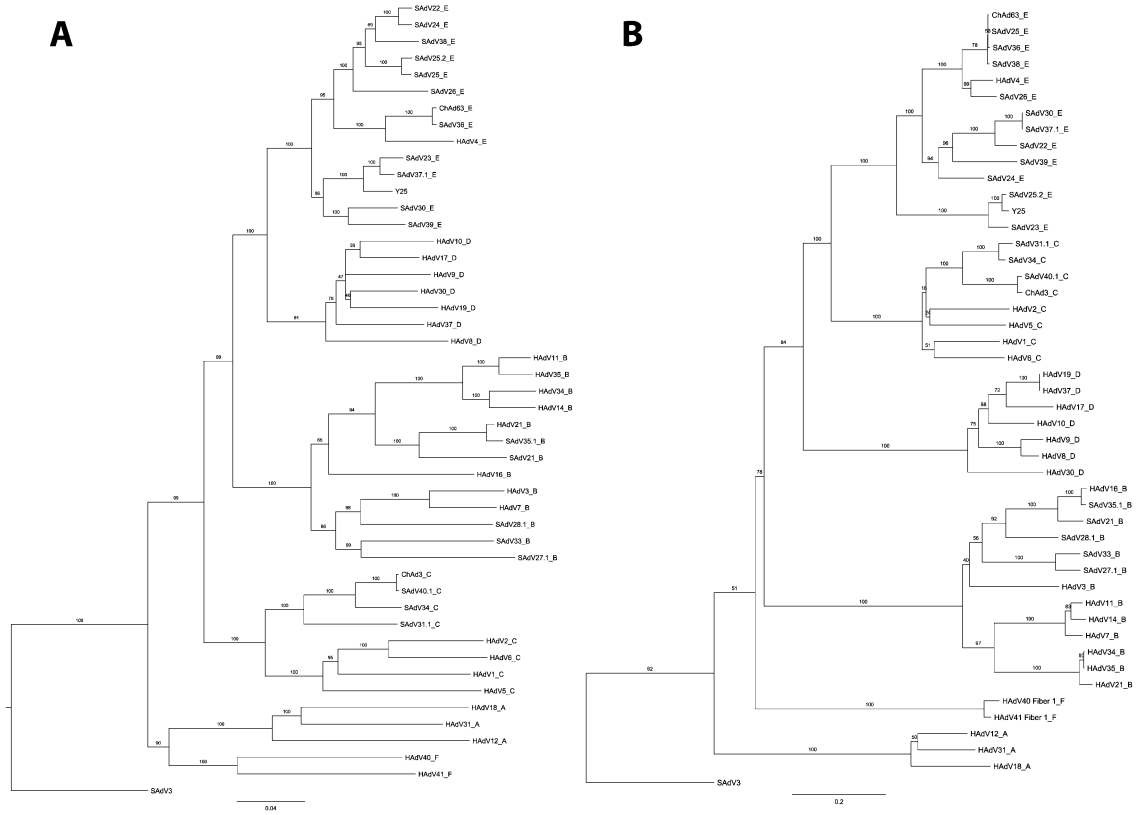


Figure 3. Adenovirus phylogenetic trees.

Phylogenetic trees based on alignment of nucleotide sequences of A) the hexon protein and (B) the fibre protein of different human (HAdV-) and chimpanzee (SAdV-) adenovirus serotypes including Y25. Reproduced from Dicks et al 2012 [33].

Humans and other mammals are known to be the natural hosts for adenoviruses of the *Mastadenovirus* genus. However, chimpanzees remain the only natural host known for Chimpanzee adenovirus Y25 [37, 38]. Simian hosts may persistently shed the wild type virus from the gastrointestinal tract leading to latent or persistent infection [39]. Adenoviruses are normally transmitted via aerosolized droplets or faecal-oral spread. In their natural host, clinically apparent simian adenovirus infections of the respiratory tract are characterized by cough. Keratoconjunctivitis and diarrhoea may also occur. Most animals, but especially adults recover within a week to 10 days. Except in neonates, mortality is generally low. Clinical presentation of Chimpanzee adenovirus Y25 specifically is unknown for their natural host. There is some evidence of cross-species transmission between human and simian adenoviruses from antibody and genetic diversity studies. There is one report of clinical disease in humans with limited onward human-to-human transmission from a new world monkey adenovirus. The clinical implications of human infection from wild type Chimpanzee adenovirus Y25 remain unknown [30, 33, 40-43].

Adenoviruses replicate as linear, extra-chromosomal DNA elements in the nucleus, and have a low risk of integration into the host genome as they remain in an episomal state in the nucleus [44-46]. The European Medicines Agency (EMA) considers adenoviruses as non-integrating vectors. Cytoplasm-evolved genes were not optimised for nuclear expression in ChAdOx1 vectored vaccines. Transcriptomics and proteomics data of ChAdOx1 nCoV-19 gene expression in human cell lines show that rare transgene transcripts with aberrant splice patterns can be detected at a very low level. However, no protein is transcribed from them. Aberrant splicing, therefore, seems to be a theoretical concern only [47].

Epithelial and endothelial cells expressing the Coxsackievirus and Adenovirus Receptor (CAR) are cell entry receptors for ChAdOx1 [48]. Other simian adenoviruses (ChAd63 and ChAd68), and AdHu 4 of species E use CAR for cell entry. The regions of the fibre and penton proteins responsible for these interactions are very highly conserved amongst species E adenoviruses, so the type and number of cells infected by E1/E3 deleted vaccine vectors derived from these viruses are not predicted to differ [49-51].

For most viral pathogens, neutralising antibodies can block viruses by interfering with their binding to host cell receptors or inhibiting changes in the viral protein needed for entry. Antibodies can also bind viruses to cells of the immune system via the fragment crystallizable gamma (Fcγ) receptors on the cell surface and internalisation of viruses typically results in their degradation. However, instead of conferring protection, antibodies can sometimes cause enhancement of disease severity in an infected person if antibody binding enables entry of the virus into its target cell, and if the virus has the capacity to evade destruction and produce more viruses after Fcγ receptor-mediated entry [52]. The enhancement of disease by antibody-dependent mechanisms has been described clinically following repeat infection (dengue haemorrhagic fever due to infection with a heterologous dengue virus serotype) and vaccination (live-attenuated dengue vaccine and formalin-inactivated measles and respiratory syncytial virus vaccines in the 1960s) [53-56]. Vaccine enhancement of disease can result from both antibody disease enhancement (ADE) and deleterious T cell responses (Th2 biased) [57]. No disease enhancement has been described for adenoviruses infections. However, enhancement of HIV infection acquisition has been previously reported with an Ad5 vectored vaccine expressing HIV antigens [21,

58]. No evidence of ADE to dengue has been found *in vitro* for a ChAdOx1 vectored vaccine expressing Zika virus antigens [59]. ChAdOx1 vectored vaccines expressing Nipah, MERS-CoV and SARS-CoV-2 antigens have been used in pre-clinical challenge or natural transmission studies in mice, camels, and non-human-primates (NHP) with no evidence of disease enhancement [60-64]. There is no evidence of COVID-19 enhancement following immunisation with Ad5, Ad26 or ChAdOx1 vectored vaccines.

The high prevalence of immunity to human adenoviruses, potentially leading to impaired immune responses, prompted the consideration of simian adenoviruses as vectors. There is low-level natural immunity to the ChAdOx1 vector as previously assessed in British and Gambian adults using virus neutralisation assays against Chimpanzee Y25. The percentage of individuals having a clinically relevant neutralising titre (defined as a 50% neutralisation titre >200) were 0% for Y25 in UK adults (n=100); and 9% for Y25 in Gambian adults (n=57) [33]. However, the prevalence of ChAdOx1 vector immunity is likely to dramatically increase with widespread deployment of ChAdOx1 nCoV-19. Replication-competent adenoviral vectors encode significantly more vector antigens in addition to the transgene product, which means the adaptive immune response will be directed towards both transgene and vector. This can negatively affect the desired immune response to the vaccine antigen through competition for major histocompatibility complex (MHC) binding sites or cytokines needed for activation, differentiation, and proliferation of T and B cells [18]. In contrast, replication-deficient vectors allow for sustained antigen presentation by reducing the vector's ability to induce death of the infected cells and inducing fewer immune responses to structural vector antigens, with most of the expressed

protein derived from the vaccine antigen. The impact of pre-existing ChAdOx1 immune responses induced from the COVID-19 vaccine on future ChAdOx1 candidates remains unknown.

ChAdOx1 has a low risk of reversion to virulence or recombination with wildtype viruses or other agents. Reversion to virulence would require the acquisition of a functional E1 region. Recombination with wild type Y25 is possible but unlikely as Y25 and other simian adenoviruses are not widely distributed in the human population. Recombination with other adenovirus species would require significant homology in the E1 flanking regions. These regions contain essential packaging motifs and other genes, therefore the number of possible recombination events to generate a replication-competent virus is very small. Recombination with E1 producer cell lines during the manufacturing process is theoretically possible but testing for recombinant-competent adenoviruses is conducted at all times ahead of vaccine release alongside examination for the presence of virus-induced cytopathic effect. [33, 65].

Standard toxicology studies have been conducted in mice ahead of first-in-human trials of ChAdOx1 vectored vaccines, and multiple animal models (other than mice) for immunogenicity testing have been developed depending on the target disease (e.g. camel for MERS-CoV; sheep/cattle/goat for Rift Valley fever), as well NHPs, hamsters, ferrets, guinea pigs, and cats [60-64, 66-68]. A developmental and reproductive toxicology (DART) study has been completed in mice for ChAdOx1 nCoV-19 with no detrimental effects observed in pregnancy, embryofoetal development, parturition or postnatal development [69].

Biodistribution studies in mice have been conducted for ChAdOx1 vectored vaccines expressing hepatitis B and SARS-CoV-2 antigens. No shedding was detected in urine or faecal samples. The virus was primarily detected at the injection site immediately after injection, and at draining lymph nodes. Distribution to some samples of other tissues (liver, lung, spleen, bone marrow, heart, liver, ovary, and testes) was noted on day 2. The levels of ChAdOx1 vector DNA and the number of tissues with detectable levels of ChAdOx1 vector DNA decreased from Day 2 to later timepoints (day 29 and day 56), indicating elimination [70]. Biodistribution studies are more informative when a replication-competent virus is administered since the amount of virus present in the subject (experimental animal or human volunteer) will increase following injection, and some viruses have a known propensity to accumulate in specific organs. However, replication-deficient viruses are known to infect cells at the injection site, and although some infectious viral particles may drain to local lymph nodes and travel through the blood to other sites in the body, concentrations of virus at these sites are very low after dilution in the blood and other tissues.

There are now 35 clinical trials of ChAdOx1 vectored vaccines expressing 16 inserts from 15 diseases and enrolling up to 37,000 participants. Before this work began, only 160 participants had been enrolled in clinical trials of influenza, tuberculosis and prostate cancer. Over 2 billion doses of ChAdOx1 nCoV-19 have now been distributed globally.

Table 3. Clinical trials of ChAdOx1 vectored vaccines

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
UK	FLU004	ChAdOx1 NP+M1	18-50	IM	5×10^8 vp	3	[71]
					5×10^9 vp	3	
					2.5×10^{10} vp	3	
					5×10^{10} vp	6	
UK	FLU005	ChAdOx1 NP+M1	18-50	IM	2.5×10^{10} vp	69	[72]
UK	TB034	ChAdOx1 85A	18-50	IM	5×10^9 vp	6	[73]
		ChAdOx1 85A	18-50	IM	2.5×10^{10} vp	36	
Switzerland	TB039	ChAdOx1 85A	18-55	Aerosol	1×10^9 vp	3	NCT04121494
				Aerosol	5×10^9 vp	3	
				Aerosol	1×10^{10} vp	3	
				Aerosol/IM	1×10^{10} vp	30	
Uganda	TB042	ChAdOx1 85A	12-49	IM	5×10^9 vp	6	NCT03681860
					2.5×10^{10}	30	

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
UK	VANCE01	ChAdOx1.5T4	18 – 75	IM	2.5×10^{10} vp	34	[74]
UK	ADVANCE	ChAdOx1.5T4	≥18	IM	2.5×10^{10} vp	23	NCT03815942
UK	VAC067	ChAdOx1 LS2	18-45	IM	5×10^{9} vp	3	NCT03203421
					2.5×10^{10} vp	10	
UK	VAMBOX	ChAdOx1 MenB.1	18-50	IM	2.5×10^{10} vp	3	ISRCTN46336916
					5×10^{10} vp	26	
UK	MERS001	ChAdOx1 MERS	18-50	IM	$5x10^{9}$ vp	6	[75].
					$2.5x10^{10}$ vp	14	NCT03399578
					$5x10^{10}$ vp	9	
Saudi Arabia	MERS002	ChAdOx1 MERS	18-50	IM	$5x10^{9}$ vp	6	NCT04170829
					$2.5x10^{10}$ vp	9	

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
					$5x10^{10}$ vp	9	
UK	CHIK001	ChAdOx1 Chik	18-50	IM	5×10^{9} vp	6	[76]
					2.5×10^{10} vp	9	
					5×10^{10} vp	9	
UK	ZIKA001 (ongoing)	ChAdOx1 Zika	18-50	IM	5×10^{9} vp	6	NCT04015648
					2.5×10^{10} vp	9	
					5×10^{10} vp	9	
Mexico	CHIKA01 (ongoing)	ChAdOx1 Chik	18-50	IM	5×10^{9} vp	12	NCT04440774
					2.5×10^{10} vp	12	
					5×10^{10} vp	12	
		ChAdOx1 Zika	18-50	IM	5×10^{9} vp	12	
					2.5×10^{10} vp	12	
					5×10^{10} vp	12	
		ChAdOx1 Zika & ChAdOx1 Chik co-administration	18-50	IM	5×10^{9} vp (×2)	12	

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
					2.5×10^{10} vp (×2)	12	
					5×10^{10} vp (×2)	12	
UK	HBV001 (ongoing)	ChAdOx1-HBV	18-65	IM	2.5×10^{9} vp	11	NCT04297917
					2.5×10^{10} vp	Up to 41	
South Korea, Taiwan, UK	HBV002 (ongoing)	ChAdOx1-HBV			2.5×10^{10} vp	Up to 48	NCT04778904
Belgium, UK		ChAdOx1-HPV	25-55	IM	2×10^{8} vp	Up to 19	NCT04607850
					2×10^{9} vp	Up to 35	
					2×10^{10} vp	Up to 35	
Spain	AELIX002	ChAdOx1.HTI	18-60	IM	5×10^{10} vp	30	NCT03204617
Spain	AELIX003 (ongoing)	ChAdOx1.HTI	18-60	IM	5×10^{10} vp	Up to 60 (expected)	NCT04364035
UK	HIV-Core0051 (ongoing)	ChAdOx1.HTI	18-65	IM	5×10^{10} vp	Up to 10	NCT04563377

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
UK	HIV-Core0052 (ongoing)	ChAdOx1.tHIVconsv1	18-65	IM	5×10^9 vp	Up to 3	NCT04586673
					5×10^{10} vp	Up to 10	
UK	COV001 (ongoing)	ChAdOx1 nCoV-19 (AZD1222)	18-55	IM	5×10^{10} vp	533	[77, 78]
UK	COV002 (ongoing)	ChAdOx1 nCoV-19	≥18	IM	$2.2 - 5.0 \times 10^{10}$ vp	≈ 5435	[78, 79]
Brazil	COV003 (ongoing)	ChAdOx1 nCoV-19	18-55	IM	5×10^{10} vp	5406	[78, 80]
Kenya	COV004 (ongoing)	ChAdOx1 nCoV-19	≥18	IM	5×10^{10} vp	400	PACTR202005681895696
South Africa	COV005 (ongoing)	ChAdOx1 nCoV-19	18-65	IM	5×10^{10} vp	1063	[81]
UK	COV006 (ongoing)	ChAdOx1 nCoV-19	6-17		5×10^{10} vp	Up to 221	ISRCTN15638344

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
USA, Peru, Chile		ChAdOx1 nCoV-19 (AZD1222)	≥18	IM	5×10^{10} vp	21,635	[82]
India		ChAdOx1 nCoV-19 (Covishield or AZD1222)	≥18	IM	5×10^{10} vp	≈ 1,300	CTRI/2020/08/027170
Japan		ChAdOx1 nCoV-19 (AZD1222)	≥18	IM	5×10^{10} vp	≈ 128	NCT04568031
UK	COV008 (ongoing)	ChAdOx1 nCoV-19	18-55	Intranasal	5×10^{9} vp	Up to 6	NCT04816019
					2×10^{10} vp	Up to 18	
					5×10^{10} vp	Up to 30	
UK	RVF001 (ongoing)	ChAdOx1 RVF	18-50	IM	5×10^{9} vp	3	NCT04754776
					2.5×10^{10} vp	Up to 6	
					5×10^{10} vp	Up to 6	
Uganda	RVF002 (ongoing)	ChAdOx1 RVF	18-50	IM	5×10^{9} vp	Up to 4	NCT04672824
					2.5×10^{10} vp	Up to 10	
					5×10^{10} vp	Up to 10	
UK	PLAVAC (ongoing)	ChAdOx1 Plague	18-55	IM	5×10^{10} vp	Up to 45	ISRCTN41077863

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers (Received ChAdOx1)	Publication / Registration Number
UK	EBL07 (ongoing)	ChAdOx1 biEBOV	18-55	IM	5×10^{9} vp	Up to 6	NCT05079750
					2.5×10^{10} vp	Up to 6	
					5×10^{10} vp	Up to 14	

Trials using ChAdOx1 nCoV-19 post conditional approval not included

Table 4. Current ChAdOx1 pipeline and clinical development stage

Disease	Pre-Clinical	Phase I/II	Phase III
Emerging Pathogens			
COVID-19	✓	✓	✓
MERS-CoV	✓	✓	
Chikungunya	✓	✓	
Zika	✓	✓	
Rift Valley Fever	✓	✓	
Marburg	✓		
Ebola (Sudan and Zaire)	✓		
Lassa	✓		
CCHF	✓		
Nipah	✓		
Plague	✓	✓	
Others			
Universal Influenza	✓	✓	
Meningitis B	✓	✓	
HIV	✓	✓	
Malaria	✓	✓	
Tuberculosis	✓	✓	
HBV (Therapeutic)	✓	✓	
HPV (Therapeutic)	✓	✓	
Prostate Cancer (Therapeutic)	✓	✓	

1.4 Regulatory frameworks

Although platforms can offer manufacturing advantages, regulatory agencies evaluate submissions as whole products for human use rather than as separate components. No special regulatory considerations are currently being given to platform-based products for human use. It is expected, however, that with increased use of platforms such as ChAdOx1, regulatory bodies become more comfortable with the safety and efficacy profiles of the vaccines generated, which

will translate into more rapid approvals with each subsequent submission [1]. The COVID-19 pandemic has proven that alternatives to the lengthy standard approval processes for vaccines are possible in the context of health emergencies without compromising on safety requirements and assessments. These alternatives include the animal rule, emergency use, and conditional marketing authorisations.

1.4.1 Animal Rule

The USA Food and Drug Administration (FDA) established regulations commonly known as the Animal Rule in 2002. The Animal Rule allows for approval of drugs, vaccines, and licensure of biological products when it would be unethical to conduct human efficacy studies, and field effectiveness trials are not feasible. These regulations are intended for products developed to prevent or reduce life-threatening or serious conditions caused by exposure to lethal or permanently disabling toxic chemical, biological, radiological, or nuclear substances. Under the Animal Rule, efficacy is established based on adequate and well-controlled studies in animal models of the human disease or condition of interest, and safety is evaluated under the pre-existing requirements for drugs, vaccines, and biological products [83].

For approvals relying on animal efficacy data, NHPs (or at least 2 other alternative animal models) are vaccinated at different doses and then challenged with the pathogen of interest to establish a protective titre. Human immunogenicity studies are then conducted to check if the same titres can be achieved. There are, however, significant disadvantages with this approach including: a) the fact that high dose challenge animal models may not replicate natural human exposure;

and b) humans are significantly bigger in size and have more blood volume compared to NHPs, so different antibody titres may be required to achieve the same level of protection.

The first vaccine approved under the animal rule was BioThrax® (Anthrax Vaccine Adsorbed) in November 2015 [84]. The first licensed adenovirus vectored vaccine (Zabdeno®) is an Ad26 based Ebola approved in July 2020 by the European Medicines Agency (EMA) under exceptional circumstances. The EMA approval was based on human immunogenicity data from over 3,300 humans and animal efficacy data was extrapolated from animal studies [85].

1.4.2 Emergency Use and Conditional Marketing Authorisation

Regulatory agencies across the globe, such as the EMA, FDA and multiple other national regulators made full use of the provisions under their respective legislation for emergency situations to speed up the procedure for approval of COVID-19 vaccines in record time, whilst fully ensuring that all requirements in terms of efficacy, quality and safety are fully assessed.

'Rolling reviews' introduced by regulators worldwide have been instrumental in speeding up the approval process for COVID-19 vaccines, allowing regulators to assess data as they become available during ongoing development, and before vaccine developers submit a request for marketing authorisation, instead of waiting until trials have finished. Therefore, when a marketing authorisation is requested, the formal assessment can proceed much faster, as the pre-clinical, chemistry, manufacturing & control (CMC), and clinical safety and immunogenicity data had already been scrutinised ahead of efficacy results becoming available, as part of the rolling review process.

Conditional Marketing Authorisation (CMA) is a pragmatic tool for fast-track approval of a medicine that fulfils an unmet medical need. CMAs have been issued to 4 COVID-19 vaccines by the EMA to date (Table 5). CMAs ensure that the safety, efficacy and quality of vaccines are demonstrated, and their benefits significantly outweigh their risks whilst allowing vaccine developers to submit additional data after the marketing authorisation is granted [86].

Table 5. COVID-19 Vaccines approved by EMA.

Manufacturer	EMA Approval Date
Pfizer (Comirnaty)	21 Dec 2020
Moderna (Spikevax)	06 Jan 2021
AstraZeneca (Vaxzevria)	29 Jan 2021
Janssen	11 Mar 2021

Emergency use authorisation (EUA) is an authorisation for temporary use of an unauthorised vaccine or product. It allows for use of a medicine under specific conditions as long as emergency circumstances apply. Several EUA have been granted to COVID-19 vaccines by multiple countries, but they are not the same as CMAs. A CMA follows a robust framework that provides safeguards whereas EUAs may not. The CMA ensures that pharmacovigilance, manufacturing controls, and post-approval obligations apply in a legally binding manner and are evaluated by EMA's scientific committees continuously, and regulatory action can be taken if necessary [86].

Under a CMA, liability is with the holder of the marketing authorisation, whereas in EUA administrative and civil liability from the manufacturer is removed when this emergency use is recommended or required by an individual country. EUA, however, is a useful tool in emergency situations, provided that additional

enhanced vaccine effectiveness, coverage, safety and impact monitoring activities are put in place [86].

1.4.3 Vaccine platform technologies regulatory framework for veterinary vaccines

Whilst no framework currently exists for regulatory approvals of vaccines based on platform technologies for human use, the EMA has introduced the concept of Vaccine Platform Technology Master File (PTMF) for the first time in 2021. This is a new concept in EU legislation for veterinary vaccines that anticipates the unnecessary re-submission and re-evaluation of data relating to the platform technology used in other previously authorised veterinary vaccine based on the same platform. This avoids the re-evaluation of parts of the dossier already assessed and certified in the first PTMF. The adoption of PTMFs is expected to speed the development and licensing procedures of new veterinary vaccines, including vaccines against emerging diseases. A full dossier would be required for the first product based on a particular platform, and an application for a PTMF could be submitted in parallel. Although the concept currently only applies to veterinary vaccines, it can be adapted for human products in the future.

1.5 Vaccine safety

Vaccines are held to very high safety standards as they are mass administered to cohorts of mostly healthy individuals who may receive no immediate health benefit, particularly when the incidence of the target infectious disease is low [87]. The risk-benefit assessments (and public perception of benefits) for prophylactic vaccines are substantially different to drug products developed to treat ill patients. As a general principle, it would be unacceptable for vaccines to introduce

significant burden of side effects, even when illness itself can lead to severe or fatal outcomes. Acceptance of side effects depends on their frequency and severity and may vary with time, based on how the side effects compare with symptoms induced by the disease itself [88].

Vaccine safety is monitored from pre-clinical toxicity studies to all steps of clinical development stages and through post licensure surveillance. This ensures that safety concerns are quickly identified should they arise, and the benefits of immunisation towards society (such as the prevention and containment of epidemics, decreased healthcare associated costs, and improved productivity), and benefits to the individual (prevention of severe disease, disability, and death from illness) outweigh the risks to the person who might suffer an adverse reaction. The balance of benefits and risks is dynamic and may also change over time as new data emerge, pathogens evolve, and new products become available [88]. Rare (≥1 in 10 000 and <1 in 1000) and very rare (<1 in 10 000) adverse reactions are seldomly captured throughout the vaccine clinical development phases, highlighting the need for continuous post marketing safety surveillance.

Reactogenicity refers to a subset of reactions that happen soon after vaccination and are a physical expression of the inflammatory response to immunisation. In clinical trials, information on expected signs and symptoms after vaccination is actively sought (or ‘solicited’). These symptoms include pain, redness, and swelling for injected vaccines (intramuscular, subcutaneous, or intradermal), and systemic symptoms, such as fever, myalgia, headache, and malaise. The broader term ‘safety’ profile refers to all adverse events (AEs) that could potentially be caused/triggered or worsened at any time after vaccination, and includes AEs, such as anaphylactic reactions (which usually occur rapidly after immunisation

and require swift recognition and management), diseases diagnosed after vaccination and immune-mediated events (usually occurring days or weeks after immunisation) [87].

Adverse events following immunisation (AEFI) require a comprehensive investigation to distinguish between events that can be potentially causally related to immunisation, and those which are merely coincidental to the vaccination procedure. Allegations that vaccines may cause an AEFI must be either confirmed or refuted based on scientific evidence. Misleading data can rapidly undermine confidence (for an individual product or vaccines in general), and lead to groundless suspension or withdrawal of a vaccine from the market; ultimately causing decreased coverage and disease resurgence [88].

Safety reporting is often heterogenous amongst different manufacturers with different groups using different grading toxicity scales depending on target population. Examples of toxicity grading scales include the “FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials”, The US National Cancer Institute “Common Terminology Criteria for Adverse Events (CTCAE)”, and the US NIH “Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS)”. There is no ‘one size fits all’ approach and modifications from these toxicity grading scales are often required to reflect different ethnic groups and medical practices across the world. The Brighton Collaboration is a network whose aim is to establish clear case definitions for individual events following vaccination, and to thereby ensure high-quality data collection and reporting consistency [89, 90]. Despite its launch over 20 years ago in 2000, the collaboration only became more widely known after the COVID-19 pandemic, following endorsements of its

standardised AEFI and Adverse Events of Special Interest (AESI) case definitions from the WHO Global Advisory Committee on Vaccine Safety, CEPI and the US CDC.

1.6 Hypothesis, aims and thesis outline

Traditional vaccine development strategies are costly and time consuming which makes them unsuitable for rapid responses in the context of outbreaks and pandemics. Platform technologies provide substantial manufacturing advantages and have the potential to significantly reduce these limitations to clinical development in global health emergencies. This project consists of clinical assessments of ChAdOx1 vectored vaccines against emerging pathogens, examining its suitability as a platform technology for accelerated R&D and response to epidemics. The overall objectives of this thesis are to characterise the safety, immunogenicity and efficacy profiles of ChAdOx1 vectored vaccines against four viral pathogens representing global public health threats.

The specific hypothesis relating to this work are that ChAdOx1 vectored vaccines are:

a) Safe, with no vaccine related serious adverse reactions (SARs), an acceptable reactogenicity profile with AEs being predominantly mild or moderate and serious adverse events (SAEs) balanced across control and intervention arms.

b) Immunogenic, with geometric mean T cell responses measured by IFN-γ ELISPOTs increased at least 3-fold from baseline to 14 days post vaccine administration, and binding antibodies geometric mean titres (GMT) increased at least 4-fold from baseline to day 28 post enrolment.

c) Efficacious, with minimum 50% efficacy against symptomatic disease regardless of severity.

Chapter two describes similarities and discrepancies between the methods used for the conduct and analysis of all clinical trials reported as part of this work.

Chapters three, four and five describe the first-in-human assessments (safety and immunogenicity profiles) of the chikungunya, Zika and MERS-CoV constructs.

Chapter six describes the assessment of safety, immunogenicity, and protective efficacy against symptomatic COVID-19 of the SARS-CoV-2 construct

Chapter seven describe reflections on lessons learned from the clinical development programme of the Oxford-AstraZeneca COVID-19 vaccine and an overall discussion of ChAdOx1 vectored vaccines included in this work.

Finally, chapter eight summarises the output of this thesis and discusses future work required in order to assess the usefulness of ChAdOx1 vectored vaccines in the next public health emergency.

Chapter 2: Material and Methods

2.1 First-in-human clinical trials of ChAdOx1 vectored vaccines

Three first-in-human, dose-escalation, clinical trials of ChAdOx1 vectored vaccines are included here. These studies are safety and immunogenicity assessments of ChAdOx1 vectored vaccines expressing antigens from chikungunya (CHIK001), Zika (ZIKA001), and MERS-CoV (MERS001) viruses.

MERS001 and CHIK001 were conducted before the COVID-19 pandemic. Two-thirds of participants in the ZIKA001 trial were recruited between Oct 2019 and Mar 2020 (before COVID-19 travel restrictions were in place). Recruitment into the ZIKA001 trial was halted for over 1 year, as a result of COVID-19 and resumed in Aug 2021.

2.1.1 Rationale for selected doses

Doses administered in these trials have been selected based on previous clinical experience with the ChAdOx1 adenovirus vector expressing different inserts (influenza, tuberculosis, and prostate cancer antigens), and other similar adenovirus vectored vaccines (ChAd63).

ChAdOx1 was administered for the first time as part of a 3+3 dose-escalation study (FLU004) of the vector encoding the influenza antigens nucleoprotein (NP) and matrix 1 (M1). ChAdOx1 NP+M1 was then safely administered at doses ranging from 5×10^8 to 5×10^{10} vp. A review of the data identified an optimal dose of 2.5×10^{10} vp balancing immunogenicity and reactogenicity. This dose was subsequently given to approximately 150 volunteers in 4 phase I studies at

the Jenner Institute (FLU005, TB034 VANCE01 and VAC067) where ChAdOx1 vectored vaccines have been demonstrated to be well tolerated. The vast majority of adverse events (AEs) have been mild-moderate and there had been no serious adverse reactions (SARs) in any of these trials.

Other simian adenovirus vectors (ChAd63, ChAd3 and PanAd3) have been safely administered at doses up to 2×10^{11} vp with an optimal dose of 5×10^{10} vp being selected, balancing immunogenicity and reactogenicity [91-93].

As these are first-in-human assessments of the CHIKV, ZIKV, and MERS-CoV antigenic inserts, the first dose of ChAdOx1 vectored vaccines proposed in these studies was 5×10^{9} vp and, therefore, are at least 10-fold less than what these new inserts are expected to be tolerated (5×10^{10} vp). Doses were gradually increased aiming to provide an optimal dose of ChAdOx1 considering the tolerability, reactogenicity and immunogenicity profiles.

2.1.2 Trial design

These were first-in-human, single-dose, open-label, dose-escalation, uncontrolled and non-randomised phase I clinical trials to assess the safety and immunogenicity profiles of the ChAdOx1 Chik, ChAdOx1 Zika, and ChAdOx1 MERS vaccine candidates in healthy volunteers aged 18-50 years old. Vaccines were administered intramuscularly into the deltoid.

Clinical trial protocols were written based on EMA guidelines for first-in-human studies. The trials were conducted according to the principles of the current revision of the Declaration of Helsinki and Good Clinical Practice (GCP).

Participants were recruited and vaccinated at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Oxford. All participants were healthy adults with

negative pre-vaccination tests for HIV antibodies, hepatitis B surface antigen, and hepatitis C antibodies. A negative urinary pregnancy test was required at screening and immediately before enrolment for all female subjects. There were 3 study groups (5×10^9, 2.5×10^{10} and 5×10^{10} vp) and up to 24 volunteers were enrolled in each of these trials (Table 6). Blood samples were drawn, and clinical assessments conducted for safety as well as immunology endpoints prior to vaccination at day 0 and subsequently at 2-, 7-, 14-, 28-, 56-, and 182-days following enrolment.

Table 6. Study groups for CHIK001, ZIKA001, and MERS001

Group	**Single Dose ChAdOx1 <antigen>**	**Route**
Group 1 (*n*=6)	5×10^9 vp	IM
Group 2 (*n*=9)	2.5×10^{10} vp	IM
Group 3 (*n*=9)	5×10^{10} vp	IM

2.1.3 Eligibility criteria

2.1.3.1 Common inclusion criteria for CHIK001, ZIKA001, and MERS001:

1. Healthy adults aged 18 to 50 years
2. Able and willing (in the Investigator's opinion) to comply with all study requirements
3. Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner or access this medical history electronically

4. For females only, willingness to practice continuous effective contraception during the study and a negative pregnancy test on the day(s) of screening and vaccination

5. Agreement to refrain from blood donation during the study

6. Provide written informed consent

2.1.3.2 Additional inclusion criteria for ZIKA001

7. Agreement to refrain from receipt of any flavivirus vaccine throughout the duration of the study (e.g., investigational or licensed yellow fever, Japanese encephalitis, tick-borne encephalitis or dengue virus vaccines).

2.1.3.3 Common exclusion criteria for CHIK001, ZIKA001, and MERS001

1. Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned use during the study period

2. Prior receipt of an investigational vaccine likely to impact on the interpretation of the trial data (e.g., adenovirus vectored vaccine).

3. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate

4. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids were allowed)

5. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine

6. Any history of hereditary angioedema, acquired angioedema, or idiopathic angioedema.

7. Any history of anaphylaxis in relation to vaccination

8. Pregnancy, lactation, or willingness/intention to become pregnant during the study

9. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)

10. History of serious psychiatric condition likely to affect participation in the study

11. Bleeding disorder (e.g., factor deficiency, coagulopathy, or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture

12. Any other serious chronic illness requiring hospital specialist supervision

13. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week

14. Suspected or known injecting drug abuse in the 5 years preceding enrolment

15. Seropositive for hepatitis B surface antigen (HBsAg)

16. Seropositive for hepatitis C virus (antibodies to HCV)

17. Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis

18. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data

19. Inability of the study team to contact the volunteer's GP to confirm medical history and safety to participate or access this medical history electronically

2.1.3.4 Additional exclusion criteria

CHIK001

20. Prior exposure to CHIKV (serology will be requested at the discretion of the investigator)

21. Travel to a CHIKV endemic region throughout the duration of the participant's enrolment in the study and within the preceding 30 days.

ZIKA001

22. Has a history of chronic or acute severe neurologic condition. Including: neurologic and neuroinflammatory sisorders: ADEM, including site-specific variants, cranial nerve disorders (including paralyses/paresis), Guillain-Barré syndrome ([GBS] including Miller Fisher syndrome and other variants), Immune-mediated peripheral neuropathies and plexopathies, optic neuritis, multiple sclerosis, narcolepsy, transverse myelitis, meningitis, or meningoencephalitis.

23. Seropositivity or cross-reactivity to Zika virus or dengue virus (serology will be requested at the discretion of the investigator).

24. Travel to a Zika virus, and/or dengue virus, endemic region in the 30 days prior to screening, until enrolment and in the first 6 months of the participant's enrolment in the study (participants had to consult the clinical team regarding international travel plans)

MERS001

25. Prior exposure to MERS-CoV (serology was requested at the discretion of the investigator)

26. History of allergic reaction to aminoglycoside antibiotics

2.1.3.5 Exclusion criteria added to ZIKA001 following the national rollout of COVID-19 vaccines and safety signal from ChAdOx1 nCoV-19

27. Planned receipt of another adenoviral vectored vaccine (e.g., Oxford/AstraZeneca or Janssen COVID-19 vaccines) within 90 days after the vaccination with the ChAdOx1 Zika.

28. Prior receipt of any vaccines administered ≤30 days before enrolment and/or planned receipt of a vaccine ≤30 days after enrolment EXCEPT for protein, RNA (or other non-adenovirus based) COVID-19 vaccinations which may be given within 14 days of the trial vaccine.

29. History of confirmed major thrombotic event (including cerebral venous sinus thrombosis, deep vein thrombosis, pulmonary embolism), history of antiphospholipid syndrome, or history of heparin-induced thrombocytopenia.

2.1.4 Safety assessments

2.1.4.1 Sequence of enrolment and dose escalation procedures

Participants were first allocated to group 1 (low dose). For safety reasons, the first volunteer in each group was vaccinated ahead of any other volunteers and the profile of AEs reviewed after 48 hours (±24h) post-vaccination. Provided there were no safety concerns, as assessed by the chief investigator (CI) and the local safety monitor (LSM), another 2 volunteers were vaccinated at the same dose after at least 48 hours had elapsed following the first volunteer being vaccinated, and at least 1 hour apart from each other. An independent safety review was conducted by the LSM after vaccination of the first three volunteers. This review included an assessment of the profile of AEs and the results of safety blood tests at day 7 post-vaccination. The CI and LSM were asked to provide the decision on whether to proceed with vaccinations of the remaining participants in the group and the first volunteer to receive the next incremental dose in the next group.

2.1.4.2 Adverse events data collection

Participants were observed in the clinic for 1 hour after the vaccination procedure and were asked to record any AEs using electronic diaries during a 28-day follow-up period. Swelling at the injection site was objectively assessed by a member of the study team during study visits.

Expected and protocol-defined local site reactions (injection-site pain, warmth, redness, and pruritus) and systemic symptoms (malaise, myalgia, arthralgia, fatigue, nausea, headache, feverishness, and temperature) were recorded for 7 days. Unsolicited AEs were recorded for 28 days, and serious adverse events (SAEs) were recorded throughout the follow-up period.

Table 7. CHIK001, ZIKA001, and MERS001 Solicited AEs

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

The severity of AEs was graded using the following criteria: (a) mild (short-lived or mild symptoms with no limitation to usual activity); (b) moderate (mild-to-moderate limitation in usual activity); (c) severe (considerable limitation in activity, medication, or medical attention required). Unsolicited AEs were reviewed for causality by an independent clinician and events considered possibly, probably, or definitively related to the study vaccine were reported. Laboratory AEs were graded using site-specific toxicity tables that were adapted from the US FDA toxicity grading scale.

Table 8. CHIK001, ZIKA001, and MERS001 Severity grading scale for local solicited AEs

Adverse Event	Grade	Intensity
Erythema at injection site*	1	>3 - ≤50 mm
	2	>50 - ≤100 mm
	3	>100 mm
Swelling at injection site	1	>3 - ≤20 mm
	2	>20 - ≤50 mm
	3	>50 mm
Ulceration/necrosis of skin at injection site	1	-
	2	-
	3	Any

*erythema or swelling ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event.

Table 9. CHIK001, ZIKA001, and MERS001 Severity grading scale for physical observations

	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Fever (oral)	37.6°C - 38.0°C	38.1°C – 39.0°C	>39.0°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130
Bradycardia (bpm)**	50 – 54	40 – 49	<40
Systolic hypertension (mmHg)	141 - 159	160 – 179	≥180
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80
Diastolic hypertension (mmHg)	91 - 99	100 – 109	≥110

*Taken after ≥10 minutes at rest

**Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes.

***Only if symptomatic (e.g., dizzy/ light-headed)

2.1.5 Immunogenicity assessments

2.1.5.1 Enzyme-linked immunospot (ELISpot)

Cellular immune responses were quantified at selected timepoints using an *ex vivo* ELISpot for Interferon Gamma (IFN-γ). Peripheral blood mononuclear cells (PBMC) were separated from whole blood within 4h of venepuncture, stimulated with synthetic overlapping peptides. The peptides were divided into peptide pools spanning the entire vaccine insert. Data were analysed according to a quality-control standard operational procedure (SOP). Summed responses to the antigen peptide pools are reported. The lower limit of detection (LLD) for the essay was established as 4 spot forming cells (SFC) per peptide pool.

For CHIK001, PBMC were stimulated with 125 synthetic peptides (20mers overlapping by 10 amino acids), divided into 13 peptide pools spanning the entire vaccine insert of the CHIKV structural antigens: Capsid (3 pools = 26 peptides), E3 (1 pool = 6 peptides), E2 (4 pools = 42 peptides), 6k (1 pool = 6 peptides) and

E1 (4 pools = 45 peptides). The lower limit of detection for the assay was 52 SFCs for summed responses to the 13 CHIKV structural antigen peptide pools.

For ZIKA001, PBMC were stimulated with 66 synthetic peptides (20mers overlapping by 10 amino acids), divided into 8 peptide pools spanning the entire vaccine insert of the ZIKV pre-membrane (2 pools = 16 peptides) and envelope (6 pools = 50 peptides). The lower limit of detection for the assay was 32 SFCs for summed responses to the 8 ZIKV antigen peptide pools. The following antibodies were used for the ELISpot assay in both CHIK001 and ZIK001: anti-human IFN-y-capture IgG1 mouse monoclonal Ab (dilution 1:100) and anti-human IFN-y-biotinylated, detection mouse IgG1 monoclonal Ab (dilution 1:1000).

For MERS001, PBMC were stimulated with 275 synthetic peptides (15mers overlapping by 10 amino acids). The peptides were divided into 14 pools spanning the entire vaccine insert of the MERS-CoV Spike protein (S1 = 4 pools; receptor binding domain [RBD] = 3 pools; S2 = 6), including the tissue plasminogen activator (tPA) leader sequence (tPA = 1 pool). Peptides were pooled into 13 pools for the MERS-CoV spike protein containing 18 or 21 peptides, plus a single pool of five peptides for the tPA. The LLD for the assay was 56 SFCs for summed responses to the 14 MERS-CoV spike peptide pools.

2.1.5.2 Enzyme-linked immunosorbent assay (ELISA)

Total anti-antigen immunoglobulin G (IgG) was measured using a standardized in-house indirect ELISA against E2 for chikungunya and spike for MERS-CoV.

For CHIK001, 1 µg/ml of CHIKV E2-recombinant protein in phosphate-buffered saline (PBS) was used to coat Nunc-immuno 96-well plates. Plates were

incubated at 4 °C for 18 h overnight [94, 95]. Coated plates were washed six times with PBS-Tween followed by blocking with casein for 1 h at room temperature (RT). Serum samples were diluted at 1:100 or 1:500 in casein to fit within the linear range of a standard curve prepared as indicated below, and then added to individual wells in triplicates. Plates were incubated at RT for 2 h, washed, as described, and then incubated at RT for 1 h with an alkaline phosphatase-conjugated goat anti-human IgG (gamma-chain specific, Sigma). Plates were developed by adding 4-nitrophenyl phosphate (Sigma) in diethanolamine substrate buffer (Thermo Scientific). A standard curve was prepared from a serum sample of a convalescent individual, following a 2-fold serial dilution starting at 1:100 and generating 10 standard points to which arbitrary ELISA units (EUs) were assigned. The optical density (OD) values of the standard points were fitted to a 4-parameter hyperbolic curve against the arbitrary EUs using the BioTek Gen5 v3.09 software and the parameters estimated from the standard curve were used to convert absorbance values of individual test samples into EU. Each ELISA plate contained the samples in triplicates, an internal positive control at 1:1600 dilution of the standard pool in triplicates, 10 standard points in duplicates, and four blank wells. Absorbance reading at 405 nm was performed using an ELx808 microplate reader (BioTek). The assay cut-off was determined from the analysis of the 24 pre-vaccinated (Day 0) samples of the trial volunteers. The seropositive cut-off was determined mathematically using the mean plus 3 standard deviations of the EU values reported for the 24 samples assayed. This value defined the threshold from which detection was feasible. A cut-off value of 51.1 EU was used as the analytical sensitivity of this assay.

Two commercially available ELISA kits were used to validate negative serum samples that had a higher background on the in-house E2 ELISA at baseline. The anti-CHIKV IgG ELISA kit from Abcam (ab177835) and the anti-CHIKV IgG ELISA kit from Euroimmun (EI 293a-9601G) were both performed according to the manufacturer's instructions.

For MERS001, Total anti-MERS spike IgG was measured using a standardised in-house indirect ELISA. Nunc MaxiSorp 96-well plates (Life Technologies, Paisley, UK) were coated with 1 μg/mL of full-length recombinant clamp MERS spike protein (supplied by Keith Chappell, The School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia) in PBS and incubated at 4°C for 18 h overnight. The coated plates were washed six times with phosphate buffered saline-Tween and then blocked with casein for 1h at room temperature. Plasma samples diluted to fall within the linear range of the curve (typically 1/500 in casein) were then added to individual wells on the plates. This step was followed by incubation of the plates at room temperature for 2 h and washing of the plates, as initially described. The plates were then incubated at room temperature for 1 h with a secondary antibody, alkaline phosphatase-conjugated goat anti-human IgG (γ-chain specific). After a final wash, plates were developed by adding 4-nitrophenyl phosphate in diethanolamine substrate buffer (Fisher Scientific UK, Loughborough, UK). The standard curve used on each plate was derived from a pool of volunteers' sera containing high-titre anti-MERS IgG. Endpoint titre determined by ELISA was used to identify the volunteer samples with the highest anti-MERS IgG titres after vaccination. A 1/100 dilution of the standard pool was used in a two-fold serial dilution to produce ten standard points that were assigned arbitrary ELISA units. The optical density values of the

standard points were fitted to a four-parameter hyperbolic curve against the arbitrary ELISA units using GEN5 software (version 3.04; BioTek Instruments, Winooski, VT, USA), and the parameters estimated from the standard curve were used to convert absorbance values of individual test samples into ELISA units. Each ELISA plate consists of the samples and internal positive control (1/800 dilution of the standard pool, corresponding to standard 4) in triplicates, ten standard points in duplicates, and four blank wells. The optical density reading of the plates at 405 nm was done with an ELx808 microplate reader (BioTek Instruments).

2.1.5.3 Neutralisation assays

2.1.5.3.1 Live and pseudovirus neutralisation - MERS001

Neutralising antibodies (NAb) against live MERS-CoV were measured by use of paired serum samples obtained at day 0 and day 28. Assays were conducted by research collaborators at the University of Marburg, Germany. Induction of virus-neutralising antibodies was confirmed according to previously published protocols [96]. Briefly, serum samples were tested for their capacity to neutralise MERS-CoV (EMC/2012 isolate) infections in vitro with 100 50% tissue culture infective doses ($TCID_{50}$) in Huh-7 cells. Sera were incubated for 1h with the virus and then added to the cells and incubated for 4 days. A recombinant human monoclonal antibody directed against the RBD of MERS-CoV S1 (MRO-895LC Creative Biolabs, Shirley, NY, USA) served as a neutralisation control. Full neutralisation was observed at a dilution of 1/512 (with or without one dilution step acceptable as inter-assay variation), which corresponds to a concentration of 0.2 µg/mL [97]. Neutralisation titres were calculated as reciprocal values of geometric mean titres of four replicates. A titre of 8 was considered positive.

For the pseudovirus neutralisation assay, MERS-CoV EMC/2012, KOR/KNIH/002, and England-1 (endoplasmic reticulum retention signal-deleted, amino acids 1–1338) pseudotyped lentiviral particles were generated and titrated using lentivirus-associated p24 ELISA Kit (Cell Biolabs, San Diego, CA, USA) [98]. Assays were conducted by research collaborators at International Vaccine Institute (IVI). Two-fold diluted serum was incubated with 2×10^6 viral particles of pseudotyped virus for 1h at 37°C and added to 786-O cells, incubated for 6h, and replaced with fresh medium; luciferase-reporter activity was measured 3 days later. A commercially available neutralising monoclonal antibody (40069-R723, Sino Biological, Beijing, China) was used as a positive control. All of the samples were analysed in duplicates. Data were expressed as geometric mean with SDs.

2.1.5.3.2 Plaque reduction neutralisation test (PRNT) - CHIK001

Induction of serum neutralizing antibodies was evaluated with a PRNT on monolayers of Vero cells (Vero ATCC CCL-81) cultured in 12-well plates using standard methods [99, 100]. Assays were conducted by research collaborators at the University of Texas Medical Branch (UTMB). Neutralizing antibody titres were recorded for four CHIKV lineages, including the chikungunya strains CHIKV-LR (Indian Ocean Lineage, IOL), SV-0444 (Asian Lineage), 37997 (West African Lineage), and YO111213 (Asian/American Lineage), all obtained from the World Reference Centre for Emerging Viruses and Arboviruses at UTMB [99]. Titres were quantified as the highest serum dilution that inhibited plaque formation in 50% ($PRNT_{50}$). Seroconversion was considered positive in samples with reciprocal titres of $PRNT_{50} \geq 10$ [101]. Limits of detection were between 10 and 1280, and any samples without a detectable titre were listed as either 5 or 2560.

2.1.6 Statistical analysis

These studies were not designed to test a specific hypothesis and are intended to obtain preliminary descriptive estimates of safety, reactogenicity and immunogenicity of ChAdOx1 CHIK, ChAdOx1 Zika, and ChAdOx1 MERS in healthy adults. The sample size allows an estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups.

With 6 or 9 subjects in each dose group, the chance of observing at least one AE of probability 25% or more is approximately 82.2% and 92.5% respectively. With 24 subjects across the three dosing cohorts, the chance of observing at least one AE of 10% probability or more is at least 92%. Therefore, if no AEs of a given type occur across the 3 different doses, we can be confident that any dose-independent event would occur in fewer than 10% of people.

Table 10. Probability of observing an AE for different event rates

True Event Rate (%)	N	Probability of Observation (%)	N	Probability of Observation (%)	N	Probability of Observation (%)
1	6	5.9	9	8.6	24	21.4
5		26.5		37		70.8
10		46.9		61.3		92
15		62.3		76.8		98
20		73.8		86.6		99.5
25		82.2		92.5		99.9

Safety endpoints are described as frequencies alongside 95% confidence intervals (CI). The association between the frequency of moderate or severe solicited AEs and group allocation (groups 2 and 3 or 1 and 3) is reported as relative risk (RR) with the respective 95% CI and p value (Fisher's exact test).

Correlations between a) T cell responses at day 14 and binding antibody levels against the antigen of interest at day 28; b) binding and neutralising antibody titres at day 28; were calculated using simple linear regression methods from their log-transformed data and reported with their respective r^2 and p value for a regression line slope different than zero.

T cell ELISpot and binding (and neutralising when applicable) antibody data are described as their geometric means within each group and fold increase from baseline. Non-parametric paired Wilcoxon, Kruskal-Wallis or Friedman tests were used to compare differences in immune responses between timepoints and groups with Dunn's correction for multiple comparisons, as appropriate.

Statistical analysis was done using GraphPad Prism version 9.1 or above (GraphPad Software Inc., California, USA).

2.2 Clinical trials of ChAdOx1 nCoV-19

2.2.1 Rationale for selected dose

The dose to be administered in COV001 and COV002 has been selected based on clinical experience with the ChAdOx1 adenovirus vector expressing the MERS-CoV Spike insert (MERS001 trial).

ChAdOx1 MERS had been given to 31 participants at doses ranging from 5×10^9 vp to 5×10^{10} vp. Despite higher reactogenicity observed at 5×10^{10} vp, this dose was safe, with self-limiting AEs and no SARs recorded. The 5×10^{10} vp dose was the most immunogenic in terms of inducing neutralising antibodies against MERS-CoV using a live virus assay. Given the immunology findings and safety profile observed with a ChAdOx1 vectored vaccine against MERS-CoV, the 5×10^{10} vp dose was chosen for ChAdOx1 nCoV-19.

The Clinical BioManufacturing Facility (CBF), who manufactured and tested batches 02P20-01 and 02P20-02 for the COV001 trial, determined vp/mL for Advent manufactured batch(es). This was done using a spectrophotometry-based methodology documented in their internal SOP (SOP A104). The doses to be administered in COV002, on Advent manufactured batches, were determined by both methods (Abs260 and qPCR). Overseas studies conducted on Advent batch(es) were dosed based on Advent's qPCR method. The University of Oxford also conducted several for information only tests for the ChAdOx1 nCoV-19 batch(es) manufactured by Advent to demonstrate comparability between the different manufacturing processes and suitability of a reconstitution process involving dilution of the product with 0.9 % (w/v) saline that were managed using local clinical trial SOPs available at each site/location.

For Advent Lot Number K.0007 the concentration is 1.7×10^{11} vp/mL (qPCR) which has been assessed by the CBF as equivalent to 3.89×10^{11} vp (Abs 260).

An analytical comparability assessment of ChAdOx1 nCoV-19 (AZD1222) manufactured by CBF, Advent and Cobra Biologics was conducted using a comprehensive set of physicochemical and biological release and characterization tests. In order to support the analytical comparability assessment, A260 testing of Advent's process (K.0007, K.0008, and K.0009 lots) was performed, where corrections to the absorbance due to excess polysorbate 80 were made to compensate for polysorbate 80 concentrations above the formulation target of 0.1% (w/v).

Differences in strength-related attributes (i.e., virus particle concentration, virus genome concentration, and infectious virus concentration) are noted. These

differences in strength are further examined for potential impact on clinical dosing. The target clinical dosage of CBF's product is 5×10^{10} viral particles per dose based on vp/mL concentration determined by UV spectroscopy (A260), whereas that of Advent's product is 5×10^{10} viral genome copies per dose based on vg/mL concentration determined by qPCR. The target clinical dosage of Symbiosis' product is $3.5 – 6.5 \times 10^{10}$ viral particles per dose based on the vp/mL concentration determined by A260, with a 0.5 mL dosing volume. This dosing range is based on a target 5×10^{10} viral particles per dose and a ± 30% range to take into account process and method variabilities. The planned clinical dosage of Symbiosis' product is compared to that of CBF and Advent products, the resulting Symbiosis' product dosage at 0.5 mL for lot 20481A is somewhat lower in total viral particle per dose (20% from the lower range limit), slightly higher in total viral genome copies per dose (12% from the higher range limit), and slightly lower in total infectious particle per dose (8% from the lower range limit). These differences are considered to be comparable to or within the variabilities from the analytical methods used in concentration determination (A260, qPCR, and infectivity) and the dosing volumes during clinical administration. In summary, with a 0.5 mL dosing volume for Symbiosis' product, strength difference from CBF and Advent products is not expected to have significant clinical impact in terms of reactogenicity and immunogenicity/efficacy.

Manufacturing of ChAdOx1 nCoV-19 is also taking place at Serum Institute of India (SII). The vaccine is manufactured under the named ChAdOx1 nCoV-19 (Covishield). The target clinical dose for ChAdOx1 nCoV-19 (Covishield) is nominal 5×10^{10} viral particles per dose based on vp/mL concentration determined by UV spectroscopy. An analytical comparability assessment of

ChAdOx1 nCoV-19 manufactured by Advent, Cobra Biologics and SII was conducted using a comprehensive set of physicochemical and biological release and characterisation tests. The planned clinical dosage of SII's product is compared to that of Cobra and Advent products, with a dosing volume of 0.5 mL.

Table 11. Clinical strengths of ChAdOx1 nCoV-19 drug product for different manufacturers

Strength Attribute	Process 1 - CBF		Process 2 - Advent				Process 3 - Cobra	Process 4 - SII
	02P20-01	02P20-02	K.0007	K.0008	K.0009	K.0011	20481A	412007
					Concentration			
Virus particle concentration (A_{260}) (vp/mL)	1.49×10^{11}	1.22×10^{11}	3.12×10^{11}	3.16×10^{11}	2.45×10^{11}	1.4×10^{11}	0.8×10^{11}	0.9×10^{11}
Virus genome concentration (qPCR) (vg/mL)	1.7×10^{11}	Not tested	1.7×10^{11}	2.1×10^{11}	1.4×10^{11}	1.5×10^{11}	1.3×10^{11}	2.08×10^{11}
AZ qPCR (vg/mL)	1.37×10^{11}	Not tested	1.38×10^{11}	1.42×10^{11}	1.12×10^{11}	0.67×10^{11}	0.77×10^{11}	0.796×10^{11}
AZ ddPCR (vg/mL)	1.17×10^{11}	Not tested	1.29×10^{11}	1.27×10^{11}	1.01×10^{11}	0.59×10^{11}	0.71×10^{11}	
Infectious particle concentration (ifu/mL) [a]	2.6×10^{9}	Not tested	2.9×10^{9}	3.0×10^{9}	2.4×10^{9}	1.3×10^{9}	1.3×10^{9}	1.69×10^{9}
AZ Infectivity (ifu/mL)	2.13×10^{9}	Not tested	1.89×10^{9}	2.04×10^{9}	2.06×10^{9}	1.09×10^{9}	1.28×10^{9}	1.33×10^{9}
P:I (vp/ifu)				70	58			53.25
					Target Clinical Dosage			
Equivalent DP volume per dose (mL)	0.34	0.41	0.294	0.235	0.356	0.5	0.5	0.5
Dosing of virus particle (vp/dose)	5.1×10^{10}	5.0×10^{10}	9.2×10^{10}	7.4×10^{10}	8.7×10^{10}	7×10^{10}	4.0×10^{10}	4.5×10^{10}

Strength Attribute	Process 1 - CBF		Process 2 - Advent				Process 3 - Cobra	Process 4 - SII
Dosing of viral genome (vg/dose)	5.8×10^{10}	NA	5.0×10^{10}	4.9×10^{10}	5.0×10^{10}	7.5×10^{10}	6.5×10^{10}	10.4×10^{10}
AZ qPCR (vg/dose)	4.7×10^{10}	NA	4.1×10^{10}	3.3×10^{10}	4.0×10^{10}	3.35×10^{10}	3.9×10^{10}	3.98×10^{10}
AZ ddPCR (vg/dose)	4.0×10^{10}	NA	3.8×10^{10}	3.0×10^{10}	3.6×10^{10}	2.95×10^{10}	3.5×10^{10}	
Dosing of infectious particle (ifu/dose)	8.8×10^{8}	NA	8.5×10^{8}	7.1×10^{8}	8.5×10^{8}	6.5×10^{8}	6.5×10^{8}	8.45×10^{8}
AZ Infectivity (ifu/dose)	7.2×10^{8}	NA	5.6×10^{8}	4.8×10^{8}	7.3×10^{8}	5.45×10^{8}	6.4×10^{8}	

ifu = infectious units; NA = not applicable; vp = virus particle; vg = virus genome

[a] Testing performed using the Advent infectivity assay.

2.2.2 Trial design

2.2.2.1 COV001

A Phase I/II, single-blinded, controlled, individually randomised study in healthy adults aged 18-55 years recruited in the UK. ChAdOx1 nCoV-19 or licensed MenACWY was administered via an intramuscular injection into the deltoid. The study assessed the efficacy, safety, and immunogenicity of ChAdOx1 nCoV-19.

There were 4 study groups with up to 540 volunteers in each of the single-dose vaccine arms (ChAdOx1 nCoV-19 or licensed MenACWY) in groups 1, 2 & 4 combined and 10 participants in group 3 with an overall sample size of up to 1090 (Table 12). Randomisation took place at an intervention to control ratio of 1:1. Only participants enrolled in groups 1, 2 and 4 were randomised. Participants in group 3 were not randomised or blinded.

Staggered enrolment applied to the first volunteers receiving the IMP. Participants were first recruited in groups 1 and 3. Once groups 1 and 3 were fully recruited, subsequent volunteers were enrolled in groups 2 and 4. Safety was assessed in real-time and interim reviews were scheduled after 1, 4, and up to 54 participants received the IMP. Randomisation blocks ensured there was at least 1 control for each participant receiving the IMP, so these safety reviews took place after 2, 8, and up to 98 participants were enrolled in the study overall (groups 1 and 3).

Table 12. Study groups for COV001

Group	Number of Volunteers	Dose	Route
1a	44	5×10^{10} vp ChAdOx1 nCoV-19	IM
1b	44	MenACWY	IM
2a	Up to 206	5×10^{10} vp ChAdOx1 nCoV-19	IM
2b	Up to 206	MenACWY	IM
3	10	Homologous prime-boost 5×10^{10} vp ChAdOx1 nCoV-19 (4 weeks interval)	IM
4a	Up to 290	5×10^{10} vp ChAdOx1 nCoV-19	IM
4b	Up to 290	MenACWY	IM

2.2.2.2 COV002

A Phase 2/3, participant-blinded individually randomised controlled trial in adults in the UK, administering either a single dose or two-doses of ChAdOx1 nCoV-19 or licensed MenACWY vaccine via IM injection. The study assessed the efficacy, safety, and immunogenicity of ChAdOx1 nCoV-19 in UK adults aged 18 and above (with no upper age limit). All data from participants with PCR (or other nucleic acid amplification test) -positive swabs were assessed for inclusion in the primary efficacy analysis by two blinded assessors who independently reviewed each case, to classify each for inclusion in the primary outcome.

Enrolment into Groups 1, 4, 5 & 6 started after a data safety monitoring board (DSMB) review of all available data from animal studies and at least 4 weeks' worth of safety and immunogenicity data were accumulated from the first 54

participants receiving ChAdOx1 nCoV-19 in COV001. A minimum of 7 days of safety data from group 1 was reviewed by the DSMB prior to enrolment of participants into group 2. Participants were randomised to ChAdOx1 nCoV-19/MenACWY on a 3:1:3:1 ratio in groups 1 and 7, and 5:1:5:1 ratio in groups 2 and 8, 1:1 in groups 4, 5a, 5b, 5c, 6, 9 and 10, and 5:1 in group 5d. Groups 5e and 5f are open-label and therefore, volunteers were not randomised.

Safety was assessed in real-time. The DSMB periodically assessed safety and efficacy data every 4-8 weeks and/or as required.

Participants were followed throughout the study to record adverse events and episodes of virologically confirmed symptomatic COVID-19 cases. Participants were tested for COVID-19 if they presented with a new onset of fever (Temperature ≥37.8°C) OR cough OR shortness of breath OR anosmia/ageusia.

Moderate and Severe COVID-19 disease were defined using WHO clinical criteria (Figure 4) [102].

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

Figure 4. WHO COVID-19 Clinical progression scale

Reproduced from WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research [102].

Table 13. Study groups for COV002

Group	Vaccine	*n*	Age group
Group 1	a1) Single dose ChAdOx1 nCOV19 vaccine: 2.2×10^{10} vp	30	56 – 69 years
Safety &	(qPCR)		
Immunogenicity	a2) Single-dose MenACWY	10	
	b1) Two dose ChAdOx1 nCOV19 vaccine (4-6 weeks	30	
	apart): 2.2×10^{10} vp (qPCR) prime-boost		
	b2) Two-dose MenACWY (4-6 weeks apart)	10	
Group 2	a1) Single dose ChAdOx1 nCOV19 vaccine: 2.2×10^{10} vp	50	≥70 years
Safety &	(qPCR)		
Immunogenicity	a2) Single-dose MenACWY (4-6 weeks apart)	10	
	b1) Two dose ChAdOx1 nCOV19 vaccine (4-6 weeks	50	
	apart): 2.2×10^{10} vp (qPCR)		
	b2) Two-dose MenACWY	10	
Group 3	Group removed	0	Children

Group	Vaccine	*n*	Age group
Group 4	a1) Single dose ChAdOx1 nCoV19 vaccine: 2.2 ×10^{10}vp	up to 1775	18 – 55 years
Efficacy	(qPCR)		
	a2) MenACWY	up to 1775	
	b1) Two dose ChAdOx1 nCOV19 vaccine (4-6 weeks	up to 50 (from	
	apart): 2.2 ×10^{10} vp (qPCR)	4a1)	
	b2) Two dose MenACWY	up to 50 (from	
		4a2)	
	c1) Two dose ChAdOx1 nCOV19 vaccine (at least 4	up to 1725	
	weeks apart): 2.2 ×10^{10} vp (qPCR) prime and 0.5mL (3.5	(from 4a1)	
	– 6.5 × 10^{10} vp, Abs 260, corrected for PS80) boost OR 5		
	×10^{10} vp (qPCR) boost		
	c2) Two dose MenACWY	up to 1725	
		(from 4a2)	
Group 5	a1) Single dose ChAdOx1 nCoV19 vaccine: 2.2 × 10^{10} vp	50	18-55 years
Batch	(qPCR)		
comparison	a2) MenACWY	50	
	b1) Single dose ChAdOx1 nCoV19 vaccine: 5 × 10^{10} vp	up to 25	
Safety &	(qPCR)		
Immunogenicity	b2) Men ACWY MenACWY	up to 25	
	(B-cell immunology only)		
	c1) Single dose ChAdOx1 nCoV19 vaccine: 5 × 10^{10} vp	up to 25	
	(qPCR)		
	c2) MenACWY	up to 25	
	(B and T cell immunology)		
	d1) Two-dose ChAdOx1 nCoV19 vaccine (4-6 weeks	up to 50	
	apart): 0.5mL (3.5 – 6.5 × 10^{10} vp, Abs 260, corrected for		
	PS80)		
	d2) Men ACWY	up to 10	
	e) Two-dose ChAdOx1 nCoV-19 vaccine (4-6 weeks	up to 15	
	apart): 0.5mL (Covishield 9 × 10^{10} vp/mL)		
	f) Two-dose ChAdOx1 nCoV-19 vaccine (4-6 weeks	up to 15	
	apart): 0.25mL prime and 0.5mL boost (Covishield 9 ×		
	10^{10} vp/mL)		
Group 6	a1) ChAdOx1 nCoV19 vaccine: 5 × 10^{10} vp (qPCR)	up to 3000	18 – 55 years
Efficacy	a2) MenACWY	up to 3000	
	b1) Two dose ChAdOx1 nCoV-19 vaccine (at least 4	up to 3000	
	weeks apart): 5 × 10^{10} vp (qPCR) prime and 0.5mL (3.5 –	(from 6a1)	
	6.5 × 10^{10} vp, Abs 260, corrected for PS80) boost OR 5 ×		
	10^{10} vp (qPCR) boost		

Group	Vaccine	*n*	Age group
	b2) Two dose MenACWY	up to 3000 (from 6a2)	
Group 7 *Efficacy*	a1) Single-dose ChAdOx1nCOV19 vaccine: $5x10^{10}$vp (qPCR)	30	56 – 69 years
	a2) Single-dose MenACWY	10	
	b1) Two dose ChAdOx1nCOV19 vaccine (4-6 weeks apart): 5×10^{10} vp (qPCR)	30	
	b2) Two-dose MenACWY (4-6 weeks apart)	10	
Group 8 *Efficacy*	a1) Single-dose ChAdOx1nCOV19 vaccine: 5×10^{10} vp (qPCR)	50	≥70 years
	a2) Single-dose MenACWY	10	
	b1) Two dose ChAdOx1nCOV19 vaccine (4-6 weeks apart): 5×10^{10} vp (qPCR) prime and 0.5mL boost ($3.5 – 6.5 \times 10^{10}$ vp, Abs 260, corrected for PS80) OR 5×10^{10} vp (qPCR) boost	50	
	b2) Two-dose MenACWY (4-6 weeks apart)	10	
Group 9 *Safety & Immunogenicity*	a1) Two dose ChAdOx1 nCOV19 vaccine (4-6 weeks apart): 0.5mL ($3.5 – 6.5 \times 10^{10}$ vp, Abs 260, corrected for PS80)	approx. 500	56 – 69 years
	a2) Two dose MenACWY	approx. 500	
Group 10 *Safety & Immunogenicity*	a1) Two dose ChAdOx1 nCOV19 vaccine (4-6 weeks apart): 0.5mL ($3.5 – 6.5 \times 10^{10}$ vp, Abs 260, corrected for PS80)	approx. 500	≥70 years
	a2) Two dose MenACWY	approx. 500	
Group 11 *Safety & Immunogenicity*	Two dose ChAdOx1 nCOV19 vaccine (4-6 weeks apart): 0.5mL ($3.5 – 6.5 \times 10^{10}$ vp, Abs 260, corrected for PS80)	up to 60	18-55 (non-ChAdOx1 naïve)

Multiple amendments have been done for both COV001 and COV002 and these trials are still ongoing. For the purposes of this thesis, the data presented here represents a subset from both these trials, as described in Table 13.

2.2.3 Eligibility criteria

2.2.3.1 COV001 and COV002 Inclusion criteria:

1. Healthy adults aged 18-55 years (COV001).

2. Adults aged 18 - 55 years (groups 4, 5, 6 and 11) – COV002
3. Adults aged 56-69 years (groups 1, 7, and 9) – COV002
4. Adults aged 70 years and older (groups 2, 8, and 10) – COV002
5. Able and willing (in the Investigator's opinion) to comply with all study requirements (participants must not rely on public transport or taxis).
6. Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner and access all medical records when relevant to study procedures.
7. 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 and vaccination.
8. Agreement to refrain from blood donation during the study.
9. Provide written informed consent.

2.2.3.2 COV001 Exclusion Criteria

1. Prior receipt of any vaccines (licensed or investigational) ≤30 days before enrolment

2. Planned receipt of any vaccine other than the study intervention within 30 days before and after each study vaccination with the exception of the licensed seasonal influenza vaccination and the licensed pneumococcal vaccine. Participants were encouraged to receive these vaccinations at least 7 days before or after their study vaccine.

3. 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)

4. Administration of immunoglobulins and/or any blood products within the 3 months preceding the planned administration of the vaccine candidate.

5. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent severe infections and use of immunosuppressant medication within the past 6 months, except topical steroids or short-term oral steroids (course lasting <14 days).

6. Any autoimmune conditions, except mild psoriasis, well-controlled autoimmune thyroid disease, vitiligo, or stable coeliac disease not requiring immunosuppressive or immunomodulatory therapy.

7. History of allergic disease or reactions likely to be exacerbated by any component of the ChAdOx1 nCoV-19 or MenACWY vaccines.

8. Any history of angioedema.

9. Any history of anaphylaxis.

10. Pregnancy, lactation, or willingness/intention to become pregnant during the study.

11. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).

12. History of serious psychiatric condition likely to affect participation in the study (e.g., ongoing severe depression, history of admission to an in-patient psychiatric facility, recent suicidal ideation, history of suicide attempt, bipolar disorder, personality disorder, alcohol and drug dependency, severe eating disorder, psychosis, use of mood stabilisers or antipsychotic medication).

13. Bleeding disorder (e.g., factor deficiency, coagulopathy, or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture.

14. Any other serious chronic illness requiring hospital specialist supervision.

15. Chronic respiratory diseases, including mild asthma (resolved childhood asthma was allowed)

16. Chronic cardiovascular disease (including hypertension), gastrointestinal disease, liver disease (except Gilberts Syndrome), renal disease, endocrine disorder (including diabetes) and neurological illness (excluding migraine)

17. Seriously overweight (BMI≥40 Kg/m2) or underweight (BMI≤18 Kg/m2)

18. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week.

19. Suspected or known injecting drug abuse in the 5 years preceding enrolment.

20. Any clinically significant abnormal finding on screening biochemistry, haematology blood tests or urinalysis.

21. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.

22. History of laboratory-confirmed COVID-19.

23. New onset of fever or a cough or shortness of breath or anosmia/ageusia since February 2020. This exclusion criteria was replaced with ‘Seropositivity for SARS-CoV-2 before enrolment’ when a reliable test became available.

24. Those who have been at high risk of exposure before enrolment, including but not limited to: close contacts of confirmed COVID-19 cases, anyone who had to self-isolate as a result of a symptomatic household member, frontline healthcare professionals working in accident and emergency (A&E), intensive care unit (ICU) and other higher-risk areas. When a reliable test became available, this exclusion criteria was replaced with seropositivity for SARS-CoV-2 before enrolment.

25. Living in the same household as any vulnerable groups at risk of severe COVID-19 disease (as per PHE guidance)

2.2.3.3 Exclusion Criteria for COV002

1. Participation in COVID-19 prophylactic drug trials for the duration of the study. Note: Participation in COVID-19 treatment trials was allowed in the event of hospitalisation due to COVID-19. The COV002 study team was informed as soon as possible.

2. Participation in SARS-CoV-2 serological surveys where participants were informed of their serostatus for the duration of the study. Note: Disclosure of serostatus post enrolment may accidentally unblind participants to group allocation. Participation in COV002 could only be allowed if volunteers were kept blinded to their serology results from local/national serological surveys

3. Receipt of any vaccine (licensed or investigational) other than the study intervention within 30 days before and after each study vaccination, with the exception of the licensed seasonal influenza vaccination and the licensed pneumococcal vaccination. Participants were encouraged to receive these vaccinations at least 7 days before or after their study vaccine.

4. Prior or planned receipt of an investigational or licensed vaccine or product likely to impact on interpretation of the trial data (e.g., adenovirus vectored vaccines, any coronavirus vaccines). This exclusion criteria did not apply to group 11, as recruitment was targeted at those volunteers who previously received a ChAdOx1 vectored vaccine.

5. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.

6. Any confirmed or suspected immunosuppressive or immunodeficient state; asplenia; recurrent severe infections and use of immunosuppressant medication within the past 6 months, except topical steroids or short-term oral steroids (course lasting ≤14 days)

7. History of allergic disease or reactions likely to be exacerbated by any component of ChAdOx1 nCoV-19 or MenACWY

8. Any history of angioedema.

9. Any history of anaphylaxis.

10. Pregnancy, lactation, or willingness/intention to become pregnant during the study.

11. Current diagnosis of or treatment for cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).

12. History of serious psychiatric condition likely to affect participation in the study.

13. Bleeding disorder (e.g., factor deficiency, coagulopathy, or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture.

14. Continuous use of anticoagulants, such as coumarins and related anticoagulants (i.e., warfarin) or novel oral anticoagulants (i.e., apixaban, rivaroxaban, dabigatran and edoxaban)

15. Suspected or known current alcohol or drug dependency.

16. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.

17. Severe and/or uncontrolled cardiovascular disease, respiratory disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder, and neurological illness (mild/moderate well-controlled comorbidities were allowed)

18. History of laboratory-confirmed COVID-19 (except groups 5d, 5e, 5f, 9, 10 and 11).

19. Seropositivity to SARS-CoV-2 before enrolment (except groups 5d, 5e, 5f, 9, 10 and 11)

2.2.4 Safety assessments

Participants were observed in the clinic for 30–60 min after the vaccination procedure and were asked to record any adverse events using electronic diaries during the 28-day follow-up period. Expected and protocol-defined local site reactions (injection site pain, tenderness, warmth, redness, swelling, induration, and itch) and systemic symptoms (malaise, muscle ache, joint pain, fatigue, nausea, headache, chills, feverishness [i.e., a self-reported feeling of having a fever], and objective fever defined as an oral temperature of 38°C or higher) were recorded for 7 days. All other events were recorded for 28 days, and serious adverse events are recorded throughout the follow-up period.

Table 14. COV001 and COV002 Solicited AEs

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

The severity of AEs were graded with the following criteria: mild (transient or mild discomfort for <48 h, no interference with activity, and no medical intervention or therapy required), moderate (mild to moderate limitation in activity [some assistance might be needed] and no or minimal medical intervention or therapy required), severe (marked limitation in activity [some assistance usually required] and medical intervention or therapy required), and potentially life-threatening

(requires assessment in the emergency department or hospitalisation). Unsolicited adverse events were reviewed for causality by two clinicians blinded to group allocation, and events considered to be possibly, probably, or definitely related to the study vaccines were reported. Laboratory AEs graded by use of site-specific toxicity tables, which were adapted from the US FDA toxicity grading scale.

Table 15. COV001 and COV002 Severity grading scale for local solicited AEs

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
	4	A&E visit or hospitalization
Tenderness	1	Mild discomfort to touch
	2	Discomfort with movement
	3	Significant discomfort at rest
	4	A&E visit or hospitalization
Erythema at injection site*	1	2.5 - 5 cm
	2	5.1 - 10 cm
	3	>10 cm
	4	Necrosis or exfoliative dermatitis
Induration/Swelling at injection site	1	2.5 – 5 cm and does not interfere with activity
	2	5.1 - 10 cm or interferes with activity
	3	>10 cm or prevents daily activity
	4	Necrosis

Table 16. COV001 and COV002 Severity grading scale for physical observations

Vital Signs	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 Potentially Life-threatening
Fever (oral)	38.0°C - 38.4°C	38.5°C – 38.9°C	39.0°C - 40°C	> 40°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130	A&E visit or hospitalisation for arrhythmia
Bradycardia (bpm)**	50 – 54	45 – 49	<45	A&E visit or hospitalisation for arrhythmia
Systolic hypertension (mmHg)	141 - 150	151 – 155	≥155	A&E visit or hospitalization for malignant hypertension
Diastolic hypertension (mmHg)	91 - 95	96 – 100	>100	A&E visit or hospitalization for malignant hypertension
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80	A&E visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 - 20	21-25	>25	Intubation

*Taken after ≥10 minutes at rest

**When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes.

***Only if symptomatic (e.g., dizzy/ light-headed)

The following adverse events of special interest (AESI) were proposed

1. Disease enhancement following vaccination with ChAdOx1 nCoV-19 were monitored. Severe COVID-19 disease wwas defined using clinical criteria. Detailed clinical parameters were collected from medical records and aligned with agreed definitions as they emerged. These were likely to include but were not limited to, oxygen saturation, need for oxygen therapy, respiratory rate, need for ventilatory support, imaging, and blood test results, amongst other clinically relevant parameters.
2. Acute respiratory distress, pneumonitis, acute cardiac injury, arrhythmia, septic-shock like syndrome and acute kidney injury related to COVID-19

disease was monitored from medical records review of hospitalised participants.

3. Neurological events of > Grade 2 severity
4. Eosinophilia as a marker of skewed Th2 responses was routinely monitored in participants attending their COVID-19 testing and follow-up visits. Marked eosinophilia of ≥ 1.5 x10^9/L was reported as an SAE.
5. AESI relevant to vaccination in general was also monitored such as: generalised convulsion, GBS, acute disseminated encephalomyelitis (ADEM), thrombocytopenia, anaphylaxis, vasculitides in addition to serious solicited AEs will be monitored.

After approval for emergency use and safety signal detection, the following AESIs were added

6. Cerebral venous sinus thrombosis
7. Heparin-induced thrombocytopenia
8. Major thrombosis with concurrent thrombocytopenia

2.2.5 Immunogenicity assessments

2.2.5.1 IFN-γ ELISpot

A total of 253 synthetic peptides (15mers overlapping by 10 amino acids) spanning the entire vaccine insert, including the tPA leader sequence were used to stimulate PBMC. Peptides were pooled into 12 pools for the SARS-CoV-2 spike protein (S1 = 6 pools; S2 = 6 pools), and a single pool for the tPA leader. The LLD was 52 SFC for summed responses to the 13 SARS-CoV-2 Spike antigen and tPA leading sequence peptide pools.

2.2.5.2 ELISA

Anti-SARS-CoV-2 spike IgG was measured by a multiplex immunoassay on the MSD platform at PPD Laboratories. The assay sequences were based on the ancestral sequences from Wuhan, China. The LLD was 33 EU/mL.

2.2.5.3 Pseudovirus neutralisation

Antibody neutralisation was measured with a lentivirus-based pseudovirus particle expressing the D614 SARS-CoV-2 spike protein. The pseudovirus neutralising antibody assay was validated at Monogram Biosciences. The LLD for pseudovirus neutralising antibody is 40 (ID_{50}).

2.2.6 Statistics

Safety endpoints are described as frequencies (%) with 95% CIs. Geometric means with 95% CI are presented for immunological endpoints and fold increase for T cell responses Participants were analysed according to the vaccine they received. To assess the relationship between responses on different assays, linear regression was used to analyse log-transformed post-baseline values.

Non-parametric paired Wilcoxon or Kruskal-Wallis tests were used to compare differences in immune responses between timepoints and groups with Dunn's correction for multiple comparisons, as appropriate. Mann Whiney test was used to compare controls and ChAdOx1 nCoV-19 responses.

Sample sizes were nominal for these immunogenicity subgroups and no power calculations were done. Statistical analyses were done using GraphPad Prism version 9.1 or above (GraphPad Software Inc., California, USA) and OpenEpi (version 3.01).

Chapter 3: Safety and immunogenicity of ChAdOx1 Chik in UK healthy adult volunteers

3.1 Authorship statement

Immunogenicity data presented in this chapter has been generated by Prof Arturo Reyes-Sandoval's and Prof Sarah Gilbert's group at the Jenner Institute (binding antibody and cellular immunogenicity data), and Prof Shannan Rossi's group at the UTMB (neutralising antibody data). This work has been published in Folegatti, P.M., et al. Nature Communications, 2021.

3.2 Background

Chikungunya fever is an arbovirus disease caused by the chikungunya virus (CHIKV), a mosquito-borne alphavirus. Alphaviruses are a genus of enveloped, positive-sense, single-stranded RNA viruses, belonging to the *Togaviridae* family. The disease typically consists of an acute illness characterised by fever, rash, myalgia and generally symmetrical and peripheral, often incapacitating, polyarthralgia and/or polyarthritis, which usually lasts weeks to months or even years in some cases [103]. Fatal disease is rare but has been observed in neonates and elderly individuals, as well as in those with comorbidities such as diabetes or cardiovascular, respiratory, and neurologic disorders.

CHIKV has been identified in 115 countries in Asia, Africa, Oceania, Europe and the Americas [104, 105]. The virus was first isolated during an outbreak on the Makonde Plateau in Tanzania in 1952–53. Outbreaks were subsequently identified in Asia during the 1950s and 1960s. Over the past 50 years, numerous

CHIKV re-emergence has been documented in both Africa and Asia at irregular intervals between outbreaks. In 2004, CHIKV emerged in Kenya and spread to Comoros, where 5,000 cases were reported. In 2005–2006, the outbreak spread to other islands in the Indian Ocean with an estimated 300,000 cases of CHIKV infections, resulting in 237 deaths [106] and an estimated economic cost of €43.9 million [107]. Since 2005, India, Indonesia, Maldives, Myanmar, and Thailand have reported over 1.9 million cases. The virus reached Europe in 2007, leading to its first autochthonous epidemic outbreak in the northeast of Italy. CHIKV has since spread to the Caribbean with the first case reported in Saint Martin in 2013, and local transmission now confirmed in 47 countries and territories in the World Health Organization Region of the Americas (PAHO). In 2015, over 1 million suspected cases of chikungunya have been recorded in the Caribbean islands and Latin American countries with 191 deaths during the same period. Canada, Mexico, and the USA have also recorded imported cases. In 2017, autochthonous cases were again reported in Europe (France and Italy) for the first time since 2014. Chikungunya outbreaks have also been reported in Bangladesh (2017), Sudan (2018), Yemen (2019) and more recently in Chad and Cambodia (2020) [105, 108-111].

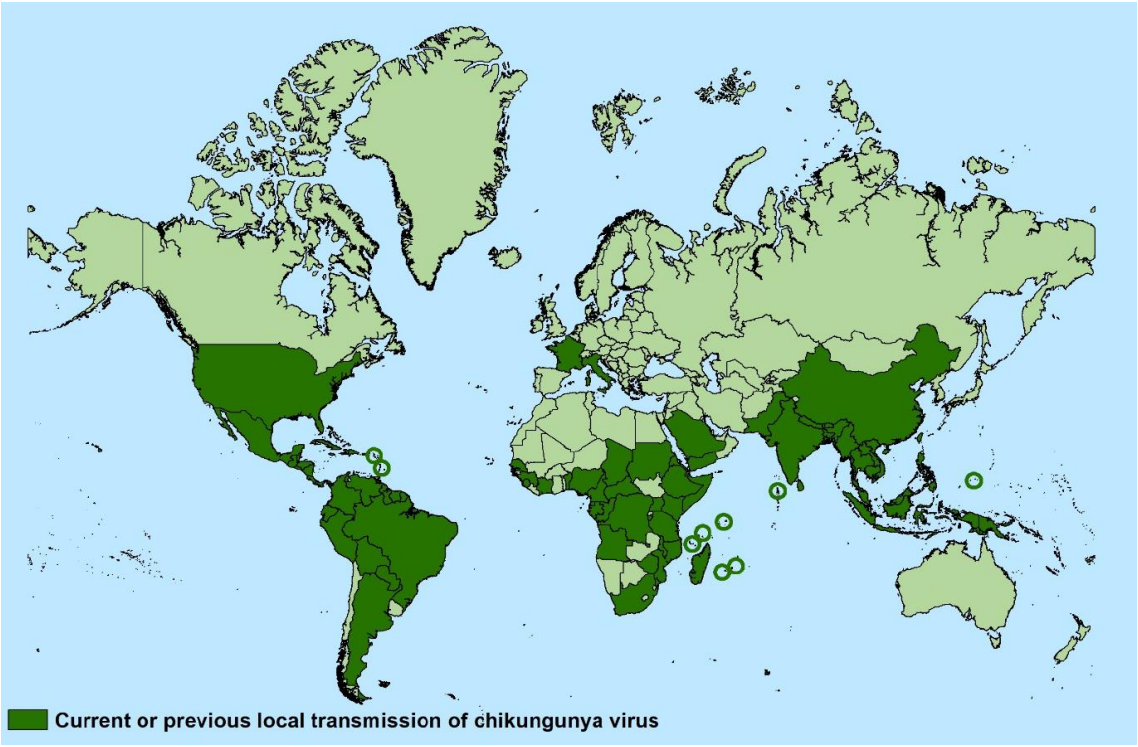


Figure 5: Geographical distribution of chikungunya.

Countries and territories where chikungunya cases have been reported (as of 30th October 2020). Source: CDC website [104].

Transmission occurs through the bite of infected female *Aedes* mosquitos which are widely distributed across the globe. *A. aegypti* are found in the tropics and subtropics, whereas *A. albopictus* have a wider distribution (also being found in temperate regions). *A. albopictus* has spread from Asia to become established in areas of Africa, Europe, and the Americas, causing the spread of CHIKV to new geographical regions [108, 112].

CHIKV is listed as a priority pathogen by the UK Vaccine Network, the US NIAID (category B), and CEPI. It has been designated as a serious condition requiring action by the WHO to promote R&D to control future outbreaks.

3.3 Progress towards a CHIKV vaccine

Vector control measures are frequently complex and have proven to be insufficient in the containment of arbovirus infections outbreaks. Effective mosquito control strategies often rely on adequate staffing levels (entomologists, social scientists, operational vector-control staff), technical expertise at decentralised levels of services, availability of funding, adequate geographical coverage, insecticide use (with their subsequent emerging resistance patterns), community engagement, capacity building and monitoring & evaluation. These requirements are frequently unmet, even in developed countries [113].

There are currently no available CHIKV specific treatments or licensed vaccines, and clinical management is based on symptom relief. Chronic arthralgia can lead to persistent incapacitation, requiring long-term treatment with nonsteroidal anti-inflammatory and immunosuppressive drugs. Several classes of direct-acting and host-targeting antivirals have been reported to have *in vitro* activity against CHIKV. However, *in vivo* efficacy has not yet been evaluated in animal models for most of these drugs. Clinical trials of chloroquine in CHIKV-infected patients failed to prove it as an effective treatment [114, 115].

Humans are thought to be the only amplification hosts during urban transmission. Therefore, the most cost-effective means of controlling the spread of the infection is by vaccination [116]. There are several vaccine candidates in pre-clinical development but only a few have entered clinical trials. The first live-attenuated vaccine, known as 181/clone25 or TSIGSD-218, was highly immunogenic but produced arthralgia in some vaccines and its further development was discontinued because of safety concerns after reversal of the attenuation had been reported both in mice and humans [117-119]. VLP [120], live measles-

vectored [12], inactivated vaccines (NCT04566484), and mRNA encoded monoclonal antibody [121] have successfully elicited immune responses in phase I and II clinical trials. A single-dose live vaccine attenuated by partial deletion of the gene encoding the non-structural replicase complex protein nsP3 has reached phase III, and publication of the results are awaited (NCT04546724). However, VLP and inactivated vaccines usually require the support of adjuvants and/or multiple injections to induce seroconversion and reach high antibody titres, increasing the costs and limiting their use in low-income countries. The use of replication-competent recombinant live attenuated virus carries the risks of inadequate attenuation causing disseminated disease, particularly in immunocompromised hosts. 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.

3.4 ChAdOx1 Chik Vaccine

CHIKV has a positive-strand RNA genome of approximately 12kb in length. A large 5′ ORF encodes for a non-structural (ns) polyprotein designated as P1234. The second ORF encodes for the precursors of viral structural proteins known as Capsid (C), Envelope (E - E3, E2 & E1), and the 6K protein (Figure 6).

ChAdOx1 Chik was engineered to express the full structural polyprotein genome of CHIKV that includes the Capsid, E3, E2, E1, and the 6K proteins. The synthetic gene was designed through a consensus sequence from 3 different CHIKV lineages (West African [WA], East/Central/South Africa [ECSA], and Asian) [94]. The cassette is well characterised and is known to elicit CHIKV specific immune responses in several pre-clinical and clinical vaccine studies [101, 122].

Specifically, ChAdOx1 Chik has been shown to be efficacious against lethal challenge and able to induce strong immune responses in animal models [68, 94].

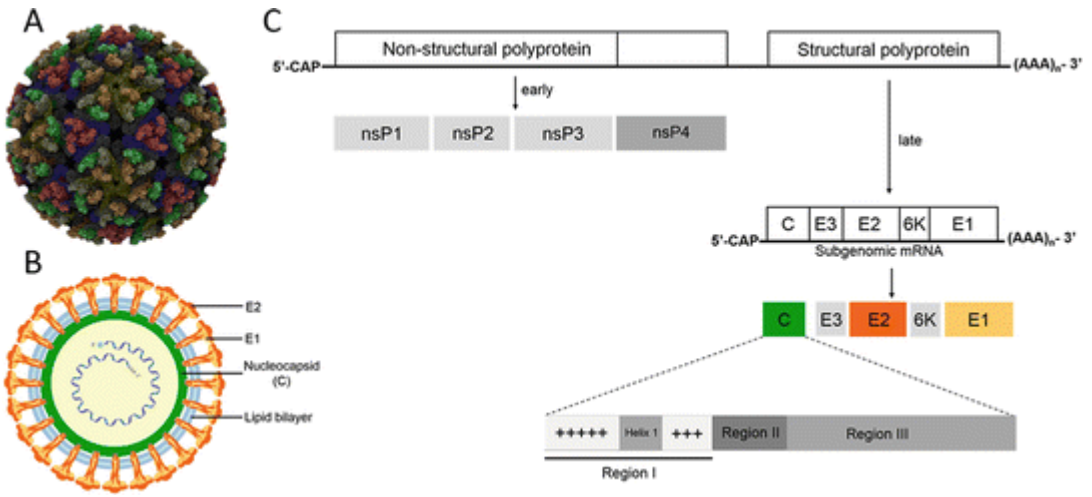


Figure 6: Genomic structure of chikungunya virus.

A) CHIKV virion; (B) Schematic representation of CHIKV particle section; (C) Schematic representation of the CHIKV genome, which encodes 2 ORFs. The non-structural polyprotein encodes non-structural proteins nsP1, nsP2, nsP3, and nsp4. The structural polyprotein is translated from a sub-genomic mRNA and encodes the capsid protein (c), envelope glycoproteins E1 and E2 and accessory proteins E3 and 6K. Capsid is segmented into three separate regions. Region I contain many positively charged residues as well as uncharged amino acids in helix 1. Region II is responsible for RNA binding and region III interacts with the E2 glycoprotein. Reproduced from Metz S.W., Pijlman G.P. (2016) Function of chikungunya virus structural proteins. In: Okeoma C. (eds) Chikungunya Virus. [123].

3.5 Methods

3.5.1 Trial design, objectives, participants, and oversight

This was a first-in-human, dose-escalation, open-label, non-randomized and uncontrolled clinical study of 24 healthy male and female subjects aged 18–50 years old.

The primary objective was to assess the safety and tolerability of ChAdOx1 Chik in healthy volunteers, measured as described in the general methods chapter. The secondary objective was to assess CHIKV structural antigen-specific humoral and cellular immune responses induced by ChAdOx1 Chik as measured by ELISA, plaque reduction neutralization test (PRNT) and *ex vivo* IFN-γ ELISpot).

Eligible volunteers were recruited at CCVTM, Oxford, UK. Screening for previous CHIKV exposure was conducted on participants with significant travel history to CHIKV-endemic areas, using a commercial ELISA kit (Anti-CHIKV IgG Human ELISA Kit, Abcam ab177835) and participants were excluded if positive. Full details of the eligibility criteria are described in the general methods chapter. A LSM provided independent safety oversight.

3.5.2 Study approvals

Written informed consent was obtained in all cases, and the trial was conducted in accordance with the principles of the Declaration of Helsinki and GCP. This study was approved within the UK by the Medicines and Healthcare Products Regulatory Agency (MHRA reference 21584/0394/001-0001) and the South-Central Oxford A Research Ethics Committee (REC reference 18/SC/0004). Vaccine use was authorized by the Genetically Modified Organisms Safety Committee (GMSC) of the Oxford University Hospitals National Health Service (OUH-NHS) Trust (GMSC reference number GM462.18.102). The trial was registered at www.clinicaltrials.gov (identifier: NCT03590392). The first participant was enrolled on 02 October 2018 and the last participant was enrolled on 01 April 2019.

3.5.3 Trial procedures

ChAdOx1 Chik was administered as a single intramuscular injection into the deltoid at a low dose of 5×10^9 vp (group 1), intermediate dose of 2.5×10^{10} vp (group 2), and high dose of 5×10^{10} vp (group 3). A staggered-enrolment approach was used for the first 3 participants in each group and interim-safety reviews were conducted prior to dose escalation (details provided in general methods chapter).

Blood samples were drawn, and clinical assessments conducted for safety as well as immunology endpoints prior to vaccination at day 0 and subsequently at 2-, 7-, 14-, 28-, 56-, and 182-days following enrolment. AE data collection procedures were done as described in the general methods section.

3.5.4. Statistics

This study was not designed to test a specific hypothesis and was intended to obtain preliminary descriptive estimates of safety, reactogenicity and immunogenicity of ChAdOx1 Chik in healthy adults. The sample size allows an estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups.

Safety and immunogenicity endpoints were analysed as described in the general methods chapter.

3.5.5 Immunogenicity assessments

3.5.5.1 ELISA

In-house ELISA was conducted at days 0, 14, 28, 56 and 182, and as described in the general methods chapter

3.5.5.2 $PRNT_{50}$

Neutralisation was assessed at days 0, 14, 28 and 182, and as described in the general methods chapter.

3.5.5.3 ELISpot

Cellular immune responses were quantified at the selected timepoints (days 0, 14, 28, 56 and 182) using an *ex vivo* ELISpot for IFN-γ, as described in the general methods chapter.

3.6 Results

3.6.1 Study population

Between 18 July 2018 and 18 October 2019, 24 healthy adult subjects received a single dose of ChAdOx1 Chik at 5×10^9, 2.5×10^{10} or 5×10^{10} vp (Figure 7). Baseline characteristics are summarized in Table 17.

3.6.2 Vaccine safety

ChAdOx1 Chik was safe at doses up to 5×10^{10} vp with no serious adverse reactions (SAR) reported. A total of 112 local and systemic solicited AEs were reported. The vast majority of solicited AEs were mild (79/112; 70.54%, 95%CI 61.53–78.18) or moderate (27/112; 24.11%, 95% CI 17.13–32.8) and self-limiting in nature. All solicited AEs were completely resolved within 7 days and 94.64% of them had their onset within the first 72 h post-vaccination (51.79% at D0, 39.29% at D1, and 3.57% at D2). Injection site pain was the most common local AE, reported by 79.17% of participants and was predominantly mild in severity. Fatigue was the most common systemic AE followed by headache, myalgia, and feverishness. The frequencies of local and systemic solicited AEs reported during the first 7 days are summarized in Table 18. Median duration of solicited AEs is

summarized in Table 19. Only one SAE was reported (traumatic hip fracture) but was deemed not related to ChAdOx1 Chik.

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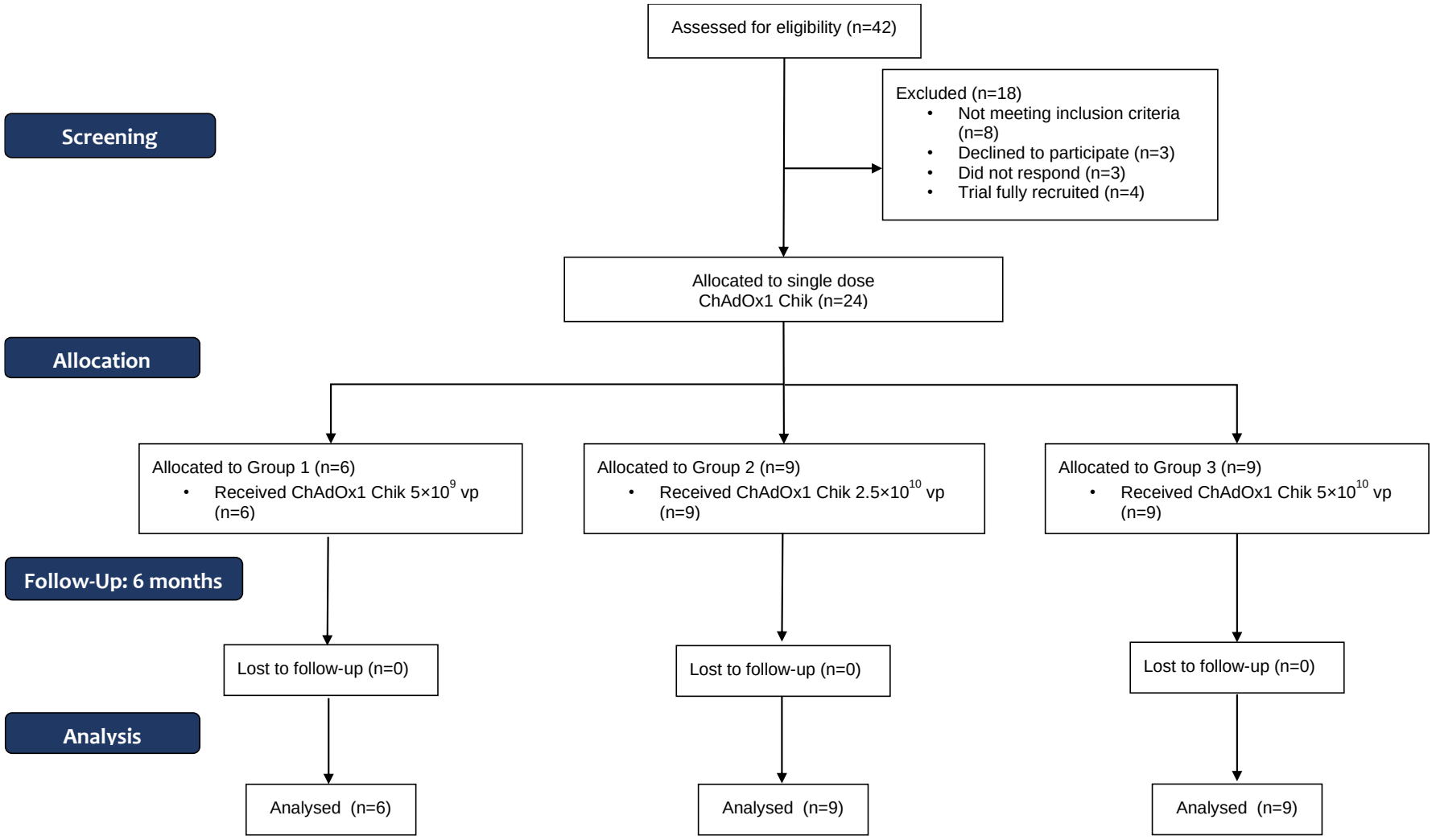


Figure 7: CHIK001 CONSORT diagram

Table 17. CHIK001 Summary of baseline characteristics

Variable	Group 1 (n=6)	Group2 (n=9)	Group 3 (n=9)	All Groups (n=24)
Age				
Median	43	26	24	29
Range	20-45	18-41	21-43	18-45
Sex				
Male – n (%)	2 (33.33)	2 (22.22)	2 (22.22)	6 (25)
Female – n (%)	4 (66.67)	7 (77.78)	7 (77.78)	18 (75)
Ethnicity				
White – n (%)	6 (100)	5 (55.56)	8 (88.89)	19 (79.17)
Mixed[a] n (%)	-	1 (11.11)		1 (4.17)
Latin American n (%)	-	3 (33.33)	1 (11.11)	4 (16.67)

[a]Mixed: White and Asian

Table 18. CHIK001 Number of participants reporting local and systemic solicited AEs.

	ChAdOx1 Chik 5x10^{9} vp (*n* = 6)				**ChAdOx1 Chik 2.5x10^{10} vp (*n* = 9)**				**ChAdOx1 Chik 5x10^{10} vp (*n* = 9)**			
	Any	Mild	Moderate	Severe	Any	Mild	Moderate	Severe	Any	Mild	Moderate	Severe
Any symptom	4 (67%)	4 (67%)	0	0	9 (100%)	6 (67%)	3 (33%)	0	9 (100%)	4 (44%)	3 (33%)	2 (22%)
Any local symptom	3 (50%)	3 (50%)	0	0	9 (100%)	8 (89%)	1 (11%)	0	7 (78%)	5 (56%)	2 (22%)	0
Pain	3 (50%)	3 (50%)	0	0	9 (100%)	8 (89%)	1 (11%)	0	7 (78%)	5 (56%)	2 (22%)	0
Pruritus	0	0	0	0	0	0	0	0	0	0	0	0
Warmth	0	0	0	0	4 (44%)	4 (44%)	0	0	3 (33%)	3 (33%)	0	0
Swelling	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	0	0	0	0	0	0	0	0	1 (11%)	1 (11%)	0	0
Any systemic symptom	2 (33%)	2 (33%)	0	0	9 (100%)	6 (67%)	3 (33%)	0	9 (100%)	4 (44%)	3 (33%)	2 (22%)
Fever	0	0	0	0	2 (22%)	2 (22%)	0	0	2 (22%)	2 (22%)	0	0
Feverishness	1 (17%)	1 (17%)	0	0	6 (67%)	3 (33%)	3 (33%)	0	7 (78%)	4 (44%)	2 (22%)	1 (11%)
Arthralgia	0	0	0	0	3 (33%)	3 (33%)	0	0	4 (44%)	0	3 (33%)	1 (11%)
Myalgia	1 (17%)	1 (17%)	0	0	7 (78%)	6 (67%)	1 (11%)	0	6 (67%)	2 (22%)	3 (33%)	1 (11%)
Headache	1 (17%)	1 (17%)	0	0	7 (78%)	6 (67%)	1 (11%)	0	6 (67%)	3 (33%)	1 (11%)	2 (22%)
Fatigue	2 (33%)	2 (33%)	0	0	5 (56%)	5 (56%)	0	0	8 (89%)	4 (44%)	4 (44%)	0
Nausea	0	0	0	0	2 (22%)	1 (11%)	1 (11%)	0	3 (33%)	1 (11%)	2 (22%)	0
Malaise	1 (17%)	1 (17%)	0	0	6 (67%)	6 (67%)	0	0	5 (56%)	1 (11%)	3 (33%)	1 (11%)

Table 19. CHIK001 Duration of AEs

AE	Median	IQR	Maximum duration
Pain at injection site	3.00	2-3	7.00
Pruritus	-	-	-
Warmth	1.00	1-1	3.00
Erythema	1.00	1-1	1.00
Swelling	-	-	-
Fever	1.00	1-1	1.00
Feverishness	2.00	1-3	3.00
Arthralgia	2.00	1-2	2.00
Myalgia	2.00	1-2	3.00
Headache	2.00	1-3	5.00
Fatigue	1.00	1-2	5.00
Nausea	1.00	1-2	2.00
Malaise	2.00	1-2.75	3.00
Overall	2.00	1-3	7.00

Four participants reported a short-lived temperature above 37.5 °C within the first 72 h post-vaccination (two in the intermediate-dose group and two in the high-dose group). The highest temperature recorded was 38 °C (classified as mild). All febrile episodes resolved within 24 h.

The proportion of moderate and severe AEs was significantly higher in group 3 compared with group 2 (RR 3.643, 95%CI 1.817–7.666, $p < 0.001$), but there were no safety concerns, despite the higher reactogenicity.

Unsolicited AEs in the 28 days following vaccination considered possibly, probably, or definitively related with ChAdOx1 Chik were predominantly mild in nature and resolved within the follow-up period (Table 20). Unsolicited AEs of

note include shivering/chills (one severe at D0 and D1, resolved by D2; one moderate at D0, resolved by D1 and one mild at D1, resolved by D2; all in Group 3); insomnia (one severe at D2, resolved by D3—Group 3) and Lower Back Pain (one severe at D0, resolved by D2—Group 2). Laboratory AEs considered at least possibly related to the study intervention were self-limiting and predominantly mild in severity (Table 21).

Table 20. CHIK001 Unsolicited AEs related with ChAdOx1 Chik

Subject ID	MEDDRA PT	MEDDRA PT CODE	Day of Onset	Duration (days)	Max. Severity
00101012	Dizziness	10013573	0	1	1
00101016	Muscle spasms	10028334	4	1	1
00101006	Cough	10011224	4	2	1
00101018	Oropharyngeal pain	10068319	0	2	1
00101018	Cough	10011224	0	8	1
00101017	Chills	10008531	1	0	1
00101019	Chills	10008531	0	1	2
00101027	Chills	10008531	0	2	3
00101027	Back pain	10003988	0	2	2
00101027	Muscular weakness	10028372	1	1	2
00101027	Sleep disorder	10040984	2	1	3
00101029	Hypoaesthesia	10020937	0	3	2
00101037	Hunger	10020466	0	1	1
00101031	Coordination abnormal	10010947	0	1	2
00101030	Back pain	10003988	0	2	3
00101030	Nasal congestion	10028735	2	1	1
00101041	Rhinorrhoea	10039101	3	3	2
00101011	Somnolence	10041349	0	1	1
00101027	Hypoaesthesia	10020937	0	1	1

Table 21. CHIK001 Laboratory AEs.

	Id	Group	Event	Severity	Timepoint	Resolved by	Comment
Haematology	101014	2	Leukopenia	Mild	D2	D7	
	101014	2	Neutropenia	Mild	D2	D7	
	101018	3	Lymphopenia	Mild	D2	D7	
	101019	3	Leucopenia	Mild	D2	D7	
	101019	3	Neutropenia	Mild	D2	D7	
	101029	3	Leukopenia	Mild	D2	D7	
	101029	3	Neutropenia	Mild	D2	D7	
	101030	2	Leukopenia	Mild	D2	D7	
	101030	2	Neutropenia	Moderate	D2	D28	
	101036	2	Leukopenia	Mild	D2	D7	
	101036	2	Neutropenia	Mild	D2	D7	
Biochemistry	101003	1	Hyperbilirubinemia	Moderate	D2	Not resolved	Participant has known Gilbert's Sd. Bilirubin=28 at D28 (baseline bilirubin=24)
	101014	2	Hypoalbuminemia	Mild	D2	Not resolved	Alb=33 at baseline; 31 at D2; 32 at D7; 31 at D28; 31 at D56. Although meets toxicity grading scale criteria (mild), this was not considered to be clinically significant
	101017	3	Hyperbilirubinemia	Moderate	D2	D7	Highest Bilirubin=34. Normal ALT

3.6.3 Immunogenicity

3.6.3.1 Humoral immunogenicity

Neutralizing antibody titres by $PRNT_{50}$ were measured from all 24 participants. All doses were highly immunogenic upon a single immunization, reaching a 100% seroconversion rate as early as 14 days against isolates representative of 3 CHIKV lineages (Table 22): Indian Ocean Lineage (IOL), West African Lineage (WAf), and Asian Lineage (As). $PRNT_{50}$ titres to La Réunion (IOL), 37997 (WAf), and SV-0444 (As), were significantly higher than baseline and persisted throughout the 182-day follow-up period. $PRNT_{50}$ to YO111213 (Asian–American lineage - AsAm) revealed a 100% seroconversion rate on day 28 with lower seroconversion rates on days 14 and 182 (91.6% and 83.3%, respectively). Geometric mean titres (GMT) peaked at day 28 for IOL (285.5, 95% CI 161.2–504.3), WAf (369.7, 95% CI 217.2–629.3) and AsAm (71.3, 95% CI 49.6–102.4); whereas titres peaked at day 56 for As (75.3, 95% CI 48.0–118.3). Highest neutralization was observed against IOL and WAf, with maximum $PRNT_{50}$ values of 1280, 1280 and 2560 at low, intermediate, and high vaccine dosages respectively. In comparison, maximum $PRNT_{50}$ GMT for each dose group were 160, 320, and 1280 for As, and 320, 640, and 320 for AsAm.

Table 22. CHIK001 $PRNT_{50}$ against CHIKV lineages (all groups combined).

	Seroconversion and $PRNT_{50}$							
	Indian Ocean (La Reunion)		West African (37997)		Asian (SV044-95)		Asian/American (YO-111213)	
	Seroconversion	GMT (95% CI)	Seroconversion	GMT (95% CI)	Seroconversion	GMT (95% CI)	Seroconversion	GMT (95% CI)
Day 0	0%	5 (5.0–5.0)	0%	5 (5.0–5.0)	0%	5 (5.0–5.0)	0%	5 (5.0–5.0)
Day 14	100%	164.7 (102.3–265.2)	100%	226.3 (139.9–365.9)	100%	47.6 (29.4–77.1)	91.6%	40 (25.5–62.6)
Day 28	100%	285.1 (161.2–504.3)	100%	369.7 (217.2–629.3)	100%	67.3 (46.1–98.2)	100%	71.3 (49.6–102.4)
Day 182	100%	213.6 (126.4–360.8)	100%	229.7 (125.4–420.8)	100%	95.1 (56.2–161.1)	83.3%	42.4 (21.9–82.2)

Table 23. CHIK001 $PRNT_{50}$ against CHIKV Indian Ocean lineage per dose

	GMT $PRNT_{50}$			*p* value*
	Low dose	Medium dose	High dose	
Day 0	5 (5-5)	5 (5-5)	5 (5-5)	NA
Day 14	113.1 (37.54-341)	160 (66.13-387.1)	217.7 (86.15-550.2)	0.5516
Day 28	226.3 (62.87-814.4)	274.3 (105.8-710.9)	345.6 (97.83-1221)	0.8225
Day 182	127 (42.48-379.7)	296.3 (111.6-786.9)	217.7 (77.3-613.3)	0.3323

*Kruskal-Wallis test

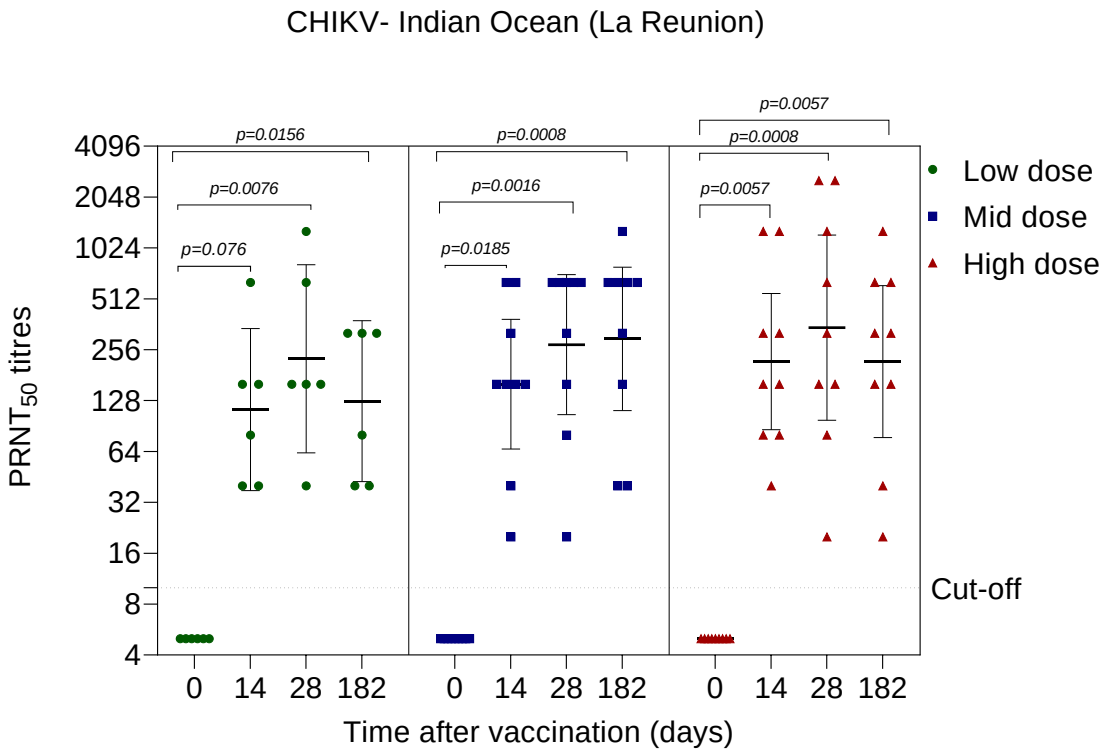


Figure 8. CHIK001 $PRNT_{50}$ values against Indian Ocean lineage per dose

Black lines represent geometric means with 95% confidence intervals (error bars). P values represent comparisons from baseline using Friedman test with Dunn's correction for multiple comparisons.

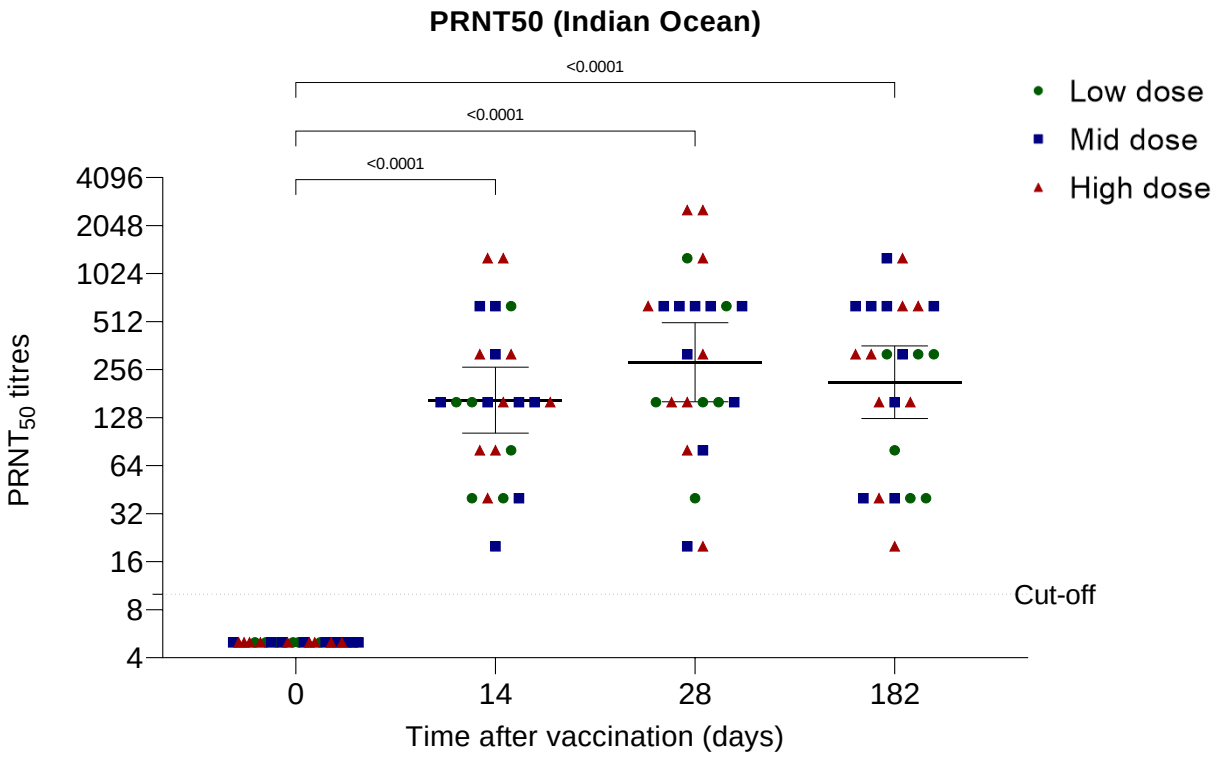


Figure 9. CHIK001 $PRNT_{50}$ values against Indian Ocean lineage (all doses combined)

Black lines represent geometric means with 95% confidence intervals (error bars). P values represent comparisons from baseline using Friedman test with Dunn's correction for multiple comparisons.

A single ChAdOx1 Chik dose induced high-antibody titres against CHIKV E2 protein. Geometric mean ELISA units at the peak response were 80.99 (95% CI 38.65–169.7); 205.90 (95% CI 92.66–457.6); 169.70 (95% CI 71.94–400.3) for the low, intermediate, and high dose, respectively (Table 24). The levels of anti-E2 antibodies showed a steady increase over time, reaching maximum seroconversion on day 182: 66.66% (4/6) for the low vaccine dose, 100% (9/9) for the intermediate dose and 77.77% (7/9) for the high dose (Table 24). Antibodies reached significantly higher levels than baseline as early as day 14 for the high-dose group, day 28 for the intermediate-dose group, and day 56 for the low-dose group . It was observed that the calculated cut-off threshold (mean

on day 0 + 3 SDEV), was influenced by 4 participants with a relatively high ELISA background at baseline. Therefore, to validate these 4 samples as negative, two commercially available ELISA kits were used. Both anti-CHIKV IgG ELISA kits from Abcam and Euroimmun confirmed that none of the participants that had a high background in the in-house ELISA were seropositive for CHIKV at baseline (data not shown).

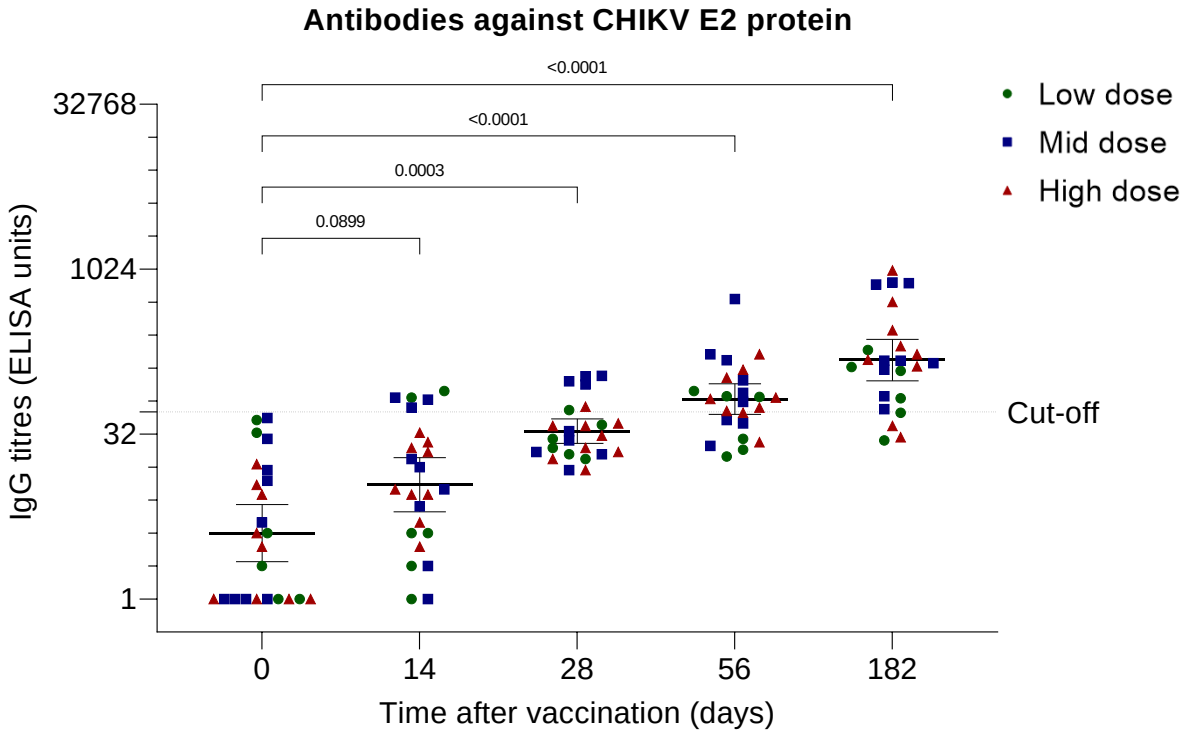


Figure 10. CHIK001 CHIKV IgG responses by standardised ELISA to E2 protein (all doses combined).

Black lines represent geometric means and 95% CI (error bars). p values for Friedman test with Dunn's correction

Table 24. CHIK001 CHIKV IgG response by standardized ELISA to E2 protein.

	ELISA							
Day	**Low dose (*n* = 6)**		**Intermediate dose (*n* = 9)**		**High dose (*n* = 9)**		**Overall (n=24)**	
	Seropositive *n* (%)	**GMT (95% CI)**	**Seropositive *n* (%)**	**GMT (95% CI)**	**Seropositive *n* (%)**	**GMT (95% CI)**	**Seropositive n (%)**	**GMT (95% CI)**
0	0 (0%)	4.741 (0.806–27.87)	0 (0%)	4.725 (1.392–16.04)	0 (0%)	3.009 (1.231–7.354)	0 (0%)	3.993 (2.191-7.275)
14	2 (33.33%)	7.475 (1.075–51.95)	3 (33.33%)	13.03 (4.078–41.66)	0 (0%)	12.14 (6.449–22.84)	5 (20.83%)	11.04 (6.241-19.54)
28	1 (16.66%)	28.83 (19.10–43.52)	4 (44.44%)	44.51 (23.90–82.90)	1 (11.11%)	29.18 (21.07–40.42)	6 (25%)	34.08 (26.38-44.04)
56	3 (50%)	41.67 (21.51–80.74)	6 (66.66%)	87.10 (42.56–178.2)	7 (77.77%)	70.02 (45.76–107.1)	16 (66.67)	66.75 (48.37-92.09)
182	4 (66.66%)	80.99 (38.65–169.7)	9 (100%)	205.9 (92.66–457.6)	7 (77.77%)	169.7 (71.94–400.3)	20 (83.33)	151.7 (98.17-234.3)

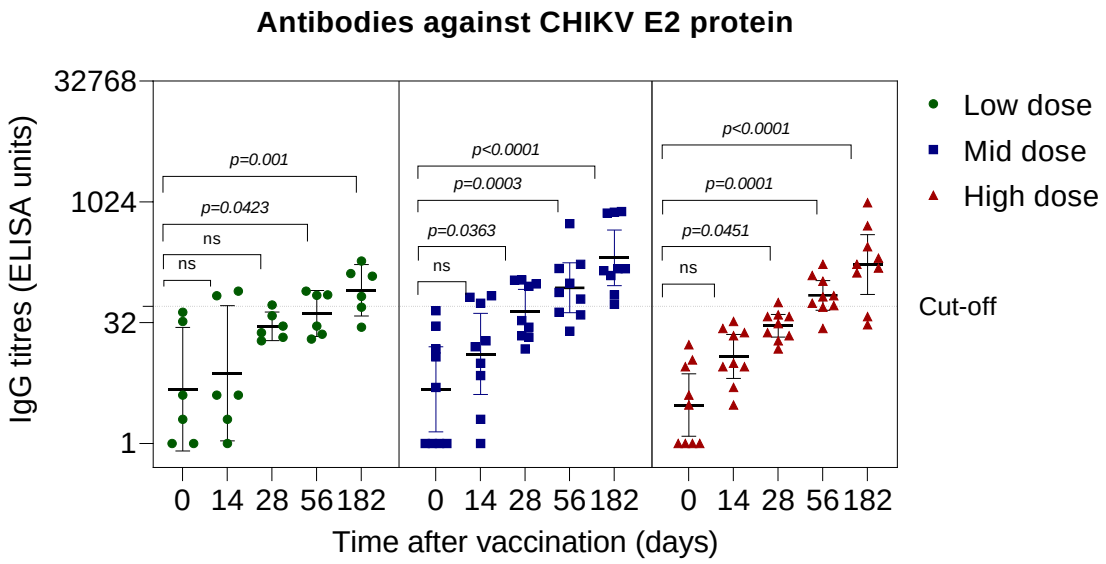


Figure 11. CHIK001 CHIKV IgG responses by standardised ELISA to E2 protein.

Black lines represent geometric means and 95% CI (error bars). *p* values for Friedman test with Dunn's correction

3.6.3.2 Cellular immunogenicity

Total ELISpot responses were quantified at days 0 (baseline), 14, 28, 56, and 182 after vaccination. At baseline, the geometric mean of IFN-γ SFC per million PBMC was 180.1 (95% CI 149.9–216.4), across all dosage groups. IFN-γ SFC, peaked at day 14 post-vaccination (1031, 95% CI 748.9–1420) and overall remained significantly higher than baseline throughout the follow-up period (*p* = <0.0001 Friedman test). However, no statistical significance was achieved when differences from baseline at day 182 were assessed at each dose group separately. Overall geometric means during follow-up were as 541.1(95%CI 411.6–711.13) on day 28; 398.2 (95%CI 298.6–530.9) on day 56; and 352.8 (95%CI 270.1–460.8) on day 182.

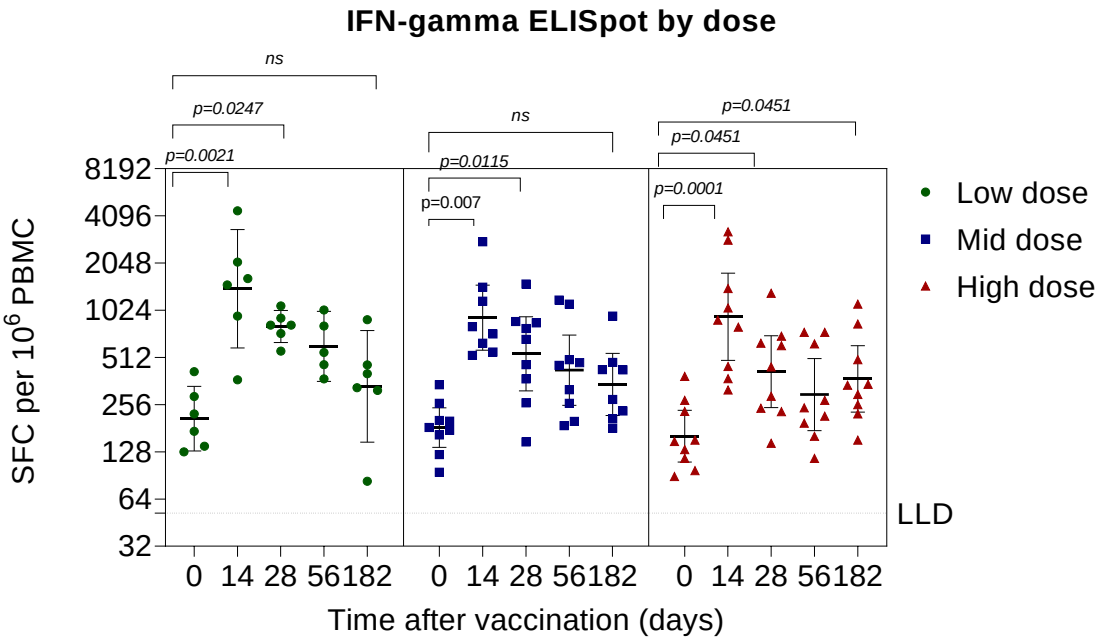


Figure 12. CHIK001 T cell responses over time per dose.

Black lines represent geometric means and 95% CI (error bars). p values for Friedman test with Dunn's correction.

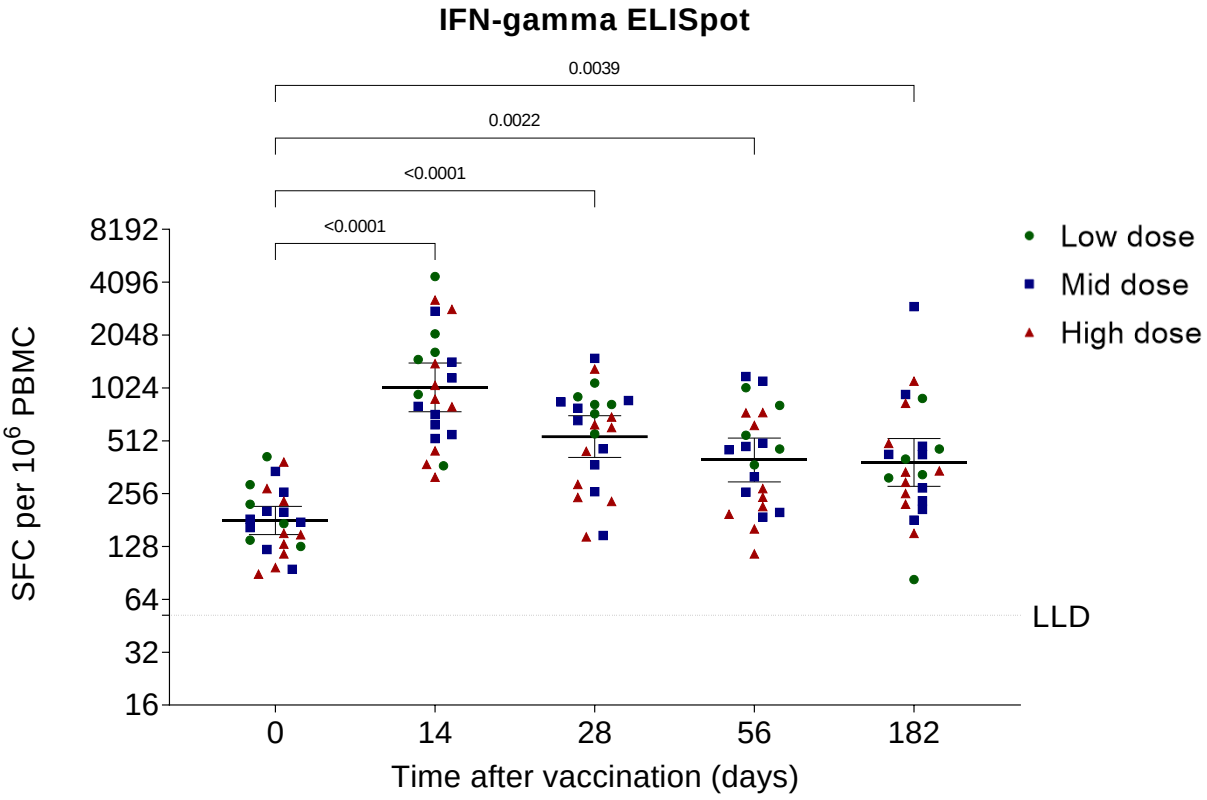


Figure 13. CHIK001 T cell responses over time all doses combined.

Black lines represent geometric means and 95% CI (error bars). p values for Kruskal Wallis test with Dunn's correction.

Table 25. CHIK001 Fold change in geometric mean T cell responses

	Geometric mean T cell responses (95%CI)			Fold change	
	D0	**D14**	**D182**	**D0 vs D14**	**D0 vs D182**
Low dose	208.5 (129.8-334.9)	1405 (588.6-3354)	335.1 (147.8-759.8)	6.74	1.60
Mid dose	182.6 (136.6-244.1)	918.6 (570.3-1480)	488.6 (235.6-1014)	5.03	2.67
High dose	161.2 (110.3-235.5)	930.1 (490.2-1765)	373.1 (229.1-607.6)	5.77	2.31
Overall	180.1 (149.9-216.4)	1031 (748.9-1420)	427.9 (317.2-577.3)	5.72	2.37

3.6.3.3 Correlations between ELISA, neutralising antibodies, and T cell responses

There was a trend towards a correlation between IgG titres measured by E2 ELISA and neutralising antibody titres at day 28 (Figure 14). No significant

correlations were found between binding or neutralising antibody titres at day 28 and T cell responses at day 14 (Figure 15 and Figure 16).

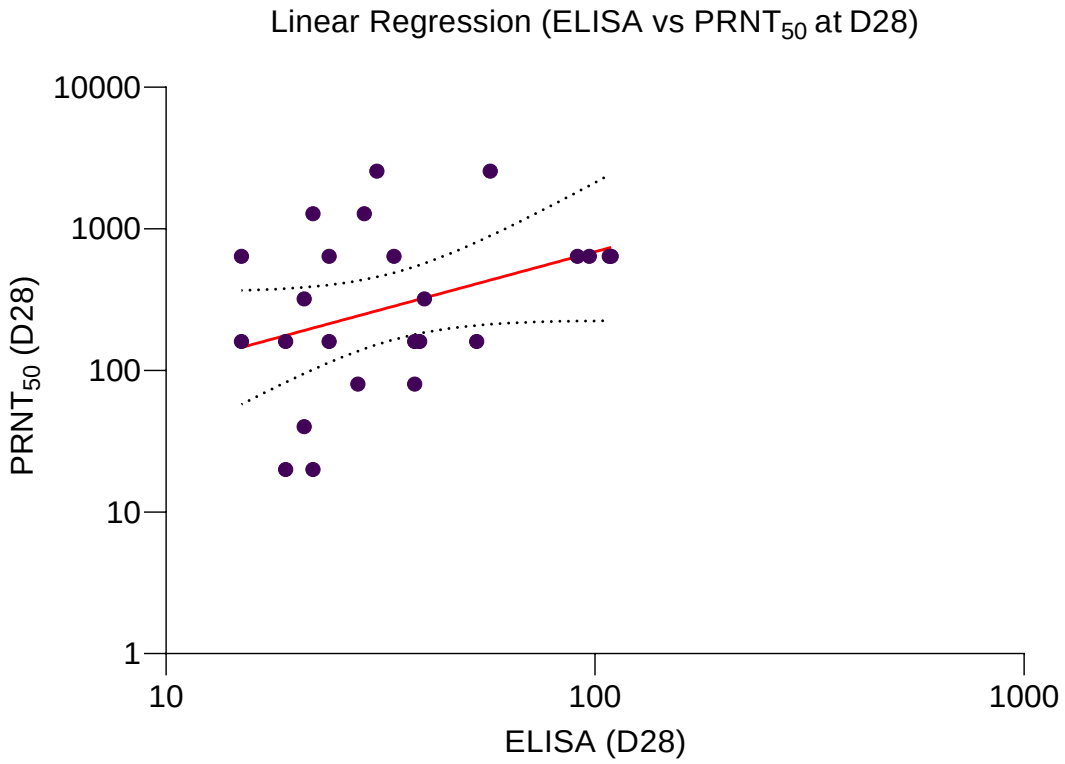


Figure 14. CHIK001 Linear regression (ELISA vs $PRNT_{50}$)

Linear regression of logged values for IgG titres (E2 ELISA) and $PRNT_{50}$ (La Reunion) at day 28 (r^2=0.1357, *p*=0.0766).

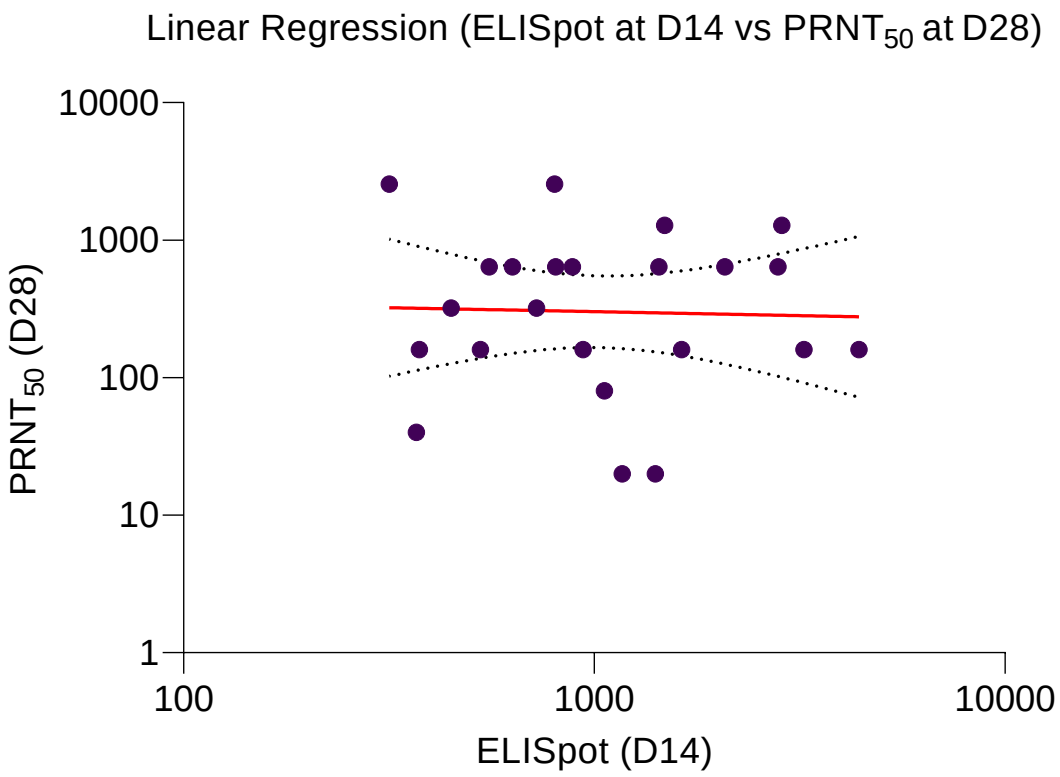


Figure 15. CHIK001 Linear regression (ELISPOT vs $PRNT_{50}$)

Linear regression of logged values for ELISpots at day 14 and $PRNT_{50}$ (La Reunion) at day 28 (r2=0.001004, p=0.8859).

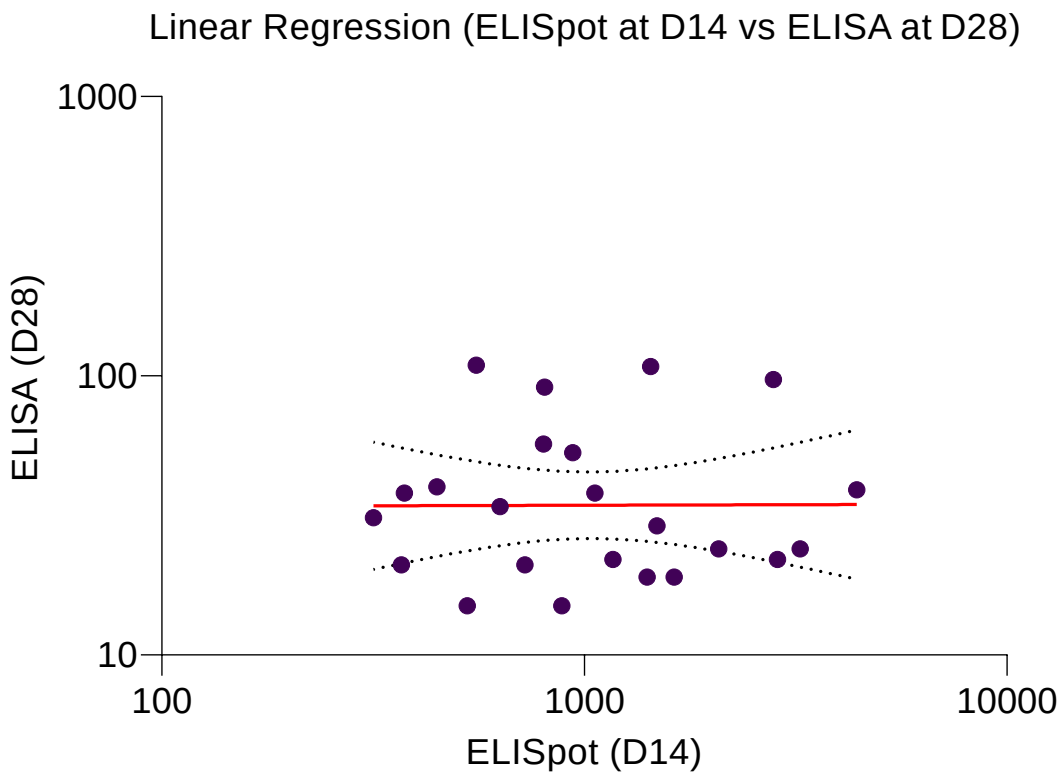


Figure 16. CHIK001 Linear regression (ELISpots vs ELISA)

Linear regression of logged values for ELISpots at day 14 and IgG titres (E2 ELISA units) at day 28 (r2=0.00002204, p=0.9830).

3.7 Discussion

These findings demonstrate that the candidate ChAdOx1 Chik vaccine given as a single dose was safe and well-tolerated across all doses tested. Greater reactogenicity was observed at the highest dose of 5×10^{10} vp. No SAR to ChAdOx1 Chik occurred. The majority of AEs reported were mild or moderate in severity, and all were self-limiting. Transient cases of leukopenia (n=5), neutropenia (n=5), and lymphopenia (n=1) were observed. Most of these were mild and resolved by day 7, one was moderate and resolved by day 28. None of

them were severe. The profile of AEs reported here is similar to that reported for other ChAdOx1 vectored vaccines expressing different antigens [71, 75].

CHIKV is responsible for swift outbreaks which affect large populations and rapidly spread to neighbouring regions. This highlights the requirement for vaccines with the capacity to promptly induce immune responses within days of immunisation. Nevertheless, most vaccines tested in clinical trials use prime-boost regimens, requiring several weeks to induce immune responses.

Animal and epidemiological studies have shown that protection from CHIKV disease is associated with the induction of neutralising antibodies [124, 125], primarily directed against structural proteins [124, 126, 127]. VLP and measles-vectored candidate vaccine developers have reported their vaccines is shown to induce neutralising antibodies 4 weeks following second vaccinations [12, 120]. Recently, a single dose live-attenuated CHIKV vaccine based on the ECSA lineage have reported 100% homologous seroconversion at day 14, but no data on heterologous lineages were shown [128]. The data shown here demonstrate that ChAdOx1 Chik achieves similar seroconversion rates by $PRNT_{50}$ at 14 days after a single administration, with evidence of cross-protective functional antibodies against 4 distinctive CHIKV lineages. Only one other chikungunya vaccine candidate has reported broad cross-neutralizing responses against isolates from the 3 CHIKV genotypes (WA, ECSA, & Asian), and the Indian Ocean sub-lineage [129].

The role of T cell immunity in clearance of CHIKV is controversial, and not well understood. Although activation of CHIKV-specific CD4+ and/or CD8+ T cells has been observed upon natural infection [130-132], some authors have rejected the

hypothesis that they might have a protective role and suggested they could exacerbate disease instead [133-135]. ChAdOx1 Chik induced a CD4+ IFN-γ+ biased cellular response towards CHIKV E1 and E2 proteins but had no significant effect on CD8+ T cells (data shown in Folegatti et al. Nature Communications 2021).

Limitations of this study include the relatively short follow-up period of six months, small sample size and open-labelled, nonrandomized, uncontrolled study design. Generalization of the study findings is limited, as this is a first-in-human trial of healthy volunteers. Further studies should be conducted in older and younger age groups, adults with comorbidities, and in populations considered to be at risk of developing chronic arthritis following CHIKV infection.

In conclusion, ChAdOx1 Chik was shown to be safe and well-tolerated at all tested doses. A single dose administration provided evidence of rapid stimulation of T cell responses and induction of functional neutralising antibodies at high titres across multiple CHIKV lineages, even at the lowest dose. ChAdOx1 Chik constitutes an attractive and affordable option for chikungunya prevention in low-income countries. The ability to induce robust cellular and humoral immunity upon a single administration portraits ChAdOx1 Chik as a suitable candidate to curb outbreaks around the world. The results of this first-in-human clinical trial support clinical development progression into phase 1b and 2 trials in CHIKV-endemic regions, such as those in Latin America, India, and Africa.

Chapter 4: Safety and immunogenicity of ChAdOx1 Zika in UK healthy adult volunteers

4.1 Authorship statement

Immunogenicity data presented in this chapter has been generated by Prof Arturo Reyes-Sandoval's group at the Jenner Institute (cellular immunogenicity data).

4.2 Background

The Zika virus (ZIKV) caught the world's attention as it spread through the Americas in 2015-16 with links to serious neurological conditions such as Guillain-Barré syndrome (GBS) in adults, microcephaly and other congenital malformations known as congenital Zika syndrome (CZS) in new-borns [136, 137]. The virus belongs to the *Flaviviridae* family of arthropod-borne RNA viruses. It is named after the Zika Forest in Uganda, from which it was first identified in 1947 (ZIKV 776 strain) [138], with the first human infection described in Nigeria in 1954 [139]. Symptoms of ZIKV infection include self-limiting acute febrile illnesses with fever, headache, arthralgia, myalgia, fatigue, and rash [136, 140, 141], although 75-80% of cases are asymptomatic.

Transmission of ZIKV happens through the bite of an infected *Aedes* mosquito, sexually, maternofoetal/vertically [142], through lactation, and blood transfusion [143, 144]. ZIKV RNA has been reported to persist for up to 41 days in serum, 32 days in urine, and 120 days in semen [145].

There are two ZIKV lineages, named African and Asian. Neither lineage was thought to have caused major concerns prior to 2007 when limited clinical cases

had been reported in sub-Saharan Africa and, South and South-East Asia. Seroprevalence studies have since suggested that ZIKV was widely circulating in these areas and was likely under-reported partially due to misdiagnosis as dengue, chikungunya or other arboviral infections which are also spread by *Aedes* mosquitoes and have similar clinical presentations.

The first major ZIKV outbreak was reported on the island of Yap, Micronesia in 2007, also corresponding to the first cases outside of Africa or Asia and where 73% of inhabitants were estimated to have been infected, showing mild illness only [146]. This indicated the spread of ZIKV in immunologically naïve populations was increasingly likely through global travel and indeed, further spread of a related strain of ZIKV (Asian lineage, strain JN860885.1) to Cambodia in 2010 preceded an outbreak in French Polynesia in 2013 (Asian lineage, strain H/PF/2013) where 28,000 ZIKV infections were estimated – 11% of the total population [147]. These French Polynesian cases included the first identification of perinatal transmission of the virus [148] and a concomitant causal link between ZIKV infection and GBS [149]. A link to central nervous system malformations in foetuses and infants associated with the 2013 outbreak was announced in November 2015 [136, 150, 151].

ZIKV subsequently spread in small imported outbreaks to Cook Islands, New Caledonia and Easter Island in 2014 [149] and in 2015, Vanuatu, Solomon Islands, Samoa and Fiji [141], all linked to travel from French Polynesia. It is postulated that the virus arrived in Brazil in 2013 [143, 152], with autochthonous transmission of the French Polynesian-linked Asian ZIKV lineage (15095_BR_2015) first reported in the North-Eastern city of Natal in March 2015 [153], and in a further 18 states by December 2015 [141]. Colombia reported

initial ZIKV infections in October 2015 followed by Suriname, Venezuela, Guatemala, Honduras, Mexico, Paraguay, Panama, French Guiana, El Salvador, Haiti, Puerto Rico, and Martinique [141]. A ZIKV outbreak was declared by PAHO in December following the rapid spread of the disease and in Brazil, a spike of GBS cases and an increase in cases of new-born microcephaly to ~99.7 per 100,000 live births in 2015, from an average of 5.7 per 100,000 in 2014 [154]. Prior flavivirus infection, varying virulence, genetic and environmental factors [155], and micro-evolution of ZIKV [141] have been proposed as an explanation to the increased severity of symptoms from those seen in the Yap (2007) and Cambodia (2010) outbreaks, to those of French Polynesia (2013-14) and the America's (2015-current).

Although the status of public health emergency of international concern with respect to ZIKV in the Americas has been lifted by the WHO after a decline attributed to herd immunity [156], the spread of ZIKV is predicted to follow that of chikungunya and dengue viruses to regions where *Aedes* mosquitoes are prevalent [157]. This includes immunologically naïve populations with effects similar to those seen in the Pacific and Americas outbreaks. Eighty-six countries or territories have reported cases of ZIKV [144] and the WHO has confirmed the urgent unmet need for an efficacious ZIKV vaccine, primarily to be aimed at women of child-bearing potential [158].

In keeping with this stance, the WHO R&D Blueprint and the NIAID list ZIKV as a priority pathogen for accelerated research. At present, there are no licensed therapeutics or prophylactic vaccines.

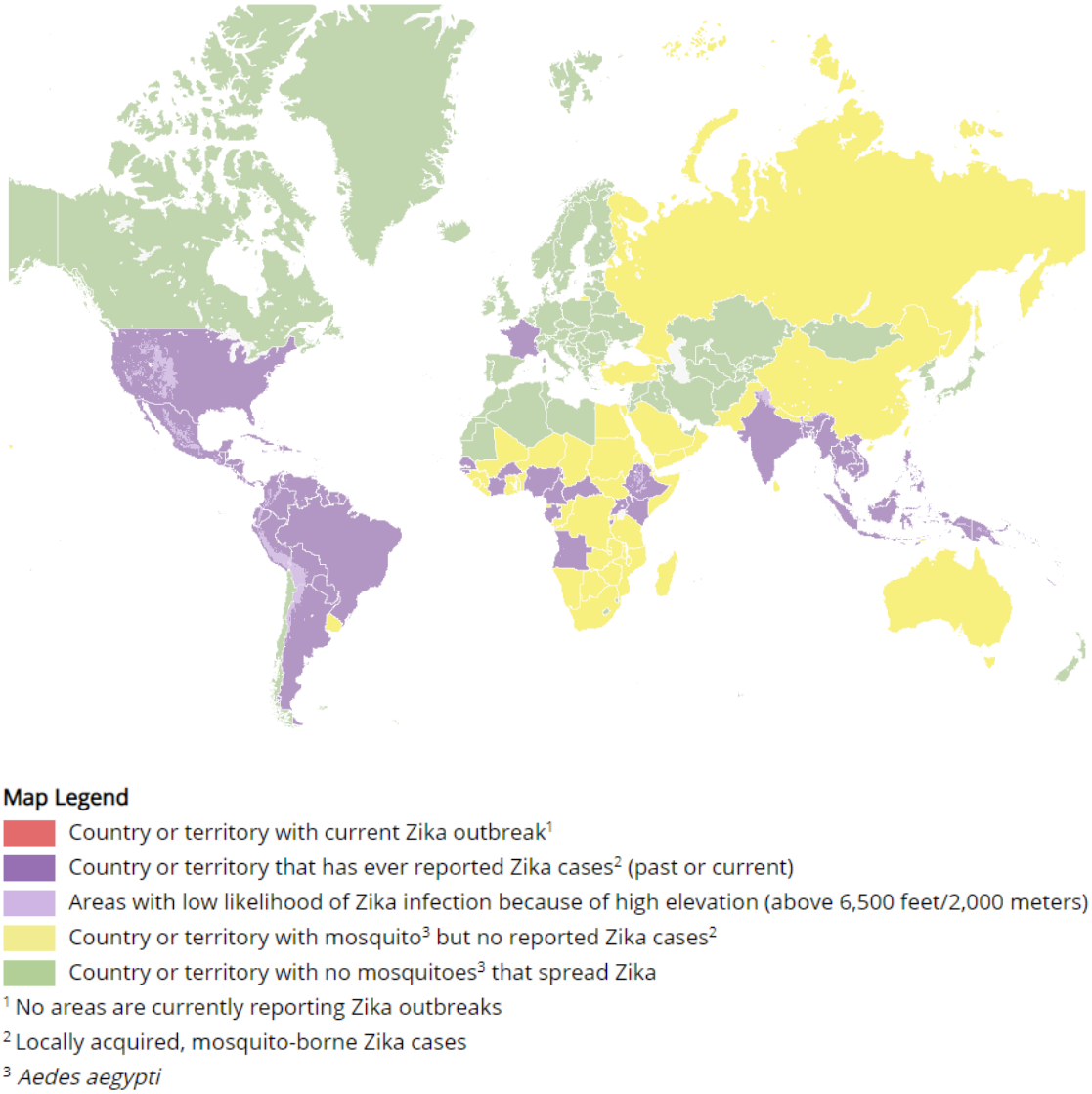


Figure 17. Countries reporting Zika cases

Source: CDC, available at https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika

4.3 Progress towards a ZIKV vaccine

Exceptional progress has been made since the WHO declared ZIKV a public health emergency, including the description of the ZIKV structure [159, 160], the expansion of sequenced genomes of several ZIKV isolates and in particular the development of preclinical ZIKV challenge models in mice [161, 162] and non-human primates [163, 164]. These models have proven useful to explore the

efficacy of many ZIKV vaccine developments and many vaccine platforms such as subunit envelope protein, VLP, live attenuated virus, inactivated virus, naked DNA vaccines, liposome-encapsulated RNA vaccines and viral vectored-based vaccines [59, 165-169].

As with CHIKV, vector control measures for ZIKV are frequently complex and have proven to be insufficient in the containment of arbovirus infections outbreaks. Effective mosquito control strategies often rely on adequate staffing levels (entomologists, social scientists, operational vector-control staff), technical expertise at decentralised levels of services, funding availability, adequate geographical coverage, insecticide use (and emerging resistance patterns), community engagement, capacity building and monitoring & evaluation. These requirements are frequently unmet, even in developed countries [113].

There are currently no licensed vaccines against ZIKV, but several vaccine candidates are in pre-clinical and clinical development. Thirteen candidates have entered phase I clinical trials and only 1 of those entered phase II [170].

VLP vaccines usually require the support of adjuvants and multiple injections to induce seroconversion and reach high antibody titres, increasing the costs and limiting their use in low-income countries. DNA vaccines also require multiple doses. The use of replication-competent recombinant live attenuated virus carries the risks of inadequate attenuation causing disseminated disease, particularly in immunocompromised hosts. Replication deficient vectors, however, avoid that risk while maintaining the advantages of native antigen presentation, the ability to express multiple antigens and elicitation of T cell immunity.

An increased incidence of GBS is associated with ZIKV infection but it is currently unknown if vaccines based on structural pre-membrane and envelope proteins would lead to a greater risk of GBS than that expected for any given vaccine (1 in 10^5-10^6) [171]. A hypothetical though yet unproven risk of antibody-dependent enhancement (ADE) exists in ZIKV vaccinees subsequently infected with DENV. ADE is not observed clinically with any flavivirus besides DENV. There have been no reports of GBS or ADE from the existing trials of candidate Zika vaccines.

4.4 ChAdOx1 Zika vaccine

The ZIKV Asian lineage (mostly French Polynesian-related strains) has been responsible for the explosive Zika outbreaks seen since 2013 in the Pacific Islands and Americas. The structural antigens of ChAdOx1 Zika consist of a consensus sequence derived from all Asian ZIKV genetic sequences reported in the literature and deposited in GenBank by November 2015. The immunogen shows 99% similarity to Asian lineage ZIKV, responsible for the America's epidemic and 97% similarity to African lineage ZIKV. The ChAdOx1 Zika gene cassette contains the ZIKV precursor of membrane protein (prM) and envelope protein with a deletion of the 3' transmembrane domain, which encodes amino acids 729–794 of ZIKV (EΔTM), cloned into the ChAdOx1 vaccine platform, and expressed under the control of the CMV promoter [59] (Figure 18). Pre-clinical testing has shown the vaccine induces strong immune responses and was fully protective in mice [59].

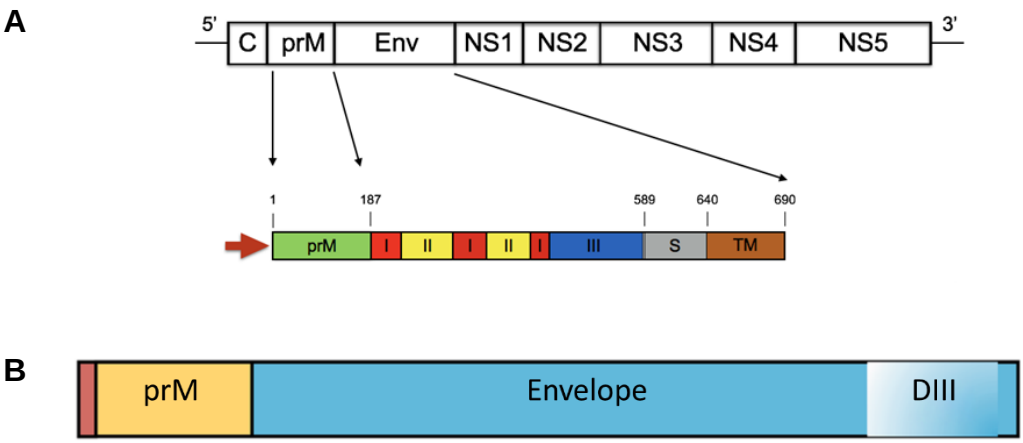


Figure 18. A) ZIKV genomic structure. B) ChAdOx1 Zika insert

The prM-EΔTM insert of ChAdOx1 Zika contains no C-terminal transmembrane domain, but does include the DIII domain associated with cellular receptor binding and contains epitopes for neutralising antibodies [172].

4.5 Methods

4.5.1 Trial design, objectives, participants, and oversight

This was a first-in-human, dose-escalation, open-label, non-randomized and uncontrolled clinical study of 24 healthy male and female subjects aged 18–50 years old.

The primary objective was to assess the safety and tolerability of ChAdOx1 Zika in healthy volunteers, measured as described in the general methods chapter. The secondary objective was to assess ZIKV antigen-specific humoral and cellular immune responses induced by ChAdOx1 Zika as measured by ELISA, a neutralisation assay and *ex vivo* IFN-γ ELISpot.

Eligible volunteers were recruited at CCVTM, Oxford, UK (Figure 19). All participants were healthy adults with negative pre-vaccination tests for HIV antibodies, hepatitis B surface antigen, and hepatitis C antibodies. A negative

urinary pregnancy test was required at screening and immediately before enrolment for all female subjects. Screening for previous ZIKV and DENV exposure was conducted on all participants, using commercial ELISA kits (anti-Zika virus IgG ELISA [EI 2668-9601 G] and anti-dengue virus IgG ELISA [EI 266b-9601 G]) from Euroimmun and participants were excluded if positive. Full details of the eligibility criteria are described in the general methods chapter. A LSM provided independent safety oversight.

4.5.2 Study approvals

Written informed consent was obtained in all cases, and the trial was conducted in accordance with the principles of the Declaration of Helsinki and GCP. This study was approved within the UK by the MHRA (reference 21584/0411/001-0001) and the South-Central Oxford A Research Ethics Committee (REC reference 19/SC/0074). Vaccine use was authorized by the GMSC of OUH-NHS Trust (GMSC reference number GM462.19.118). The trial was registered at www.clinicaltrials.gov (identifier: NCT04015648). The first participant was enrolled on 21 October 2019 and the last participant was enrolled on 28 September 2021.

4.5.3 Trial procedures

ChAdOx1 Zika was administered as a single intramuscular injection into the deltoid at a low dose of 5×10^9 vp (group 1), intermediate dose of 2.5×10^{10} vp (group 2), and high dose of 5×10^{10} vp (group 3). A staggered-enrolment approach was used for the first 3 participants in each group and interim-safety reviews were conducted prior to dose escalation (details provided in general methods chapter).

Blood samples were drawn, and clinical assessments conducted for safety as well as immunology endpoints prior to vaccination at day 0 and subsequently at 2, 7, 14, 28, 56-, 90-, 182- and 364-days following enrolment. AE data collection procedures were conducted as described in the general methods chapter.

4.5.4. Statistics

This study was not designed to test a specific hypothesis and was intended to obtain preliminary descriptive estimates of safety, reactogenicity and immunogenicity of ChAdOx1 Zika in healthy adults. The sample size allows an estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups.

Safety and immunogenicity outcomes were analysed as described in the general methods chapter.

4.5.5 Immunogenicity assessments

4.5.5.1 Humoral immunogenicity

Due to significant delays imposed by the COVID-19 pandemic on recruitment timelines, binding (ELISA) and neutralising antibody data have not yet been generated. Assays will be run once samples from all participants in the study have been drawn.

Two commercially available ELISA kits were used to validate the seronegativity of serum samples at baseline. The anti-Zika Virus IgG ELISA (EI 2668-9601 G) and anti-dengue virus IgG ELISA (EI 266b-9601 G) from Euroimmun were both performed according to the manufacturer's instructions.

4.5.5.2 ELISpot

Cellular immune responses were quantified at selected timepoints using an *ex vivo* ELISpot for IFN-γ, as described in the general methods chapter.

4.6 Results

4.6.1 Study population

Between 21 October 2019 and 28 September 2021, 24 healthy adult subjects received a single dose of ChAdOx1 Zika at 5×10^9, 2.5×10^{10} or 5×10^{10} vp (Figure 19). Baseline characteristics are summarized in Table 26.

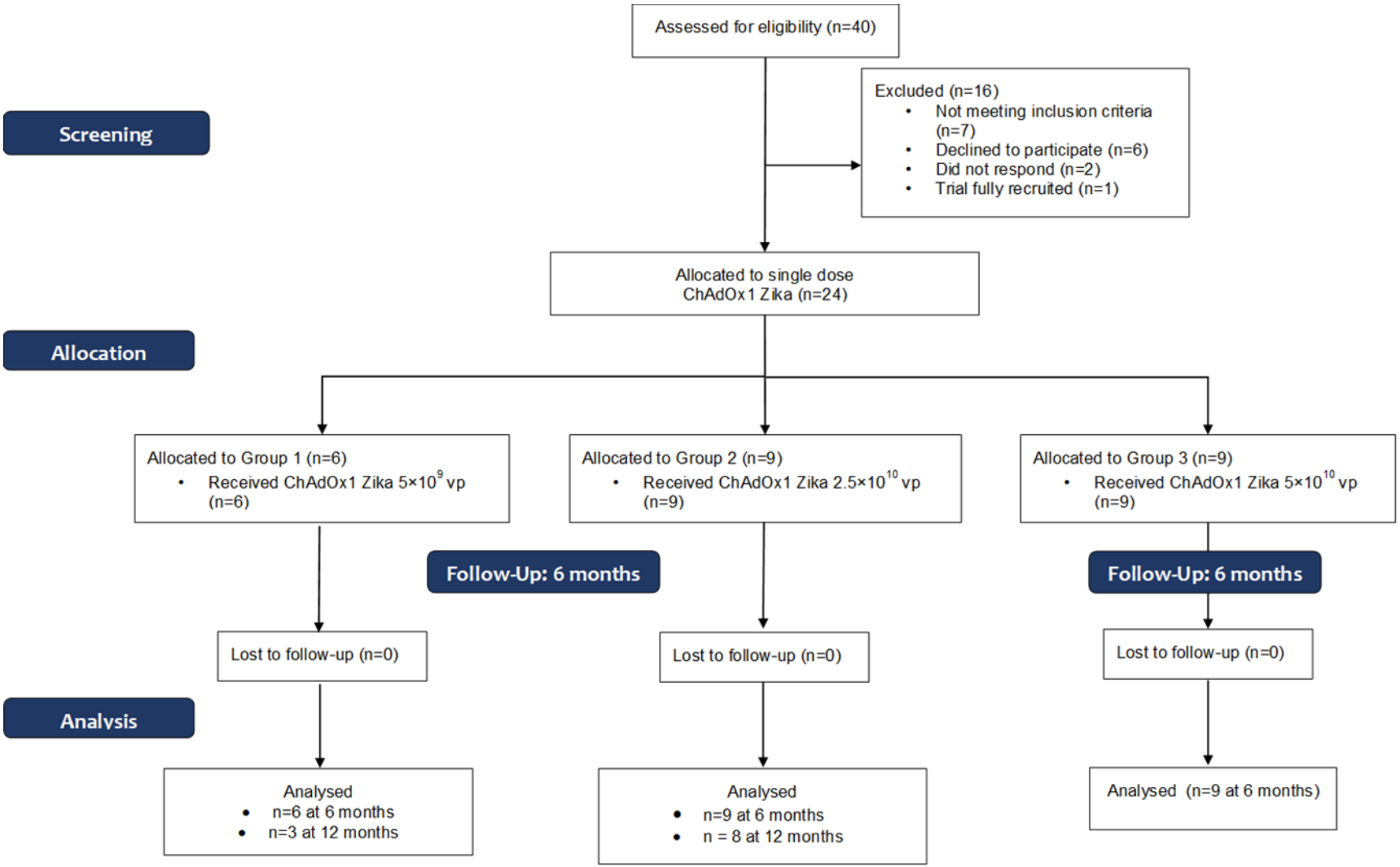


Figure 19: ZIKA001 CONSORT diagram

Table 26. ZIKA001 Summary of baseline characteristics

Variable	Group 1 (n=6)	Group2 (n=9)	Group 3 (n=9)	All Groups (n=24)
Age				
Median	20.5	24	29	25
Range	18-35	19-43	24-38	18-43
Sex				
Male – n (%)	3 (50%)	3 (33.33%)	2 (22.22)	8 (33.33%)
Female – n (%)	3 (50%)	6 (66.67%)	7 (77.78)	16 (66.67%)
Ethnicity				
White – n (%)	6 (100)	7 (77.78%)	8 (88.89%)	21 (87.5)
Asian n (%)	-	2 (22.22%)	1 (11.11%)	3 (12.5)

4.6.2 Vaccine safety

ChAdOx1 Zika was safe at doses up to 5×10^{10} vp with no SAR reported. A total of 118 local and systemic solicited AEs were reported. The vast majority of solicited AEs were mild (85/118, 72.03% 95%CI 63.34-79.34%) or moderate (28/118; 23.73%, 95% CI 16.96–32.16) and self-limiting in nature. All solicited AEs were completely resolved within 7 days and 89.83% of them had their onset within the first 72 h post-vaccination (31% at D0, 47% at D1, and 11% at D2). Injection site pain was the most common local AE, reported by 87.5% of participants and was predominantly mild in severity. Headache was the most common systemic AE followed by fatigue, myalgia, malaise, and feverishness. The frequencies of local and systemic solicited AEs reported during the first 7 days are summarized in Table 27. Median duration of solicited AEs is summarized in Table 28. There were 2 SAEs reported, but both events were deemed not related to ChAdOx1 Zika.

Table 27. ZIKA001 Number of participants reporting local and systemic solicited AEs.

	ChAdOx1 Zika $5x10^9$ vp (*n* = 6)				**ChAdOx1 Zika $2.5x10^{10}$ vp (*n* = 9)**				**ChAdOx1 Zika $5x10^{10}$ vp (*n* = 9)**			
	Any	Mild	Moderate	Severe	Any	Mild	Moderate	Severe	Any	Mild	Moderate	Severe
Any symptom	6 (100%)	4 (67%)	2 (33%)	0	9 (100%)	4 (44%)	3 (33%)	2 (22%)	9 (100%)	4 (44%)	3 (33%)	2 (22%)
Any local symptom	6 (100%)	5 (83%)	1 (17%)	0	7 (78%)	4 (44%)	3 (33%)	0	9 (100%)	6 (67%)	3 (33%)	0
Pain	6 (100%)	5 (83%)	1 (17%)	0	7 (78%)	4 (44%)	3 (33%)	0	8 (89%)	5 (56%)	3 (33%)	0
Pruritus	1 (17%)	1 (17%)	0	0	1 (11%)	1 (11%)	0	0	1 (11%)	1 (11%)	0	0
Warmth	1 (17%)	1 (17%)	0	0	2 (22%)	2 (22%)	0	0	3 (33%)	3 (33%)	0	0
Swelling	0	0	0	0	1 (11%)	1 (11%)	0	0	0	0	0	0
Erythema	1 (17%)	1 (17%)	0	0	0	0	0	0	1 (11%)	1 (11%)	0	0
Any systemic symptom	6 (100%)	5 (83%)	1 (17%)	0	8 (89%)	4 (44%)	2 (22%)	2 (22%)	9 (100%)	5 (56%)	2 (22%)	2 (22%)
Fever	0	0	0	0	0	0	0	0	2 (22%)	0	2 (22%)	0
Feverishness	3 (50%)	3 (50%)	0	0	5 (56%)	3 (33%)	2 (22%)	0	4 (44%)	1 (11%)	2 (22%)	1 (11%)
Arthralgia	1 (17%)	1 (17%)	0	0	2 (22%)	2 (22%)	0	0	2 (22%)	1 (11%)	1 (11%)	0
Myalgia	1 (17%)	1 (17%)	0	0	6 (67%)	6 (67%)	0	0	5 (56%)	4 (44%)	0	1 (11%)
Headache	6 (100%)	5 (83%)	1 (17%)	0	5 (56%)	3 (33%)	1 (11%)	1 (11%)	7 (78%)	3 (33%)	4 (44%)	0
Fatigue	3 (50%)	3 (50%)	0	0	6 (67%)	4 (44%)	2 (22%)	0	6 (67%)	3 (33%)	2 (22%)	1 (11%)

	ChAdOx1 Zika $5x10^9$ vp (*n* = 6)				ChAdOx1 Zika $2.5x10^{10}$ vp (*n* = 9)				ChAdOx1 Zika $5x10^{10}$ vp (*n* = 9)			
Nausea	2 (33%)	2 (33%)	0	0	3 (33%)	3 (33%)	0	0	4 (44%)	3 (33%)	1 (11%)	0
Malaise	2 (33%)	2 (33%)	0	0	6 (67%)	2 (22%)	1 (11%)	1 (11%)	6 (67%)	4 (44%)	2 (22%)	0

Table 28. ZIKA001 Duration of AEs

AE	Median	IQR	Maximum duration
Pain at injection site	2	1-4	7
Pruritus	1	1-1	1
Warmth	1.5	1-3.5	5
Swelling	1	1-1	1
Erythema	4	1-7	7
Fever	1.5	1-2	2
Feverishness	1	1-2.75	5
Arthralgia	1	1-1	1
Myalgia	1	1-1.75	4
Headache	1.5	1-2.25	5
Fatigue	2	2-5	7
Nausea	1	1-1.5	6
Malaise	1	1-2	6
Overall	1	1-3	7

Two participants reported a short-lived temperature above 37.5 °C within the first 72 h post-vaccination (both in the high-dose group). The highest temperature recorded was 38.9 °C (classified as moderate). All febrile episodes resolved within 24 h.

The proportion of moderate and severe AEs was not significantly higher in group 3 compared with group 2 (RR 1.558, 95%CI 0.8669–2.896, p = 0.1422), but it was significantly higher in group 3 compared with group 1 (RR 5.510 95%CI 1.648 to 20.51, p = 0.0030)

Unsolicited AEs in the 28 days following vaccination considered possibly, probably, or definitively related with ChAdOx1 Zika were predominantly mild in

nature and resolved within the follow-up period (Table 29). Unsolicited AEs of note include chills (one severe and one moderate at D1, resolved by D2; all in Group 3); dizziness (one severe at D2, resolved by D5; one moderate at D2, resolved by D3; and one mild at D1, resolved by D2) and one severe (self-limiting) episode of paraesthesia. Laboratory AEs considered at least possibly related to the study intervention were self-limiting and predominantly mild in severity (Table 30). There were 2 SAEs reported on the same subject (suicide attempt and appendicitis), and both events were deemed unrelated with ChAdOx1 Zika.

Table 29. ZIKA001 Unsolicited AEs related with ChAdOx1 Chik

Subject ID	MEDDRA PT	MEDDRA PT CODE	Day of Onset	Duration (days)	Max. Severity
00101007	Oropharyngeal pain	10068319	4	4	1
00101013	Administration site bruise	10075094	1	7	1
00101013	Cough	10011224	2	1	1
00101015	Productive cough	10036790	3	12	1
00101015	Rhinorrhoea	10039101	3	7	1
00101018	Paraesthesia	10033775	2	1	3
00101018	Tachycardia	10043071	2	1	1
00101018	Dizziness	10013573	2	1	2
00101020	Dizziness	10013573	0	5	3
00101020	Nasal congestion	10028735	2	1	1
00101011	Diarrhoea	10012735	1	1	1
00101011	Epistaxis	10015090	1	1	1
00101022	Oropharyngeal pain	10068319	3	5	1
00101023	Back pain	10003988	2	1	1
00101030	Injection site muscle weakness	10084588	0	3	2
00101030	Pain in extremity	10033425	0	3	1
00101029	Musculoskeletal stiffness	10052904	0	1	1
00101032	Chills	10008531	1	1	3
00101037	Chills	10008531	1	1	2
00101037	Dizziness	10013573	1	1	1

Table 30. ZIKA001 Laboratory AEs.

	Id	Group	Event	Severity	Timepoint	Resolved by
Haematology	101001	1	Lymphopenia	Mild	D2	D7
	101020	2	Lymphopenia	Mild	D2	D7
	101021	2	Anaemia	Mild	D7	D28
	101023	2	Neutropenia	Mild	D2	D7
	101027	2	Neutropenia	Mild	D2	D7
	101038	3	Leucopenia	Mild	D2	D7
	101038	3	Lymphopenia	Moderate	D2	D7
	101036	3	Lymphopenia	Mild	D2	D7
	101029	3	Leucopenia	Mild	D2	D7
	101029	3	Neutropenia	Mild	D2	D7
	101029	3	Lymphopenia	Mild	D2	D7

4.6.3 Cellular immunogenicity

Cellular immunogenicity was measured from fresh PBMCs by an *ex vivo* IFN-γ ELISpot assay, using pools of peptides spanning the pre-membrane and envelope ZIKV proteins as stimuli. The total responses were quantified at days 0 (baseline), 7, 14, 28, 56, 90, 182, and 364 after vaccination. However, due to COVID-19 travel restrictions, half of the volunteers in group 1 were unable to attend their late timepoints at days 182 and 364, and the other half were unable to attend days 56 and 90. In group 2, only 1 volunteer attended their day 56 visit and none of them could make the day 90 timepoint. One volunteer in group 2 also did not attend their day 28 visit, and one volunteer in group 3 missed their day 14 due to COVID-19 self-isolation guidance at the time. Only data up to day 28 is presented for group 3, as follow-up is still ongoing.

At baseline, the geometric mean of IFN-γ SFC per million PBMC was 55.85 (95% CI 44.72 – 69.75), across all dosage groups. ChAdOx1 Zika significantly increased the number of IFN-γ SFC, peaking at day 14 post-vaccination (411.2,

95% CI 291.9 – 579.3) and remained significantly higher than baseline until day 28 (313.1, 95% CI 198.5 – 493.6) (Figure 21).

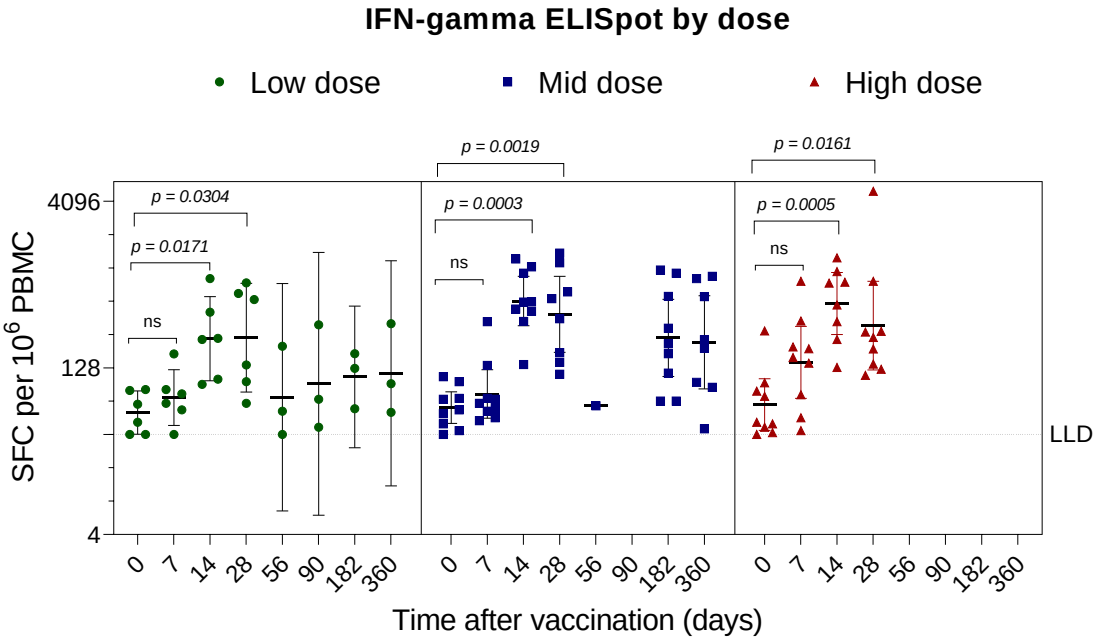


Figure 20: ZIKA001 T cell responses over time.

Black lines represent geometric means and 95% CI (error bars). p values for Kruskal-Wallis test with Dunn's correction.

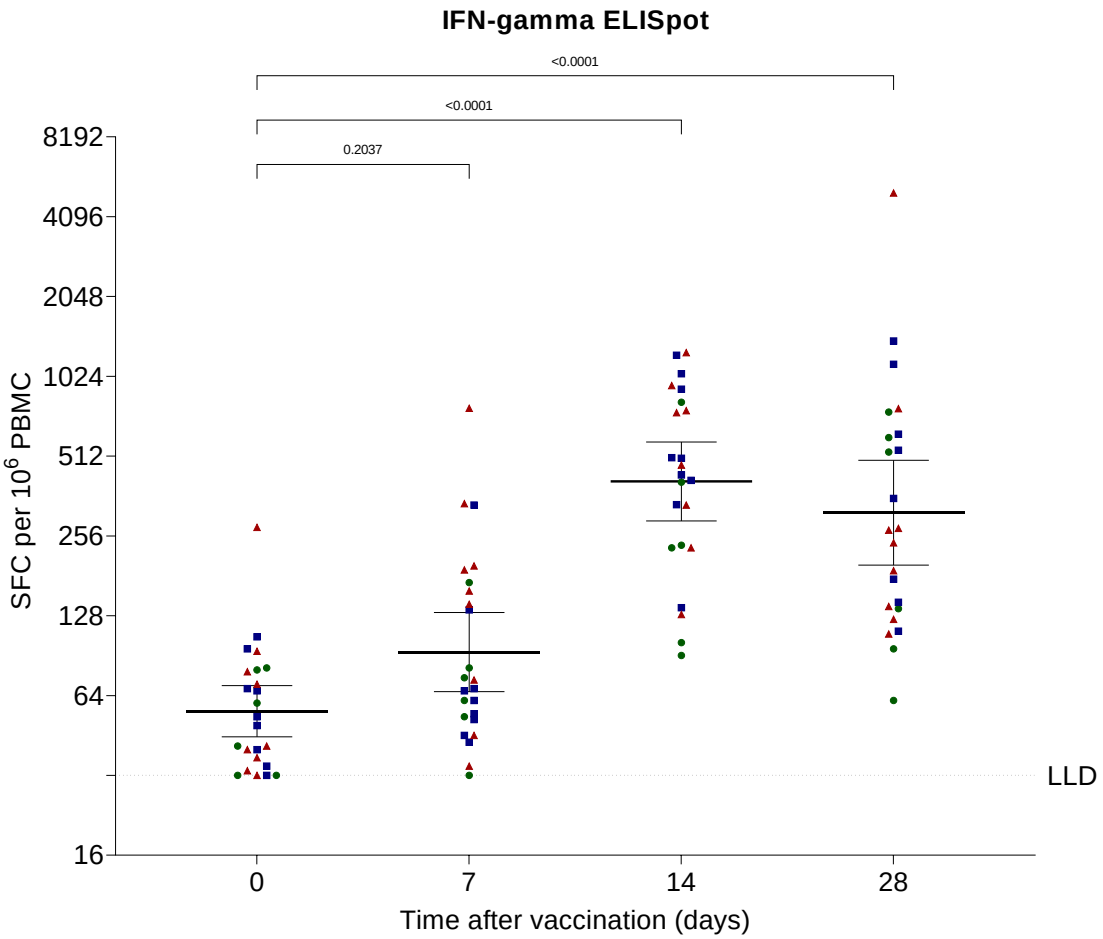


Figure 21. ZIKA001 T cell responses over time all doses combined.

Black lines represent geometric means and 95% CI (error bars). p values for Kruskal-Wallis test with Dunn's correction.

Table 31. ZIKA001 Fold change in geometric mean T cell responses

	Geometric mean T cell responses (95%CI)				Fold change		
	D0	**D7**	**D14**	**D28**	**D0 vs D7**	**D0 vs D14**	**D0 vs D28**
Low dose	50.46	69.05	234.7	240.1	1.37	4.65	4.76
	(32.13 – 79.14)	(38.68 – 123.3)	(97.83 – 562.8)	(77.56 – 743.5)			
Mid dose	56.01	74.29	513.7	389.7	1.33	9.17	6.96
	(40.38 – 77.68)	(44.85 – 123.1)	(306.8 – 860.1)	(176.6 – 859.8)			
High dose	59.59	143.3	487.5	307.5	2.4	8.18	5.16
	(34.84 -	(67.85 – 302.8)	(255.5 – 930.2)	(122.3 – 773.6)			
Overall	55.85	93.33	411.2	313.1	1.67	7.36	5.6
	(44.72 – 69.75)	(66.20 – 131.6)	(291.9 – 579.3)	(198.5 – 493.6)			

4.7 Discussion

These findings demonstrate that the candidate ChAdOx1 Zika vaccine given as a single dose was safe and well-tolerated across all doses tested. Greater reactogenicity was observed at the highest dose of 5×10^{10} vp. No SAR to ChAdOx1 Zika occurred. The majority of AEs reported were mild or moderate in severity, and all were self-limiting. Transient cases of leukopenia (n=2), neutropenia (n=3), and lymphopenia (n=6) were observed. Most of these were mild and resolved by day 7, one was moderate and resolved by day 7. None of them were severe. The profile of AEs reported here is similar to that reported for other ChAdOx1 vectored vaccines expressing different antigens [71, 75, 76].

There is an increasing body of (pre-clinical) evidence showing that both CD4 and CD8 T cell responses, rather than antibody driven immunity, can mediate protection against ZIKV infection [173-178]. A single dose of ChAdOx1 Zika was able to induce a robust IFN-γ ELISpot responses with an overall 7.36-fold increase from baseline at day 14, which persisted higher than baseline until day 28.

Limitations of this study include the relatively short follow-up period of six months, small sample size and an open-labelled, nonrandomized, uncontrolled study design. Generalisation of the study findings is limited, as this is a first-in-human study of healthy volunteers. Further studies should be conducted in older and younger age groups, adults with comorbidities, and in populations considered to be at risk of developing ZIKV infection in order to better assess the risks of disease enhancement, as previously observed with DENV vaccines [53].

The theoretical risks of ZIKV vaccine-induced immune-mediated neuroinflammatory events, such as GBS for example, remain unknown. Special consideration should be given to GBS in the context of this being identified as a safety signal following the widespread use of adenoviral vectored vaccines during the COVID-19 pandemic. Therefore, enhanced surveillance for GBS is recommended during further clinical development stages for ChAdOx1 Zika.

In conclusion, ChAdOx1 Zika was shown to be safe and well-tolerated at all tested doses. A single dose administration provided evidence of rapid stimulation of T cell responses, even at the lowest dose. Preliminary data generated from this study supported the initiation of an ongoing phase Ib clinical trial in Mexico, where both ChAdOx1 Chik and ChAdOx1 Zika are undergoing clinical assessment as standalone or co-administration strategies in endemic countries. Clinical humoral immunogenicity data is awaited, and further clinical development of ChAdOx1 Zika will depend on its ability to induce binding and neutralising antibodies against ZIKV, in addition to T cell responses shown here. Efficacy assessments for ZIKV vaccines are as challenging as for CHIKV. A phase IIb trial of a DNA vaccine candidate conducted by NIAID with over 2,300 volunteers across 17 sites in the Americas accrued only 3 cases during the 2-years follow-up period (NCT03110770), suggesting alternatives to licensure pathways may be required.

Chapter 5: Safety and immunogenicity of ChAdOx1 MERS in healthy adult UK volunteers

5.1 Authorship statement

Immunogenicity data presented in this chapter has been generated by Prof Sarah Gilbert's group at the Jenner Institute (binding antibody and cellular immunogenicity data), and research collaborators at Marburg (live virus neutralising antibodies data) and IVI (pseudo neutralising data). This work has been published in Folegatti, P.M. et al. The Lancet Infectious Diseases, 2020.

5.2 Background

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes an emerging zoonotic viral respiratory disease that was first described in 2012 [179] and is now endemic in Saudi Arabia. It has since spread to different countries in the Middle East and other regions, with 2574 laboratory-confirmed cases of MERS-CoV infection including 886 deaths in 27 countries reported, as of June 2021 [180], including the largest outbreak outside the Middle East in South Korea

Clinical presentation of MERS-CoV infections varies from asymptomatic to severe acute respiratory distress and death. Common clinical symptoms include fever, cough, and shortness of breath. Pneumonia is a common finding, but it might not always be present. Gastrointestinal symptoms, including diarrhoea, have also been reported. Severe illness can cause respiratory failure that requires mechanical ventilation and support in an intensive care unit. MERS-CoV has a reported case fatality rate of approximately 35%, although mild and

asymptomatic cases are highly likely to be under-reported, translating into probable overestimates on MERS-CoV mortality rates. The virus appears to cause more severe disease in the elderly, immunosuppressed and those with chronic diseases such as cardiovascular disease and diabetes [181-184].

MERS-CoV poses a significant threat to public health security based on its epidemic potential and lack of currently available effective countermeasures and has been listed as a priority pathogen for R&D by the WHO, NIAID (category C), the Center for Disease Control and Prevention (CDC), the UK Health Security Agency and many other global agencies and expert groups.

The disease is caused by a coronavirus and is spread by droplet infection of the airways. Dromedary camels are a recognised source of zoonotic infections and occupational exposure is associated with seroconversion [185]. However, only 40% of primary cases have been associated with a direct camel contact [186]. Human to human transmission, especially in hospital settings, has been responsible for the majority of cases seen in recent outbreaks [187], although, no sustained human to human transmission has been recorded to date (overall $R_0<1$ for MERS-CoV). The role of asymptomatic or mild cases in transmission patterns remains controversial and unclear. No specific treatment options or licensed vaccines are currently available.

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, whereas the S protein is involved in receptor binding, mediating virus

entry into host cells during CoVs infection via different receptors [188]. MERS-CoV belongs to the phylogenetic lineage C of the genus *Betacoronavirus* and it recognises the dipeptidyl peptidase 4 (DPP4) host receptor, which is well conserved between camels and humans [189]. It is the sixth CoV known to cause human infections and the first human virus within lineage C. The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), which was responsible for the 2002-2003 SARS global epidemic and the and SARS coronavirus 2 (SARS-CoV-2), which is responsible for the current outbreak, belong to lineage B (genus *Betacoronavirus*). HCoV-OC43 and HCoV-HKU1 are representatives of lineage A (genus *Betacoronavirus*) whilst HCoV-229E and HCoV-NL63 belong to the genus *Alphacoronavirus*. HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63 are globally distributed and generally associated with mild respiratory symptoms, accounting for up to a third of all common cold cases [190].

5.3 Progress towards a MERS-CoV vaccine

Global efforts to develop a coronavirus vaccine faded in the aftermath of the SARS-CoV global epidemic but has since gained renewed momentum in the face of the current MERS-CoV and SARS-CoV-2 outbreaks. Most of the developed vaccines were based on the S surface glycoprotein, the primary target for neutralizing antibodies during any natural coronavirus infection. Several preclinical and clinical studies showed that the SARS-CoV S1 protein subunit, and specifically the RBD at its core, could serve as a dominant target for neutralising antibodies in mice, non-human primates, and humans. S1, therefore, became the basis for a number of promising SARS-CoV vaccine candidates [191].

The S1 protein subunit and the Receptor Binding Domain (RBD) have also been the basis for several MERS-CoV vaccine candidates. Both constructs have elicited neutralising antibodies of high potency across multiple viral strains. Despite their demonstrated immunogenicity in animal models and anticipated safety in humans, RBD or S1-subunit based vaccine candidates are limited in their epitope breadth. Vaccine candidates that elicit a more diverse antibody repertoire, as well as a robust cellular immune response, may offer the advantage of broader and more durable protection [191].

Live attenuated viruses have historically been among the most immunogenic platforms available, as they can 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 moderately immuno-compromised adults with co-morbidities have suffered the most severe MERS-CoV disease, 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 immunosuppressed. 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 [191].

Several recombinant DNA, protein and viral vectored MERS-CoV candidate vaccines have been developed and tested in animal models (mice, non-human primates, and camels) with varied efficacy results. Recently, a recombinant MVA encoding the full-length Spike protein antigen (S) showed partial efficacy by

significantly reducing MERS-CoV viral shedding in a camel challenge study. [192, 193]

The first MERS-CoV vaccine to be used in humans entered a phase I dose-ranging safety study in January 2016. The GLS-5300, a DNA plasmid vaccine that expresses the MERS-CoV spike (S) glycoprotein, was administered to 75 healthy adult volunteers in the USA, by the Walter Reed Army Institute of Research. The vaccine was shown to be safe and immunogenic, and its development has now progressed into phase 2 trials [194].

Despite increasing efforts from affected countries with support from WHO to improve infection prevention and control (IPC) measures, new MERS-CoV cases continue to arise in the Arabian Peninsula several years after it was first described [180]. The nonspecific clinical features of MERS-CoV quite often lead to delayed diagnosis and increased exposure in healthcare facilities contributing to the persistence of nosocomial outbreaks, which is further complicated by the lack of effective treatment options available and sub-optimal adherence to IPC and isolation practices [195]. Considering the complexities around the implementation of overall control measures for MERS-CoV and the numerous challenges in filling knowledge gaps required to achieve it, vaccination remains the key cost-effective strategy to tackle the global MERS-CoV threat.

5.4 MERS-CoV spike protein as a vaccine antigen

The spike (S) protein is a type I, trimeric, transmembrane glycoprotein located at the surface of the viral envelope of CoVs, giving rise to spike-shaped protrusions from the virion. S is 1353 amino acids in length, heavily glycosylated (with 21 predicted N-linked glycosylation sites) and consists of a large ectodomain and a

short cytosolic tail. The S proteins of CoVs can be divided into two functional subunits: the N-terminal S1 and the C-terminal S2. S1 and S2 are responsible for cellular receptor DPP4 binding (MERS-CoV) via the RBD and fusion of virus and cell membranes respectively, thereby mediating the entry of MERS-CoV into the target cells [188, 196, 197]. The RBD of MERS-CoV has a core structure, which is homologous to that of the SARS-CoV, and a receptor-binding motif, which is specific to MERS-CoV, determining receptor recognition and viral pathogenesis [198]. The roles of S in receptor binding and membrane fusion make it an ideal target for vaccine and antiviral development, as it is the main target for neutralising antibodies [199].

5.5 ChAdOx1 MERS vaccine

The simian adenovirus vectored vaccine ChAdOx1 MERS expresses a codon-optimised coding sequence for the full-length Spike protein from MERS-CoV isolate Camel/Qatar_2_2014 (GenBank: KJ650098.1). Preclinical work has shown that the vaccine is highly immunogenic in animal models, being able to induce both cellular and humoral responses (polyfunctional $CD8^+$ T cells and neutralising antibodies) [66]. A single dose of ChAdOx1 MERS also conferred protective efficacy in transgenic human DPP4 mice against lethal MERS-CoV challenge [60], which supported progression into clinical development. Immunogenicity and partial protective efficacy in a natural transmission model in dromedary camels have also been reported [64].

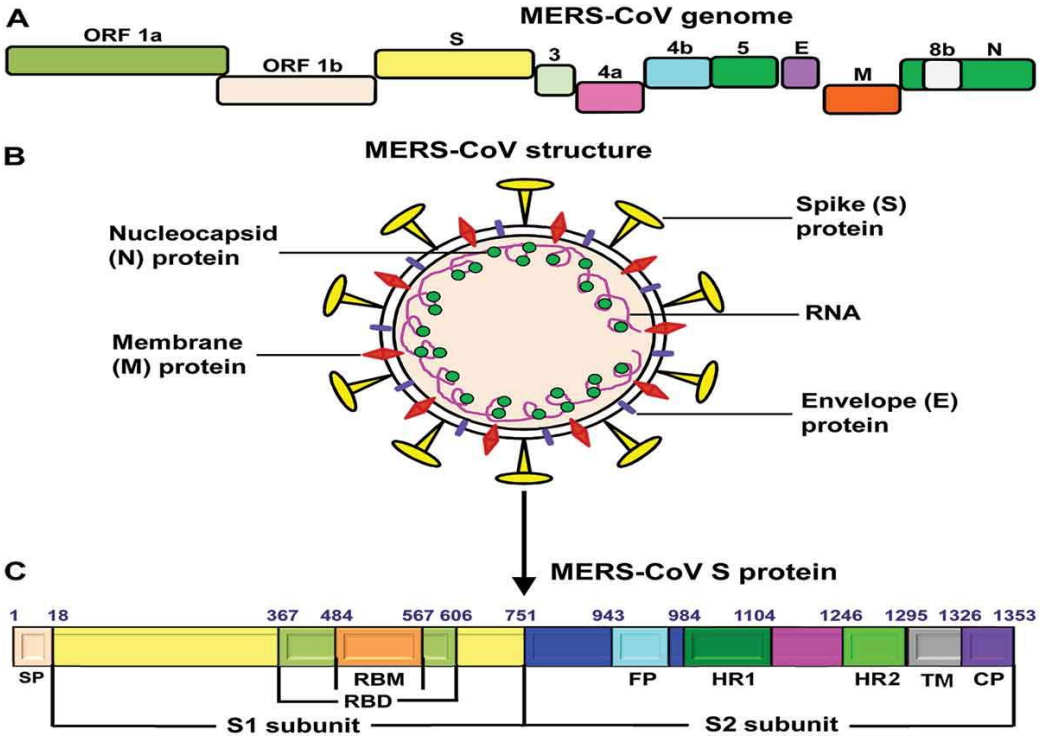


Figure 22: MERS-CoV structure.

Reproduced from Lanying Du; Wanbo Tai; Yusen Zhou; Shibo Jiang; Expert Review of Vaccines [200]

5.6 Methods

5.6.1 Trial design, objectives, participants, and oversight

This was a first-in-human, dose-escalation, open-label, non-randomised, uncontrolled clinical study of 24 healthy male and female subjects aged 18–50 years old.

The primary objective was to assess the safety and tolerability of ChAdOx1 MERS in healthy volunteers, measured as described in the general methods chapter. The secondary objective was to assess MERS-CoV antigen-specific humoral and cellular immune responses induced by ChAdOx1 MERS as

measured by ELISA, virus-neutralising antibody assays, and ELISpot from baseline to 12 months.

Eligible volunteers were recruited at CCVTM, Oxford, UK (Figure 23). All participants were healthy adults with negative pre-vaccination tests for HIV antibodies, hepatitis B surface antigen, and hepatitis C antibodies. A negative urinary pregnancy test was required at screening and immediately before enrolment for all female subjects. Participants were assessed for their likelihood of being previously exposed to MERS-CoV based on travel history and occupational exposure and were excluded if likely to have been previously exposed. Full details of the eligibility criteria are described in the general methods chapter. A LSM provided independent safety oversight.

5.6.2 Study approvals

Written informed consent was obtained from all participants, and the trial was done in accordance with the principles of the Declaration of Helsinki and GCP. This study was approved in the UK by the MHRA (reference 21584/0381/001-0001) and the South Central—Oxford A Research Ethics Committee (reference 17/SC/0552). Vaccine use was authorised by the GMSC of OUH-NHS Trust (reference number GM462.18.101). This study was registered with ClinicalTrials.gov, NCT03399578.

5.6.3 Trial procedures

ChAdOx1 MERS was administered as a single intramuscular injection into the deltoid at 5×10^9 vp (low-dose group), 2.5×10^{10} vp (intermediate-dose group), and 5×10^{10} vp (high-dose group). A staggered-enrolment approach was used

for the first three participants in each group, and interim safety reviews were done before dose escalation (details provided in the general methods chapter).

Blood samples were drawn, and clinical assessments were done for safety as well as immunology endpoints before vaccination at day 0 and subsequently at days 2, 7, 14, 28, 56, 182, and 364 after vaccination. AE data collection was as described in the general methods chapter.

5.6.4 Statistics

This study was not designed to test a specific hypothesis and was intended to obtain preliminary descriptive estimates of safety, reactogenicity and immunogenicity of ChAdOx1 MERS in healthy adults. The sample size allows an estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups.

Safety and immunogenicity endpoints are reported as described in the general methods chapter.

5.6.5 Immunogenicity assessments

5.6.5.1 ELISA

Binding antibody responses were measured using an in-house ELISA at days 0,14, 28, 56, 182 and 364, and as described in the general methods chapter.

5.6.5.2 NAbs (live and pseudovirus)

Live and pseudovirus Nabs against heterologous strains were measured at days 0 and 28, as described in the general methods chapter.

5.6.5.3 ELISpot

Cellular immune responses were quantified at selected timepoints using an *ex vivo* ELISpot for IFN-γ, as described in the general methods chapter.

5.7 Results

5.7.1 Study population

Between March 14 and Aug 15, 2018, 24 healthy adults were enrolled: 6 were assigned to the low-dose group, 9 to the intermediate-dose group, and 9 to the high-dose group (Figure 23). See Table 32 for baseline characteristics.

All participants received a single dose of ChAdOx1 MERS according to their group allocation. All participants were available for follow-up at 6 months, but 5 (1 in the low-dose group, 1 in the intermediate-dose group, and 3 in the high-dose group) were lost to follow-up at 12 months (Figure 23).

Table 32. MERS001 Summary of baseline characteristics

Variable	**Group 1 (n=6)**	**Group2 (n=9)**	**Group 3 (n=9)**	**All Groups (n=24)**
Age				
Median	25.5	29	23	24.5
Range	19-37	23-43	20-47	19-47
Sex				
Male – n (%)	2 (33.33)	2 (22.22)	3 (33.33)	7 (29.17)
Female – n (%)	4 (66.67)	7 (77.78)	6 (66.67)	17 (70.83)
Ethnicity				
White – n (%)	6 (100)	9 (100)	9 (100)	24 (100)

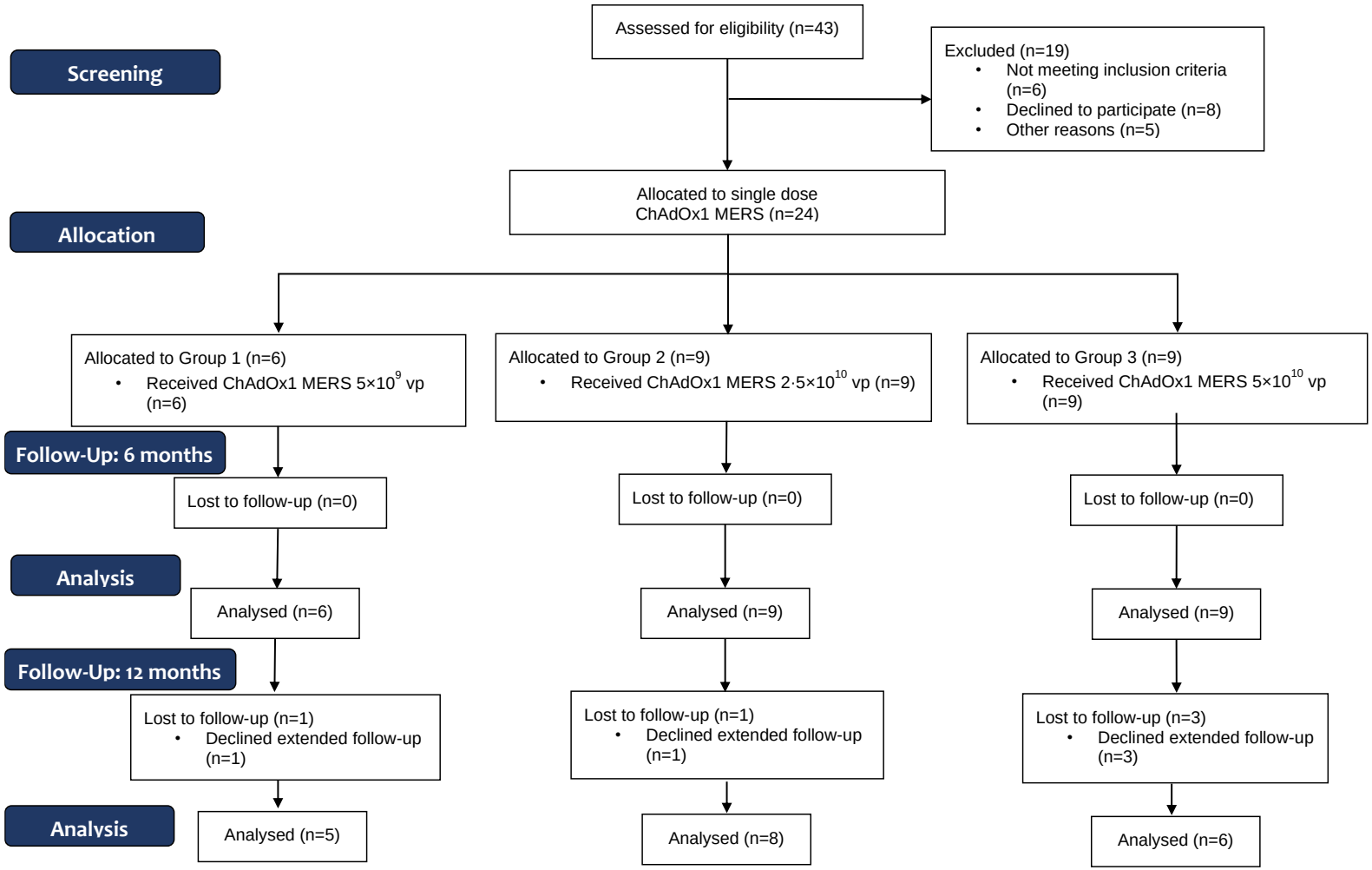


Figure 23. MERS001 CONSORT diagram

5.7.2 Vaccine safety

ChAdOx1 MERS was safe at doses up to 5×10^{10} vp with no SARs reported during 12 months of follow-up. 124 local and systemic solicited adverse events were reported from enrolment to 7 days after vaccination (Table 33). One SAE reported (bowel obstruction) was deemed to be not related to ChAdOx1 MERS. 92 (74%, 95% CI 66–81) solicited AEs were mild, 31 (25%, 95% CI 18–33) were moderate, and all were self-limiting. All solicited AEs were completely resolved 6 days after vaccination. 119 (96%) had their onset within the first 72 h after vaccination (66 [53%] at day 0, 48 [39%] at day 1, and five [4%] at day 2). Injection site pain was the most common local AE, reported by 18 (75%) of 24 participants and was predominantly mild in severity (Table 33). Fatigue was the most common systemic AE, followed by headache and malaise. Median duration of solicited AEs is summarised in Table 34.

Table 33. MERS001 Number of participants reporting local and systemic solicited AEs.

	ChAdOx1 MERS 5x10^9 vp (*n* = 6)				**ChAdOx1 MERS 2.5x10^{10} vp (*n* = 9)**				**ChAdOx1 MERS 5x10^{10} vp (*n* = 9)**			
	Any	Mild	Moderate	Severe	Any	Mild	Moderate	Severe	Any	Mild	Moderate	Severe
Any symptom	6 (100%)	5 (83%)	1 (17%)	0	7 (78%)	5 (56%)	2 (22%)	0	9 (100%)	0	8 (89%)	1 (11%)
Any local symptom	4 (67%)	4 (67%)	0	0	7 (78%)	7 (78%)	0	0	9 (100%)	5 (56%)	4 (44%)	0
Pain	2 (33%)	2 (33%)	0	0	7 (78%)	7 (78%)	0	0	9 (100%)	5 (56%)	4 (44%)	0
Pruritus	2 (33%)	2 (33%)	0	0	1 (11%)	1 (11%)	0	0	2 (22%)	2 (22%)	0	0
Warmth	1 (17%)	1 (17%)	0	0	1 (11%)	1 (11%)	0	0	2 (22%)	2 (22%)	0	0
Swelling	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	2 (33%)	2 (33%)	0	0	1 (11%)	1 (11%)	0	0	2 (22%)	2 (22%)	0	0
Any systemic symptom	6 (100%)	5 (83%)	1 (17%)	0	7 (78%)	5 (56%)	2 (22%)	0	9 (100%)	1 (11%)	7 (78%)	1 (11%)
Fever	0	0	0	0	1 (11%)	1 (11%)	0	0	5 (56%)	1 (11%)	3 (33%)	1 (11%)
Feverishness	1 (17%)	1 (17%)	0	0	4 (44%)	4 (44%)	0	0	7 (78%)	4 (44%)	3 (33%)	0
Arthralgia	1 (17%)	1 (17%)	0	0	2 (22%)	2 (22%)	0	0	5 (56%)	5 (56%)	0	0
Myalgia	2 (33%)	2 (33%)	0	0	4 (44%)	4 (44%)	0	0	7 (78%)	5 (56%)	2 (22%)	0
Headache	4 (67%)	3 (50%)	1 (17%)	0	5 (56%)	5 (56%)	0	0	7 (78%)	2 (22%)	5 (56%)	0
Fatigue	4 (67%)	4 (67%)	0	0	6 (67%)	5 (56%)	1 (11%)	0	7 (78%)	3 (33%)	4 (44%)	0
Nausea	0	0	0	0	3 (33%)	2 (22%)	1 (11%)	0	5 (56%)	4 (44%)	1 (11%)	0
Malaise	1 (17%)	1 (17%)	0	0	5 (55%)	4 (44%)	1 (11%)	0	6 (67%)	1 (11%)	5 (56%)	0

Table 34. MERS001 Duration of AEs

AE	Median	IQR*	Maximum duration
Pain at injection site	3.5	1-4.25	6
Pruritus	1	1-1	1
Warmth	1	1-1.75	2
Erythema	1	1-1	1
Fever	1	1-1	1
Feverishness	1.5	1-2	3
Arthralgia	1	1-2	4
Myalgia	1	1-2.5	4
Headache	2	1-2	5
Fatigue	2	1-3	4
Nausea	1	1-3	5
Malaise	1	1-2	2
Overall	1	1-2	6

Six participants reported a short-lived temperature above 37.5°C within the first 72h post-vaccination (1 in the intermediate dose group and 5 in the high dose group). One participant in group 3 had a temperature of 39.6°C (classed as severe) on the day of vaccination. This episode resolved within 24h.

The proportion of moderate and severe AEs was significantly higher in group 3 compared to group 2 (RR 5·83, 95%CI 2·11 –17·42, p< 0·001), but there were no safety concerns despite the higher reactogenicity.

Unsolicited AEs in the 28 days following vaccination considered possibly, probably, or definitely related with ChAdOx1 MERS were predominantly mild in

nature and resolved within the follow-up period (Table 35). Laboratory AEs considered at least possibly related to the study intervention were self-limiting and predominantly mild in severity (Table 36).

Table 35. MERS001 Unsolicited AEs considered related to ChAdOx1 MERS

Subject ID	MEDDRA PT	MEDDRA PT CODE	Day of Onset	Duration (days)	Max. Severity
00101006	Nasal congestion	10028735	1	10	1
00101006	Rhinorrhoea	10039101	1	8	1
00101006	Diarrhoea	10012735	5	1	1
00101006	Nasopharyngitis	10028810	10	1	1
00101011	Back pain	10003988	0	2	1
00101011	Lymphadenopathy	10025197	1	6	1
00101011	Odynophagia	10030094	1	6	1
00101039	Orthostatic hypotension	10031127	0	1	2
00101012	Photophobia	10034960	1	1	1
00101012	Ear pain	10014020	1	1	1
00101012	Arthralgia	10003239	1	1	1
00101012	Arthralgia	10003239	1	5	1
00101010	Decreased appetite	10061428	1	1	1
00101010	Diarrhoea	10012735	2	1	2
00101010	Vomiting	10047700	4	2	2
00101013	Dizziness	10013573	2	1	1
00101027	Dizziness	10013573	0	1	1
00101027	Back pain	10003988	0	2	1
00101027	Neck pain	10028836	1	1	1
00101027	Abdominal pain upper	10000087	1	1	1
00101017	Neck pain	10028836	1	1	1

Subject ID	MEDDRA PT	MEDDRA PT CODE	Day of Onset	Duration (days)	Max. Severity
00101015	Feeling cold	10016326	0	1	1
00101015	Rash	10037844	1	2	1
00101020	Nausea	10028813	7	2	1
00101019	Productive cough	10036790	1	1	1
00101019	Sneezing	10041232	1	1	1
00101019	Ear pain	10014020	6	2	1
00101032	Chills	10008531	0	1	2
00101032	Feeling cold	10016326	0	1	2
00101032	Decreased appetite	10061428	1	1	1
00101032	Alopecia	10001760	7	50	2
00101037	Chills	10008531	0	1	1
00101037	Infrequent bowel movements	10059158	2	1	1
00101037	Headache	10019211	13	1	1
00101033	Headache	10019211	7	2	1
00101038	Chills	10008531	0	1	2
00101038	Eczema	10014184	26	1	1
00101038	Pityriasis rosea	10035114	26	56	3

Table 36. MERS001 Laboratory AEs considered related to ChAdOx1 MERS

ID	Group	Event	Severity	Onset	Resolved by
101013	2	Neutropenia	Mild	D7	D28
101014	3	Neutropenia	Moderate	D2	D7
101015	3	Leucopenia	Mild	D2	D7
101015	3	Lymphopenia	Mild	D2	D7
101020	3	Leucopenia	Mild	D2	D7
101020	3	Neutropenia	Mild	D2	D7
101024	2	Leucopenia	Mild	D2	D7
101024	2	Neutropenia	Mild	D2	D7
101032	3	Neutropenia	Mild	D2	D7
101035	2	Neutropenia	Mild	D2	D7
101038	3	Neutropenia	Mild	D2	D7
101039	1	Anaemia	Mild	D7	D28

5.7.3 Humoral immunogenicity

A single dose of the vaccine-induced a strong antibody response in 22 (92%) participants across all groups, which persisted for 1 year after vaccination. Antibody responses increased rapidly after vaccination, peaking 28 days after vaccination for all groups. Seroconversion occurred in 18 (75%) of 24 participants at 14 days after vaccination, increasing to 22 (92%) participants up to 56 days after vaccination. Seropositivity was maintained in 13 (68%) of 19 participants up to 1 year after vaccination (geometric mean 381·1 ELISA units, 95% CI 251·2–578·3). Antibody responses peaked at day 28, with the high-dose group producing the highest antibody response. IgG titres increased significantly at day 28 and day 56 after vaccination with ascending doses of ChAdOx1 MERS

compared to baseline, and no significant differences in anti-MERS IgG were found between the dose groups at these timepoints . Detectable anti-MERS IgG titres were observed in 4 (17%) of 24 participants at baseline. However, this baseline response did not inhibit the antibody responses to the vaccine, as evident by the increased IgG titres observed for these participants at day 28 and day 56 after vaccination.

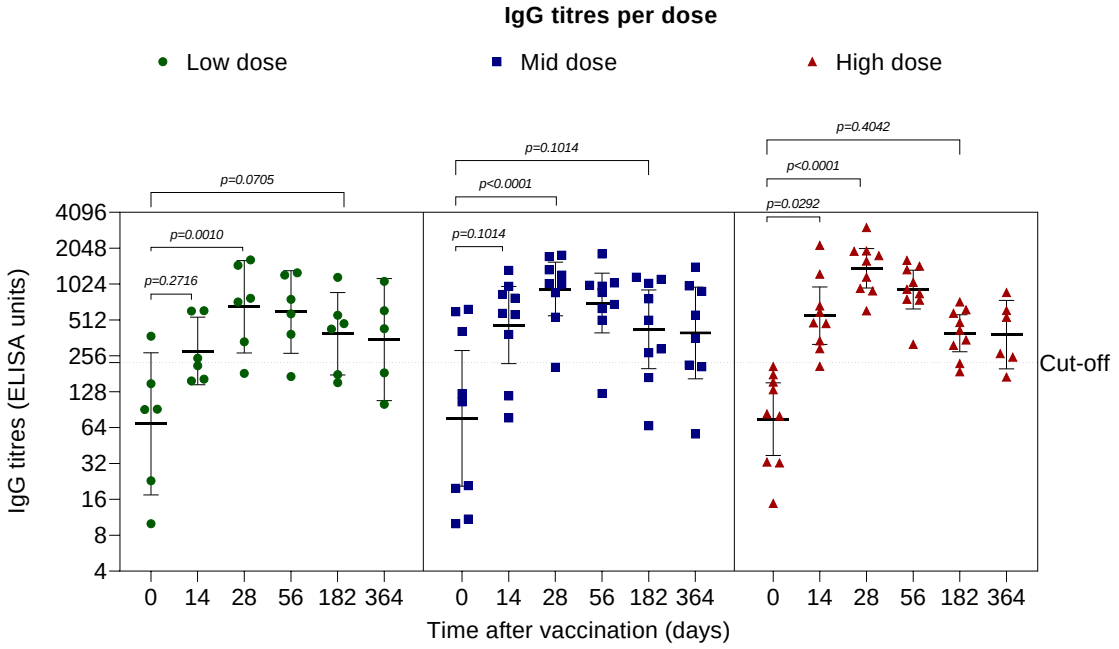


Figure 24. MERS001 MERS-CoV IgG responses per dose.

Black lines represent geometric means and 95% CI (error bars). p values for Friedman test with Dunn's correction.

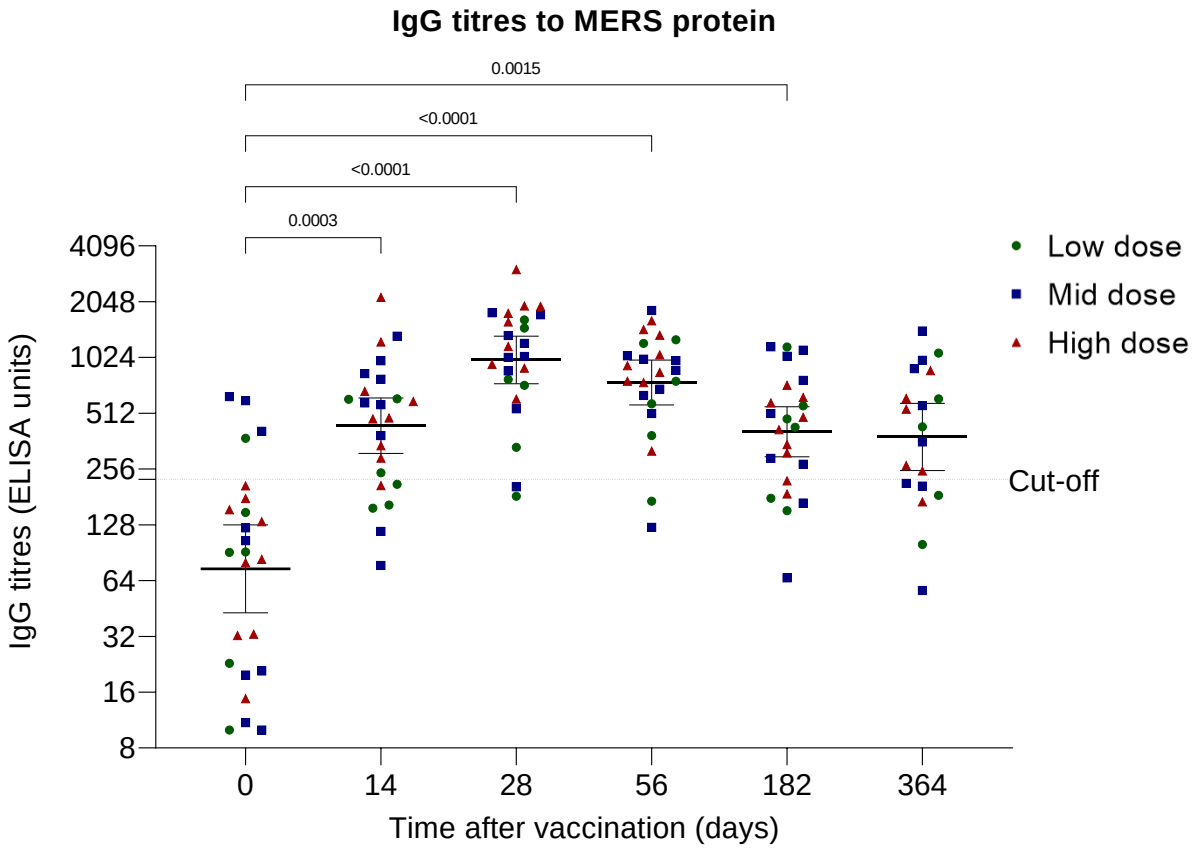


Figure 25. MERS001 Total IgG titres.

Dark lines represent geometric means and 95% CI (error bars). p values for Friedman test with Dunn's correction.

Table 37. MERS001 IgG response by ELISA and seropositivity by group.

	ELISA							
Day	**Low dose (*n* = 6)**		**Intermediate dose (*n* = 9)**		**High dose (*n* = 9)**		**Overall (n=24)**	
	Seropositive *n* (%)	**GMT (95% CI)**	**Seropositive *n* (%)**	**GMT (95% CI)**	**Seropositive *n* (%)**	**GMT (95% CI)**	**Seropositive *n* (%)**	**GMT (95% CI)**
0	1 (17%)	68.83 (17.43-271.7)	3 (33%)	78.58 (20.65-284)	0	75.41 (37.37-152.2)	4 (17%)	74.14 (42.92-128.1)
14	3 (50%)	281.3 (146.5-539.9)	7 (78%)	464.2 (220.4-977.6)	8 (89%)	556.4 (319.3-969.7)	18 (75%)	438.3 (310.5-618.9)
28	5 (83%)	660.6 (270.8-1612)	8 (89%)	930.5 (554.3-1562)	9 (100%)	1391 (950.2-2036)	22 (92%)	993.1 (739.0-1334)
56	5 (83%)	597.2 (269.1-1325)	8 (89%)	710.2 (399.3-1263)	9 (100%)	920.9 (633.6-1338)	22 (92%)	749.7 (567.6.1-990.1)
182	4 (67%)	392.2 (177-869.3)	7 (78%)	427.2 (199.8-913.1)	8 (89%)	395.9 (276.8-566.1)	19 (80%)	406.4 (297.8-554.5)
364	3* (60%)	350.4 (107.9-1138)	5* (63%)	398.5 (164.5-965.2)	5* (83%)	385.1 (198.9-745.8)	13* (68%)	381.1 (251.2-578.3)

* 1 participant at the low dose, 1 at the intermediate dose and 3 participants at high dose did not consent to the extended 1-year follow-up.

Neutralising antibodies against heterologous live MERS-CoV were measured from 22 participants (5 in the low-dose group, 8 in the intermediate-dose group, and 9 in the high-dose group). Virus neutralising antibody titres were measured at day 0 and were negative, and at day 28. Neutralising antibodies were detected only in the low-dose group (1 of 5 participants) and high-dose group (4 [44%, 95% CI 19–73] of 9 participants), with only the high-dose group producing a significant increase in neutralising antibody titres compared to baseline (p=0.0156, Wilcoxon test).

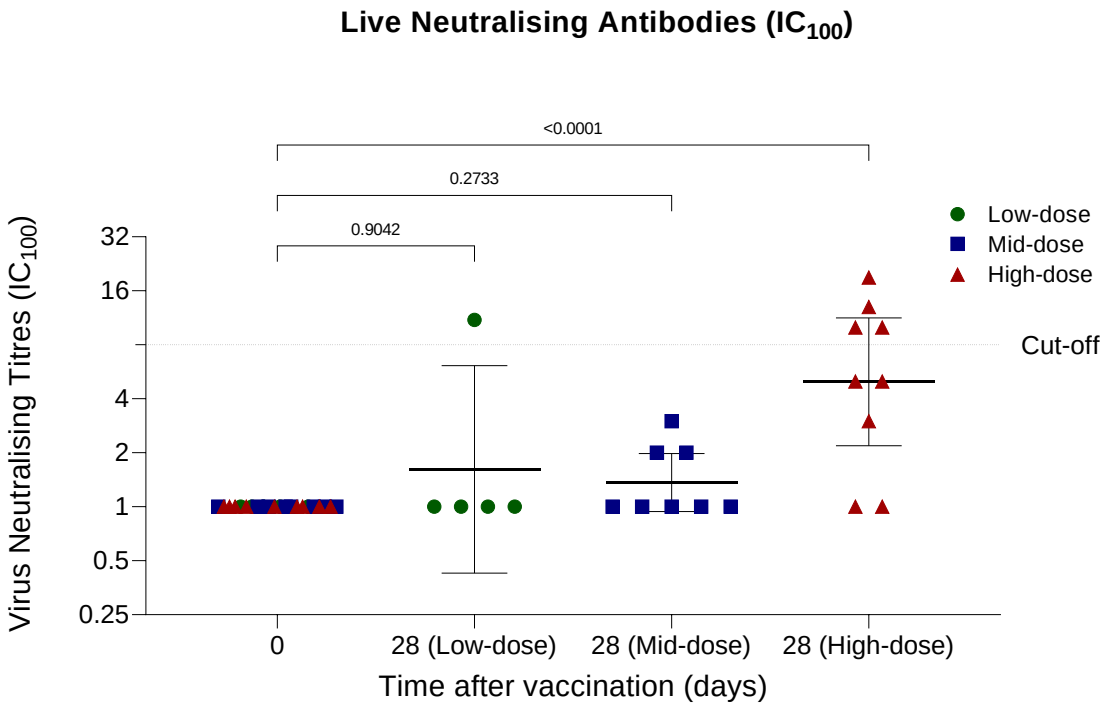


Figure 26. MERS001 Live virus neutralising antibodies (IC_{100}).

Black lines represent geometric mean and 95% confidence intervals (error bars). P values for Kruskal-Wallis' test with Dunn's correction for multiple comparisons.

Neutralisation tests of three pseudotyped lentiviruses expressing spike glycoprotein from three different MERS-CoV strains were negative at day 0, but

17 (71% [95% CI 49–87]) of 24 samples were positive at day 28 when tested against EMC/2012 and KOR/KNIH/002, and 19 (79% [95% CI 58–93]) were positive when tested against England-1 at day 28.

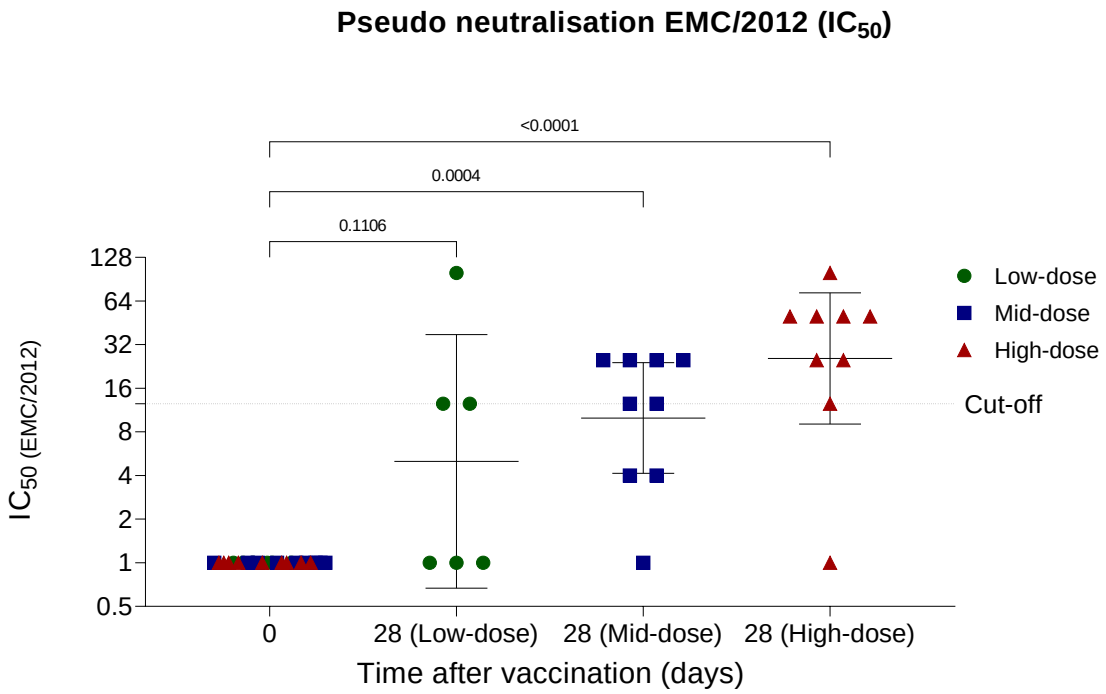


Figure 27. MERS001 Pseudovirus neutralisation (IC_{50})

Black lines represent geometric mean and 95% confidence intervals (error bars). P values for Kruskal-Wallis' test with Dunn's correction for multiple comparisons.

5.7.4 Cellular immunogenicity

Cellular immunogenicity to ChAdOx1 MERS was assessed by *ex-vivo* ELISpot. High frequencies of interferon-γ-secreting T cells recognising MERS spike peptides were elicited (Figure 28).

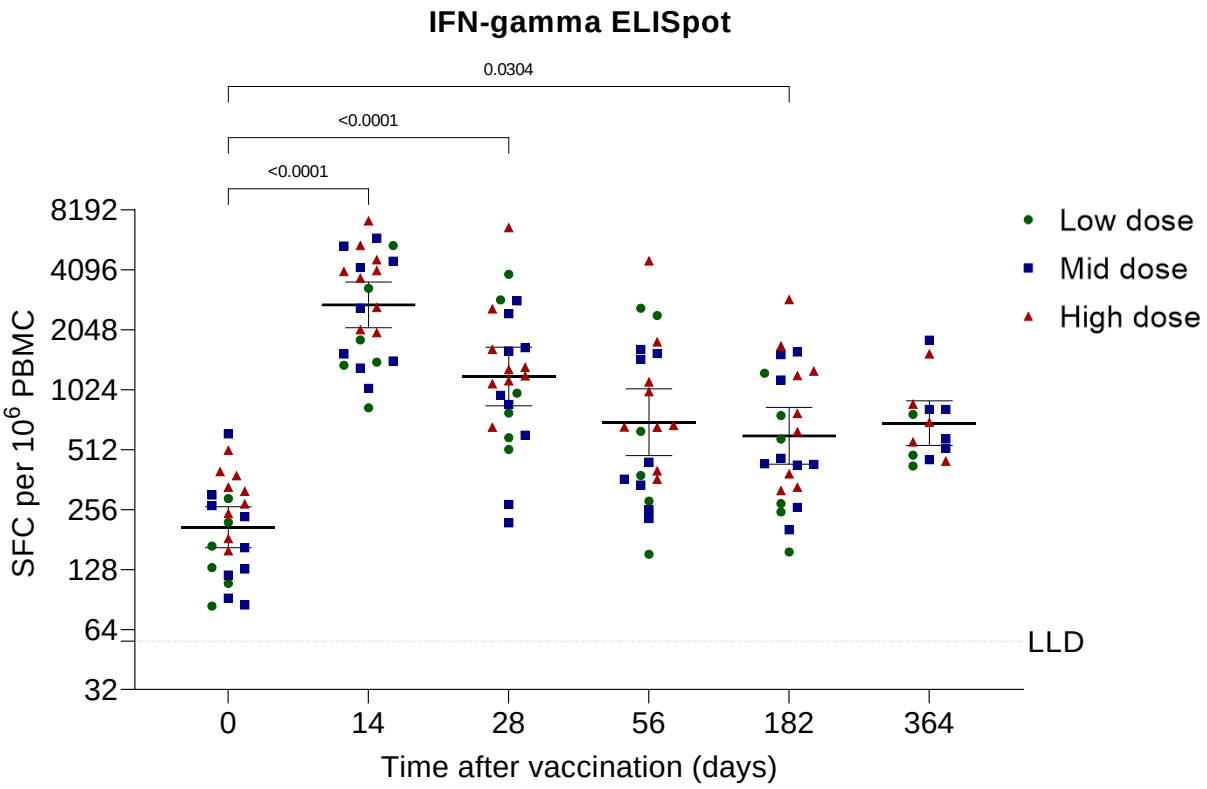


Figure 28. MERS001 Combined IFN-γ ELISpot responses over time

Dark lines represent geometric mean and 95% confidence intervals (error bars). P values for Friedman's test with Dunn's correction for multiple comparisons.

Responses peaked at 14 days after vaccination for all groups (geometric mean 1932 SFCs [95%CI 949.7–3931] for the low-dose group, 2567 SFCs [95%CI 1524–4323] for the intermediate-dose group, and 3657 SFCs [95%CI 2620–5105] for the high-dose group; Figure 29). No significant effect of vaccine dose on magnitude of response was detected at any timepoint. The increase in T cell responses persisted to 1 year after vaccination at over 3 times higher than baseline (geometric mean 695·6 SFCs [95% CI 537·5–900·2], p=0·0002 Wilcoxon test, day 364 vs day 0), although fewer samples were available at day 364. T cell responses to both the RBD (within S1) and S2 persisted to day 364 after vaccination.

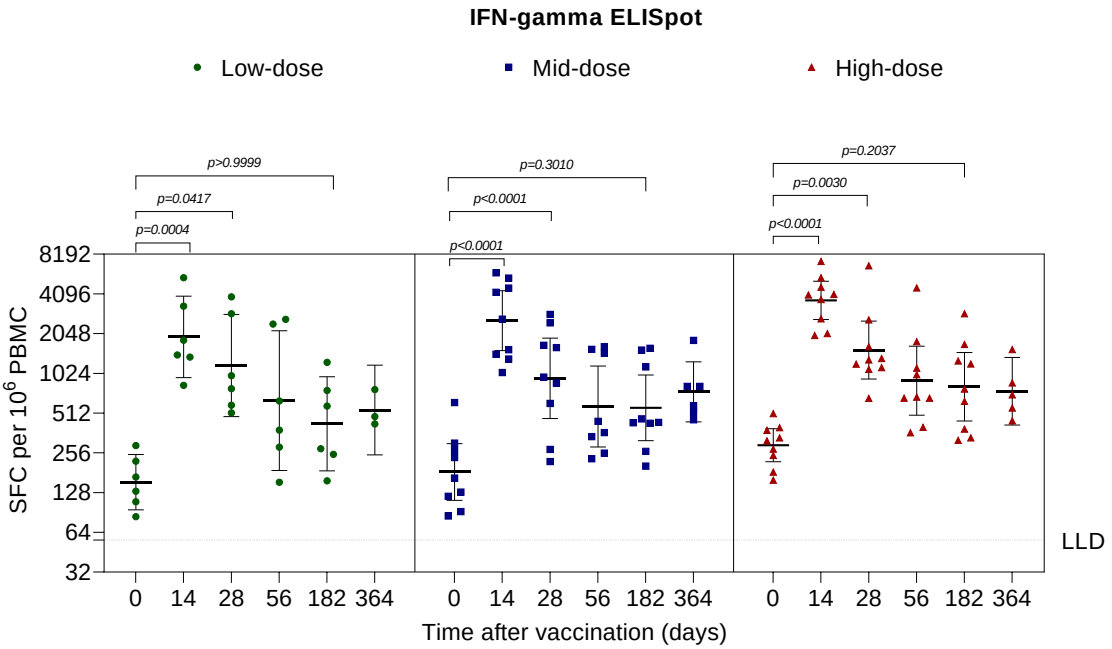


Figure 29. MERS001 IFN-γ ELISpot responses per dose over time

Dark lines represent geometric mean and 95% confidence intervals (error bars). P values for Friedman's test with Dunn's correction for multiple comparisons

Table 38. MERS001 Fold change in geometric mean T cell responses

	Geometric mean T cell responses (95%CI)			Fold change	
	D0	**D14**	**D182**	**D0 vs D14**	**D0 vs D182**
Low dose	153.3 (94.62-248.2)	1932 (949.7-3931)	424.7 (187.2-963.8)	12.6	2.8
Mid dose	183.5 (111.9-300.7)-	2567 (1524-4323)	561.6 (316.5-996.5)	14	3
High dose	292.4 (219.4-389.5)	3657 (2620-5105)	810.8 (446.7-1472)	12.5	2.8
Overall	208.9 (164.9-264.6)	2731 (2097-3556)	601.1 (432.9-834.4)	13	2.9

5.7.5 Correlations between ELISA, neutralising antibodies, and T cell responses

There was a modest positive correlation between IgG titres measured by ELISA and live virus neutralising antibody titres at day 28 (Figure 30). No significant correlations were found between binding or pseudovirus neutralising antibody titres at day 28 (Figure 31) and T cell responses at day 14 (Figure 32, Figure 34).

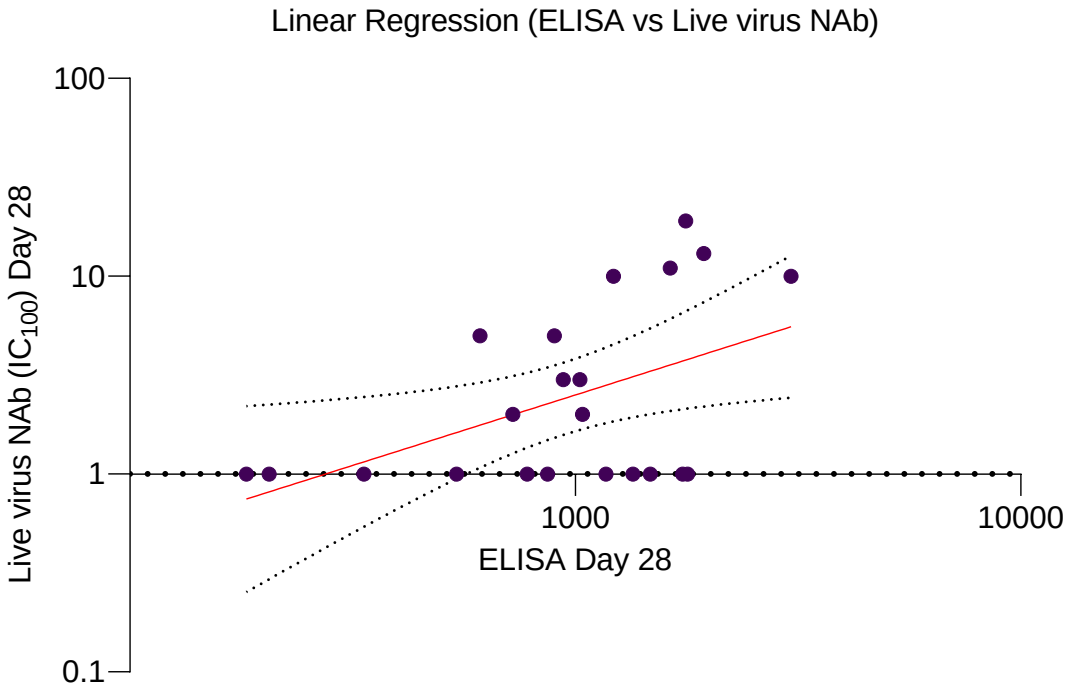


Figure 30. MERS001 Linear regression (ELISA vs live virus IC_{100})

Linear regression of logged values for IgG titres (ELISA) and Virus Neutralisation Titres (IC_{100}) at day 28 (r^2=0.2306, p=0.0237).

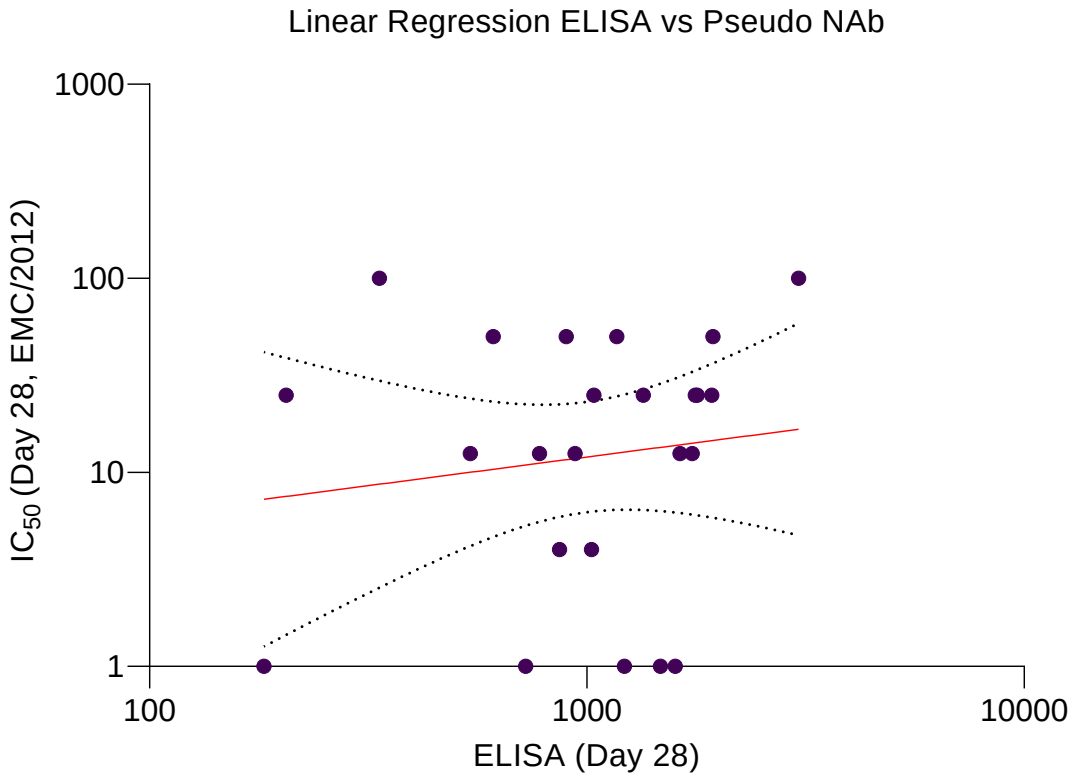


Figure 31. MERS001 Linear regression (ELISA vs Pseudovirus IC_{50})

Linear regression of logged values for IgG titres (ELISA) at day 28 and pseudovirus neutralisation (IC_{50}, EMC/2012) at day 28 (r^2=0.01830, p=0.5285).

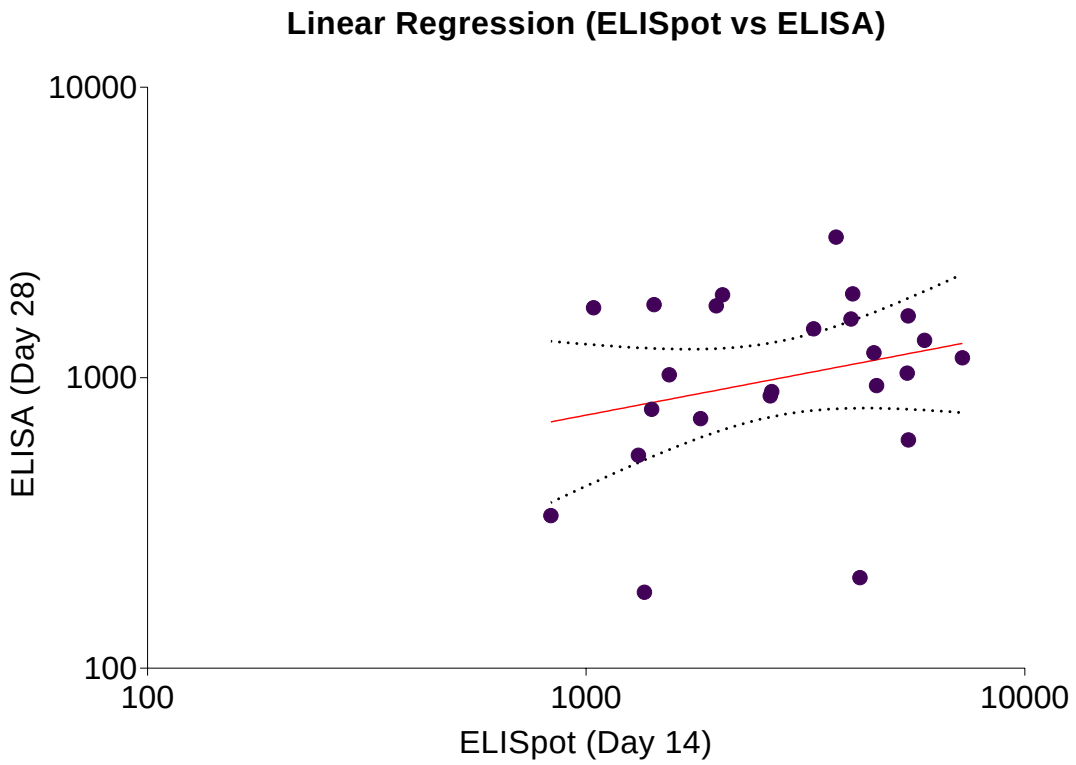


Figure 32. MERS001 Linear regression (ELISpots vs ELISA)

Linear regression of logged values for ELISpots at day 14 and IgG titres (ELISA units) at day 28 (r^2=0.06618, p=0.2249).

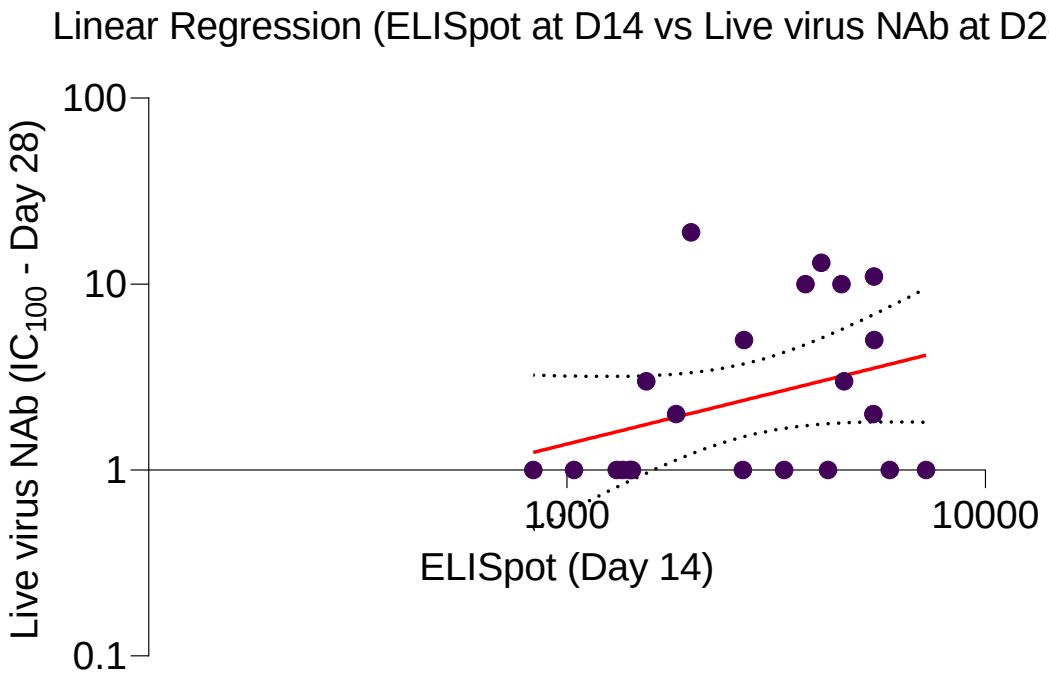


Figure 33. MERS001 Linear regression (ELISpots vs live virus IC_{100})

Linear regression of logged values for ELISpots at day 14 and IgG titres (ELISA units) at day 28 (r^2= 0.1169, p= 0.1194).

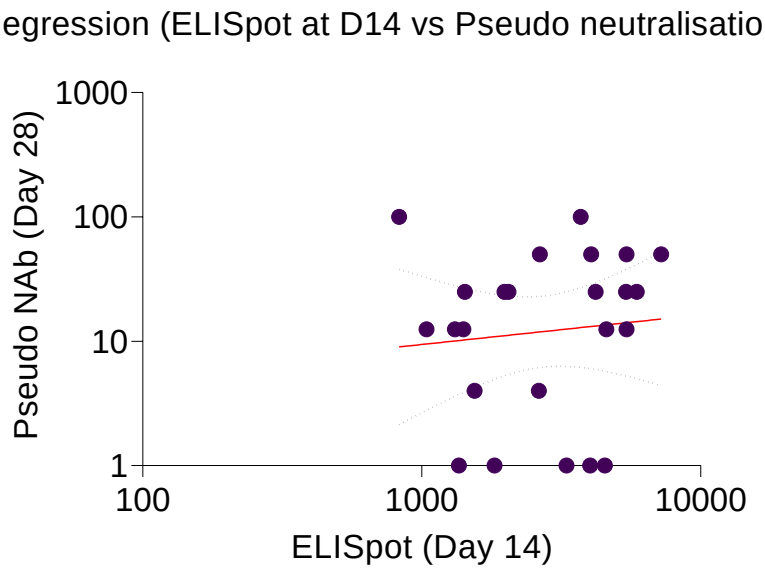


Figure 34. MERS001 Linear regression (ELISpots vs Pseudovirus NAbs)

Linear regression of logged values for ELISpots at day 14 and IgG titres (ELISA units) at day 28 (r^2= 0.1169, p= 0.1194).

5.8. Discussion

In this study, the candidate ChAdOx1 MERS vaccine given as a single dose was safe and well-tolerated in all 3 groups, although a higher reactogenicity profile was observed at the 5 × 10^{10} vp dose, with 5 out of 9 participants in that group reporting short-lived fever above 37.5°C. No serious adverse reactions occurred during the follow-up period. The majority of AEs reported were mild or moderate in severity and all AEs were self-limiting. The profile of AEs reported in this trial is similar to another ChAdOx1 vectored vaccine expressing influenza A antigens [72, 201] and other closely related simian adenoviruses, such as ChAdOx2, ChAd3 and ChAd63 vectored vaccines expressing different antigens. [202-204]

Safety concerns around the use of full-length coronavirus spike glycoproteins as a vaccine antigen have been raised following historical reports of immunopathology and 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 [205-207]. To date, there has been one report of lung immunopathology following MERS-CoV challenge in mice immunised with an inactivated MERS-CoV candidate vaccine [208]. However, in preclinical studies of ChAdOx1 immunisation and MERS-CoV challenge, no antibody-dependent enhancement was observed in transgenic mice expressing human DPP4, dromedary camels or non-human primates [60, 61, 64].

The vaccine was immunogenic at all doses, inducing seroconversion in most participants and T cell responses in all, with responses demonstrating good durability up to one year post-vaccination. Onset of detectable immune responses was rapid, with T cell responses reaching a peak 14 days after vaccination and antibodies at 28 days. A small number of participants had positive antibody or T cell responses (but never both) prior to vaccination. This could be due to cross-reactivity with other known human coronaviruses, such as HCoV-229E, HCoV-HKU1, HCoV-NL63 and HCoV-OC43, all of which circulate worldwide and can cause lower respiratory tract infections [209]. The prevalence of seropositivity to these four viruses in the UK is unknown, but a cross-sectional survey in the Netherlands found that 100% of children had seroconverted to at least one of these by the age of 10 years, and it is likely that the rate is similar in the UK [210]. With the emergence of SARS-CoV-2, pre-existing cross-reactive immune responses to human coronaviruses is likely to be an area of further investigation. Importantly, pre-existing T cell or antibody responses did not affect vaccine immunogenicity and no neutralising antibodies were detected prior to

vaccination. Alternatively, assay optimisation may be required to better establish a threshold for positivity.

Correlates of protection for MERS-CoV are currently unknown. Neutralising antibodies targeting different epitopes of the Spike glycoprotein have been associated with protection against MERS-CoV challenge in animal models [211]. A single dose of ChAdOx1 MERS was able to elicit NAbs against live MERS CoV in 44% (4/9, 95%CI 19-73%,) of participants receiving the high dose. Between 71% (17/24) and 79% (19/24) of all participants produced neutralising antibodies in assays employing pseudotyped lentiviruses expressing the Spike protein from three different strains of MERS-CoV, suggesting maintained neutralising activity across heterologous variants from the vaccine strain. In MERS survivors CD8+ T cell responses were found to correlate with less severe disease and lower virus shedding [212]. ChAdOx1 MERS vaccination resulted in significant increases in MERS S-specific T cell responses which were maintained for at least a year after vaccination at 3-fold higher than baseline.

MERS-CoV vaccines are required for both camels and humans. ChAdOx1 MERS has been tested in dromedary camels in Saudi Arabia and was shown to significantly reduce viral shedding, which could potentially translate into reduced zoonotic transmission [64]. The One Health vaccine development approach deployed here, by which the same vaccine is co-developed for humans and susceptible animal species, allows vaccine efficacy to be tested in an appropriate animal model, which could support licensure of the vaccine for humans. Target groups include people who are occupationally exposed to camels and healthcare workers. However, severe, and fatal cases of MERS-CoV disproportionally affect individuals over the age of 50 and with comorbidities. Therefore, it is paramount

that vaccines developed against MERS-CoV are suitable for administration in older age groups in the context of an outbreak. The use of replication-deficient vectors avoids the risks of inadequate attenuation of replication-competent vaccines which could potentially lead to disseminated disease in immunocompromised hosts. Immunogenicity of ChAdOx1 vectored vaccine against influenza and SARS-CoV-2 have been demonstrated in older adults [72, 79]. For use in outbreaks, rapid onset of immunity after a single dose as demonstrated here is highly desirable.

CEPI is supporting the clinical development of 4 novel vaccines against MERS, all of which express the full-length Spike protein. In addition to ChAdOx1 MERS, a DNA vaccine and two other viral vectored vaccines (MVA and measles vectors are being developed) [96, 194, 213]). Data from early clinical trials will support the use of one or more of these vaccines in the Middle East, or as a stockpile suitable for outbreak response in any country. ChAdOx1 MERS is now out licensed to Vaccitech plc who are planning to further its commercial development.

Limitations of this study include the small sample size and the open-label, non-randomised and uncontrolled trial design. Long term safety and immunogenicity findings should be interpreted with caution considering 5/24 participants declined the 12-month extended follow-up. Further research is required to better understand the importance of pre-existing immunity and cross-reactivity to other coronaviruses in the context of emerging coronaviruses outbreaks. Nonetheless, this study provides valuable information on reactogenicity and immunogenicity of the first clinical use of ChAdOx1 MERS.

In conclusion, ChAdOx1 MERS was safe and well-tolerated at all tested doses. A single dose was able to elicit both humoral and cellular responses against MERS-CoV. The results of this first-in-human clinical trial support clinical development progression into Phase Ib and II trials in the Middle East. Healthy adults, healthcare workers, people who are occupationally exposed to camels and older age groups with comorbidities will be recruited and assessed for safety and immunogenicity of ChAdOx1 MERS to be given as a single or two-dose administration regimen [75].

Chapter 6: Safety and immunogenicity of ChAdOx1 nCoV-19 in UK adult volunteers, and efficacy in the UK and Brazil.

6.1 Authorship statement

Immunogenicity data presented in this chapter has been generated by Prof Sarah Gilbert's group at the Jenner Institute (binding antibody and cellular immunogenicity data), and collaborators at Monogram Biosciences (pseudo-neutralising antibodies data). This work has been published in Folegatti, P.M. et al. Lancet 2020; Ramasamy MN*, Minassian AM*, Ewer KJ*, Flaxman AL*, Folegatti PM*, et al. Lancet 2021; Voysey M*, Clemens SAC*, Madhi SA*, Weckx LY*, Folegatti PM*, et al. Lancet 2021; Voysey M*, Costa Clemens SA*, Madhi SA*, Weckx LY*, Folegatti PM*, et al. Lancet 2021.

6.2 Background

In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a live animal and seafood wholesale market in Wuhan, China and was later confirmed to be infected with a novel coronavirus, initially known as 2019-nCoV [214]. 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 the Middle East respiratory syndrome (MERS-CoV), a member of the lineage C betacoronavirus [215]. 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, with the first case reported outside China (in Thailand) on 13th January 2020. The WHO declared the COVID-19 outbreak a public health emergency of international concern on 30th January 2020, which evolved into a pandemic, declared on 11th March 2020. As of 30th September 2021, over 233,136,147 cases have been reported to the WHO, with 4,771,408 deaths.

SARS-CoV-2 belongs to the phylogenetic lineage B of the genus *Betacoronavirus* and it recognises the angiotensin-converting enzyme 2 (ACE2) as the entry receptor [216]. 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 ongoing COVID-19 pandemic has caused major disruption to healthcare systems with significant socioeconomic impacts. Containment measures have failed to stop the spread of the virus. There are now monoclonal antibodies and antivirals available for COVID-19 treatment and a global effort for accelerated vaccine development has seen several candidates being approved for emergency use in record time.

Live attenuated viruses have historically been among the most immunogenic platforms available, as they can present multiple antigens across the viral life cycle in their native conformations. However, manufacturing live-attenuated viruses requires complex containment and biosafety measures. The yield of SARS-CoV-2 in culture at the outset of the pandemic was unknown, potentially

limiting global supply. 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 immunosuppressed. 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 [191].

6.3 Progress towards a SARS-CoV-2 vaccine

Several SARS-CoV-2 vaccines are now approved for use in various countries. In April 2020, when clinical trials of ChAdOx1 nCoV-19 began, there were none. As of 30th September 2021, there were still 121 vaccines in clinical development and 194 candidates undergoing pre-clinical testing [217].

The WHO global target product profile of critical characteristics for pre-qualification of a COVID-19 vaccine requires candidates to be targeted at the most at-risk groups, including older adults; have a favourable safety profile; provide efficacy as measured by prevention of virologically confirmed disease or transmission, or both, and to provide at least 6 months of protection for individuals at ongoing risk of exposure to SARS-CoV-2 [218].

Table 39. COVID-19 vaccines approved

Vaccine manufacturer	Platform	WHO Pre-qualification	Number of Countries
Pfizer/Biontech	mRNA	31 Dec 2020	136
Oxford-AstraZeneca / SII	Simian adenoviral vector (ChAdOx1)	15 Feb 2021	182
Janssen	Human adenoviral vector (Ad26)	12 Mar 2021	54
Moderna	mRNA	30 April 2021	75
Sinopharm	Inactivated	02 May 2021	74
Sinovac	Inactivated	01 June 2021	41
Gamaleya Institute	Human adenoviral vector (Ad5 and Ad26)	Ongoing	47
CanSino	Human adenoviral vector (Ad5)	Ongoing	7
Bharat Biotech	Inactivated	Ongoing	9
Anhui Zhifei Longcom (China)	Subunit protein	-	2
Vector Institute (Russia)	Subunit protein	-	2
Center for Genetic Engineering and Biotechnology (Cuba)	Subunit protein	-	3
Barkat Pharmaceutical (Iran)	Inactivated	-	1
Research Institute for Biological Safety Problems (Kazakhstan)	Inactivated	-	1
Medigen (Taiwan)	Subunit protein	-	1
Chumakov Center (Russia)	Inactivated	-	1
Minhai Biotechnology Co (China)	Inactivated	-	1
Zydus Cadila (India)	DNA	-	1

Source: COVID-19 Vaccine Tracker https://covid19.trackvaccines.org/ (as of 30th Sep 2021)

6.4 Spike protein as a vaccine antigen

As with MERS-CoV, the roles of S in receptor binding and membrane fusion make it an ideal target for vaccine and antiviral development, as it is the main target for neutralising antibodies. Most COVID-19 vaccines under development are based on the spike protein.

6.5 ChAdOx1 nCoV-19 vaccine

The 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 N-terminal 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) [66]. ChAdOx1 nCoV-19 lacks the proline substitutions used by other COVID-19 vaccine developers to stabilise the antigen structure in its prefusion state. Data from native-like post-translational processing and assembly on ChAdOx1 nCoV-19 reveal the expression of S proteins on the surface of cells adopting the trimeric prefusion conformation, with no proline substitutions required [219].

In rhesus macaques, a single vaccination with ChAdOx1 nCoV-19 induced humoral and cellular immune responses. Protection against lower respiratory tract infection was observed in vaccinated non-human primates after high-dose SARS-CoV-2 challenge [63].

6.6 Methods

6.6.1 Trial design, objectives, participants, and oversight

Efficacy trials of ChAdOx1 nCoV-19 (COV001, COV002 and COV003) were originally designed as single-dose studies. Following preliminary neutralising antibody results from the small cohort receiving the prime-boost regimen in COV001, all studies were amended to incorporate a booster dose. An independent DSMB provided safety oversight across these trials. A COVID-19 case was defined as any virologically confirmed symptomatic case presenting with at least any of the following: new onset of fever (T≥37.8^{o} C), cough, shortness of breath, anosmia or ageusia

6.6.1.1 COV001

This phase 1/2, participant-blinded, multicentre, randomised controlled trial was done at five centres in the UK (CCVTM, University of Oxford; NIHR Southampton Clinical Research Facility, University Hospital Southampton NHS Foundation Trust, Southampton; Clinical Research Facility, Imperial College London; St Georges University of London and University Hospital NHS Foundation Trust; and University Hospitals Bristol and Weston NHS Foundation Trust). Healthy adult participants aged 18–55 years were recruited through local advertisements.

All participants underwent a screening visit where a full medical history and examination was taken in addition to blood and urine tests (HIV; hepatitis B and C serology; full blood count; kidney and liver function tests; and urinary screen for blood, protein, and glucose and a pregnancy test done in women of childbearing potential). Volunteers with a history of laboratory-confirmed SARS-CoV-2 infection; those at higher risk for SARS-CoV-2 exposure pre-enrolment

(i.e., front-line healthcare workers working in emergency departments, intensive care units, and COVID-19 wards, and close contacts of confirmed COVID-19 cases), and those with a new onset of fever, cough, shortness of breath, and anosmia or ageusia since 1st February 2020, were excluded from the study. An amendment to the study protocol (amendment date April 21, 2020) allowed for recruitment of healthcare workers with a negative SARS-CoV-2 serology at screening, once an antibody test became available. Full details of the eligibility criteria are described in the general methods chapter.

Written informed consent was obtained from all participants, and the trial was done in accordance with the principles of the Declaration of Helsinki and GCP.

Participants were randomly assigned (1:1) to receive either the ChAdOx1 nCoV-19 vaccine or the MenACWY vaccine. MenACWY was used as a comparator vaccine to maintain blinding of participants who experienced local or systemic reactions, since these reactions are frequently seen with ChAdOx1 vectored vaccinations given at the proposed dose. Use of saline as a placebo would risk unblinding participants as those who had notable reactions would know they were in the ChAdOx1 nCoV-19 vaccine group.

Randomisation lists, using block randomisation stratified by study group and study site, were generated by the study statistician. Block sizes of 2 and 4 were chosen to align with the study group sizes and sequence of enrolment, and varied across study groups. Computer randomisation was done with full allocation concealment within the secure web platform used for the study electronic case report form (REDCap version 9.5.22; Vanderbilt University, Nashville, TN, USA). The trial staff administering the vaccine prepared vaccines out of sight of the

participants and syringes were covered with an opaque material until ready for administration to ensure blinding of participants. Clinical investigators and the laboratory team remained blinded to group allocation. This study was registered with ClinicalTrials.gov, NCT04324606, and with ISRCTN, 15281137.

The co-primary outcomes were to assess efficacy as measured by cases of symptomatic virologically confirmed COVID-19 and safety of the vaccine as measured by the occurrence of serious adverse events. Secondary outcomes include safety, reactogenicity (occurrence of local and systemic reactogenicity signs and symptoms for 7 days after vaccination; occurrence of unsolicited adverse events for 28 days after vaccination; change from baseline for safety laboratory measures), immunogenicity profiles of ChAdOx1 nCoV-19 (cellular and humoral), and efficacy against hospital-attended COVID-19, and death.

6.6.1.2 COV002

This was a single-blind, multicentre, randomised, controlled, phase 2/3 trial, the safety and efficacy of the ChAdOx1 nCoV-19 vaccine, with sequential age-escalation immunogenicity sub-studies. The study was run at 20 centres in the UK (for efficacy) with 2 of those sites selected for the immunogenicity cohorts (NIHR Southampton Clinical Research Facility, University Hospital Southampton/NHS Foundation Trust, and CCVTM, University of Oxford). A batch comparison immunogenicity cohort (group 5) was introduced to ensure immune responses were comparable across the many different batches used in the study and included participants aged 18-55 receiving either ChAdOx1 nCoV-19 or control vaccine.

Participants were recruited in an age-escalation manner. Firstly, recruiting adults aged 18–55 years, then adults aged 56–69 years, and finally adults aged 70 years and older with controlled medical comorbidities. Participants aged 65 years and older with a Dalhousie Clinical Frailty Score of 4 or higher were excluded. Study groups are detailed in the general methods chapter.

A minimum of 2 weeks of safety and immunogenicity data were reviewed by the DSMB before recruitment to each successive age cohort. The 56–69 years and 70 years and older groups were randomly assigned to receive either one dose or two doses of vaccine and were then randomly assigned to receive the experimental or the control vaccine.

All participants underwent a screening visit in which a full medical history, targeted examination, blood test for SARS-CoV-2 exposure, and a urinary pregnancy test in women of childbearing potential were done. Volunteers who were seropositive to SARS-CoV-2 before enrolment were excluded from immunogenicity cohorts. Participants included in the immunogenicity cohorts had additional safety tests (blood tests for HIV, hepatitis B and C serology, full blood count, and kidney and liver function tests). Full details of eligibility criteria are in the general methods chapter.

Written informed consent was obtained from all participants, and the trial is being done in accordance with the principles of the Declaration of Helsinki and GCP. An independent DSMB reviewed all interim safety reports.

Participants were randomly assigned to receive either the ChAdOx1 nCoV-19 vaccine or the quadrivalent MenACWY, as per COV001. Participants aged 18–55 years were randomly assigned (1:1) in the low-dose cohort and (5:1) in the

standard-dose cohort to receive either ChAdOx1 nCoV-19 or MenACWY. For 18–55 years cohorts, participants were given two doses of the study vaccine. Participants aged 56–69 years were randomly assigned (3:1:3:1) to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY. Participants aged 70 years or older were randomly assigned (5:1:5:1) to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY.

Randomisation lists, using block randomisation stratified by age and dose group and study site, were generated by the study statistician. Block sizes were chosen to align with the age group and dose group sizes. Computer randomisation was done with full allocation concealment within the secure web platform used for the study electronic case report form (REDCap version 9.5.22). The trial staff administering the vaccine prepared vaccines out of sight of the participants and syringes were covered with an opaque material until ready for administration to ensure masking of participants. Participants, clinical investigators, and the laboratory team remained masked to group allocation for the duration of the study. However, trial staff administering the vaccine were unmasked.

The coprimary outcomes of the trial were to assess efficacy as measured by the number of cases of symptomatic, virologically confirmed COVID-19 and safety of the vaccine as measured by the occurrence of serious adverse events. Secondary outcomes include safety, reactogenicity, and immunogenicity profiles of ChAdOx1 nCoV-19 in older adults (aged 56–69 years and ≥70 years), efficacy against asymptomatic, severe, non-severe COVID-19, and death. This study was registered with ClinicalTrials.gov, NCT04400838, and with ISRCTN, 15281137.

6.6.1.3 COV003

COV003 was a single-blind phase 3 study in Brazil that began on June 23, 2020. Recruitment was targeted at those at higher risk of exposure to SARS-CoV-2, including healthcare workers at 6 sites across Brazil. Participants were aged 18 years or older. Participants were randomised 1:1 to receive ChAdOx1 nCoV-19 at a target dose of 5×10^{10} vp ($3.5–6.5 \times 10^{10}$ vp) or a control vaccine (MenACWY prime followed by saline placebo boost) with administration up to 12 weeks apart (target 4 weeks). Study outcomes were as per efficacy cohorts in COV002. Only efficacy data from COV003 is shown here.

6.6.2 Study approvals

6.6.2.1 COV001

The study was approved in the UK by the MHRA (reference 21584/0424/001-0001) and the South-Central Berkshire Research Ethics Committee (reference 20/SC/0145). Vaccine use was authorised by GMSCs at each participating site.

6.6.2.2 COV002

The study was approved in the UK by the MHRA (reference 21584/0428/001-0001) and the South-Central Berkshire Research Ethics Committee (reference 20/SC/0179). Vaccine use was authorised by GMSCs at each participating site.

6.6.2.3 COV003

The study was approved in Brazil by the national regulator (Agencia Nacional de Vigilancia Sanitaria – ANVISA) and both local and national ethics committees Vaccine use was authorised by GMSCs at each participating site

6.6.3 Trial procedures

6.6.3.1 COV001

ChAdOx1 nCoV-19 was administered at a dose of 5×10^{10} viral particles. The MenACWY vaccine was provided by the UK Department of Health and Social Care and administered as per summary of product characteristics (SmPC) at the standard dose of 0.5 mL. Vaccines were administered as a single intramuscular injection into the deltoid.

Participants were recruited and followed up according to groups. Participants were recruited first for groups 1 and 3, then group 2, and then group 4. Group 1 (the phase 1 component of the study) consisted of participants who had intensive early follow-up visits for safety and immunogenicity purposes at days 3, 7, 14, 28, and 56 after vaccination. Group 2 consisted of participants who had higher blood volumes drawn for humoral and cellular immunogenicity assessment than group 4, which consisted of participants who had a serum sample drawn for humoral immunology assessments only. Group 3 consisted of ten participants who were enrolled in a non-randomised prime-boost group and received a booster ChAdOx1 nCoV-19 administered 28 days after the first dose. These participants were not blinded and had extensive follow-up for safety and immunogenicity purposes, as per group 1, after each dose. A staggered-enrolment approach was used for the first 2, 6, and 90 participants recruited in groups 1 and 3 and interim safety reviews with the independent DSMB were done before proceeding with vaccinations in larger numbers of volunteers. Volunteers were considered enrolled into the trial at the point of vaccination.

Participants in all groups had blood samples drawn and clinical assessments for safety as well as immunology at days 0 and 28 and were also followed up at days

184 and 364. A later amendment to the protocol (amendment date June 22, 2020) provided for additional testing of booster vaccinations.

In 2 of the 5 trial sites (Oxford and Southampton), a protocol amendment (amendment date May 6, 2020) was implemented to allow prophylactic paracetamol to be administered before vaccination and participants were advised to continue with 1g of paracetamol every 6h for 24h to reduce vaccine-associated reactions. All participants enrolled after the protocol amendment at these two sites were given prophylactic paracetamol and randomised equally to the vaccine or control arms of the study. AE data collection was as described in the general methods chapter.

6.6.3.2 COV002

ChAdOx1 nCoV-19 was administered as a single-dose or two-dose regimen (minimum 4 weeks apart) at either a low dose (2.2×10^{10} vp) or a standard target dose of 5×10^{10} vp ($3.5–6.5 \times 10^{10}$ vp). It was administered as an intramuscular injection into the deltoid. The MenACWY vaccine was provided by the UK Department of Health and Social Care and administered as per SmPC at the standard dose.

Safety data from animal studies and the COV001 clinical trial were reviewed by the DSMB before recruitment of participants. Participants were observed in clinic for a minimum of 15min after the vaccination procedure in case of any immediate AEs.

Participants were instructed to complete a diary card to record solicited local and systemic adverse reactions for 7 days after each dose. Protocol-defined solicited

local and systemic AEs were as per COV001. SAEs events were recorded throughout the follow-up period of 1 year after the last dose of the vaccine.

The severity of adverse events was graded as per COV001. Participants in immunogenicity cohorts had clinical and immunogenicity assessments at 0, 7, 14, and 28 days after their prime and booster vaccinations.

6.6.4 Statistics

For the purposes of this thesis, immunogenicity data were combined across different age groups and a description and comparison between different dosing levels and regimens is presented here across COV001 and COV002.

6.6.4.1 COV001

Safety and immunogenicity outcomes were analysed as described in the general methods chapter.

The sample size for the study was determined by the number of doses of vaccine that were available for use after the initial clinical manufacturing process. Sample sizes for efficacy are based on the number of primary outcome events that accrue.

6.6.4.2 COV002

Safety and immunogenicity outcomes were analysed as described in the general methods chapter.

Sample sizes were determined based on number of events needed to accrue for efficacy outcomes.

6.6.4.3 Efficacy

Vaccine efficacy (VE) was calculated as (1 – RR) x 100%, where RR is the relative risk of symptomatic infection (ChADOx1 nCoV-19: Control) and 95% confidence intervals are presented using the Taylor series method. Cases up to 7th December 2020 were included (before the COVID-19 Pfizer vaccine was introduced in the UK). Efficacy results are presented after a single standard dose (as originally designed) or 2 standard doses for COV001 and COV002 combined, and COV003 separately. Only cases occurring after 21 days from prime or 14 days from boost vaccinations were included.

6.6.5 Immunogenicity assessments

6.6.5.1 COV001

Cellular responses were assessed using *ex-vivo* IFN-γ ELISpot assays to enumerate antigen-specific T cells. Humoral responses at baseline and following vaccination were assessed using a standardised total IgG ELISA against SARS CoV-2 spike protein, and a pseudovirus neutralisation assay (PseudoNA IC_{50}).

6.6.5.2 COV002

Humoral and cellular responses were assessed as per COV001. Neutralising antibodies to the ChAdOx1 vector were measured using a secreted embryonic alkaline phosphatase (SEAP)-reporter assay, which measures the reciprocal of the serum dilution required to reduce in-vitro expression of vector-expressed SEAP by 50%, 24h after transduction [33].

6.7 Results

6.7.1 Study population

6.7.1 COV001

Between April 23 and May 21, 2020, 1077 participants were enrolled into the study and assigned to vaccination with either ChAdOx1 nCoV-19 (n=543) or MenACWY (n=534); 10 of these participants were enrolled in group 3 (prime-boost group), and therefore, were not randomly assigned. Eighty eight participants were included in group 1, 412 in group 2, and 567 in group 4. All randomised participants were vaccinated. One participant randomised to the MenACWY group received the ChAdOx1 nCoV-19 vaccine (Figure 35).

The median age of participants was 35 years (IQR 28–44 years), 536 (49·8%) participants were female and 541 (50·2%) were male, and the majority of participants (979 [90·9%]) were white. Baseline characteristics were similar between randomised groups (Table 40).

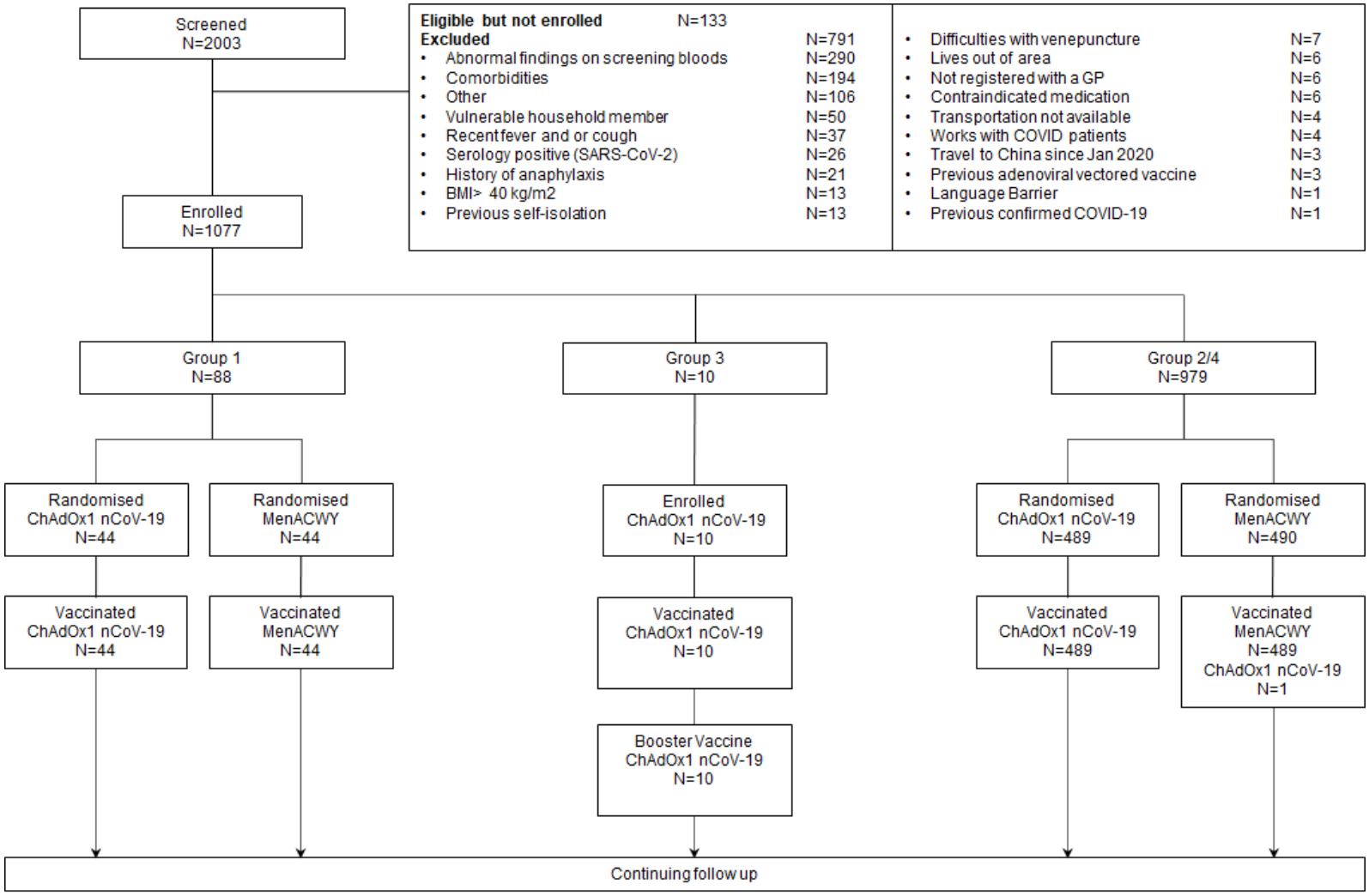


Figure 35. COV001 CONSORT diagram.

Table 40. COV001. Summary of baseline characteristics.

	ChAdOx1 N=543	**MenACWY N=534**
Female sex (n %)	265 (49%)	271 (51%)
Age, years, median [IQR]	34 [28, 43]	36 [28, 45]
Non-smoker	495 (91%)	485 (91%)
Non-drinker	89 (16%)	60 (11%)
BMI, median [IQR]	24 [22, 27]	24 [22, 27]
Ethnicity		
Arab	2 (<1%)	2 (<1%)
Black	4 (1%)	2 (<1%)
E. Asian	5 (1%)	4 (1%)
S. Asian	15 (3%)	20 (4%)
Mixed	12 (2%)	9 (2%)
Other	11 (2%)	10 (2%)
Not given	1 (<1%)	1 (<1%)
White	493 (91%)	486 (91%)

6.7.2 COV002

Between 30th May and 13th November 2020, 10,804 participants were vaccinated in COV002 (5,593 with ChAdOx1 nCoV-19 and 5,211 with MenACWY). Eight hundred participants were enrolled in immunogenicity cohorts and were assigned to the experimental vaccine or control group: 400 participants aged 18–55 years (240 assigned to ChAdOx1 nCoV-19, 160 assigned to MenACWY), 160 aged 56–69 years (120 assigned to ChAdOx1 nCoV-19, 40 assigned to MenACWY), and 240 aged 70 years and older (200 assigned to ChAdOx1 nCoV-19, 40 assigned to MenACWY). Two different doses were administered: standard dose (SD) and low dose (LD). Participants in the immunology cohorts presented here represent 4 different dosing regimens (SD, LD, SD/SD, and LD/LD given 4-6 weeks apart), recruited from the Southampton site (aged 70+), and CCVTM, Oxford. The median age in the 56–69 years group was 60·0 years (IQR 57·5–63·0) and in the

70 years and older group was 73·0 years (IQR 71·0–76·0), with the oldest participants aged 83 years.

Baseline characteristics of the participants in COV002 have been extensively published elsewhere. In summary, approximately 80% of participants in COV002 were aged 18-55, 60% were female and 90% were white.

6.7.2 Vaccine safety

6.7.2.1 COV001

328 (67%) of 487 participants in the ChAdOx1 nCoV-19 group and 180 (38%) of 477 participants in the MenACWY group reported pain after vaccination, which was mostly mild to moderate in intensity. Tenderness of mostly mild intensity was reported in the ChAdOx1 nCoV-19 group by 403 (83%) participants, and in the MenACWY group by 276 (58%) participants.

Fatigue and headache were the most commonly reported systemic reactions. Fatigue was reported in the ChAdOx1 nCoV-19 group by 340 (70%) participants and in the MenACWY group by 227 (48%) participants, whereas headaches were reported in the ChAdOx1 nCoV-19 group by 331 (68%) participants and in the MenACWY group by 195 (41%) participants.

Other systemic adverse reactions were common in the ChAdOx1 nCoV-19 group muscle ache (294 [60%]), malaise (296 [61%]), chills (272 [56%]); and feeling feverish (250 [51%]). In the ChAdOx1 nCoV-19 group, 87 (18%) participants reported a temperature of at least 38°C, and eight (2%) had a temperature of at least 39°C. In comparison, two (<1%) of those receiving MenACWY reported a fever of at least 38°C.

The severity and intensity of local and systemic reactions were highest on day 1 after vaccination. All 10 participants in the prime-boost group received their booster vaccine on day 28; solicited local and systemic reactions were measured in these participants for 7 days after both the prime and booster doses. The reactogenicity profile after the second dose appeared less severe in this subset, although the small number of participants in this group led to wide CIs.

Unsolicited adverse events in the 28 days following vaccination considered to be possibly, probably, or definitely related to ChAdOx1 nCoV-19 were predominantly mild and moderate in nature and resolved within the follow-up period. Laboratory adverse events considered to be at least possibly related to the study intervention were self-limiting and predominantly mild or moderate in severity. Transient haematological changes from baseline (neutropenia) were observed in 25 (46%) of 54 participants in the ChAdOx1 nCoV-19 group compared with three (7%) of 44 participants in the MenACWY group. There was one serious adverse event in the MenACWY group consisting of a new diagnosis of haemolytic anaemia, occurring 9 days after vaccination. The participant was clinically well throughout the study. The event was reported as a suspected unexpected serious adverse reaction relating to the MenACWY vaccine.

Table 41. COV001 Solicited adverse local and systemic reactions. Frequency and 95% CI.

Symptom	Vaccine Group	None	Mild	Moderate	Severe	Hospitalisation	Any
Pain	ChAdOx1 nCoV-19	159 (33%, 28%-37%)	258 (53%, 48%-57%)	66 (14%, 11%-17%)	4 (1%, 0%-2%)	0 (0%, 0%-1%)	328 (67%, 63%-72%)
	MenACWY	297 (62%, 58%-67%)	161 (34%, 30%-38%)	18 (4%, 2%-6%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	180 (38%, 33%-42%)
Redness	ChAdOx1 nCoV-19	472 (97%, 95%-98%)	8 (2%, 1%-3%)	6 (1%, 0%-3%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	15 (3%, 2%-5%)
	MenACWY	467 (98%, 96%-99%)	4 (1%, 0%-2%)	6 (1%, 0%-3%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	10 (2%, 1%-4%)
Warmth	ChAdOx1 nCoV-19	365 (75%, 71%-79%)	121 (25%, 21%-29%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	122 (25%, 21%-29%)
	MenACWY	377 (79%, 75%-83%)	97 (20%, 17%-24%)	3 (1%, 0%-2%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	100 (21%, 17%-25%)
Itch	ChAdOx1 nCoV-19	452 (93%, 90%-95%)	35 (7%, 5%-10%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	35 (7%, 5%-10%)
	MenACWY	452 (95%, 92%-97%)	23 (5%, 3%-7%)	2 (0%, 0%-2%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	25 (5%, 3%-8%)
Swelling	ChAdOx1 nCoV-19	466 (96%, 93%-97%)	10 (2%, 1%-4%)	10 (2%, 1%-4%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	21 (4%, 3%-7%)
	MenACWY	463 (97%, 95%-98%)	8 (2%, 1%-3%)	6 (1%, 0%-3%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	14 (3%, 2%-5%)
Induration	ChAdOx1 nCoV-19	470 (97%, 94%-98%)	13 (3%, 1%-5%)	3 (1%, 0%-2%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	17 (3%, 2%-6%)
	MenACWY	468 (98%, 96%-99%)	7 (1%, 1%-3%)	2 (0%, 0%-2%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	9 (2%, 1%-4%)
Tenderness	ChAdOx1 nCoV-19	84 (17%, 14%-21%)	333 (68%, 64%-72%)	66 (14%, 11%-17%)	4 (1%, 0%-2%)	0 (0%, 0%-1%)	403 (83%, 79%-86%)
	MenACWY	201 (42%, 38%-47%)	258 (54%, 49%-59%)	17 (4%, 2%-6%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	276 (58%, 53%-62%)
Feverishness	ChAdOx1 nCoV-19	237 (49%, 44%-53%)	99 (20%, 17%-24%)	111 (23%, 19%-27%)	40 (8%, 6%-11%)	0 (0%, 0%-1%)	250 (51%, 47%-56%)

Symptom	Vaccine Group	None	Mild	Moderate	Severe	Hospitalisation	Any
	MenACWY	439 (92%, 89%-94%)	36 (8%, 5%-10%)	2 (0%, 0%-2%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	38 (8%, 6%-11%)
Fever ≥ 38°C	ChAdOx1 nCoV-19	400 (82%, 78%-85%)	46 (9%, 7%-12%)	33 (7%, 5%-9%)	8 (2%, 1%-3%)	0 (0%, 0%-1%)	87 (18%, 15%-22%)
	MenACWY	475 (100%, 98%-100%)	2 (0%, 0%-2%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	2 (0%, 0%-2%)
Chills	ChAdOx1 nCoV-19	215 (44%, 40%-49%)	121 (25%, 21%-29%)	112 (23%, 19%-27%)	39 (8%, 6%-11%)	0 (0%, 0%-1%)	272 (56%, 51%-60%)
	MenACWY	431 (90%, 87%-93%)	42 (9%, 6%-12%)	4 (1%, 0%-2%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	46 (10%, 7%-13%)
Joint pain	ChAdOx1 nCoV-19	337 (69%, 65%-73%)	97 (20%, 16%-24%)	47 (10%, 7%-13%)	6 (1%, 0%-3%)	0 (0%, 0%-1%)	150 (31%, 27%-35%)
	MenACWY	431 (90%, 87%-93%)	37 (8%, 6%-11%)	8 (2%, 1%-3%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	46 (10%, 7%-13%)
Muscle ache	ChAdOx1 nCoV-19	193 (40%, 35%-44%)	168 (34%, 30%-39%)	107 (22%, 18%-26%)	19 (4%, 2%-6%)	0 (0%, 0%-1%)	294 (60%, 56%-65%)
	MenACWY	359 (75%, 71%-79%)	104 (22%, 18%-26%)	14 (3%, 2%-5%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	118 (25%, 21%-29%)
Fatigue	ChAdOx1 nCoV-19	147 (30%, 26%-34%)	176 (36%, 32%-41%)	134 (28%, 24%-32%)	30 (6%, 4%-9%)	0 (0%, 0%-1%)	340 (70%, 66%-74%)
	MenACWY	250 (52%, 48%-57%)	169 (35%, 31%-40%)	57 (12%, 9%-15%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	227 (48%, 43%-52%)
Headache	ChAdOx1 nCoV-19	156 (32%, 28%-36%)	168 (34%, 30%-39%)	136 (28%, 24%-32%)	27 (6%, 4%-8%)	0 (0%, 0%-1%)	331 (68%, 64%-72%)
	MenACWY	282 (59%, 55%-64%)	166 (35%, 31%-39%)	26 (5%, 4%-8%)	3 (1%, 0%-2%)	0 (0%, 0%-1%)	195 (41%, 36%-45%)

Symptom	Vaccine Group	None	Mild	Moderate	Severe	Hospitalisation	Any
Malaise	ChAdOx1 nCoV-19	191 (39%, 35%-44%)	136 (28%, 24%-32%)	124 (25%, 22%-30%)	36 (7%, 5%-10%)	0 (0%, 0%-1%)	296 (61%, 56%-65%)
	MenACWY	394 (83%, 79%-86%)	70 (15%, 12%-18%)	12 (3%, 1%-4%)	1 (0%, 0%-1%)	0 (0%, 0%-1%)	83 (17%, 14%-21%)
Nausea	ChAdOx1 nCoV-19	363 (75%, 70%-78%)	81 (17%, 13%-20%)	33 (7%, 5%-9%)	10 (2%, 1%-4%)	0 (0%, 0%-1%)	124 (25%, 22%-30%)
	MenACWY	428 (90%, 87%-92%)	41 (9%, 6%-11%)	8 (2%, 1%-3%)	0 (0%, 0%-1%)	0 (0%, 0%-1%)	49 (10%, 8%-13%)

Table 42. COV001 Solicited adverse local and systemic reactions (group 3 – Prime/Boost). Frequency and 95% CI.

Symptom	1st dose (N=10)					2nd dose (N=10)				
	None	Mild	Moderate	Severe	Hospitalisation	None	Mild	Moderate	Severe	Hospitalisation
Pain	5 (50%, 19%-81%)	4 (40%, 12%-74%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Redness	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Warmth	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Itch	9 (90%, 55%-100%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	9 (90%, 55%-100%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Swelling	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Induration	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Tenderness	5 (50%, 19%-81%)	4 (40%, 12%-74%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	5 (50%, 19%-81%)	5 (50%, 19%-81%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)

Symptom					1st dose (N=10)				2nd dose (N=10)	
Feverishness	3 (30%, 7%-65%)	2 (20%, 3%-56%)	5 (50%, 19%-81%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	1 (10%, 0%-45%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Fever ≥ 38°C	5 (50%, 19%-81%)	5 (50%, 19%-81%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Chills	2 (20%, 3%-56%)	5 (50%, 19%-81%)	3 (30%, 7%-65%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Joint pain	7 (70%, 35%-93%)	2 (20%, 3%-56%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	10 (100%, 69%-100%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Muscle ache	4 (40%, 12%-74%)	4 (40%, 12%-74%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Fatigue	5 (50%, 19%-81%)	4 (40%, 12%-74%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	6 (60%, 26%-88%)	3 (30%, 7%-65%)	1 (10%, 0%-45%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Headache	3 (30%, 7%-65%)	3 (30%, 7%-65%)	4 (40%, 12%-74%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	6 (60%, 26%-88%)	4 (40%, 12%-74%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Malaise	6 (60%, 26%-88%)	0 (0%, 0%-31%)	4 (40%, 12%-74%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)
Nausea	6 (60%, 26%-88%)	1 (10%, 0%-45%)	3 (30%, 7%-65%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	8 (80%, 44%-97%)	2 (20%, 3%-56%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)	0 (0%, 0%-31%)

Table 43. COV001 Unsolicited AEs.

		ChAdOx1 nCOV-19			MenACWY			Total	All
				Max Sev			Max Sev		
MedDRA System Organ Class	MedDRA Preferred Term	Mild	Mod	Sev	Mild	Mod	Sev	ChAd	Men	
Related Adverse Events										
Eye disorders	Eye pain	1	.	.	.	.	.	1	.	1
Gastrointestinal disorders	Abdominal distension	.	.	.	1	.	.	.	1	1
	Abdominal pain upper	1	.	.	.	1	.	1	1	2
	Diarrhoea	1	.	.	1	1	.	1	2	3
	Dry mouth	.	.	.	2	.	.	.	2	2
General disorders and administration sit	Axillary pain	.	.	.	.	1	.	.	1	1
	Chills	.	1	1	.	.	.	2	.	2
	Fatigue	1	.	.	.	.	.	1	.	1
	Feeling abnormal	1	.	.	.	.	.	1	.	1
	Feeling jittery	1	.	.	.	.	.	1	.	1
	Malaise	.	1	1	.	.	.	2	.	2
	Night sweats	.	1	.	.	.	.	1	.	1
	Pain	.	1	.	.	.	.	1	.	1
	Pyrexia	.	.	1	.	.	.	1	.	1
	Vaccination site bruising	1	.	.	1	.	.	1	1	2
	Vaccination site pain	2	.	.	.	.	.	2	.	2
Musculoskeletal and connective tissue di	Arthralgia	.	1	.	.	.	.	1	.	1
	Limb discomfort	1	1	.	.	.	.	2	.	2

		ChAdOx1 nCOV-19			MenACWY			Total	All
				Max Sev			Max Sev		
MedDRA System Organ Class	MedDRA Preferred Term	Mild	Mod	Sev	Mild	Mod	Sev	ChAd	Men	
	Muscle spasms	.	1	.	.	.	.	1	.	1
	Musculoskeletal stiffness	.	1	.	.	.	.	1	.	1
	Neck pain	.	1	.	.	.	.	1	.	1
Nervous system disorders	Abnormal sleep-related event	.	1	.	.	.	.	1	.	1
	Dysgeusia	.	.	.	1	.	.	.	1	1
	Headache	2	1	.	.	.	.	3	.	3
	Insomnia	.	.	.	.	1	.	.	1	1
	Paraesthesia	.	1	.	.	.	.	1	.	1
Psychiatric disorders	Depressed mood	.	1	.	.	.	.	1	.	1
Renal and urinary disorders	Pollakiuria	.	.	.	1	.	.	.	1	1
	Polyuria	1	.	.	.	.	.	1	.	1
Respiratory, thoracic, and mediastinal di	Cough	1	.	.	.	.	.	1	.	1
	Nasopharyngitis	1	.	.	.	.	.	1	.	1
	Oropharyngeal pain	3	.	.	.	.	.	3	.	3
	Rhinorrhoea	2	.	.	.	.	.	2	.	2
Skin and subcutaneous tissue disorders	Pruritus	.	1	.	.	1	.	1	1	2
Vascular disorders	Dizziness	1	1	.	.	.	.	2	.	2

Note: The table above describes only unsolicited AEs reported within the 28-day period for participants in Group 1. More than one MedDRA code can apply to the same event therefore the total number of codes does not equal the total number of participants or events experienced.

6.7.2.2 COV002

Reactogenicity results are presented here for a subset of individuals in the immunology cohorts. Injection-site pain and tenderness were the most common solicited local adverse reactions and occurred most frequently in the first 48 h after vaccination. In those aged 56 years or older, a standard dose of ChAdOx1 nCoV-19, whether the prime or boost vaccination, elicited a greater number of local or systemic reactions than did MenACWY. At least one local symptom was reported after the prime vaccination with standard-dose ChAdOx1 nCoV-19 by 43 (88%) of 49 participants in the 18–55 years group, 22 (73%) of 30 in the 56–69 years group, and 30 (61%) of 49 in the 70 years and older group. Similar proportions of local symptoms were reported after the boost vaccination with the standard dose of ChAdOx1 nCoV-19, with 37 (76%) of 49 participants in the 18–55 years group, 21 (72%) of 29 in the 56–69 years group, and 27 (55%) of 49 in the 70 years and older group reporting at least one local symptom. A similar pattern was seen across the age groups in participants after their prime vaccination with low-dose ChAdOx1 nCoV-19 and after the boost vaccination with the low-dose vaccine, but with fewer total adverse reactions than in the standard-dose groups. In the two-dose control groups, across both the low-dose and standard-dose cohorts, local symptoms were reported by 33 (57%) of 58 participants in the 18–55 years group, 5 (25%) of 20 in the 56–69 years group, and 7 (35%) of 20 in the 70 years and older group after the prime vaccination with MenACWY, and by 50 (86%) of 58 in the 18–55 years group, 7 (37%) of 19 in the 56–69 years group, and 4 (20%) of 20 in the 70 years and older group after the boost vaccination with MenACWY.

Fatigue, headache, feverishness, and myalgia were the most commonly solicited systemic adverse reactions. At least one systemic symptom was reported after the prime vaccination with the standard dose of ChAdOx1 nCoV-19 by 42 (86%) of 49 participants in the 18–55 years group, 23 (77%) of 30 in the 56–69 years group, and 32 (65%) of 49 in the 70 years and older group. The severity of symptoms reported in the standard-dose groups was reduced after the boost vaccination, with only 1 (1%) of 127 participants reporting a severe reaction compared with 7 (5%) of 128 participants after the prime vaccination. At least one systemic AE after the boost vaccination of standard dose of ChAdOx1 nCoV-19 was reported by 32 (65%) of 49 participants in the 18–55 years group, 21 (72%) of 29 in the 56–69 years group, and 21 (43%) of 49 in the 70 years and older group. Within 7 days after the prime vaccination with ChAdOx1 nCoV-19, the incidence of objectively measured fever was low in the 18–55 years standard-dose group (12 [24%] of 49), and no fevers were recorded in either the 56–69 years or 70 years and older standard-dose groups. No participants of any age who received the standard dose of ChAdOx1 nCoV-19 had objective fever after the boost vaccination. A similar pattern of decreasing reactogenicity with increasing age was seen in the low-dose groups.

Table 44. COV002 Summary of solicited AEs.

Symptom	Dose	Age group	Any	None	Adjusted
Pain	LD prime	18-55 years	23 (46.0%)	27 (54.0%)	0.003
		56-69 years	8 (26.7%)	22 (73.3%)	
		70+ years	4 (8.7%)	42 (91.3%)	
	SD prime	18-55 years	30 (61.2%)	19 (38.8%)	0.003
		56-69 years	13 (43.3%)	17 (56.7%)	
		70+ years	10 (20.4%)	39 (79.6%)	
	LD boost	18-55 years	8 (15.7%)	43 (84.3%)	>0.999
		56-69 years	5 (16.7%)	25 (83.3%)	
		70+ years	3 (6.7%)	42 (93.3%)	
	SD boost	18-55 years	24 (49.0%)	25 (51.0%)	0.002
		56-69 years	10 (34.5%)	19 (65.5%)	
		70+ years	5 (10.2%)	44 (89.8%)	
Redness	LD prime	18-55 years	0 (0.0%)	50 (100.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	1 (2.2%)	45 (97.8%)	
	SD prime	18-55 years	0 (0.0%)	49 (100.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	1 (2.0%)	48 (98.0%)	
	LD boost	18-55 years	1 (2.0%)	50 (98.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	2 (4.4%)	43 (95.6%)	
	SD boost	18-55 years	1 (2.0%)	48 (98.0%)	>0.999
		56-69 years	0 (0.0%)	29 (100.0%)	
		70+ years	1 (2.0%)	48 (98.0%)	
		18-55 years	9 (18.0%)	41 (82.0%)	

Symptom	Dose	Age group	Any	None	Adjusted
Warmth	LD prime	56-69 years	1 (3.3%)	29 (96.7%)	0.343
		70+ years	1 (2.2%)	45 (97.8%)	
	SD prime	18-55 years	7 (14.3%)	42 (85.7%)	
		56-69 years	2 (6.7%)	28 (93.3%)	>0.999
		70+ years	7 (14.3%)	42 (85.7%)	
	LD boost	18-55 years	3 (5.9%)	48 (94.1%)	
		56-69 years	0 (0.0%)	30 (100.0%)	>0.999
		70+ years	1 (2.2%)	44 (97.8%)	
	SD boost	18-55 years	6 (12.2%)	43 (87.8%)	
		56-69 years	4 (13.8%)	25 (86.2%)	>0.999
		70+ years	2 (4.1%)	47 (95.9%)	
Itch	LD prime	18-55 years	2 (4.0%)	48 (96.0%)	
		56-69 years	2 (6.7%)	28 (93.3%)	>0.999
		70+ years	1 (2.2%)	45 (97.8%)	
	SD prime	18-55 years	2 (4.1%)	47 (95.9%)	
		56-69 years	2 (6.7%)	28 (93.3%)	>0.999
		70+ years	2 (4.1%)	47 (95.9%)	
	LD boost	18-55 years	1 (2.0%)	50 (98.0%)	
		56-69 years	0 (0.0%)	30 (100.0%)	>0.999
		70+ years	0 (0.0%)	45 (100.0%)	
	SD boost	18-55 years	6 (12.2%)	43 (87.8%)	
		56-69 years	1 (3.4%)	28 (96.6%)	>0.999
		70+ years	1 (2.0%)	48 (98.0%)	
Swelling	LD prime	18-55 years	0 (0.0%)	50 (100.0%)	
		56-69 years	0 (0.0%)	30 (100.0%)	>0.999
		70+ years	1 (2.2%)	45 (97.8%)	
	SD prime	18-55 years	0 (0.0%)	49 (100.0%)	
		56-69 years	0 (0.0%)	30 (100.0%)	>0.999
		70+ years	2 (4.1%)	47 (95.9%)	

Symptom	Dose	Age group	Any	None	Adjusted
	LD boost	18-55 years	0 (0.0%)	51 (100.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	2 (4.4%)	43 (95.6%)	
	SD boost	18-55 years	0 (0.0%)	49 (100.0%)	>0.999
		56-69 years	0 (0.0%)	29 (100.0%)	
		70+ years	2 (4.1%)	47 (95.9%)	
Induration	LD prime	18-55 years	0 (0.0%)	50 (100.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	1 (2.2%)	45 (97.8%)	
	SD prime	18-55 years	0 (0.0%)	49 (100.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	1 (2.0%)	48 (98.0%)	
	LD boost	18-55 years	0 (0.0%)	51 (100.0%)	>0.999
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	2 (4.4%)	43 (95.6%)	
	SD boost	18-55 years	0 (0.0%)	49 (100.0%)	>0.999
		56-69 years	0 (0.0%)	29 (100.0%)	
		70+ years	1 (2.0%)	48 (98.0%)	
Tenderness	LD prime	18-55 years	36 (72.0%)	14 (28.0%)	<0.001
		56-69 years	12 (40.0%)	18 (60.0%)	
		70+ years	11 (23.9%)	35 (76.1%)	
	SD prime	18-55 years	37 (75.5%)	12 (24.5%)	0.547
		56-69 years	20 (66.7%)	10 (33.3%)	
		70+ years	24 (49.0%)	25 (51.0%)	
	LD boost	18-55 years	30 (58.8%)	21 (41.2%)	0.016
		56-69 years	11 (36.7%)	19 (63.3%)	
		70+ years	10 (22.2%)	35 (77.8%)	
	SD boost	18-55 years	30 (61.2%)	19 (38.8%)	>0.999
		56-69 years	17 (58.6%)	12 (41.4%)	

Symptom	Dose	Age group	Any	None	Adjusted
		70+ years	23 (46.9%)	26 (53.1%)	
Feverish	LD prime	18-55 years	8 (16.0%)	42 (84.0%)	>0.999
		56-69 years	4 (13.3%)	26 (86.7%)	
		70+ years	1 (2.2%)	45 (97.8%)	
	SD prime	18-55 years	21 (42.9%)	28 (57.1%)	0.008
		56-69 years	3 (10.0%)	27 (90.0%)	
		70+ years	5 (10.2%)	44 (89.8%)	
	LD boost	18-55 years	3 (5.9%)	48 (94.1%)	>0.999
		56-69 years	1 (3.3%)	29 (96.7%)	
		70+ years	3 (6.7%)	42 (93.3%)	
	SD boost	18-55 years	5 (10.2%)	44 (89.8%)	>0.999
		56-69 years	4 (13.8%)	25 (86.2%)	
		70+ years	4 (8.2%)	45 (91.8%)	
Fever	LD prime	18-55 years	0 (0.0%)	50 (100.0%)	>0.999
		56-69 years	2 (6.7%)	28 (93.3%)	
		70+ years	2 (4.3%)	44 (95.7%)	
	SD prime	18-55 years	12 (24.5%)	37 (75.5%)	<0.001
		56-69 years	0 (0.0%)	30 (100.0%)	
		70+ years	0 (0.0%)	49 (100.0%)	
	LD boost	18-55 years	0 (0.0%)	51 (100.0%)	>0.999
		56-69 years	1 (3.3%)	29 (96.7%)	
		70+ years	0 (0.0%)	45 (100.0%)	
	SD boost	18-55 years	0 (0.0%)	48 (100.0%)	-
		56-69 years	0 (0.0%)	29 (100.0%)	
		70+ years	0 (0.0%)	49 (100.0%)	
Chills	LD prime	18-55 years	6 (12.0%)	44 (88.0%)	>0.999
		56-69 years	3 (10.0%)	27 (90.0%)	
		70+ years	1 (2.2%)	45 (97.8%)	
		18-55 years	17 (34.7%)	32 (65.3%)	

Symptom	Dose	Age group	Any	None	Adjusted
	SD prime	56-69 years	3 (10.0%)	27 (90.0%)	0.004
		70+ years	2 (4.1%)	47 (95.9%)	
	LD boost	18-55 years	3 (5.9%)	48 (94.1%)	>0.999
		56-69 years	1 (3.3%)	29 (96.7%)	
		70+ years	1 (2.2%)	44 (97.8%)	
	SD boost	18-55 years	7 (14.3%)	42 (85.7%)	0.787
		56-69 years	3 (10.3%)	26 (89.7%)	
		70+ years	0 (0.0%)	49 (100.0%)	
Joint pain	LD prime	18-55 years	9 (18.0%)	41 (82.0%)	>0.999
		56-69 years	2 (6.7%)	28 (93.3%)	
		70+ years	4 (8.7%)	42 (91.3%)	
	SD prime	18-55 years	16 (32.7%)	33 (67.3%)	>0.999
		56-69 years	5 (16.7%)	25 (83.3%)	
		70+ years	7 (14.3%)	42 (85.7%)	
	LD boost	18-55 years	3 (5.9%)	48 (94.1%)	>0.999
		56-69 years	3 (10.0%)	27 (90.0%)	
		70+ years	1 (2.2%)	44 (97.8%)	
	SD boost	18-55 years	3 (6.1%)	46 (93.9%)	>0.999
		56-69 years	5 (17.2%)	24 (82.8%)	
		70+ years	4 (8.2%)	45 (91.8%)	
Muscle ache	LD prime	18-55 years	17 (34.0%)	33 (66.0%)	0.285
		56-69 years	3 (10.0%)	27 (90.0%)	
		70+ years	5 (10.9%)	41 (89.1%)	
	SD prime	18-55 years	26 (53.1%)	23 (46.9%)	0.029
		56-69 years	11 (36.7%)	19 (63.3%)	
		70+ years	9 (18.4%)	40 (81.6%)	
	LD boost	18-55 years	11 (21.6%)	40 (78.4%)	>0.999
		56-69 years	3 (10.0%)	27 (90.0%)	
		70+ years	5 (11.1%)	40 (88.9%)	

Symptom	Dose	Age group	Any	None	Adjusted
	SD boost	18-55 years	17 (34.7%)	32 (65.3%)	>0.999
		56-69 years	7 (24.1%)	22 (75.9%)	
		70+ years	9 (18.4%)	40 (81.6%)	
Fatigue	LD prime	18-55 years	27 (54.0%)	23 (46.0%)	>0.999
		56-69 years	11 (36.7%)	19 (63.3%)	
		70+ years	14 (30.4%)	32 (69.6%)	
	SD prime	18-55 years	37 (75.5%)	12 (24.5%)	0.046
		56-69 years	15 (50.0%)	15 (50.0%)	
		70+ years	20 (40.8%)	29 (59.2%)	
	LD boost	18-55 years	18 (35.3%)	33 (64.7%)	>0.999
		56-69 years	8 (26.7%)	22 (73.3%)	
		70+ years	9 (20.0%)	36 (80.0%)	
	SD boost	18-55 years	27 (55.1%)	22 (44.9%)	>0.999
		56-69 years	12 (41.4%)	17 (58.6%)	
		70+ years	16 (32.7%)	33 (67.3%)	
Headache	LD prime	18-55 years	21 (42.0%)	29 (58.0%)	0.133
		56-69 years	9 (30.0%)	21 (70.0%)	
		70+ years	6 (13.0%)	40 (87.0%)	
	SD prime	18-55 years	32 (65.3%)	17 (34.7%)	>0.999
		56-69 years	15 (50.0%)	15 (50.0%)	
		70+ years	20 (40.8%)	29 (59.2%)	
	LD boost	18-55 years	13 (25.5%)	38 (74.5%)	>0.999
		56-69 years	4 (13.3%)	26 (86.7%)	
		70+ years	4 (8.9%)	41 (91.1%)	
	SD boost	18-55 years	15 (30.6%)	34 (69.4%)	>0.999
		56-69 years	10 (34.5%)	19 (65.5%)	
		70+ years	10 (20.4%)	39 (79.6%)	
Malaise	LD prime	18-55 years	12 (24.0%)	38 (76.0%)	>0.999
		56-69 years	4 (13.3%)	26 (86.7%)	

Symptom	Dose	Age group	Any	None	Adjusted
		70+ years	6 (13.0%)	40 (87.0%)	
	SD prime	18-55 years	20 (40.8%)	29 (59.2%)	>0.999
		56-69 years	8 (26.7%)	22 (73.3%)	
		70+ years	12 (24.5%)	37 (75.5%)	
	LD boost	18-55 years	5 (9.8%)	46 (90.2%)	>0.999
		56-69 years	4 (13.3%)	26 (86.7%)	
		70+ years	2 (4.4%)	43 (95.6%)	
	SD boost	18-55 years	14 (28.6%)	35 (71.4%)	>0.999
		56-69 years	3 (10.3%)	26 (89.7%)	
		70+ years	6 (12.2%)	43 (87.8%)	
Nausea	LD prime	18-55 years	5 (10.0%)	45 (90.0%)	>0.999
		56-69 years	2 (6.7%)	28 (93.3%)	
		70+ years	3 (6.5%)	43 (93.5%)	
	SD prime	18-55 years	13 (26.5%)	36 (73.5%)	>0.999
		56-69 years	4 (13.3%)	26 (86.7%)	
		70+ years	4 (8.2%)	45 (91.8%)	
	LD boost	18-55 years	5 (9.8%)	46 (90.2%)	>0.999
		56-69 years	3 (10.0%)	27 (90.0%)	
		70+ years	1 (2.2%)	44 (97.8%)	
	SD boost	18-55 years	4 (8.2%)	45 (91.8%)	>0.999
		56-69 years	6 (20.7%)	23 (79.3%)	
		70+ years	3 (6.1%)	46 (93.9%)	

*p values from Chi-square test for trend.

6.7.2.3 SAEs

As of 30th September 2021, 656 SAEs were reported across the COV001-002-003 studies which were balanced between control and intervention arms. 7 of those events resulted in death (2 cancers, 1 road traffic accident, 1 COVID-19 related death [control group], 2 myocardial infarction episodes, and 1 sepsis associated with immunosuppressive state which was unknown before enrolment). None of the fatal events were deemed related to trial interventions. Three of the non-fatal SAEs were considered to be at least possibly related to either ChAdOx1 nCoV-19 (myelitis) or MenACWY (myelitis, haemolytic anaemia).

Three cases of transverse myelitis were initially reported as suspected unexpected serious adverse reactions, with two in the ChAdOx1 nCoV-19 vaccine study arm, triggering a study pause for review in each case. Independent clinical review of these cases indicated that 1 in the experimental group and 1 in the control group were unlikely to be related to study interventions, but a relationship remained possible in the third case. A 4th case of transverse myelitis in the control group was deemed not related to study interventions. Two multiple sclerosis episodes (both in the intervention arm) and 2 optic neuritis cases (1 in the control arm) were deemed not related to any of the study interventions.

Very rare events of anaphylaxis, angioedema, capillary leak syndrome, thrombosis with thrombocytopenia and GBS have been observed following mass administration of ChAdOx1-nCoV-19 [220]. However, none of these events have been reported to date as part of the University of Oxford COVID-19 vaccine sponsored trials. Multiple sclerosis and transverse myelitis have not been

identified to date as a safety signal, as their incidence amongst those vaccinated with ChAdOx1 nCoV-19 fall within the expected background rates.

6.7.3 Humoral immunogenicity

6.7.3.1 COV001 and COV002

6.7.3.1.1 Single Standard Dose

IgG GMT for participants aged 18 and above who received a single standard dose at day 28 (Groups 1 and 2 in COV001 and Groups 7a, 8a and 5b/c in COV002) was 1.466 in the control group (n=200, 95%CI 1.293 – 1.661) and 176.9 (n=352, 95%CI 156.3-200.2) in the ChAdOx1 nCoV-19 arm.

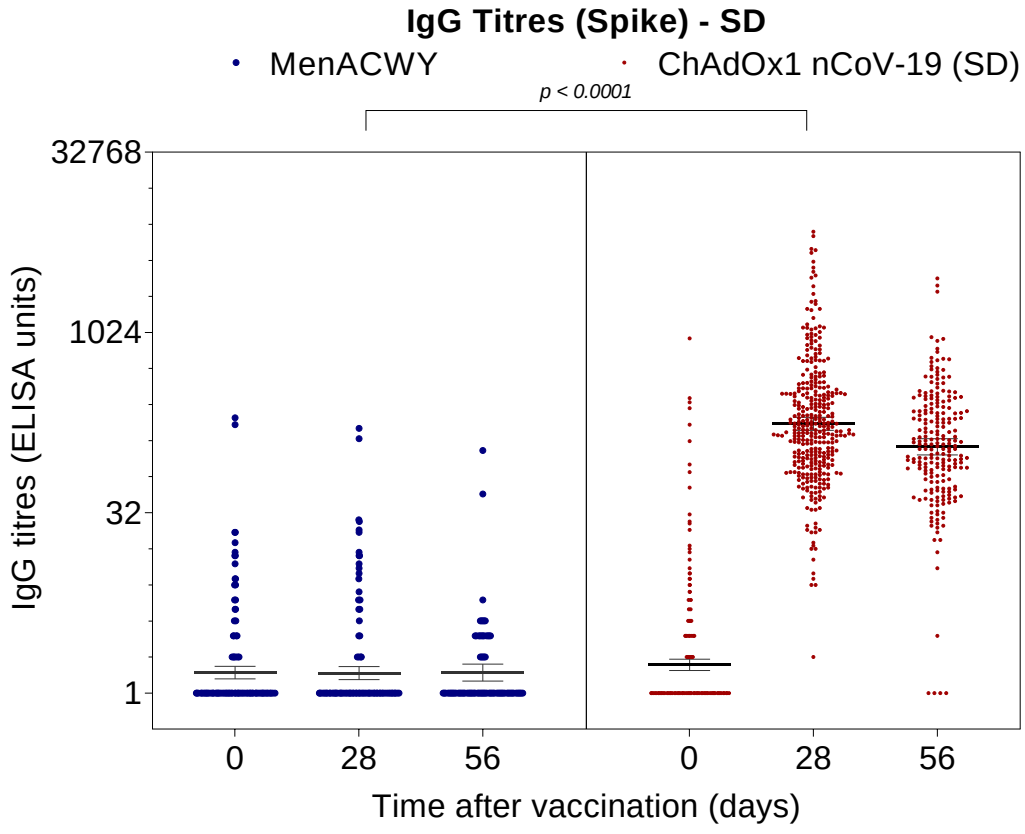


Figure 36. COV001 and COV002. IgG titres (Spike) after a single SD

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test

Pseudo-neutralisation GMT at day 28 was 42.09 in the control group (n=78, 95%CI 39.19-45.21), and 88.49 in the in the ChAdOx1 nCoV-19 arm (n=177, 95%CI 75.04-104.4).

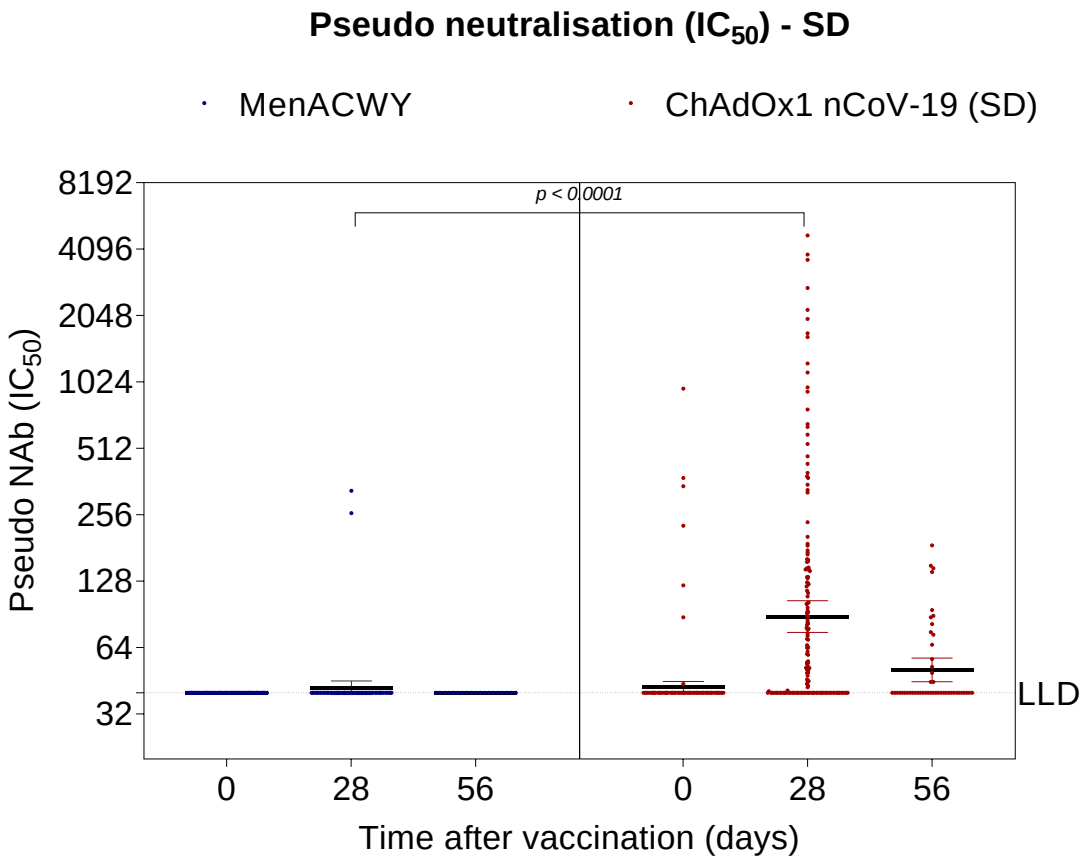


Figure 37. COV001 and COV002 Pseudo-neutralisation after single SD

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test.

6.7.3.1.2 Single Low Dose

IgG GMT for participants aged 18 and above who received a single low dose at day 28 (Groups 1a, 2a and 5a in COV002) was 1.610 in the control group (n=70, 95%CI 1.290 – 2.010) and 103.7 (n=124, 95%CI 83.83-128.2) in the ChAdOx1 nCoV-19 arm.

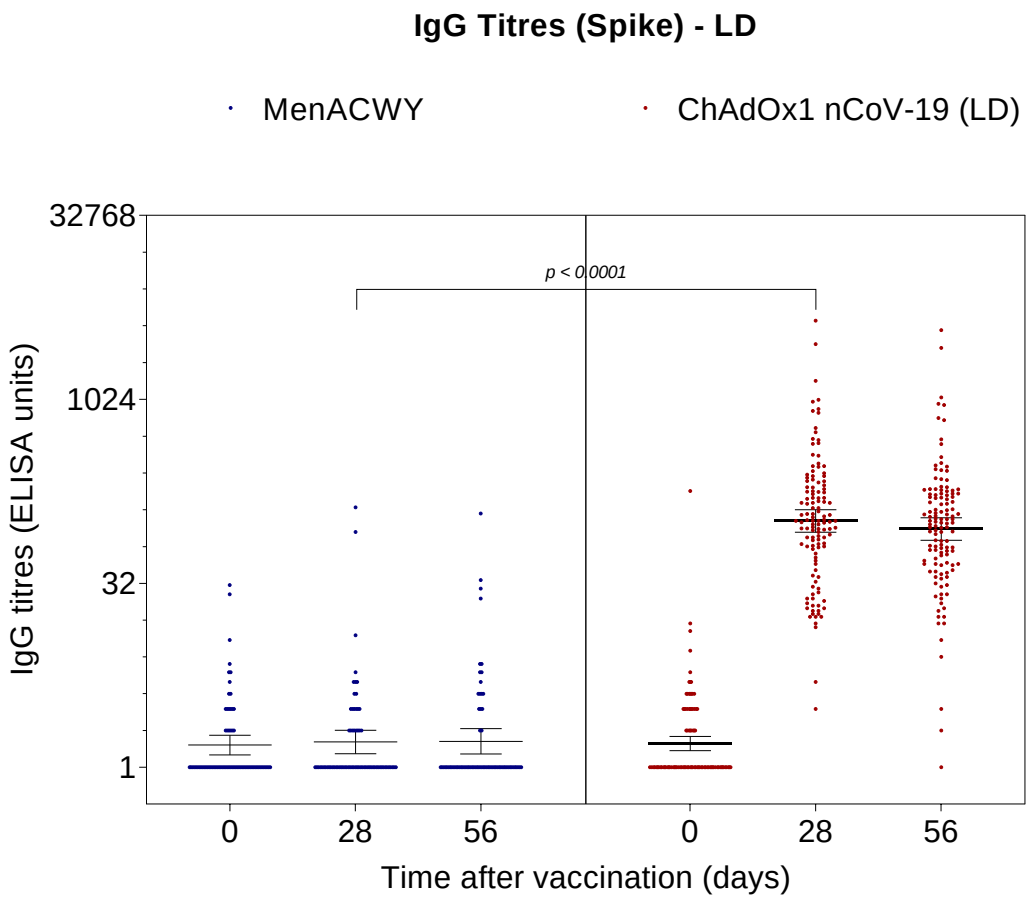


Figure 38. COV001 and COV002 IgG titres (Spike) after a single LD

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test.

Pseudo-neutralisation GMT at day 28 was 40 in the control group (n=31, 95%CI 40-40), and 68.76 in the in the ChAdOx1 nCoV-19 arm (n=118, 95%CI 57.45-82.31).

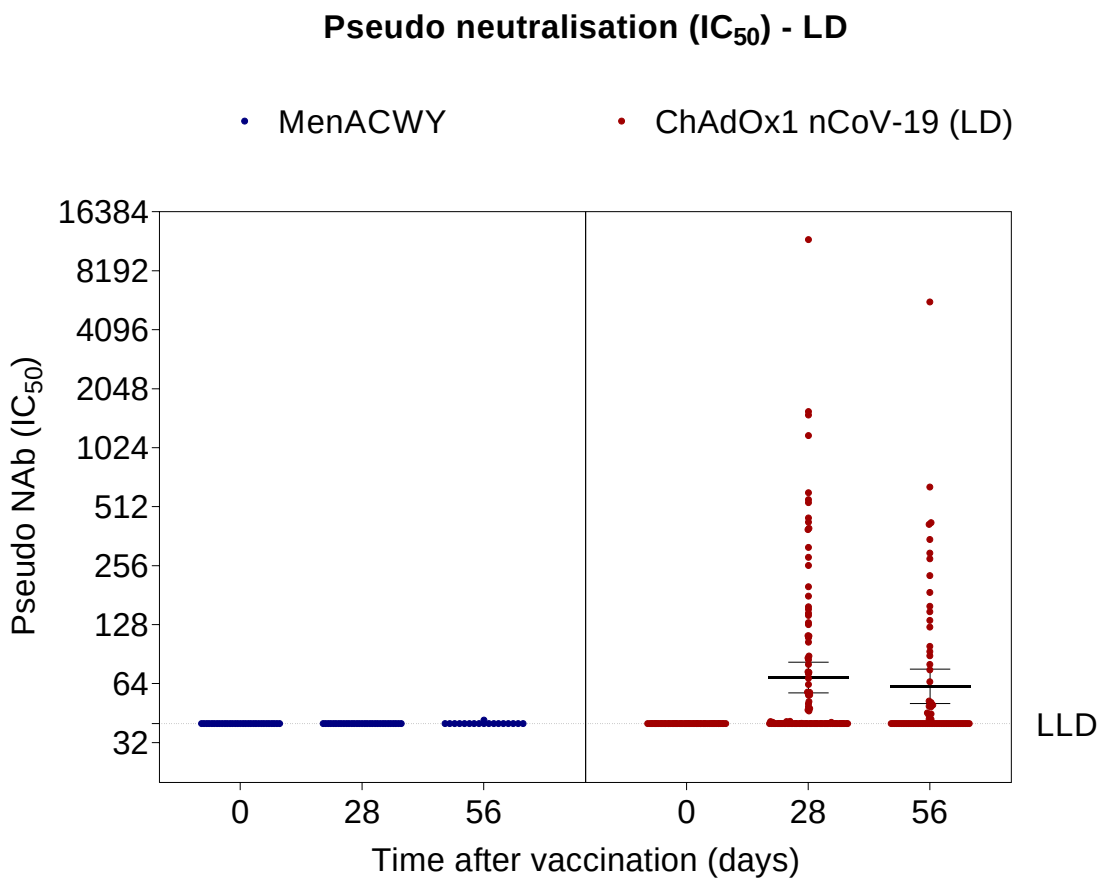


Figure 39. COV001 and COV002 Pseudo-neutralisation after single LD

Black lines represent GMT with 95% CI.

6.7.3.1.3 Two Standard Doses

IgG GMT for participants aged 18 and above who received a 2-standard dose at day 56 (Group 3 in COV001 and Groups 5d, 5e, 7b, and 8b in COV002, 4-6 weeks apart) was 2.301 in the control group (n=30, 95%CI 1.553 – 3.408) and 558 (n=148, 95%CI 476.7-653.1) in the ChAdOx1 nCoV-19 arm.

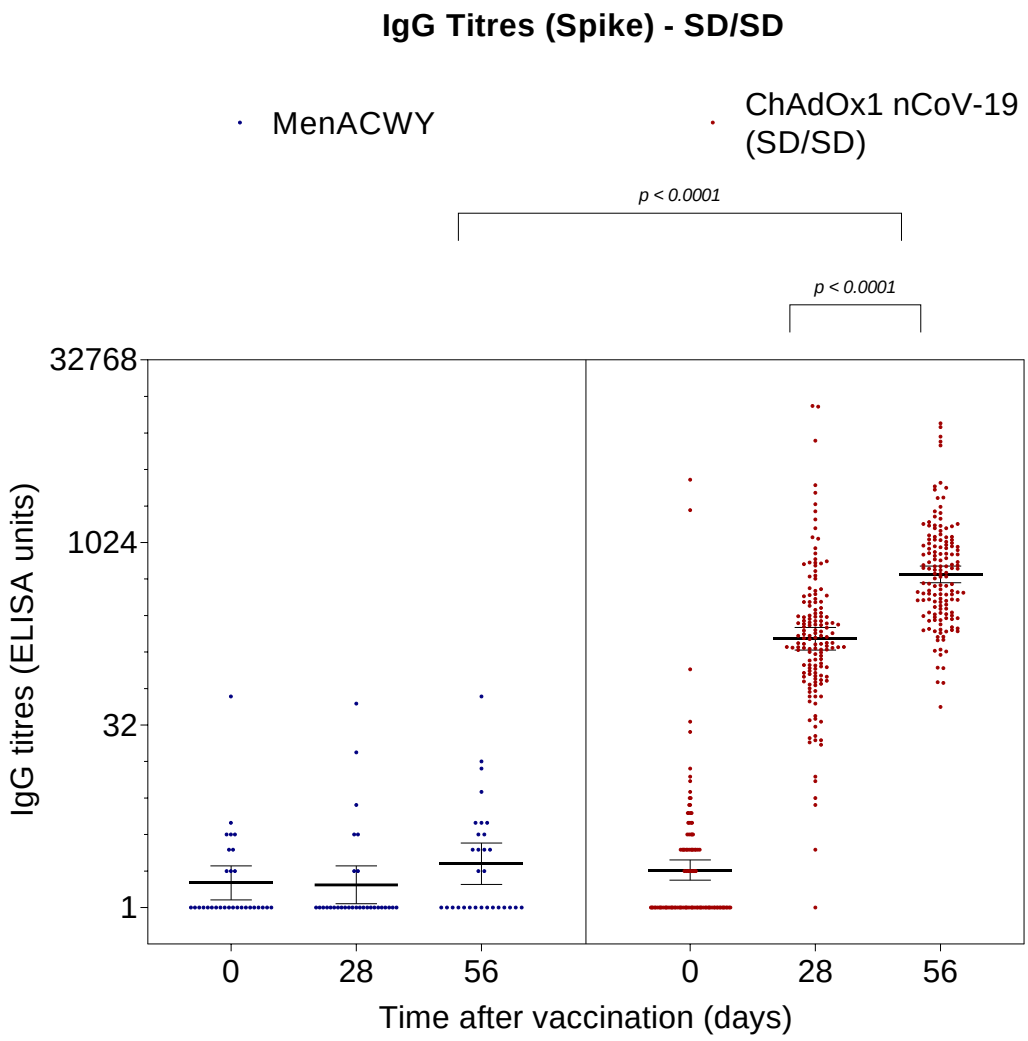


Figure 40. COV001 and COV002 IgG titres (Spike) after 2 SD (SD/SD

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test (MenACWY vs ChAdOx1 nCoV-19 at day 56) or Wilcoxon matched-pairs signed-rank test (ChAdOx1 nCoV-19 day 28 vs day 56).

Pseudo-neutralisation GMT at 14 days post boost (except Group 5e in COV002 – missing data) was 40 in the control group (n=27, 95%CI 40-40), and 172.8 in the in the ChAdOx1 nCoV-19 arm (n=127, 95%CI 146.2-204.3).

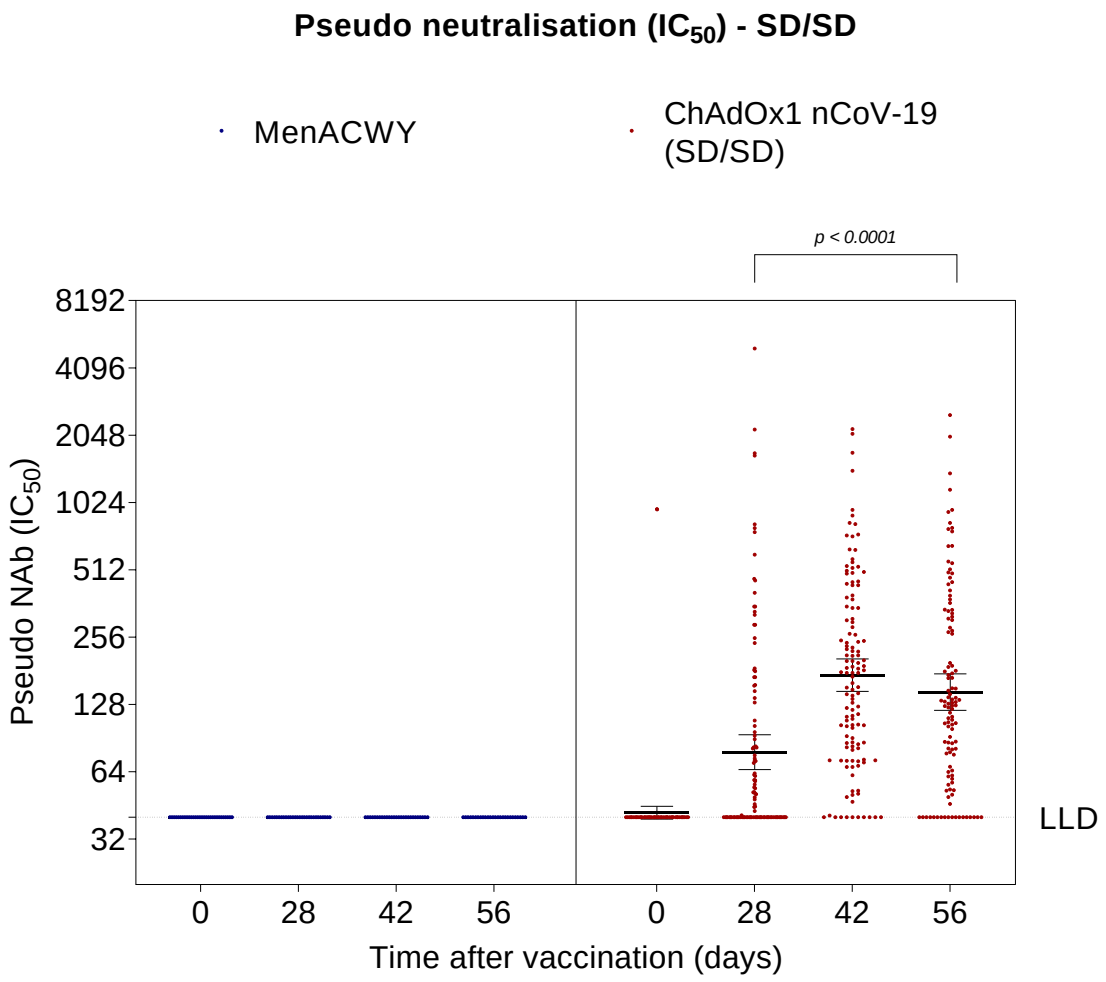


Figure 41. COV001 and COV002 Pseudo-neutralisation after 2 SD (SD/SD)

Black lines represent GMT with 95% CI. *P* value for Wilcoxon matched-pairs signed-rank test (ChAdOx1 nCoV-19 day 28 vs day 56).

6.7.3.1.4 Two Low Doses

IgG GMT for participants aged 18 and above who received a 2 low doses at day 56 (Groups 4b, 1b, and 2b in COV002, 4-6 weeks apart) was 1.777 in the control group (n=68, 95%CI 1.329 – 2.377) and 350.9 (n=121, 95%CI 284.4-433.1) in the ChAdOx1 nCoV-19 arm.

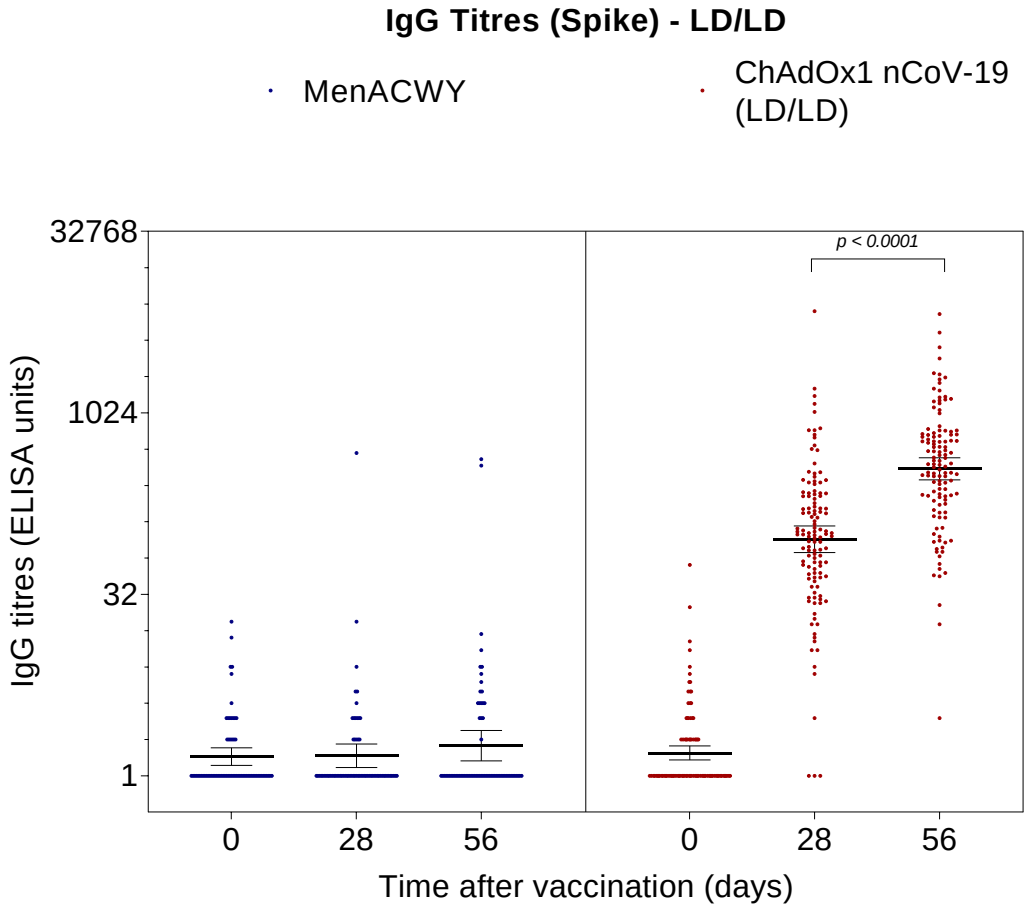


Figure 42. COV001 and COV002 IgG titres (Spike) after 2 low doses (LD/LD)

Black lines represent GMT with 95% CI. *P* value for Wilcoxon matched-pairs signed-rank test (ChAdOx1 nCoV-19 day 28 vs day 56).

Pseudo-neutralisation GMT at 14 days post boost was 40.43 in the control group (n=63, 95%CI 39.58-41.3), and 160.4 in the in the ChAdOx1 nCoV-19 arm (n=120, 95%CI 137.8-186.7).

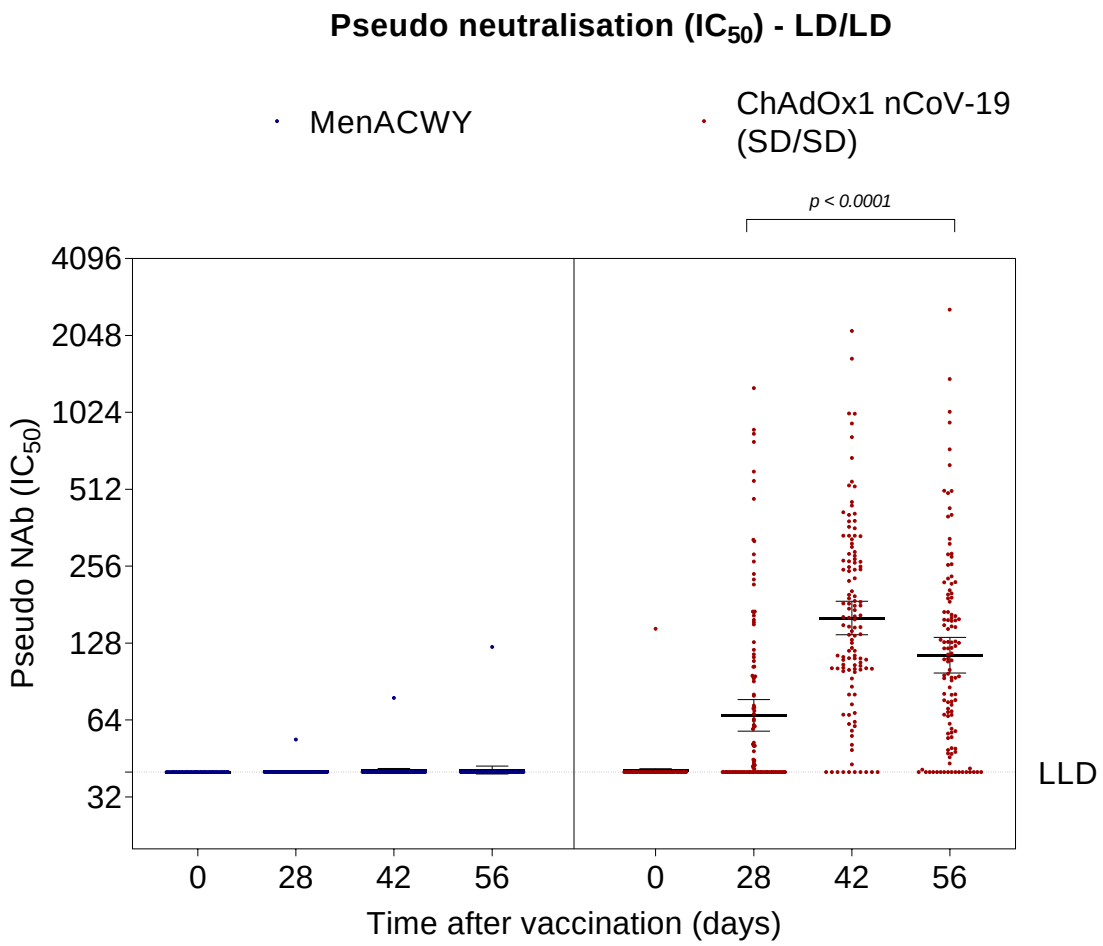


Figure 43. COV001 and COV002 Pseudo neutralisation after 2 LD (LD/LD)

Black lines represent GMT with 95% CI. *P* value for Wilcoxon matched-pairs signed-rank test (ChAdOx1 nCoV-19 day 28 vs day 56).

6.7.3.1.5 SD/SD vs LD/SD

IgG GMT for participants aged 18-55 who received either SD/SD or LD/SD at day 56 (Groups 5e and 5f in COV002, 4-6 weeks apart) was 818.9 in the SD/SD group (n=15, 95%CI 430.5 – 1558) and 728.5 (n=15, 95%CI 489.8-1083) in the LD/SD arm.

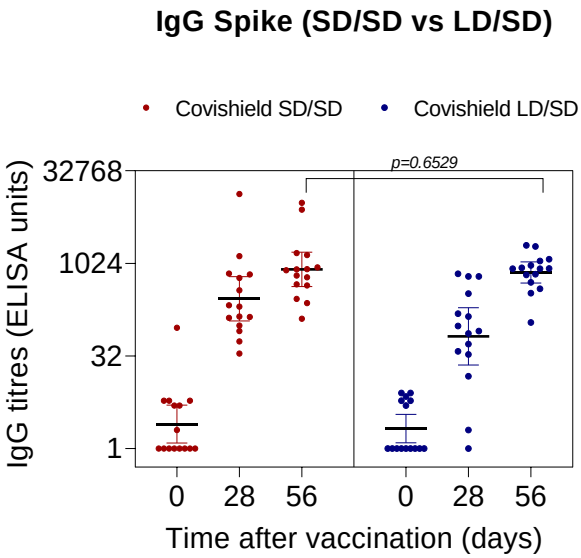


Figure 44. COV002 IgG titres (Spike) after SD or LD prime followed by SD boost

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test

6.7.3.1.6 Batch comparison

IgG GMT at day 28 (post-prime vaccination) was similar across the 4 different manufacturer's products used in the studies, with no significant differences between them.

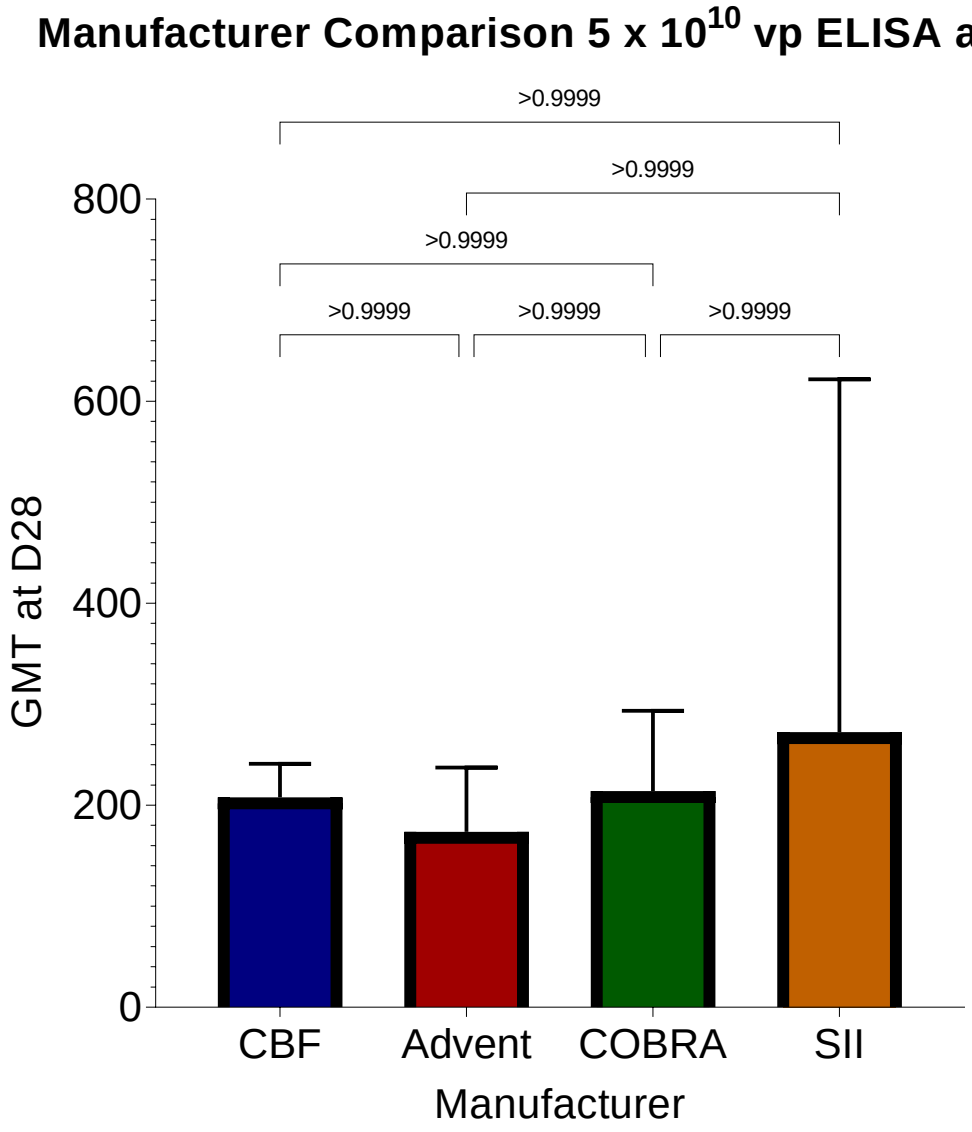


Figure 45. COV001 and COV002 IgG (Spike) at day 28 post-prime (batch comparison groups).

Black lines represent GMT with 95% CI. *P* values for Kruskal Wallis test with Dunn's correction for multiple comparisons

6.7.3.1.5 Antibody responses in ChAdOx1 primed individuals

IgG GMT at day 56 (28 days post-boost vaccination) was similar in a small number of volunteers who previously received a ChAdOx1 vectored vaccine (13-28 months before) expressing Men B and MERS-CoV antigens (Group 11 in

COV002), compared to those who were ChAdOx1 naïve (Group 3 in COV001) with no significant differences between them.

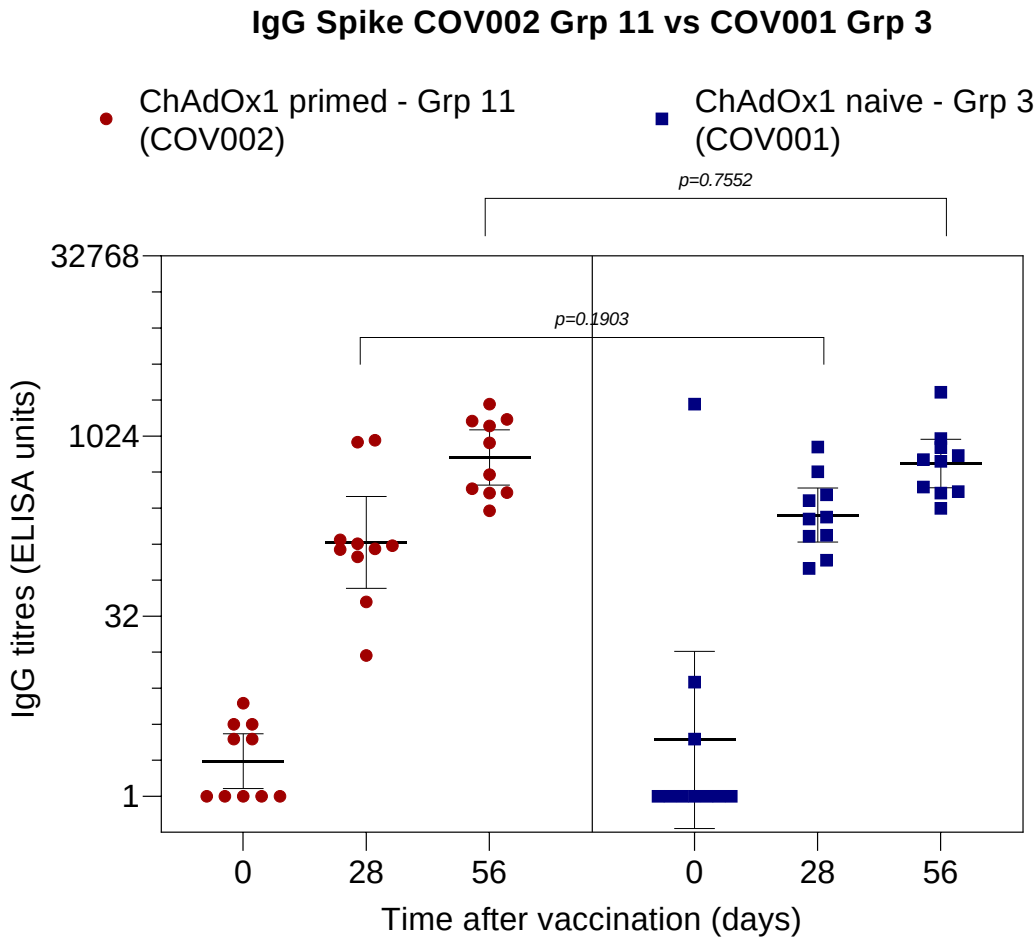


Figure 46. COV001 and COV002 ChAdOx1 primed vs ChAdOx1 naïve.

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test

Table 45. COV001 and COV002 IgG GMT (Spike) with different regimens

	IgG GMT (95%CI)					P value
Regimen	**SD**	**LD**	**SD/SD**	**LD/LD**	**LD/SD**	
Day 28	176.9 (156.3-200.2)	103.7 (83.83-128.2)	164.5 (132.5-204.3)	91.49 (71.10-117.7)	66.59 (22.77-194.8)	<0.0001
Day 56	113.6 (97.09-133)	89.04 (72.08-110)	558 (476.7-653.1)	350.9 (284.4-433.1)	728.5 (489.8-1083)	<0.0001

P value Kruskal Wallis test

Table 46. COV001 and COV002 Pseudo virus neutralisation titres (IC_{50}) with different regimens

	Pseudovirus neutralising GMT (95%CI)				P value
Regimen	**SD**	**LD**	**SD/SD**	**LD/LD**	
Day 28	88.49 (75.04-104.4)	68.76 (57.45-82.31)	78.25 (65.45-93.55)	66.67 (57.86-76.82)	0.0366
Day 56	50.69 (44.8-57.35)	61.95 (50.63-75.79)	145.2 (120.4-175.1)	114.7 (97.78-134.6)	<0.0001

P value Kruskal Wallis test

6.7.4 Cellular immunogenicity

6.7.4.1 COV001 and COV002

6.7.4.1.1 Single Standard Dose

IFN-γ ELISpot geometric mean responses for participants aged 18 and above who received a single standard dose at day 14 (Groups 1 and 2 in COV001 and Groups 7a, 8a and 5c in COV002) was 81.98 in the control group (n=81, 95%CI 70.95–94.74) and 794.6 (n=130, 95%CI 664.7-949.9) in the ChAdOx1 nCoV-19 arm.

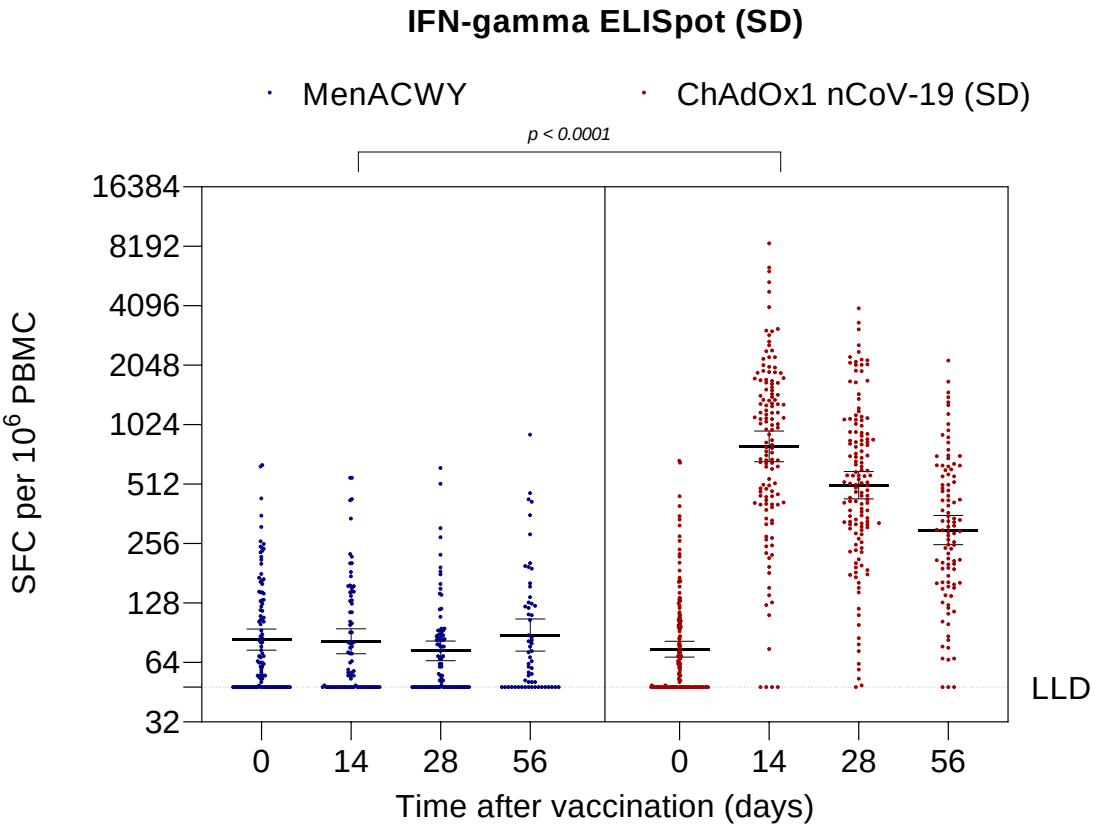


Figure 47. COV001 and COV002 IFN-γ ELISpot responses following a single SD

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test

6.7.4.1.2 Single Low Dose

IFN-γ ELISpot geometric mean responses for participants aged 18 and above who received a single low dose at day 14 (Groups 1a, 2a, and 5a in COV002) was 83.3 in the control group (n=61, 95%CI 71.20–97.47) and 1034 (n=121, 95%CI 886.7-1205) in the ChAdOx1 nCoV-19 arm.

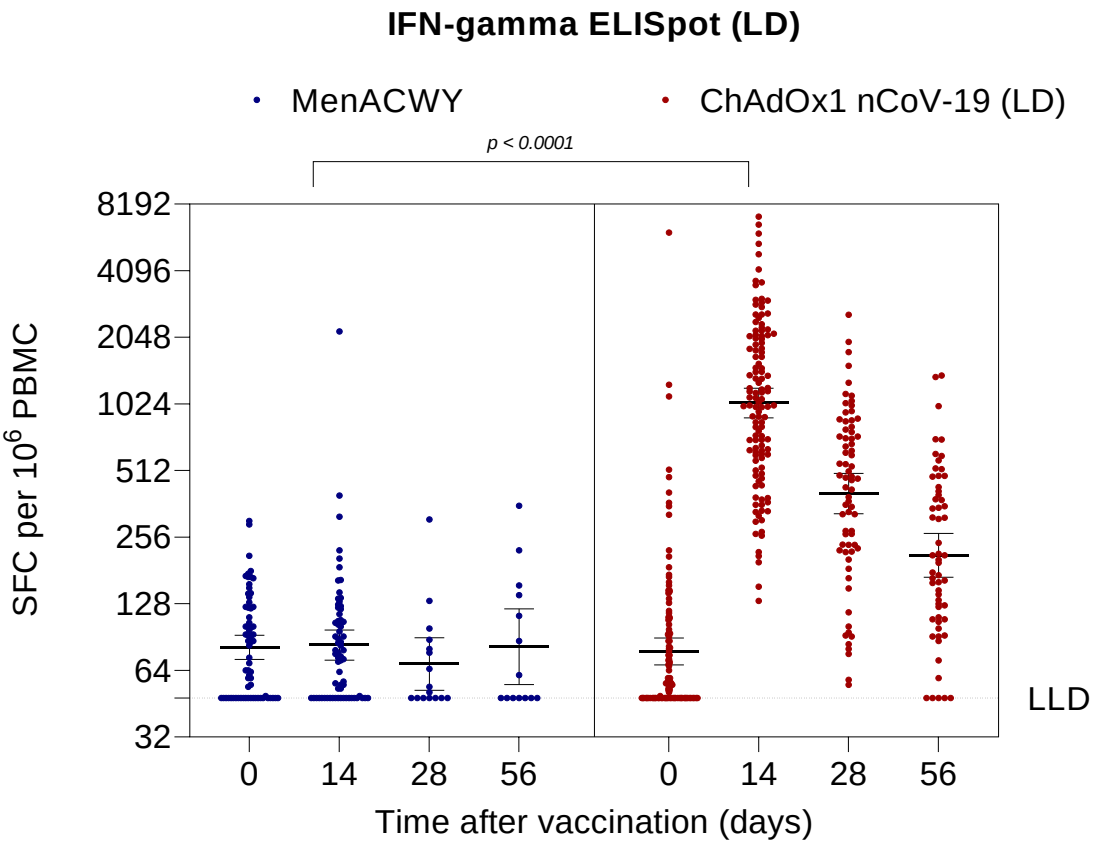


Figure 48. COV001 and COV002 IFN-γ ELISpot responses following a single LD

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test

6.7.4.1.3 Two Standard Doses

IFN-γ ELISpot geometric mean responses for participants aged 18 and above who received 2 standard doses at day 14 days post-boost (Group 3 in COV001 and Groups 5d, 5e, 7b, 8b in COV002) was 76.4 in the control group (n=23,

95%CI 61.78–94.47) and 387.1 (n=115, 95%CI 327.5-457.5) in the ChAdOx1 nCoV-19 arm. No boost in T cell responses was observed following a second dose of ChAdOx1 nCoV-19.

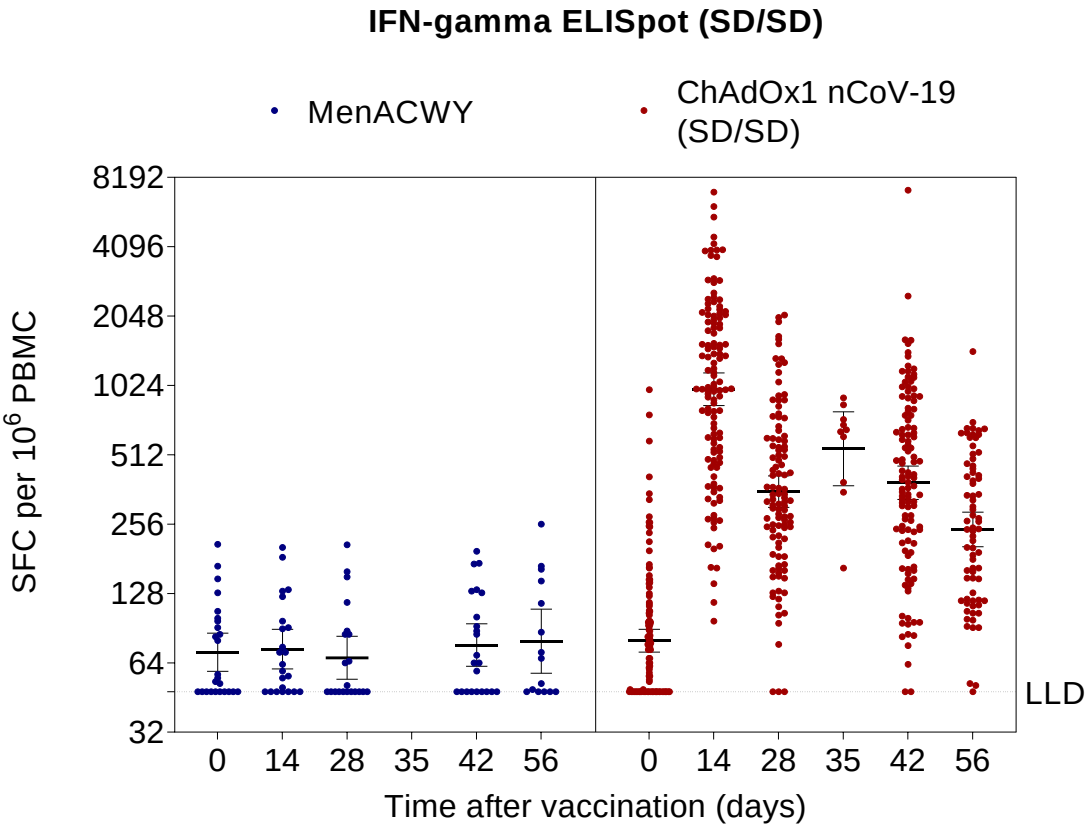


Figure 49. COV001 and COV002 IFN-γ ELISpot responses following 2 SD

Black lines represent GMT with 95% CI.

6.7.4.1.4 Two Low Doses

IFN-γ ELISpot geometric mean responses for participants aged 56 and above (missing data for those aged 18-55) who received 2 low doses at day 14 days post-boost (Groups 1b and 2b in COV002) was 68.94 in the control group (n=16, 95%CI 55.33–85.91) and 413.5 (n=65, 95%CI 331.2-516.4) in the ChAdOx1

nCoV-19 arm. No boost in T cell responses was observed following a second dose of ChAdOx1 nCoV-19.

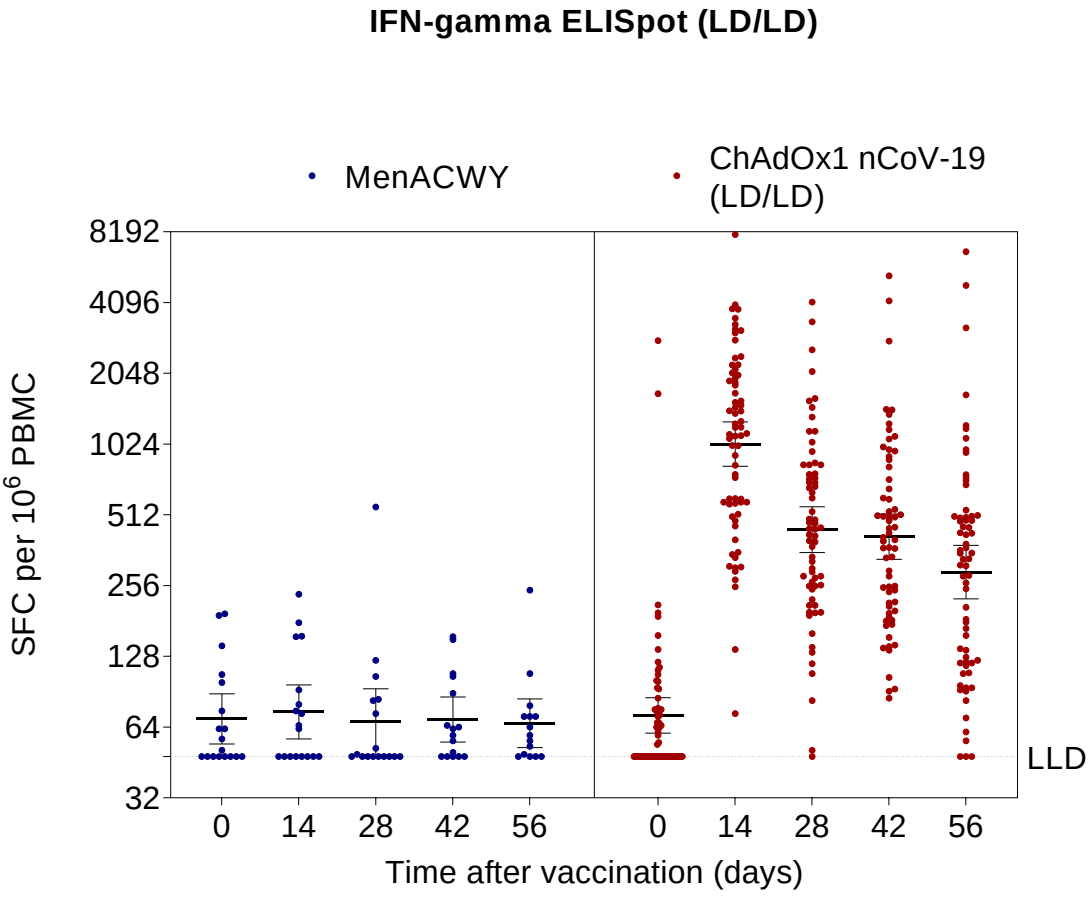


Figure 50. COV002 IFN-γ ELISpot responses following 2 LD in those aged 56+

Black lines represent GMT with 95% CI.

6.7.4.1.5 SD/SD vs LD/SD

IFN-γ ELISpot geometric mean responses for participants aged 18-55 at day 14 days post-boost (Groups 5e and 5f in COV002) was 184.4 in SD/SD group (n=12, 95%CI 110.5–307.5) and 302.4 (n=9, 95%CI 128.8-710.2) in the LD/SD group. No boost in T cell responses was observed following a second dose of ChAdOx1 nCoV-19.

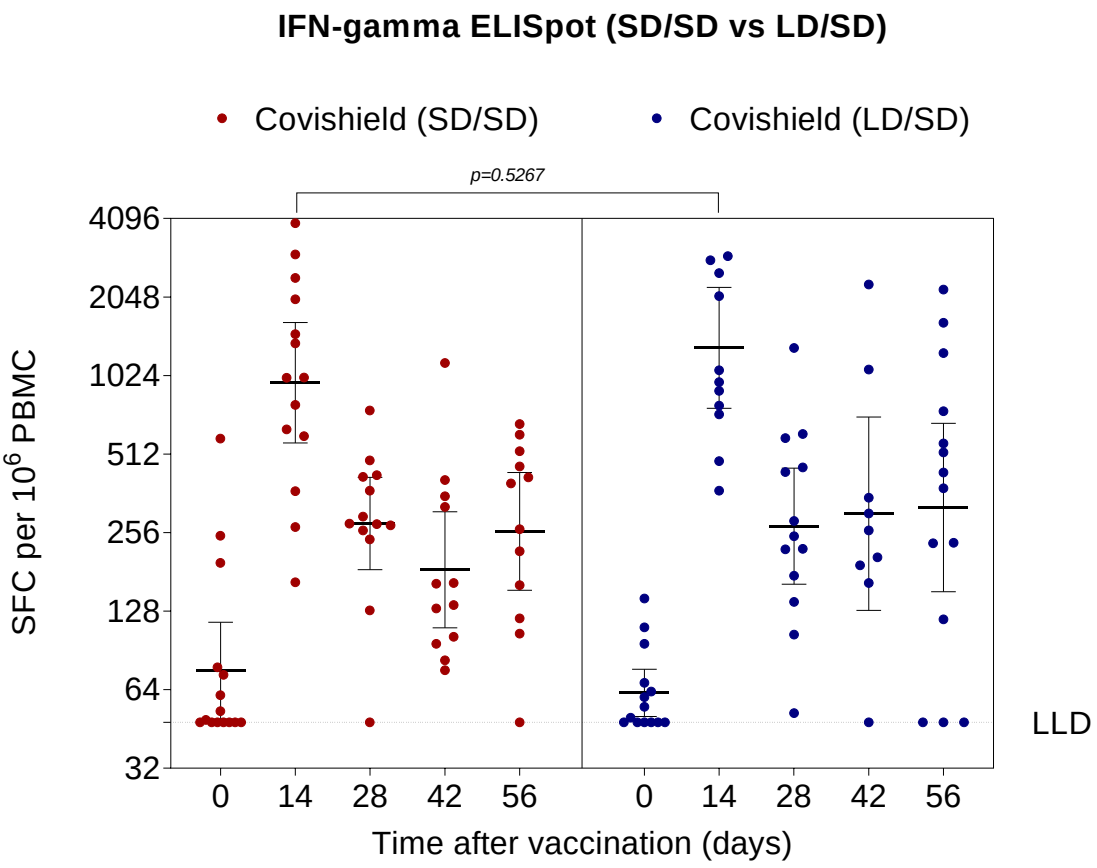


Figure 51. COV002 IFN-γ ELISpot responses following an SD/SD or LD/SD in those aged 18-55.

Black lines represent GMT with 95% CI. *P* value for Mann Whitney test

Table 47. COV001 and COV002 Geometric mean IFN-γ ELISpot responses with different regimens

			ELISpot GMT (95%CI)			P value
Regimen	**SD**	**LD**	**SD/SD**	**LD/LD**	**LD/SD**	
Day 14	794.6 (664.7-949.9)	1034 (886.7-1205)	985.4 (836.6-1161)	1023 (823.1-1272)	1308 (767.6-2228)	0.2529
Day 56	299.9 (253-355.3)	211.8 (168.5-266.2)	242.6 (204-288.5)	291.9 (224.6-379.4)	319.5 (151.9-672.1)	0.1461

P value for Kruskal Wallis test

Table 48. COV001 and COV002 Fold increase in geometric mean IFN-γ ELISpot responses with different regimens

	Fold increase ELISpot GMT from D0				
Regimen	**SD**	**LD**	**SD/SD**	**LD/LD**	**LD/SD**
Day 14	10.64	13.26	12.35	14.27	21
Day 56	4.02	2.72	3.04	4.07	5.13

6.7.5 Anti-vector Responses

GMT for anti-ChAdOx1 neutralising antibodies post-prime vaccinations for those aged 18+ who received a low dose prime were 201.2 (n=133, 95%CI 155.2-317.1), and 235.9 (n=83, 95%CI 175.5-317.1) for those who received a standard dose prime. No boosting in anti-vector responses were observed following a second dose of ChAdOx1 nCoV-19.

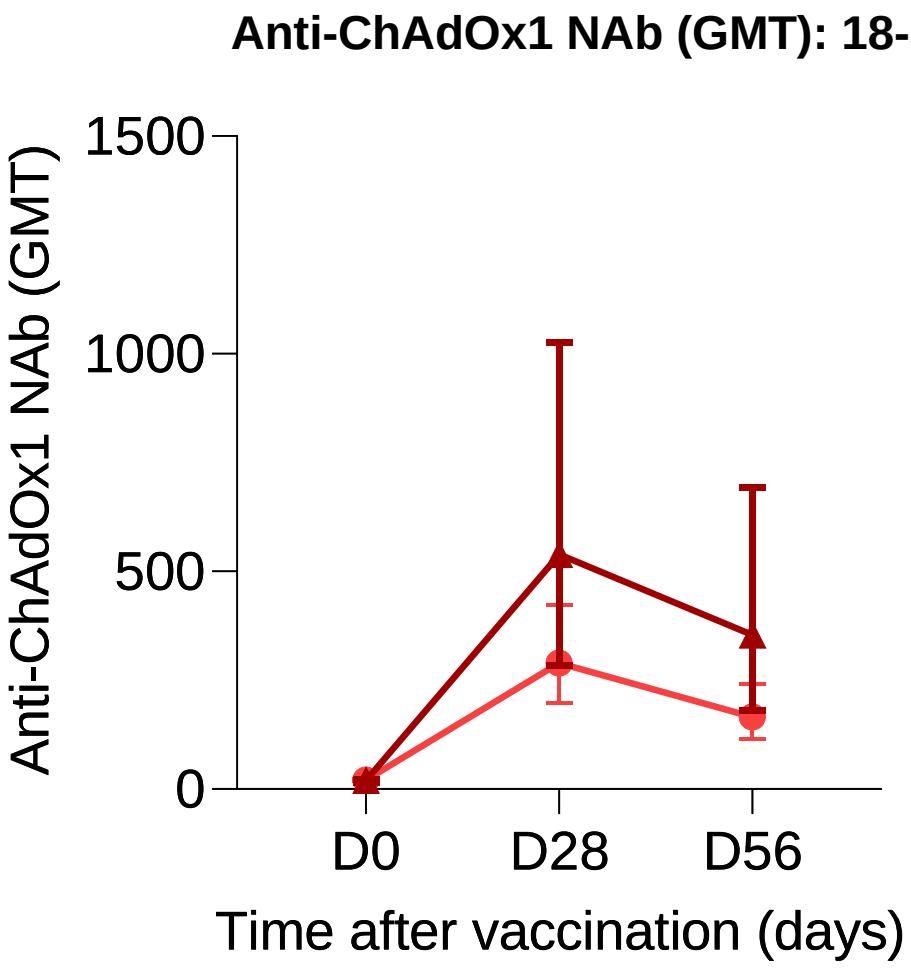


Figure 52. COV001 and COV002 geometric mean anti-ChAdOx1 NAb in those aged 18-55.

Dark red = SD, light red = LD. Black lines represent GMT with 95% CI.

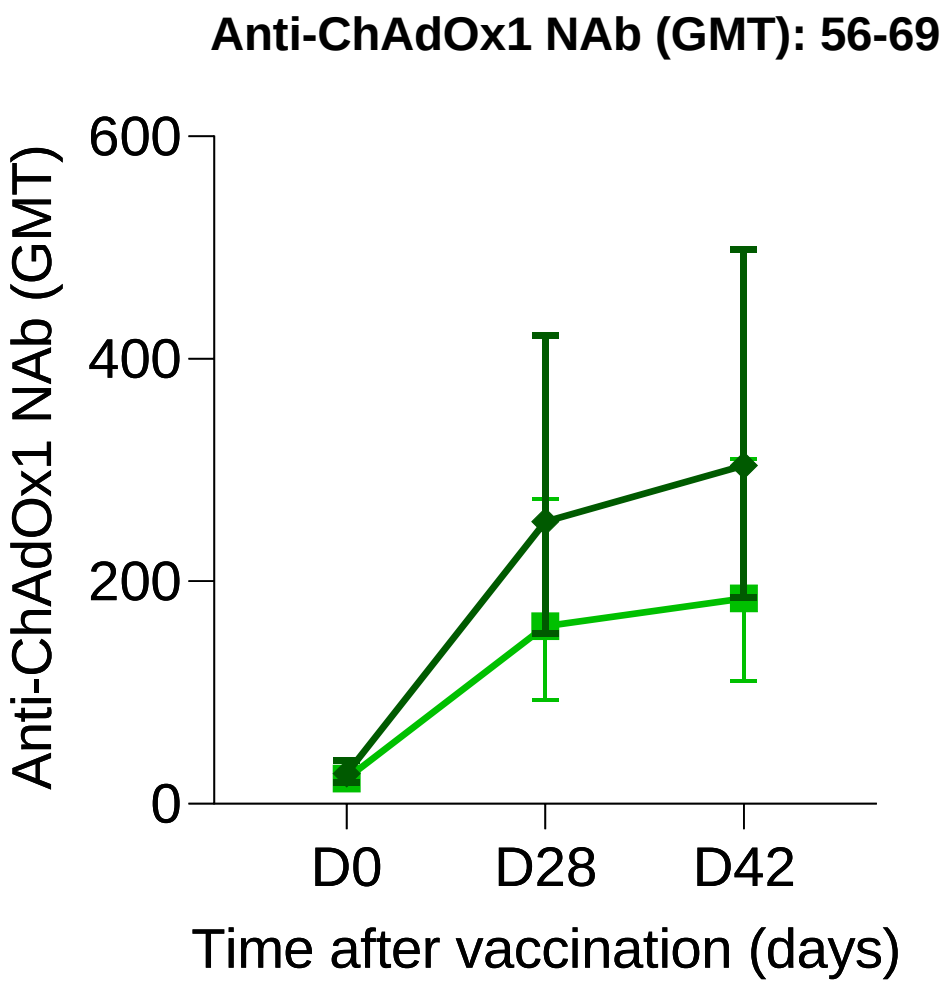


Figure 53. COV002 geometric mean anti-ChAdOx1 NAb in those aged 56-69.

Dark green = SD, light green = LD. Black lines represent GMT with 95% CI.

6.7.6 Correlations

6.7.6.1 Binding & Neutralising Ab and T cell responses

There was a moderate positive correlation between IgG titres measured by ELISA and pseudovirus neutralising antibody titres at day D56, and a weak positive correlation between T cell responses at day 14 and IgG titres at day 56.

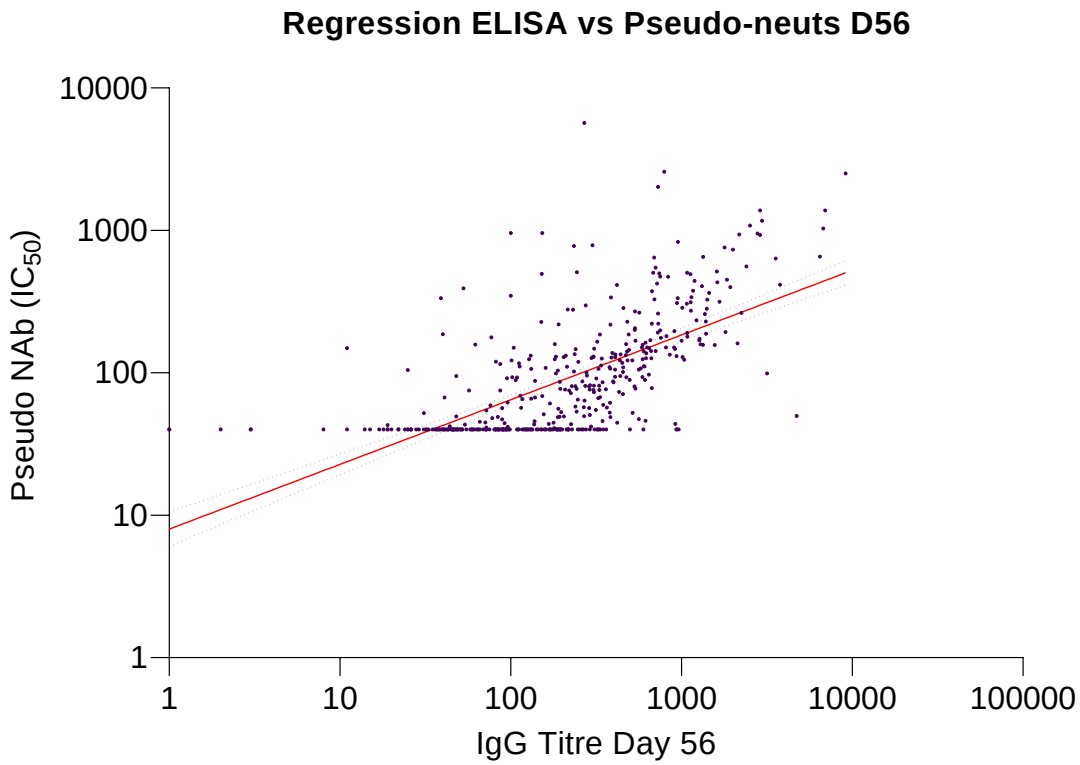


Figure 54. COV001 and COV002 Linear regression IgG titres (Spike) at day 56 vs Pseudovirus NAbs at D56

r^2 = 0.4223 p value <0.0001

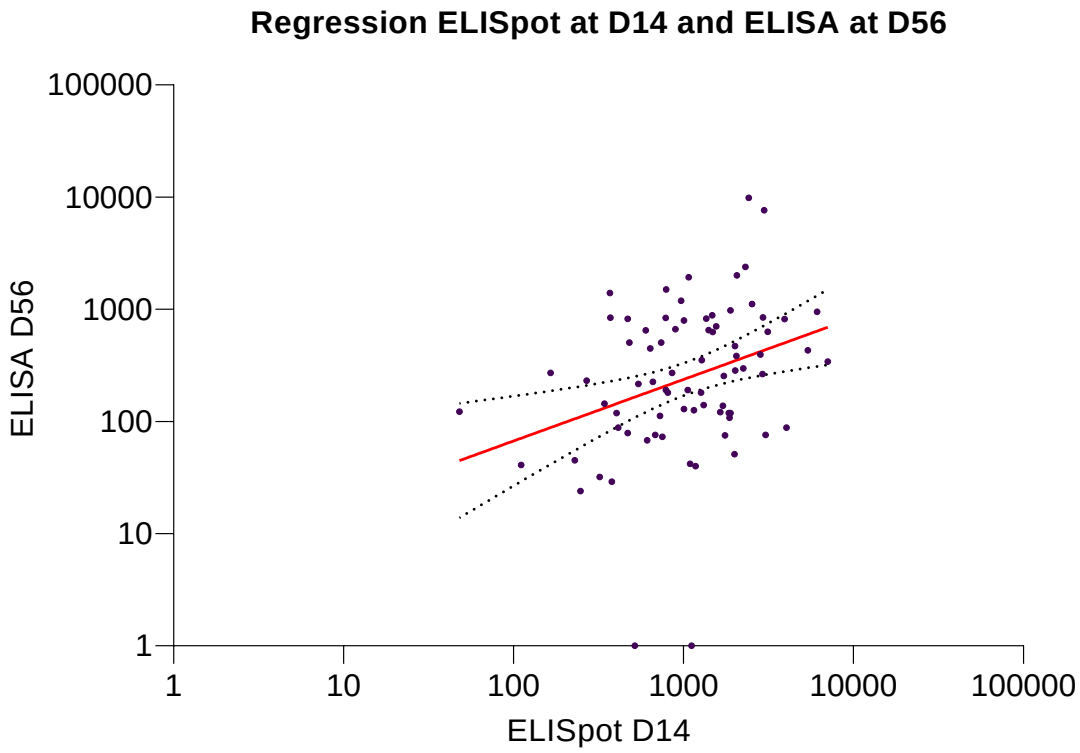


Figure 55. COV001 and COV002 Linear regression IFN-γ ELISpot at D14 vs IgG titres (Spike) at D56

r^2 = 0.1065 p value 0.0038

6.7.6.2 Anti-vector and binding, NAbs post boost

There was no correlation between anti-vector titres post-prime at day 28 and binding or neutralising antibodies at day 56.

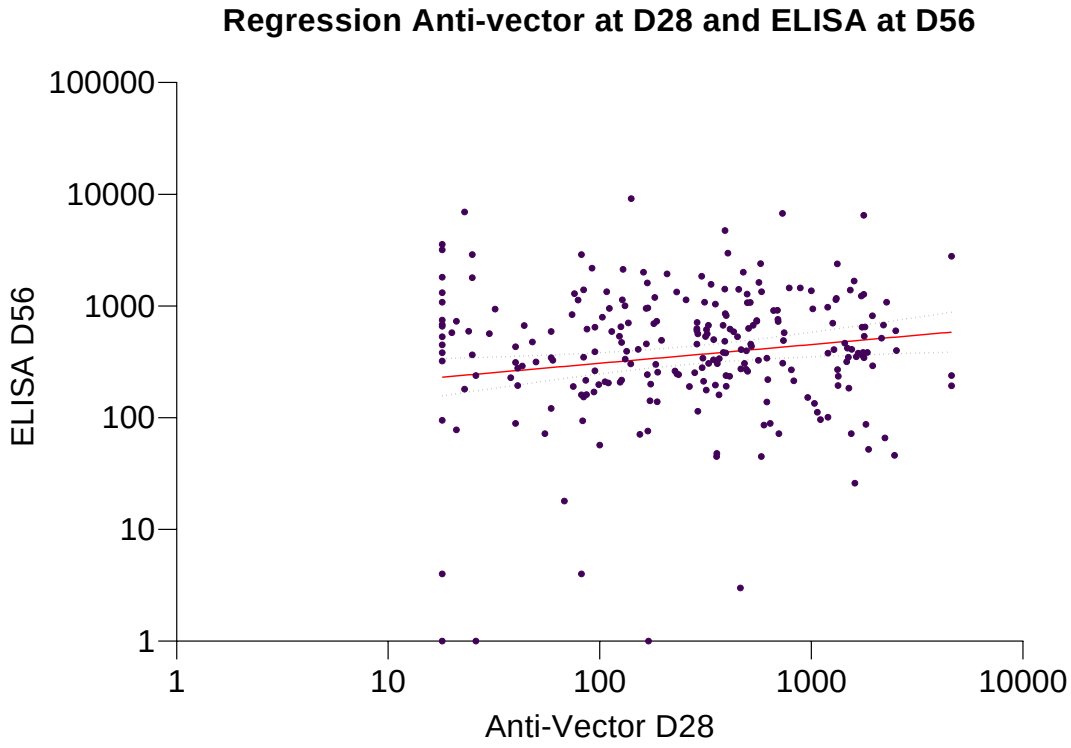


Figure 56. COV001 and COV002 Linear regression Anti-vector at D28 vs IgG titres (Spike) at day 56

r^2 = 0.02611, p value 0.0097

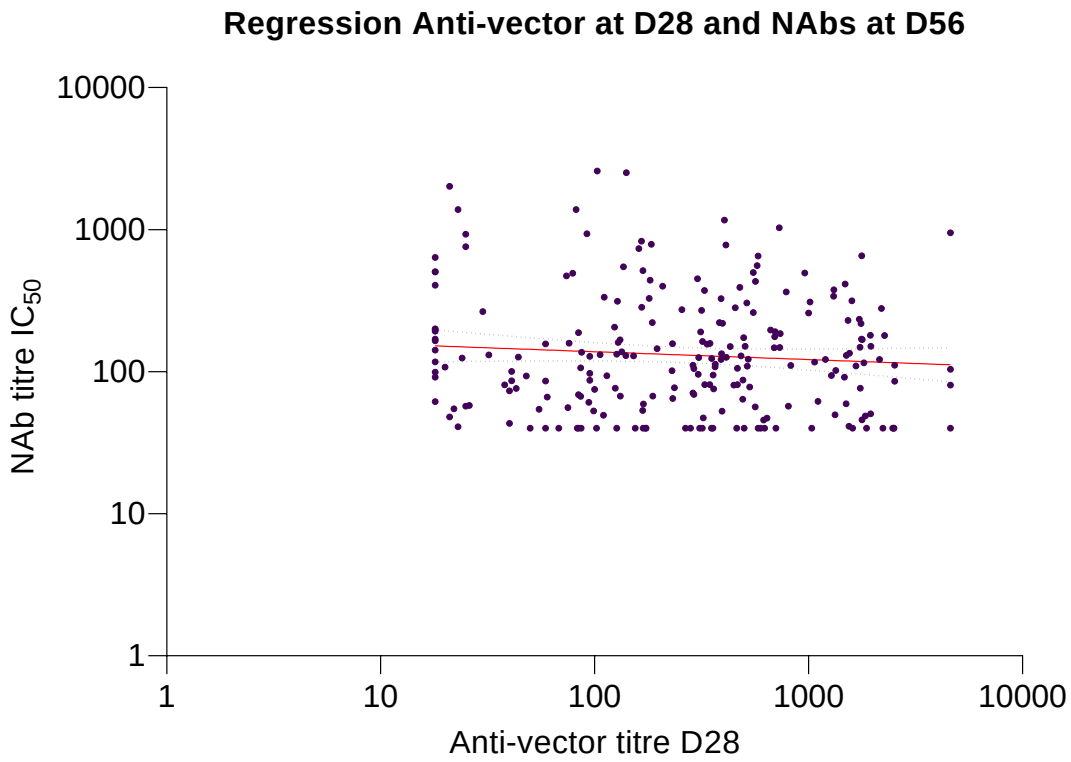


Figure 57. COV001 and COV002 Linear regression anti-vector at day 28 vs NAb at day 56

r^2=0.007049, p value 0.2076

6.7.5 Efficacy

Efficacy against symptomatic COVID-19 disease regardless of severity is summarised in Table 49. There were 15 COVID-19 hospital admissions (WHO clinical progression score ≥4) after 21 days post-prime vaccinations in COV002 and COV003. All of them were in the control group. Two COVID-19 hospital admissions happened in the ChAdOx1 nCoV-19 arm with symptom onset either at 10 days post-prime or at the same day as enrolment.

Table 49. COV001 and COV002 Efficacy against symptomatic disease

COV001/COV002	Cases/Vaccinated Controls	Cases/Vaccinated ChAdOx1 nCoV-19	VE (95% CI)	P value (Chi-square)
Single SD*	16/739	13/586	-2.5% (-111.3-50.1)	0.9474
2 SD	133/4967	23/3470	75.25% (61.55-84.06)	<0.0001
2SD <6 weeks	6/995	3/1051	52.66% (-88.7-88.13)	0.2792
2SD >=12 weeks	70/2128	6/1229	85.16 (65.94-93.53)	<0.0001

* In those who did not receive a booster (as originally designed)

Table 50. COV003 Efficacy against symptomatic disease

COV003	Cases/Vaccinated Controls	Cases/Vaccinated ChAdOx1 nCoV-19	VE (95% CI)	P value (Chi-square)
Single SD*	20/286	7/182	45% (-27.4-76.26)	0.1547
2 SD	110/4251	51/4314	54.31% (36.5-67.13)	<0.0001
2SD <6 weeks	67/3190	30/3209	55.49% (31.73-70.98)	0.0001
2SD >=12 weeks	6/120	2/127	68.5% (-0.53-93.52)	0.1286

* In those who did not receive a booster (as originally designed)

6.8 Discussion

The ChAdOx1 nCoV-19 vaccine given as a single or 2-dose regimen was safe and well tolerated, despite having a higher reactogenicity profile compared to the control vaccine, MenACWY. The majority of solicited AEs reported were mild or moderate in severity, and all were self-limiting. The profile of AEs reported here is similar to that for other ChAdOx1-vectored vaccines and other closely related simian adenoviruses, such as ChAdOx2, ChAd3, and ChAd63, expressing multiple different antigens [75, 91, 203, 204] at this dose level, as well as to some licensed vaccines [221]. ChAdOx1 nCoV-19 had a lower reactogenicity profile in older adults compared to younger adults. Fewer AEs were reported after the boost vaccination than after the prime vaccination and reactogenicity reduced with increasing age. Reactogenicity was dose-dependent across all age groups, similar to other ChAdOx1 vectored vaccines given at lower doses. By contrast with these findings, a second dose of mRNA vaccines tends to be more reactogenic than priming doses [4, 222], whereas inactivated vaccines have lower reactogenicity profiles in general [223].

Very rare events of thrombosis with thrombocytopenia syndrome (TTS) have been associated with ChAdOx1 nCoV-19 following global widespread use after conditional marketing authorisation. No TTS cases were identified as part of the clinical trials leading to ChAdOx1 nCoV-19 authorisation. Overall, SAEs in the studies were balanced between intervention and control groups, and no safety signal was identified in these studies. One case of myelitis was deemed possibly related to ChAdOx1 nCoV-19, but (at the time of writing) it has not been identified as a safety signal following vaccine roll-out [220].

A dose of 5×10^{10} vp ($3.5 - 6.5 \times 10^{10}$ vp) was chosen based on previous experience with ChAdOx1 MERS, where despite increased reactogenicity, a dose-response relationship with neutralising antibodies was observed [75]. The protocols were written when the pandemic was accelerating in the UK and a single higher dose was chosen to provide the highest chance of rapid induction of neutralising antibody. However, the 2-dose regimens tested here have been shown to induce significantly higher binding and neutralising antibody responses, with no relevant boosting of T cell responses. This data informed the decision to change course on the programme from single dose to a 2-dose regimen.

A single dose of ChAdOx1 nCoV-19 elicits an increase in spike-specific antibodies by day 28 and neutralising antibody specially after a booster dose. A clear effect of a boost vaccination on antibody titres at day 56 was seen that was unrelated to the dose regimen. A similar pattern was observed with neutralising antibody responses, with a booster effect observed in individuals who received a second dose of vaccine. Differences between GMT for both binding and neutralising antibodies were statistically significant.

Neutralising antibodies targeting different epitopes of the spike glycoprotein have been associated with protection from COVID-19 in early preclinical rhesus macaque studies [224]. There is now increasing evidence that neutralising antibodies correlate with protection against COVID-19 across different vaccine candidates [225], and a correlate of protection for ChAdOx1 nCoV-19 and other vaccines have now been established [226, 227]. The correlation of neutralising antibodies with IgG titres indicates that a standardised ELISA might be sufficient to predict protection.

T cell responses are important in controlling disease in natural infection [228] and so generation of a robust cellular immune response is a desirable feature for COVID-19 vaccines. Spike-specific T cell responses measured with ELISpot peaked at 14 days after the prime vaccination, consistent with previous studies of simian adenovirus-vectored vaccines and were similar in all groups regardless of vaccine dose. A positive, although weak, correlation between T cell responses at day 14 and IgG titres post boost was observed.

Theoretical concerns about vaccine-enhanced disease have led to a view that a type 1 T-helper (Th1)-biased CD4 response is a preferred coronavirus vaccine characteristic [229]. Disease enhancement has not been observed to date, following widespread use of COVID-19 vaccines.

Changes in immune responses induced by ChAdOx1 nCoV-19 were driven by vaccination regimen (low vs standard dose and single vs 2-dose) rather than age [79]. Hence the immunogenicity data presented here are pooled across different age groups. Severe and fatal cases of COVID-19 disproportionally affect older individuals. Therefore, it is important that vaccines developed to reduce or prevent COVID-19 are suitable for administration in older age groups.

There were no differences in immune responses across different manufacturers. The LD/SD group at 4-6 weeks interval elicited similar immune responses at 28 days post-boost, which suggests the preliminary finding of higher efficacy amongst those in COV002 who received this regimen [78] was mostly driven by longer interval between doses, rather than by enhanced immune responses.

Anti-vector immunity was low before vaccination in UK adults aged 18–55 years, with no relationship between the presence of antibodies to ChAdOx1 and immune

response to the vaccine antigen, including for those who were previously exposed to ChAdOx1 vectored vaccines. Future studies will address the potential effect of anti-vector antibodies on homologous boosting, although in the subgroup reported on here, who received two vaccinations 28 days apart, there was clear evidence of boosting of antibody response to SARS-CoV-2 spike protein.

Efficacy results varied substantially according to dose interval, with improved efficacy observed at 12 weeks compared to 4-6 weeks interval between prime and boost vaccination. The change in trial design meant that it was not possible to reliably estimate efficacy after a single dose, as originally designed. Estimates for short-term efficacy up to 3 months after a single dose, censoring cases before boosters have been reported [230]. The approach used here only included cases in those who did not receive a booster, as an attempt to establish what the vaccine efficacy would have been if the trial design remained unchanged. The small number of cases accrued in this subset does not allow for a reliable measure of efficacy after a single dose, given the wide confidence intervals. A pooled efficacy analysis across efficacy trials conducted in the UK, Brazil and South Africa has been reported [78, 230]. Cases from the South Africa study have been excluded based on different trial procedures for capturing symptomatic cases. Brazil and UK trial procedures were more similar, but volunteers were drawn from a different pool of individuals who were at higher risk of COVID-19 in Brazil compared to the UK. Most volunteers in Brazil received a second dose within 4-6 weeks, whereas in the UK, the vast majority of participants who agreed to a second dose, had within an interval greater than 6 weeks. For these reasons, efficacy data are presented separately here for Brazil and UK.

A limitation of this study is its single-blind design. However, all laboratory analyses and clinical assessments reported here were done in a blinded fashion. A further limitation is the possible variation of the severity of local reactions due to the difference in injection volumes between different batches of vaccines in the low-dose group. Finally, the selection of participants aged 70 years and older, with a median age of 73–74 years between dose groups and with few comorbidities, might not be representative of the general older population, including those living in residential care settings or older than 80 years.

In conclusion, ChAdOx1 nCoV-19 was safe, tolerated, and immunogenic, while reactogenicity was reduced with subsequent doses. A single dose elicited both humoral and cellular responses against SARS-CoV-2, with a booster immunisation augmenting neutralising antibody titres. The anti-spike antibody responses increased after a boost vaccination at an interval of 4-6 weeks, but the neutralising anti-vector antibody responses did not. There was no correlation between anti-vector antibody titres and anti-spike total IgG. Further work with larger sample sizes is required to investigate if homologous boosting with adenovirus-vectored vaccines can be done without loss of immunogenicity to the pathogen-specific transgene, given the likelihood of widespread anti-ChAdOx1 immunity driven by vaccine rollout.

CHAPTER 7: Overall assessment of ChAdOx1 and lessons learned from the COVID-19 pandemic

7.1 Overall Assessment of ChAdOx1 vectored vaccines

Taken together, the data described in this work confirm the hypothesis that ChAdOx1 vectored vaccines against emerging pathogens are safe (in the context of COVID-19 pandemic), immunogenic and effective. ChAdOx1 vectored vaccines have shown a consistent safety profile with reactogenicity, especially fever and chills, directly linked to dose, regardless of antigen expressed. AEs were predominantly mild or moderate in severity and self-limiting in nature. SAEs were balanced across control and intervention groups, but SARs (myelitis) were identified and attributed to ChAdOx1 nCoV-19 as part of clinical trials presented here and further safety signals were recognised as part of post authorisation surveillance (GBS and TTS). All ChAdOx1 constructs presented here were able to induce at least 3-fold increase by day 14 in geometric mean T cell responses measured by IFN-γ ELISpot, regardless of dose. The CHIKV, MERS-CoV and SARS-CoV-2 constructs were able to show a 4-fold GMT increase by day 28 in binding antibodies measured by ELISA (humoral responses for the ZIKV construct were not assessed). Sample sizes on ChAdOx1 vectored vaccines other than COVID-19 were not large enough to explore the contribution of differences in infectious particles from different batches, sex, or BMI on reactogenicity or immunogenicity. Efficacy of ChAdOx1 nCoV-19 was shown to be greater than 50% after two doses, although efficacy against symptomatic disease after a single dose could not be accurately determined.

A single dose of ChAdOx1 vectored vaccines was able to induce robust short-term (within first 14 days) T cell responses between 5-12-fold greater than baseline, regardless of dose. There were differences between T cell fold increase for coronavirus antigens (MERS-CoV and SARS-CoV-2 had higher fold increase) and non-coronavirus ones (chikungunya and Zika had lower fold increases). The ELISpot assays were conducted using the same methods and SOPs, so these differences are unlikely to be attributed to the assay. The MERS-CoV antigen was, however, bigger in size than CHIKV. The tPA leader sequence included in MERS-CoV and SARS-CoV-2 is absent from the CHIKV and ZIKV constructs, which could partially explain the differences observed in T cell responses, although it doesn't account for the enhanced neutralising activity observed with the CHIKV construct. Conserved epitopes across common coronaviruses could also explain the enhanced short-term T cell response for MERS-CoV and SARS-CoV-2 constructs.

A homologous second dose (given at 4-6 weeks interval) does not boost T cell responses. This might be of significance for vaccines where T cell responses are expected to play a much more significant role than antibody responses, such as for dengue and Zika for example. In this case, a heterologous boost (with an MVA construct) might be a better approach.

There are important differences in antibody assays (both binding and neutralising) conducted for the different trials reported here, making formal comparisons across different antigens inappropriate. Nonetheless, there were major differences between neutralising antibody responses elicited from the CHIKV construct, where even a single low dose of ChAdOx1 CHIK was able to induce strong neutralising antibodies compared to coronavirus constructs

(MERS-CoV and SARS-CoV-2), where a 2-dose (at the high dose regimen) were required to induce good levels of neutralising antibodies. This suggests that despite assay optimisation being required, the ability of ChAdOx1 vectored vaccines to induce binding and neutralising antibody responses is antigen/disease-specific, rather than an inherent feature of ChAdOx1 vectored vaccines overall.

The experience accumulated through planning, execution and data analysis of the three first-in-human clinical trials reported here (CHIK001, ZIKA001 and MERS001), shaped protocol design and conduct of COVID-19 vaccine studies. Adjustments to inclusion/exclusion criteria, the use of more standardised AE data collection methods, including use of Brighton Collaboration case definitions for AEs and AESIs and severity grading tables aligned with the FDA toxicity grading scales, a refinement on timing for safety and immunology assessments, dose required, sequence of enrolment, informed consent procedures and holding/stopping rules were incorporated into the COVID-19 trials based on these previous ChAdOx1 studies' findings and ethics and MHRA feedback.

The biggest advantage of ChAdOx1 vectored vaccines comes from ease of manufacture and its 'plug and play' feature with a predictable reactogenicity profile. However, pathogen-specific dose-finding and immunogenicity trials with a comparison between single and 2-dose regimens are still likely to be required, given the differences in immune responses observed across the constructs reported here. The decision to pursue a single or 2-dose regimen could also depend on pathogen characteristics (for example respiratory vs non-respiratory pathogens) where virus neutralisation and protective immunity in the airways is more difficult to induce from intramuscular injections.

7.2 Lessons learned from ChAdOx1 nCoV-19 trials

7.2.1 Pre-clinical testing

Given the previous experience with ChAdOx1 MERS, the MHRA was satisfied that a Good Laboratory Practices (GLP) toxicology study did not need to be completed prior to first-in-human and subsequent trials leading to emergency use authorisation of ChAdOx1 nCoV-19. Therefore, the COV001 and COV002 clinical trials were authorised ahead of formal planning for such studies. Other international regulators followed suit and approved clinical trials in Brazil and South Africa waiving the requirement for GLP toxicology, considering that the phase I/II trial (COV001) was already underway in the UK with no evidence of harm in human volunteers. Nonetheless, a small immunogenicity study of ChAdOx1 nCoV-19 had been conducted in mice ahead of the clinical trial authorisation submission and an NHP challenge study was underway.

There were initial concerns around hypothetical disease enhancement driven by predominantly Th2 responses in historical animal challenge studies of formalin-inactivated SARS-CoV and MERS-CoV vaccine candidates. The pre-clinical challenge studies conducted in mice and NHPs were instrumental in providing reassurances that it would be safe to proceed with clinical testing of ChAdOx1 nCoV-19. The start of the COV001 trial was subject to a review of the pre-clinical data by the MHRA and the DSMB. Evidence of disease enhancement rather than efficacy was set as the no-go criteria. Multiple pre-clinical studies were conducted in mice, ferrets, NHPs and pigs, which all showed consistent immunogenicity profiles (binding and neutralising antibodies as well as predominantly Th1 cellular responses). Efficacy results varied greatly, depending on species, virus challenge dose and route of administration [63, 231-233], highlighting that no animal models

could reliably predict efficacy in humans. All animal models were set up very fast with no standardisation intra or interspecies, and between different research groups.

Biodistribution studies had not been conducted on any ChAdOx1 vectored vaccines ahead of ChAdOx1 nCoV-19 clinical trials but were initiated in parallel. Two biodistribution studies have now been completed on ChAdOx1 vaccines encoding HBV and SARS-CoV-2 antigens, and further biodistribution studies are unlikely to be required for this platform ahead of conditional marketing approvals for future candidates. These ChAdOx1 biodistribution data confirmed the vector was present predominantly at the intramuscular injection site, with low-level DNA detection in other organs for a short period after dosing and subsequent elimination within 1 month.

Well-designed pre-clinical challenge and immunogenicity studies looking at providing evidence for (or lack of) disease enhancement and describing the profile of immune responses, especially regarding Th1 vs Th2 responses are likely to continue to be warranted ahead of clinical testing for future pathogens. These studies could potentially be combined with GLP toxicology studies in mice, optimising resources. Alternative models to NHPs would considerably reduce complexity, costs, and time ahead of clinical testing. For example, ferrets have long been considered a better model for respiratory pathogens and could potentially replace NHP studies for respiratory agents. NHPs, however, remain the only suitable species for licensure under the animal rule.

7.2.2 Manufacturing

Capacity to manufacture ChAdOx1 vectored vaccines to good manufacturing practices (GMP) and at scale for phase III trials have been one of the largest bottlenecks for the ChAdOx1 nCoV-19 vaccine development programme. Clinical trials could have progressed faster had the appropriate infrastructure been in place, avoiding slightly different manufacturing processes across different clinical manufacturing organizations (CMOs), which then had an impact on dosing strategies, logistical issues with overseas shipment of vaccines in the context of travel restrictions, as well as threats of prohibiting exports of essential products from certain countries, as was the case with ventilators in the USA.

A consistent vaccine supply would have avoided the inadvertent issue with low priming doses for groups 1-4 in COV002, which negatively impacted recruitment timelines, efficacy endpoint assessments, and ultimately delayed public access to the vaccine.

The Vaccines Manufacturing and Innovation Centre (VMIC) has been established to promote, develop, and accelerate the growth of the UK vaccine industry. It was conceived as a partnership between academic institutions (University of Oxford, Imperial College London, and The London School of Hygiene and Tropical Medicine), and industrial partners with funds from the UK Research Innovation (UKRI) as a virtual institution in 2018. However, it wasn't until the COVID-19 pandemic hit in 2020 that the actual construction of the facility started. It was hoped that once inaugurated, VMIC would be able to bridge the gap between research and expertise in manufacturing at scale, dealing with issues around supply shortages for phase III clinical trials of promising candidates. Sadly, VMIC is now up for sale in a short-sighted decision made by the UK government.

Increasing manufacturing capacity will also lead to fewer different batches being produced for clinical trial use.

7.2.2.1 Qualified assays for vaccine release suitable for phase III trials

Differences in the downstream process for the drug product between the CBF and Advent's clinical trial materials, and the use of different methods for assessing their concentration (spectrophotometry vs qPCR) translated into dosing differences stemming from inaccuracies of viral particle measurement which was influenced by higher concentrations of polysorbate 80 in the absorbance method. The wide intra-assay variability for Advent's qPCR method also highlighted the need to establish qualified assays which can reliably measure viral particles concentration across different manufacturing processes ahead of phase III pivotal trials.

7.2.2.2 Dose range

Every round of viral vector manufacturing will produce variable yields with different viral particle concentrations between batches. Agreeing on a pre-specified acceptable dose range for the same final target volume to be administered, rather than a fixed dose based on vp would also minimise the number of amendments required and replicate the final intended use of the product.

7.2.3 Trial design and conduct

7.2.3.1 Single vs double-blinded

The COV001, COV002 and COV003 studies were written as participant blinded with members of the clinical team assessing trial endpoints blinded to group allocation. The initial concern was that widespread transmission could translate

into significant staff shortages which could then compromise a double-blinded design.

The pandemic restrictions imposed across the country meant that staff working on other research projects could be seconded to the COVID-19 vaccine programme. A simplified GCP course offered by NIHR made recruitment of extra staff into clinical research more straightforward. Taken together, these measures meant that any impact on study staff absence due to COVID-19 was highly diluted, and a double-blind study design would have been feasible after all. The implementation of a blinded clinical endpoint adjudication committee minimised the inherent biases introduced by single-blinded trial designs.

7.2.3.2 Single vs 2-dose regimens and choice of comparator

Studies were initially written as a single dose and placebo-controlled. Given that the ChAdOx1 nCoV-19 was expected to be significantly more reactogenic than a saline placebo injection, and in order to maintain blinding of participants, a decision was made to replace saline with a MenACWY injection as the comparator before the clinical trial began. MenACWY was not expected to have any impact on COVID-19 and is licensed as a single dose vaccine in adults.

As immunogenicity data emerged on a 2-dose schedule from group 3 in COV001, the studies were amended to include a booster dose. A second dose of MenACWY was introduced in the UK but declined by investigators in Brazil, who opted for a saline placebo boost instead of a second off-label MenACWY dose in the control group. In retrospect, using saline as the comparator from the offset, and having a double-blind design would have improved trial efficiency.

There is now an established correlation between efficacy against symptomatic disease and neutralising antibody titres [226]. However, despite significant higher NAb titres observed in the 2-dose regimen, short-term efficacy against the wildtype SARS-CoV-2 strain was similar for 1 or 2 doses (76% within the first 3 months after a single dose vs 66.7% after 2 SD) [230]. The Janssen COVID-19 (Ad26 vector expressing full-length prefusion stabilised Spike) was initially licensed as a single-dose vaccine and had similar short-term efficacy results. Our ability to assess the appropriateness of a single-dose regimen was impaired as participants were not originally randomised to receive 1 or 2 doses, but were offered a booster dose instead, introducing a significant source of bias and substantially reducing the number of cases accrued after a single dose only.

It is now widely known that immunity driven by either SARS-CoV-2 vaccines or natural infection does wane in a relatively short timeframe [234]. A single dose regimen is no longer considered appropriate with most countries now recommending a 3-dose regimen, with potentially further boosters required for the most vulnerable. A third dose of ChAdOx1 has been shown to boost humoral responses (2-3 fold), but not in the same magnitude as mRNA platforms (20-24 fold) in those whose primary vaccination schedule consisted of 2 homologous ChAdOx1 doses [235].

A single vs 2-dose regimen has significant public health implications regarding vaccine supply, and further structured assessment comparing these 2 regimens is still warranted for other pathogens described here. As the interval between doses is now known to have a significant impact on peak NAbs titres, a second dose should be administered at a 12-week interval (minimum 8 weeks), balancing

short-term efficacy against severe disease with 1 dose, and optimal interval required for high NAbs.

7.2.3.3 Case definitions and clinical trial endpoints

Each vaccine developer came up with their own case definitions for primary efficacy endpoints. Having consistent standardised endpoint definitions for clinical disease would have made comparisons across different products less problematic.

As clinical data emerged, case definitions were updated with new COVID-19 symptoms other than those on the original WHO acute respiratory illness definition (fever and cough or shortness of breath), such as anosmia and ageusia. Other mild COVID-19 symptoms such as diarrhoea, and malaise amongst others were not incorporated into the Oxford sponsored COV trials but were included in case definitions for trials conducted by other vaccine developers. CEPI eventually proposed a standard case definition and efficacy endpoints for the studies they were funding, but the document was only released on the 25th of June 2020 when trials had been well underway since April (COV001), May (COV002) and June (COV003). The WHO Solidarity Trial protocol did not go far enough and proposed that primary outcomes should be defined as ‘virologically confirmed COVID-19 disease, regardless of severity’, with no mention to what case definition or list of symptoms should be used.

The WHO COVID-19 severity grading scale has proven to be a valid and standardised tool to measure efficacy against different disease severities (mild disease, hospitalisation, ICU admission and death). The scale was published after trials already started, so it was important to ensure detailed clinical

parameters were collected from medical records and aligned with agreed definitions as they emerged. Medical records reviews do add an extra burden to overstretched trial teams and limiting the amount of clinical data required from specific timepoints during their inpatient stay is key. Combining data collection tools on clinical parameters with others already established, such as the one proposed by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) could potentially reduce the burden on trial teams.

Vaccines against respiratory viruses usually have greater efficacy against severe disease compared to mild disease, and greater efficacy against symptomatic disease compared to asymptomatic infection. Prevention of moderate to severe disease significantly reduces the burden on healthcare services and provide more objective individual benefit, and therefore should be preferred as primary endpoints over mild and asymptomatic infection. Having a clinical endpoint adjudication committee allowed for a standardised assessment of disease severity. However, moderate to severe disease are usually less frequently observed than mild cases, and this will have an impact on the overall sample size required. Incidence rates for symptomatic cases varied substantially throughout the pandemic, reflecting the impact of non-pharmacological containment measures implemented at different waves of the epidemic. A balance between relatively rare non-mild outcomes and overall disease incidence rates should be considered, and designing trials based on number of cases required is likely to be more efficient. Although it is accepted that measuring efficacy against asymptomatic infection provides valuable evidence with significant policy implications to vaccine roll-out strategies, their assessment alongside efficacy against symptomatic disease may introduce important case ascertainment

biases, compromising overall efficacy read-outs on the primary outcomes and, therefore, should be considered after efficacy against symptomatic disease is established.

In the context of public health emergencies, the only one question that matters: is: Does the vaccine work? Proposition of multiple secondary and exploratory endpoints should be avoided or limited to specific subgroups, as they often overcomplicate trial procedures, and compromise volunteer compliance. Exploratory endpoint assessments should not come at the expense of the primary endpoint.

7.2.3.4 Multi-site and international collaboration

Initial modelling prediction suggested efficacy studies could be conducted anywhere, as widespread transmission would allow for efficacy to be rapidly detected in the UK, except that containment measures introduced dramatically decreased the number of cases. University closures had a significant impact on the ability to identify cases at the Oxford site and it soon became obvious that extra sites were required. Some of them had already been added to study protocol from the beginning as an insurance measure, which paid off as they soon had to be activated. COV002 expanded to up to 18 different sites in the UK which were selected based on the network of investigators known to each other. Some had more experience with vaccine trials than others and some struggled more than others, impacting on our ability to efficiently recruit participants at the pace required.

As cases decreased in the UK, international collaborations were instrumental to the success of the Oxford-AstraZeneca vaccine programme. Half of the efficacy

data used towards conditional approval came from partners in Brazil who generated data at trial sites we had never worked with before, and under ethics and regulatory frameworks previously unknown to Oxford.

Taken together, the experiences in the UK and international sites have highlighted the need for a clinical trial network or consortium for vaccine studies where preparedness activities with the harmonisation of core SOPs would be recommended, ahead of the next pandemic. It could also promote accelerated local approvals based on a common framework. There were substantial differences in service provision and research structure available across the different sites, which affected the ability of trials sites to execute complex trial procedures. A pragmatic approach tailored to local requirements is key to success.

Having separate international studies instead of a multi-country trial under the same protocol has proven to be highly efficient, with fast-track approvals for clinical trial start and subsequent amendments. However, if data is to be pooled across different protocols, we need to ensure same data collection methods, core case report forms (CRFs), case definitions, and endpoint procedures are used.

7.2.3.5 Database templates

Numerous changes to CRFs were required during the study, as multiple amendments were implemented and as we learned more about database requirements for approval. Safety assessments in real-time require MedDRA coding in real-time, which had it been in place would have substantially reduced the workload required when working at such a fast pace.

Previous preparedness plans have put a lot of emphasis on master protocols but having a master database template that is both investigator friendly and regulatory compliant will also make running trials under emergency situations more efficient.

7.2.3.6 Trial participants

In the absence of reliable methods for serostatus ascertainment before enrolment, recruitment of those likely to be at greater risk for SARS-CoV-2 exposure was initially avoided (e.g., healthcare workers). The idea behind the strategy was to minimise the chances of recruiting seropositive volunteers at baseline, which could compromise efficacy assessments. However, it soon became clear regulators needed data on those who were seropositive at baseline, as it would be highly impractical to check serostatus ahead of mass vaccination campaigns. There are advantages and disadvantages to prioritising recruitment of those at higher risk of exposure. The main advantage comes from obtaining efficacy read-outs quicker, given they are more likely to meet primary efficacy endpoints. However, the level of exposure (e.g., viral load) for those at greater risk could also potentially translate into impaired efficacy results that cannot be generalisable to the wider population. Either way, recruitment into efficacy cohorts should be simplified and not restricted to serostatus at baseline, as the impact of those seropositive in our ability to detect cases amongst the seronegative at enrolment was negligible. Adjustments to sample sizes to account for seropositivity at baseline seem more appropriate and efficient than screening for serostatus ahead of enrolment.

Safety and reactogenicity profiles of ChAdOx1 vectored vaccines had not been previously established for older age groups at the proposed 5×10^{10} vp dose.

Sequential age escalation enrolment as advised by the DSMB was appropriate at the beginning. A lower reactogenicity profile was observed with increasing age with no compromise to immune responses (binding antibodies and T cell responses). However, the trials selected fairly healthy sub-populations amongst those aged 56+, and so immunogenicity findings might not be a true representation of the general older age groups. Given that safety and reactogenicity profiles are now established for ChAdOx1, future trials can be conducted using a less restrictive approach towards recruitment with no need for age escalation procedures. Future trials should also ensure a higher proportion of older participants (target 30%), given most diseases disproportionately affect the elderly.

7.2.4 Parallel planning and Risk Management

Parallel planning was instrumental in obtaining answers as quickly as possible without compromising the safety of volunteers. The clinical trial applications had been in place and approved with volunteers lined-up for vaccinations ahead of the pre-clinical data becoming available. DSMB reviews were timely scheduled ahead of volunteer enrolment at different stages in the programme. It does come with a substantial financial risk, which in this case paid off.

7.2.5 Early engagement with Regulatory Authorities

Early discussions with the MHRA in the UK and ANVISA in Brazil were essential to ensure confidence in the data generated and a smooth clinical trial authorisation process, especially with its numerous subsequent amendments.

7.2.6 Communication strategy

Effective, clear, and simple communication on trial findings and safety concerns have proven to be just as important as actually running the study as it has significant implications on vaccine hesitancy.

CHAPTER 8: Conclusions and future work

ChAdOx1 has shown a consistent safety, reactogenicity and immunogenicity profile across different antigens tested, and we now have proof it is also effective. ChAdOx1 as a platform technology can substantially improve the timescale from pathogen identification to vaccine manufacturing and clinical trial initiation, but questions on risks for disease enhancement and dosing schedule remain pathogen-specific.

Much of the success of the ChAdOx1 nCoV-19 vaccine relied on previous experience (clinical and pre-clinical data) obtained from the MERS-CoV construct, rather than other ChAdOx1 vectored candidates, especially regarding target dose likely to induce neutralising activity. Creating 'prototype' vaccines against families of viruses to speed up development in case of emergency, as has been suggested by the NIAID in the USA, could be part of the solution to tackle future threats. Pre-clinical work on prototype vaccines could at least partially address the concerns over disease enhancement for a certain family of viruses as a risk mitigation approach ahead of clinical testing for novel constructs.

The implications of widespread anti-vector immunity to the utility of future ChAdOx1 vectored vaccines remain unknown, and several studies are now being planned or are underway which will try to answer this question. However, the significant increase in antibody responses following a homologous boost shown with the COVID-19 vaccine, and preliminary evidence from other ChAdOx1-primed individuals shown here suggest that anti-vector immunity may not be as relevant as previously thought. The role of 3rd and additional booster doses, as well as the use of fractional doses, remain important questions to be answered.

Risk-benefit assessments will continue to be required for ChAdOx1 vectored vaccines in the context of the safety signals identified with ChAdOx1 nCoV-19 and other adenovirus vectored vaccines (GBS and TTS). The mechanisms underlying the TTS phenomenon remain unknown, as is still unclear if these are inherent to adenovirus vectored COVID-19 vaccines or also applicable to other constructs. Reactogenicity is not trivial at 5×10^{10} vp, with fevers and chills being relatively common side effects that could be mistaken by the disease itself we are trying to protect people against. This could impact the perception of individual benefit, despite the clear benefit to wider society and against severe outcomes. Lower dose regimens with fewer side effects still require further investigation.

Pandemic preparedness plans have been developed and outlined for several years now, mostly focusing on enhanced surveillance strategies, infectious diseases modelling, and development of new therapies and vaccines, stemming from previous failures in response [236, 237]. Despite significant progress achieved throughout the past 20 years, and significant increase in funding, the world has failed to act on most of the recommendations proposed by such epidemic preparedness plans. The conclusions after reviews of public health emergencies of international concern are quite often the same: the world is ill-prepared to respond to severe pandemics or to any other similar global sustained and threatening public-health emergency.

Vaccine manufacturing has proven to be the greatest bottleneck in the speedy delivery of clinical development programmes and post-authorisation deployment. Vaccine inequity is now responsible for significant avoidable deaths in countries unable to access a fair share of the approved COVID-19 vaccines to date, despite innovative and collaborative strategies such as COVAX. Publicly funded vaccine

manufacturing facilities could help fill the gap in supply preparedness. Manufacturing capacity is now in place in developing countries for ChAdOx1 (and other adenovirus) vectored vaccines (e.g., China, India, Brazil, Mexico/Argentina), so should be feasible to develop effective vaccines for developing country's needs, which partly addresses the inequality issue. However, vaccine manufacturing capability in Africa is still urgently required.

There have been significant advances in regulatory frameworks that resulted in emergency use approval of ChAdOx1 nCoV-19 in less than 12 months from pathogen sequence release. The COVID-19 pandemic has proven that regulatory timelines can be significantly reduced with no compromise to individual's safety. The introduction of the Vaccine Platform Technology Master File (PTMF) concept for veterinary products could pave the way for streamlined human vaccines assessments.

In conclusion, ChAdOx1 has been shown to be safe, immunogenic, and effective. The benefit/risk balance remains overwhelmingly positive in the context of the very rare serious adverse reactions identified during widespread use of the ChAdOx1 nCoV-19 vaccine, compared to risks of severe COVID-19 and COVID-19 death. Evaluation of ChAdOx1-based vaccines is continuing as part of ongoing clinical trials and regulatory surveillance systems. The first ChAdOx1 vectored vaccine approved for human use, against SARS-CoV-2, received emergency use authorization in the UK on 30th December 2020, and is now approved in more than 180 countries. New prophylactic and therapeutic vaccines based on the ChAdOx1 vector are currently undergoing pre-clinical and clinical assessment, including vaccines against viral haemorrhagic fevers, Nipah, HIV, hepatitis B, amongst others.

Table 51. Key points from this work

Key points	Thesis Page
1. Reactogenicity is dose-dependent and antigen independent	90,121,144,186,195
2. Safety, immunogenicity and efficacy profiles now established at 5×10^{10} vp in adults aged 18+	195,214,220,227
3. Dose finding studies are still required, though driven by differences in humoral immune responses rather than reactogenicity.	96,153,214
4. Two-dose vs Single-dose approach pathogen dependent (respiratory vs non-respiratory)	96,153,214
5. If 2 doses required, a 12-week interval is suggested.	227
6. Anti-vector immunity is less important than previously thought	221,222,225,226
7. T cell responses are dose-independent and not boosted using a homologous regimen	103,129,156,220
8. Peak T cell responses correlate with post booster humoral responses, but not post-prime.	106, 159,224
9. ChAdOx1 use in future outbreaks: risk-benefit assessment given safety signals	131, 202
10. Next public health emergency: a) address manufacturing capacity first, b) keep it simple, c) double-blind and placebo-controlled, d) engage early (collaborators, regulators, pharma), e) have a master database, f) go for 5×10^{10} dose if no other dose-finding studies available (+/- 30%) at 1 and/or 2 doses, g) enrol 18+ with no age limit, h) consider serostatus at baseline at the point of data analysis (not enrolment).	Chapter 7

Appendix

1. Links to published manuscripts associated with this work

- **First author or shared co-first authorship**

Folegatti, P.M., et al. A single dose of ChAdOx1 Chik vaccine induces neutralizing antibodies against four chikungunya virus lineages in a phase 1 clinical trial. Nat Commun 12, 4636 (2021). https://doi.org/10.1038/s41467-021-24906-y

Clemens, S.A.C., et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 lineages circulating in Brazil. Nat Commun 12, 5861 (2021). https://doi.org/10.1038/s41467-021-25982-w

Voysey, M., et al., Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. Lancet, 2021. 397(10277): p. 881-891. https://doi.org/10.1016/S0140-6736(21)00432-3

Voysey, M., et al., Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. The Lancet, 2020. https://doi.org/10.1016/S0140-6736(20)32661-1

Barrett, J.R., et al., Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. Nature Medicine, 2020. https://doi.org/10.1038/s41591-020-01179-4

Folegatti, P.M., et al., Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind,

randomised controlled trial. The Lancet, 2020. 396(10249): p. 467-478. https://doi.org/10.1016/S0140-6736(20)31604-4

Ramasamy, M.N., et al., Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. The Lancet, 2020. 396(10267): p. 1979-1993. https://doi.org/10.1016/S0140-6736(20)32466-1

Folegatti, P.M., et al., Safety and immunogenicity of a candidate Middle East respiratory syndrome coronavirus viral-vectored vaccine: a dose-escalation, open-label, non-randomised, uncontrolled, phase 1 trial. The Lancet Infectious Diseases, 2020. https://doi.org/10.1016/S1473-3099(20)30160-2

2. Links to published manuscripts associated with this work

- **Co-author**

Bosaeed, M., et al., Safety and immunogenicity of ChAdOx1 MERS vaccine candidate in healthy Middle Eastern adults (MERS002): an open-label, non-randomised, dose-escalation, phase 1b trial. The Lancet. Microbe, 2021. https://doi.org/10.1016/S2666-5247(21)00193-2

Flaxman, A., et al., Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). The Lancet, 2021. 398(10304): p. 981-990. https://doi.org/10.1016/S0140-6736(21)01699-8

Feng, S., et al., Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nature Medicine, 2021. https://doi.org/10.1038/s41591-021-01540-1

Swanson Phillip, A., et al., AZD1222/ChAdOx1 nCoV-19 vaccination induces a polyfunctional spike protein–specific TH1 response with a diverse TCR repertoire. Science Translational Medicine, 2021. 13(620): p. eabj7211. https://doi.org/10.1126/scitranslmed.abj7211

Frater, J., et al., Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in HIV infection: a single-arm substudy of a phase 2/3 clinical trial. The Lancet HIV, 2021. 8(8): p. e474-e485. https://doi.org/10.1016/S2352-3018(21)00103-X

Emary, K.R.W., et al., Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of

a randomised controlled trial. Lancet, 2021. 397(10282): p. 1351-1362. https://doi.org/10.1016/S0140-6736(21)00628-0

Ewer, K.J., et al., T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. Nat Med, 2020. https://doi.org/10.1038/s41591-020-01194-5

Madhi, S.A., et al., Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant. N Engl J Med, 2021. 384(20): p. 1885-1898. https://doi.org/10.1056/NEJMoa2102214

3. Antigen peptide sequences

3.1 ChAdOx1 Chik

Peptide number	Peptide sequence	Chik Peptides
1	MEFIPTQTFYNRRYQPRPWT	Capsid 1
2	NRRYQPRPWTPRPTIQVIRP	
3	PRPTIQVIRPRPRPQRQAGQ	
4	RPRPQRQAGQLAQLISAVNK	
5	LAQLISAVNKLTMRAVPQQK	
6	LTMRAVPQQKPRKNRKNKKQ	
7	PRKNRKNKKQKQKQQAPQND	
8	KQKQQAPQNDPKQKKQPPQK	
9	PKQKKQPPQKKPAQKKKKPG	Capsid 2
10	KPAQKKKKPGRRERMCMKIE	
11	RRERMCMKIENDCIFEVKHE	
12	NDCIFEVKHEGKVMGYACLV	
13	GKVMGYACLVGDKVMKPAHV	
14	GDKVMKPAHVKGTIDNADLA	
15	KGTIDNADLAKLAFKRSSKY	
16	KLAFKRSSKYDLECAQIPVH	
17	DLECAQIPVHMKSDASKFTH	
18	MKSDASKFTHEKPEGYYNWH	Capsid 3
19	EKPEGYYNWHHGAVQYSGGR	
20	HGAVQYSGGRFTIPTGAGKP	
21	FTIPTGAGKPGDSGRPIFDN	
22	GDSGRPIFDNKGRVVAIVLG	
23	KGRVVAIVLGGANEGARTAL	
24	GANEGARTALSVVTWNKDIV	
25	SVVTWNKDIVTKITPEGAEE	
26	TKITPEGAEEWSLAIPVMCL	
27	WSLAIPVMCLLANTTFPCSQ	E3
28	LANTTFPCSQPPCTPCCYEK	
29	PPCTPCCYEKEPESTLRMLE	
30	EPESTLRMLEDNVMRPGYYQ	
31	DNVMRPGYYQLLKASLTCSP	
32	LLKASLTCSPHRQRRSTKDN	
33	HRQRRSTKDNFNVYKATRPY	E2 1
34	FNVYKATRPYLAHCPDCGEG	
35	LAHCPDCGEGHSCHSPVALE	
36	HSCHSPVALERIRNEATDGT	
37	RIRNEATDGTLKIQVSLQIG	

Peptide number	Peptide sequence	Chik Peptides
38	LKIQVSLQIGIKTDDSHDWT	
39	IKTDDSHDWTKLRYMDNHMP	
40	KLRYMDNHMPADAERAGLLV	
41	ADAERAGLLVRTSAPCTITG	
42	RTSAPCTITGTMGHFILARC	
43	TMGHFILARCPKGETLTVGF	E2 2
44	PKGETLTVGFTDGRKISHSC	
45	TDGRKISHSCTHPFHHDPPV	
46	THPFHHDPPVIGREKFHSRP	
47	IGREKFHSRPQHGKELPCST	
48	QHGKELPCSTYVQSTAATAE	
49	YVQSTAATAEEIEVHMPPDT	
50	EIEVHMPPDTPDRTLMSQQS	
51	PDRTLMSQQSGNVKITVNGQ	
52	GNVKITVNGQTVRYKCNCGG	
53	TVRYKCNCGGSNEGLTTTDK	E2 3
54	SNEGLTTTDKVINNCKIDQC	
55	VINNCKIDQCHAAVTNHKKW	
56	HAAVTNHKKWQYNSPLVPRN	
57	QYNSPLVPRNAELGDRKGKI	
58	AELGDRKGKIHIPFPLANVT	
59	HIPFPLANVTCRVPKARNPT	
60	CRVPKARNPTVTYGKNQVIM	
61	VTYGKNQVIMLLYPDHPTLL	
62	LLYPDHPTLLSYRNMGEEPN	
63	SYRNMGEEPNYHEEWVTHKK	E2 4
64	YHEEWVTHKKEVRLTVPTEG	
65	EVRLTVPTEGLEVTWGNNEP	
66	LEVTWGNNEPYKYWPQLSTN	
67	YKYWPQLSTNGTAHGHPHEI	
68	GTAHGHPHEIILYYYELYPT	
69	ILYYYELYPTMTVVIVSVAS	
70	MTVVIVSVASFVLLSMVGTA	
71	FVLLSMVGTAVGMCMCARRR	
72	VGMCMCARRRCITPYELTPG	
73	CITPYELTPGATVPFLLSLI	
74	ATVPFLLSLICCIRTAKAAT	
75	CCIRTAKAATYYEAAAYLWN	6k
76	YYEAAAYLWNEQQPLFWLQA	
77	EQQPLFWLQALIPLAALIVL	
78	LIPLAALIVLCNCLRLLPCC	

Peptide number	Peptide sequence	Chik Peptides
79	CNCLRLLPCCCKTLAFLAVM	
80	CKTLAFLAVMSIGAHTVSAY	
81	SIGAHTVSAYEHVTVIPNTV	E1 1
82	EHVTVIPNTVGVPYKTLVNR	
83	GVPYKTLVNRPGYSPMVLEM	
84	PGYSPMVLEMELLSVTLEPT	
85	ELLSVTLEPTLSLDYITCEY	
86	LSLDYITCEYKTVIPSPYVK	
87	KTVIPSPYVKCCGTAECKDK	
88	CCGTAECKDKSLPDYSCKVF	
89	SLPDYSCKVFTGVYPFMWGG	
90	TGVYPFMWGGAYCFCDAENT	
91	AYCFCDAENTQLSEAHVEKS	
92	QLSEAHVEKSESCKTEFASA	E1 2
93	ESCKTEFASAYRAHTASASA	
94	YRAHTASASAKLRVLYQGNN	
95	KLRVLYQGNNITVAAYANGD	
96	ITVAAYANGDHAVTVKDAKF	
97	HAVTVKDAKFIVGPMSSAWT	
98	IVGPMSSAWTPFDNKIVVYK	
99	PFDNKIVVYKGDVYNMDYPP	
100	GDVYNMDYPPFGAGRPGQFG	
101	FGAGRPGQFGDIQSRTPESK	
102	DIQSRTPESKDVYANTQLVL	
103	DVYANTQLVLQRPAAGTVHV	E1 3
104	QRPAAGTVHVPYSQAPSGFK	
105	PYSQAPSGFKYWLKERGASL	
106	YWLKERGASLQHTAPFGCQI	
107	QHTAPFGCQIATNPVRAVNC	
108	ATNPVRAVNCAVGNIPISID	
109	AVGNIPISIDIPDAAFTRVV	
110	IPDAAFTRVVDAPSLTDMSC	
111	DAPSLTDMSCEVPACTHSSD	
112	EVPACTHSSDFGGVAIIKYA	
113	FGGVAIIKYAASKKGKCAVH	
114	ASKKGKCAVHSMTNAVTIRE	E1 4
115	SMTNAVTIREAEIEVEGNSQ	
116	AEIEVEGNSQLQISFSTALA	
117	LQISFSTALASAEFRVQVCS	
118	SAEFRVQVCSTQVHCAAACH	
119	TQVHCAAACHPPKDHIVNYP	

Peptide number	Peptide sequence	Chik Peptides
120	PPKDHIVNYPASHTTLGVQD	
121	ASHTTLGVQDISTTAMSWVQ	
122	ISTTAMSWVQKITGGVGLIV	
123	KITGGVGLIVAVAALILIVV	
124	AVAALILIVVLCVSFSRH	
125	IVAVAALILIVVLCVSFSRH	

3.2 ChAdOx1 Zika

Peptide number	Peptide sequence	Zika Peptides
1	ATRRGSAYYMYLDRNDAGEA	PrM 1
2	YLDRNDAGEAISFPTTLGMN	
	ISFPTTLGMNKCYIQIMDLG	
4	KCYIQIMDLGHMCDATMSYE	
5	HMCDATMSYECPMLDEGVEP	
6	CPMLDEGVEPDDVDCWCNTT	
7	DDVDCWCNTTSTWVVYGTCH	
8	STWVVYGTCHHKKGEARRSR	
9	HKKGEARRSRRAVTLPSHST	PrM 2
10	RAVTLPSHSTRKLQTRSQTW	
11	RKLQTRSQTWLESREYTKHL	
12	LESREYTKHLIRVENWIFRN	
13	IRVENWIFRNPGFALAAAAI	
14	PGFALAAAAIAWLLGSSTSQ	
15	AWLLGSSTSQKVIYLVMILL	
16	KVIYLVMILLIAPAYSIRCI	PrM2/Env1
17	IAPAYSIRCIGVSNRDFVEG	Env 1
18	GVSNRDFVEGMSGGTWVDVV	
19	MSGGTWVDVVLEHGGCVTVM	
20	LEHGGCVTVMAQDKPTVDIE	
21	AQDKPTVDIELVTTTVSNMA	
22	LVTTTVSNMAEVRSYCYEAS	
23	EVRSYCYEASISDMASDSRC	

Peptide number	Peptide sequence	Zika Peptides
24	ISDMASDSRCPTQGEAYLDK	
25	PTQGEAYLDKQSDTQYVCKR	Env 2
26	QSDTQYVCKRTLVDRGWGNG	
27	TLVDRGWGNGCGLFGKGSLV	
28	CGLFGKGSLVTCAKFACSKK	
29	TCAKFACSKKMTGKSIQPEN	
30	MTGKSIQPENLEYRIMLSVH	
31	LEYRIMLSVHGSQHSGMIVN	
32	GSQHSGMIVNDTGHETDENR	
33	DTGHETDENRAKVEITPNSP	Env 3
34	AKVEITPNSPRAEATLGGFG	
35	RAEATLGGFGSLGLDCEPRT	
36	SLGLDCEPRTGLDFSDLYYL	
37	GLDFSDLYYLTMNNKHWLVH	
38	TMNNKHWLVHKEWFHDIPLP	
39	KEWFHDIPLPWHAGADTGTP	
40	WHAGADTGTPHWNNKEALVE	
41	HWNNKEALVEFKDAHAKRQT	Env 4
42	FKDAHAKRQTVVVLGSQEGA	
43	VVVLGSQEGAVHTALAGALE	
44	VHTALAGALEAEMDGAKGRL	
45	AEMDGAKGRLSSGHLKCRLK	
46	SSGHLKCRLKMDKLRLKGVS	
47	MDKLRLKGVSYSLCTAAFTF	
48	YSLCTAAFTFTKIPAETLHG	
49	TKIPAETLHGTVTVEVQYAG	Env 5
50	TVTVEVQYAGTDGPCKVPAQ	
51	TDGPCKVPAQMAVDMQTLTP	
52	MAVDMQTLTPVGRLITANPV	
53	VGRLITANPVITESTENSKM	
54	ITESTENSKMMLELDPPFGD	
55	MLELDPPFGDSYIVIGVGEK	

Peptide number	Peptide sequence	Zika Peptides
56	SYIVIGVGEKKITHHWHRSG	
57	KITHHWHRSGSTIGKAFEAT	
58	STIGKAFEATVRGAKRMAVL	Env 6
59	VRGAKRMAVLGDTAWDFGSV	
60	GDTAWDFGSVGGALNSLGKG	
61	GGALNSLGKGIHQIFGAAFK	
62	IHQIFGAAFKSLFGGMSWFS	
63	SLFGGMSWFSQILIGTLLMW	
64	QILIGTLLMWLGLNTKNGSI	
65	LGLNTKNGSISLMCLALGGV	
66	SLMCLALGGVLIFLSTAVSA	

3.3 ChAdOx1 MERS

Peptide number	Peptide sequence	MERS or tPA Peptides
1	MDAMKRGLCCVLLLC	tPA
2	RGLCCVLLLCGAVFV	
3	VLLLCGAVFVSASQE	
4	GAVFVSASQEIHARF	
5	SASQEIHARFRRIHS	
6	IHARFRRIHSVFLLM	Pool 1
7	RRIHSVFLLMFLLTP	
8	VFLLMFLLTPTESYV	
9	FLLTPTESYVDVGPD	
10	TESYVDVGPDSVKSA	
11	DVGPDSVKSACIEVD	
12	SVKSACIEVDIQQTF	
13	CIEVDIQQTFFDKTW	
14	IQQTFFDKTWPRPID	
15	FDKTWPRPIDVSKAD	
16	PRPIDVSKADGIIYP	
17	VSKADGIIYPQGRTY	
18	GIIYPQGRTYSNITI	
19	QGRTYSNITITYQGL	
20	SNITITYQGLFPYQG	
21	TYQGLFPYQGDHGDM	

Peptide number	Peptide sequence	MERS or tPA Peptides
22	FPYQGDHGDMYVYSA	
23	DHGDMYVYSAGHATG	
24	YVYSAGHATGTTPQK	
25	GHATGTTPQKLFVAN	
26	TTPQKLFVANYSQDV	
27	LFVANYSQDVKQFAN	
28	YSQDVKQFANGFVVR	
29	KQFANGFVVRIGAAA	
30	GFVVRIGAAANSTGT	
31	IGAAANSTGTVIISP	
32	NSTGTVIISPSTSAT	
33	VIISPSTSATIRKIY	
34	STSATIRKIYPAFML	
35	IRKIYPAFMLGSSVG	
36	PAFMLGSSVGNFSDG	
37	GSSVGNFSDGKMGRF	Pool 2
38	NFSDGKMGRFFNHTL	
39	KMGRFFNHTLVLLPD	
40	FNHTLVLLPDGCGTL	
41	VLLPDGCGTLLRAFY	
42	GCGTLLRAFYCILEP	
43	LRAFYCILEPRSGNH	
44	CILEPRSGNHCPAGN	
45	RSGNHCPAGNSHTSF	
46	CPAGNSHTSFATYHT	
47	SHTSFATYHTPATDC	
48	ATYHTPATDCSDGNY	
49	PATDCSDGNYNRNAS	
50	SDGNYNRNASLNSFK	
51	NRNASLNSFKEYFNL	
52	LNSFKEYFNLRNCTF	
53	EYFNLRNCTFMYTYN	
54	RNCTFMYTYNITEDE	
55	MYTYNITEDEILEWF	Pool 3
56	ITEDEILEWFGITQT	
57	ILEWFGITQTAQGVH	
58	GITQTAQGVHLFSSR	
59	AQGVHLFSSRYVDLY	
60	LFSSRYVDLYGGNMF	
61	YVDLYGGNMFQFATL	
62	GGNMFQFATLPVYDT	

Peptide number	Peptide sequence	MERS or tPA Peptides
63	QFATLPVYDTIKYYS	
64	PVYDTIKYYSIIPHS	
65	IKYYSIIPHSIRSIQ	
66	IIPHSIRSIQSDRKA	
67	IRSIQSDRKAWAAFY	
68	SDRKAWAAFYVYKLQ	
69	WAAFYVYKLQPLTFL	
70	VYKLQPLTFLLDFSV	
71	PLTFLLDFSVDGYIR	
72	LDFSVDGYIRRAIDC	
73	DGYIRRAIDCGFNDL	
74	RAIDCGFNDLSQLHC	
75	GFNDLSQLHCSYESF	
76	SQLHCSYESFDVESG	
77	SYESFDVESGVYSVS	
78	DVESGVYSVSSFEAK	
79	VYSVSSFEAKPSGSV	Pool 4
80	SFEAKPSGSVVEQAE	
81	PSGSVVEQAEGVECD	
82	VEQAEGVECDFSPLL	
83	GVECDFSPLLSGTPP	
84	FSPLLSGTPPQVYNF	
85	SGTPPQVYNFKRLVF	
86	QVYNFKRLVFTNCNY	
87	KRLVFTNCNYNLTKL	
88	TNCNYNLTKLLSLFS	
89	NLTKLLSLFSVNDFT	
90	LSLFSVNDFTCSQIS	
91	VNDFTCSQISPAAIA	
92	CSQISPAAIASNCYS	
93	PAAIASNCYSSLILD	
94	SNCYSSLILDYFSYP	
95	SLILDYFSYPLSMKS	
96	YFSYPLSMKSDLSVS	Pool 5
97	LSMKSDLSVSSAGPI	
98	DLSVSSAGPISQFNY	
99	SAGPISQFNYKQSFS	
100	SQFNYKQSFSNPTCL	
101	KQSFSNPTCLILATV	
102	NPTCLILATVPHNLT	
103	ILATVPHNLTTITKP	

Peptide number	Peptide sequence	MERS or tPA Peptides
104	PHNLTTITKPLKYSY	
105	TITKPLKYSYINKCS	
106	LKYSYINKCSRLLSD	
107	INKCSRLLSDDRTEV	
108	RLLSDDRTEVPQLVN	
109	DRTEVPQLVNANQYS	
110	PQLVNANQYSPCVSI	
111	ANQYSPCVSIVPSTV	
112	PCVSIVPSTVWEDGD	
113	VPSTVWEDGDYYRKQ	
114	WEDGDYYRKQLSPLE	
115	YYRKQLSPLEGGGWL	
116	LSPLEGGGWLVASGS	
117	GGGWLVASGSTVAMT	
118	VASGSTVAMTEQLQM	
119	TVAMTEQLQMGFGIT	
120	EQLQMGFGITVQYGT	
121	GFGITVQYGTDTNSV	Pool 6
122	VQYGTDTNSVCPKLE	
123	DTNSVCPKLEFANDT	
124	CPKLEFANDTKIASQ	
125	FANDTKIASQLGNCV	
126	KIASQLGNCVEYSLY	
127	LGNCVEYSLYGVSGR	
128	EYSLYGVSGRGVFQN	
129	GVSGRGVFQNCTAVG	
130	GVFQNCTAVGVRQQR	
131	CTAVGVRQQRFVYDA	
132	VRQQRFVYDAYQNLV	
133	FVYDAYQNLVGYYSD	
134	YQNLVGYYSDDGNYY	
135	GYYSDDGNYYCLRAC	
136	DGNYYCLRACVSVPV	
137	CLRACVSVPVSVIYD	Pool 7
138	VSVPVSVIYDKETKT	
139	SVIYDKETKTHATLF	
140	KETKTHATLFGSVAC	
141	HATLFGSVACEHISS	
142	GSVACEHISSTMSQY	
143	EHISSTMSQYSRSTR	

Peptide number	Peptide sequence	MERS or tPA Peptides
144	TMSQYSRSTRSMLKR	
145	SRSTRSMLKRRDSTY	
146	SMLKRRDSTYGPLQT	
147	RDSTYGPLQTPVGCV	
148	GPLQTPVGCVLGLVN	
149	PVGCVLGLVNSSLFV	
150	LGLVNSSLFVEDCKL	
151	SSLFVEDCKLPLGQS	
152	EDCKLPLGQSLCALP	
153	PLGQSLCALPDTPST	Pool 8
154	LCALPDTPSTLTPRS	
155	DTPSTLTPRSVRSVP	
156	LTPRSVRSVPGEMRL	
157	VRSVPGEMRLASIAF	
158	GEMRLASIAFNHPIQ	
159	ASIAFNHPIQVDQLN	
160	NHPIQVDQLNSSYFK	
161	VDQLNSSYFKLSIPT	
162	SSYFKLSIPTNFSFG	
163	LSIPTNFSFGVTQEY	
164	NFSFGVTQEYIQTTI	
165	VTQEYIQTTIQKVTV	
166	IQTTIQKVTVDCKQY	
167	QKVTVDCKQYVCNGF	
168	DCKQYVCNGFQKCEQ	
169	VCNGFQKCEQLLREY	
170	QKCEQLLREYGQFCS	
171	LLREYGQFCSKINQA	
172	GQFCSKINQALHGAN	
173	KINQALHGANLRQDD	
174	LHGANLRQDDSVRNL	Pool 9
175	LRQDDSVRNLFASVK	
176	SVRNLFASVKSSQSS	
177	FASVKSSQSSPIIPG	
178	SSQSSPIIPGFGGDF	
179	PIIPGFGGDFNLTLL	
180	FGGDFNLTLLEPVSI	
181	NLTLLEPVSISTGSR	
182	EPVSISTGSRSARSA	

Peptide number	Peptide sequence	MERS or tPA Peptides
183	STGSRSARSAIEDLL	
184	SARSAIEDLLFDKVT	
185	IEDLLFDKVTIADPG	
186	FDKVTIADPGYMQGY	
187	IADPGYMQGYDDCMQ	
188	YMQGYDDCMQQGPAS	
189	DDCMQQGPASARDLI	
190	QGPASARDLICAQYV	
191	ARDLICAQYVAGYKV	
192	CAQYVAGYKVLPPLM	
193	AGYKVLPPLMDVNME	
194	LPPLMDVNMEAAYTS	
195	DVNMEAAYTSSLLGS	
196	AAYTSSLLGSIAGVG	
197	SLLGSIAGVGWTAGL	
198	IAGVGWTAGLSSFAA	
199	WTAGLSSFAAIPFAQ	
200	SSFAAIPFAQSIFYR	
201	IPFAQSIFYRLNGVG	
202	SIFYRLNGVGITQQV	
203	LNGVGITQQVLSENQ	
204	ITQQVLSENQKLIAN	
205	LSENQKLIANKFNQA	Pool 10
206	KLIANKFNQALGAMQ	
207	KFNQALGAMQTGFTT	
208	LGAMQTGFTTTNEAF	
209	TGFTTTNEAFRKVQD	
210	TNEAFRKVQDAVNNN	
211	RKVQDAVNNNAQALS	
212	AVNNNAQALSKLASE	
213	AQALSKLASELSNTF	
214	KLASELSNTFGAISA	
215	LSNTFGAISASIGDI	
216	GAISASIGDIIQRLD	
217	SIGDIIQRLDVLEQD	
218	IQRLDVLEQDAQIDR	Pool 11
219	VLEQDAQIDRLINGR	
220	AQIDRLINGRLTTLN	
221	LINGRLTTLNAFVAQ	

Peptide number	Peptide sequence	MERS or tPA Peptides
222	LTTLNAFVAQQLVRS	
223	AFVAQQLVRSESAAL	
224	QLVRSESAALSAQLA	
225	ESAALSAQLAKDKVN	
226	SAQLAKDKVNECVKA	
227	KDKVNECVKAQSKRS	
228	ECVKAQSKRSGFCGQ	
229	QSKRSGFCGQGTHIV	
230	GFCGQGTHIVSFVVN	
231	GTHIVSFVVNAPNGL	
232	SFVVNAPNGLYFMHV	
233	APNGLYFMHVGYYPS	
234	YFMHVGYYPSNHIEV	
235	GYYPSNHIEVVSAYG	
236	NHIEVVSAYGLCDAA	
237	VSAYGLCDAANPTNC	
238	LCDAANPTNCIAPVN	
239	NPTNCIAPVNGYFIK	
240	IAPVNGYFIKTNNTR	
241	GYFIKTNNTRIVDEW	
242	TNNTRIVDEWSYTGS	
243	IVDEWSYTGSSFYAP	
244	SYTGSSFYAPEPITS	
245	SFYAPEPITSLNTKY	
246	EPITSLNTKYVAPQV	
247	LNTKYVAPQVTYQNI	Pool 12
248	VAPQVTYQNISTNLP	
249	TYQNISTNLPPPLLG	
250	STNLPPPLLGNSTGI	
251	PPLLGNSTGIDFQDE	
252	NSTGIDFQDELDEFF	
253	DFQDELDEFFKNVST	
254	LDEFFKNVSTSIPNF	
255	KNVSTSIPNFGSLTQ	
256	SIPNFGSLTQINTTL	
257	GSLTQINTTLLDLTY	
258	INTTLLDLTYEMLSL	
259	LDLTYEMLSLQQVVK	Pool 13
260	EMLSLQQVVKALNES	

Peptide number	Peptide sequence	MERS or tPA Peptides
261	QQVVKALNESYIDLK	
262	ALNESYIDLKELGNY	
263	YIDLKELGNYTYYNK	
264	ELGNYTYYNKWPWYI	
265	TYYNKWPWYIWLGFI	
266	WPWYIWLGFIAGLVA	
267	WLGFIAGLVALALCV	
268	AGLVALALCVFFILC	
269	LALCVFFILCCTGCG	
270	FFILCCTGCGTNCMG	
271	CTGCGTNCMGKLKCN	
272	TNCMGKLKCNRCCDR	
273	KLKCNRCCDRYEEYD	
274	RCCDRYEEYDLEPHK	
275	YEEYDLEPHKVHVH	

3.4 ChAdOx1 nCoV-19

Peptide number	Peptide sequence	Subunit	Pool
1	MFVFLVLLPLVSSQC	S1	1
2	VLLPLVSSQCVNLTT		
3	VSSQCVNLTTRTQLP		
4	VNLTTRTQLPPAYTN		
5	RTQLPPAYTNSFTRG		
6	PAYTNSFTRGVYYPD		
7	SFTRGVYYPDKVFRS		
8	VYYPDKVFRSSVLHS		
9	KVFRSSVLHSTQDLF		
10	SVLHSTQDLFLPFFS		
11	TQDLFLPFFSNVTWF		
12	LPFFSNVTWFHAIHV		
13	NVTWFHAIHVSGTNG		
14	HAIHVSGTNGTKRFD		
15	SGTNGTKRFDNPVLP		
16	TKRFDNPVLPFNDGV		
17	NPVLPFNDGVYFAST		

Peptide number	Peptide sequence	Subunit	Pool
18	FNDGVYFASTEKSNI		
19	YFASTEKSNIIRGWI		
20	EKSNIIRGWIFGTTL		
21	IRGWIFGTTLDSKTQ		2
22	FGTTLDSKTQSLLIV		
23	DSKTQSLLIVNNATN		
24	SLLIVNNATNVVIKV		
25	NNATNVVIKVCEFQF		
26	VVIKVCEFQFCNDPF		
27	CEFQFCNDPFLGVYY		
28	CNDPFLGVYYHKNNK		
29	LGVYYHKNNKSWMES		
30	HKNNKSWMESEFRVY		
31	SWMESEFRVYSSANN		
32	EFRVYSSANNCTFEY		
33	SSANNCTFEYVSQPF		
34	CTFEYVSQPFLMDLE		
35	VSQPFLMDLEGKQGN		
36	LMDLEGKQGNFKNLR		
37	GKQGNFKNLREFVFK		
38	FKNLREFVFKNIDGY		
39	EFVFKNIDGYFKIYS		
40	NIDGYFKIYSKHTPI		
41	FKIYSKHTPINLVRD		3
42	KHTPINLVRDLPQGF		
43	NLVRDLPQGFSALEP		
44	LPQGFSALEPLVDLP		
45	SALEPLVDLPIGINI		
46	LVDLPIGINITRFQT		
47	IGINITRFQTLLALH		
48	TRFQTLLALHRSYLT		
49	LLALHRSYLTPGDSS		
50	RSYLTPGDSSSGWTA		
51	PGDSSSGWTAGAAAY		
52	SGWTAGAAAYYVGYL		
53	GAAAYYVGYLQPRTF		
54	YVGYLQPRTFLLKYN		
55	QPRTFLLKYNENGTI		
56	LLKYNENGTITDAVD		

Peptide number	Peptide sequence	Subunit	Pool
57	ENGTITDAVDCALDP		
58	TDAVDCALDPLSETK		
59	CALDPLSETKCTLKS		
60	LSETKCTLKSFTVEK		
61	CTLKSFTVEKGIYQT		
62	FTVEKGIYQTSNFRV		
63	GIYQTSNFRVQPTES		4
64	SNFRVQPTESIVRFP		
65	QPTESIVRFPNITNL		
66	IVRFPNITNLCPFGE		
67	NITNLCPFGEVFNAT		
68	CPFGEVFNATRFASV		
69	VFNATRFASVYAWNR		
70	RFASVYAWNRKRISN		
71	YAWNRKRISNCVADY		
72	KRISNCVADYSVLYN		
73	CVADYSVLYNSASFS		
74	SVLYNSASFSTFKCY		
75	SASFSTFKCYGVSPT		
76	TFKCYGVSPTKLNDL		
77	GVSPTKLNDLCFTNV		
78	KLNDLCFTNVYADSF		
79	CFTNVYADSFVIRGD		
80	YADSFVIRGDEVRQI		
81	VIRGDEVRQIAPGQT		
82	EVRQIAPGQTGKIAD		
83	APGQTGKIADYNYKL		
84	GKIADYNYKLPDDFT		
85	YNYKLPDDFTGCVIA		
86	PDDFTGCVIAWNSNN		
87	GCVIAWNSNNLDSKV		5
88	WNSNNLDSKVGGNYN		
89	LDSKVGGNYNYLYRL		
90	GGNYNYLYRLFRKSN		
91	YLYRLFRKSNLKPFE		
92	FRKSNLKPFERDIST		
93	LKPFERDISTEIYQA		
94	RDISTEIYQAGSTPC		
95	EIYQAGSTPCNGVEG		
96	GSTPCNGVEGFNCYF		

Peptide number	Peptide sequence	Subunit	Pool
97	NGVEGFNCYFPLQSY		
98	FNCYFPLQSYGFQPT		
99	PLQSYGFQPTNGVGY		
100	GFQPTNGVGYQPYRV		
101	NGVGYQPYRVVVLSF		
102	QPYRVVVLSFELLHA		
103	VVLSFELLHAPATVC		
104	ELLHAPATVCGPKKS		
105	PATVCGPKKSTNLVK		
106	GPKKSTNLVKNKCVN		
107	TNLVKNKCVNFNFNG		
108	NKCVNFNFNGLTGTG		
109	FNFNGLTGTGVLTES		
110	LTGTGVLTESNKKFL		
111	VLTESNKKFLPFQQF		6
112	NKKFLPFQQFGRDIA		
113	PFQQFGRDIADTTDA		
114	GRDIADTTDAVRDPQ		
115	DTTDAVRDPQTLEIL		
116	VRDPQTLEILDITPC		
117	TLEILDITPCSFGGV		
118	DITPCSFGGVSVITP		
119	SFGGVSVITPGTNTS		
120	SVITPGTNTSNQVAV		
121	GTNTSNQVAVLYQDV		
122	NQVAVLYQDVNCTEV		
123	LYQDVNCTEVPVAIH		
124	NCTEVPVAIHADQLT		
125	PVAIHADQLTPTWRV		
126	ADQLTPTWRVYSTGS		
127	PTWRVYSTGSNVFQT		
128	YSTGSNVFQTRAGCL		
129	NVFQTRAGCLIGAEH		
130	RAGCLIGAEHVNNSY		
131	IGAEHVNNSYECDIP		
132	VNNSYECDIPIGAGI		
133	ECDIPIGAGICASYQ		
134	IGAGICASYQTQTNS		
135	CASYQTQTNSPRRAR	S2	7
136	TQTNSPRRARSVASQ		

Peptide number	Peptide sequence	Subunit	Pool
137	PRRARSVASQSIIAY		
138	SVASQSIIAYTMSLG		
139	SIIAYTMSLGAENSV		
140	TMSLGAENSVAYSNN		
141	AENSVAYSNNSIAIP		
142	AYSNNSIAIPTNFTI		
143	SIAIPTNFTISVTTE		
144	TNFTISVTTEILPVS		
145	SVTTEILPVSMTKTS		
146	ILPVSMTKTSVDCTM		
147	MTKTSVDCTMYICGD		
148	VDCTMYICGDSTECS		
149	YICGDSTECSNLLLQ		
150	STECSNLLLQYGSFC		
151	NLLLQYGSFCTQLNR		
152	YGSFCTQLNRALTGI		
153	TQLNRALTGIAVEQD		
154	ALTGIAVEQDKNTQE		
155	AVEQDKNTQEVFAQV		8
156	KNTQEVFAQVKQIYK		
157	VFAQVKQIYKTPPIK		
158	KQIYKTPPIKDFGGF		
159	TPPIKDFGGFNFSQI		
160	DFGGFNFSQILPDPS		
161	NFSQILPDPSKPSKR		
162	LPDPSKPSKRSFIED		
163	KPSKRSFIEDLLFNK		
164	SFIEDLLFNKVTLAD		
165	LLFNKVTLADAGFIK		
166	VTLADAGFIKQYGDC		
167	AGFIKQYGDCLGDIA		
168	QYGDCLGDIAARDLI		
169	LGDIAARDLICAQKF		
170	ARDLICAQKFNGLTV		
171	CAQKFNGLTVLPPLL		
172	NGLTVLPPLLTDEMI		
173	LPPLLTDEMIAQYTS		
174	TDEMIAQYTSALLAG		
175	AQYTSALLAGTITSG		9
176	ALLAGTITSGWTFGA		

Peptide number	Peptide sequence	Subunit	Pool
177	TITSGWTFGAGAALQ		
178	WTFGAGAALQIPFAM		
179	GAALQIPFAMQMAYR		
180	IPFAMQMAYRFNGIG		
181	QMAYRFNGIGVTQNV		
182	FNGIGVTQNVLYENQ		
183	VTQNVLYENQKLIAN		
184	LYENQKLIANQFNSA		
185	KLIANQFNSAIGKIQ		
186	QFNSAIGKIQDSLSS		
187	IGKIQDSLSSTASAL		
188	DSLSSTASALGKLQD		
189	TASALGKLQDVVNQN		
190	GKLQDVVNQNAQALN		
191	VVNQNAQALNTLVKQ		
192	AQALNTLVKQLSSNF		
193	TLVKQLSSNFGAISS		
194	LSSNFGAISSVLNDI		
195	GAISSVLNDILSRLD		
196	VLNDILSRLDKVEAE		10
197	LSRLDKVEAEVQIDR		
198	KVEAEVQIDRLITGR		
199	VQIDRLITGRLQSLQ		
200	LITGRLQSLQTYVTQ		
201	LQSLQTYVTQQLIRA		
202	TYVTQQLIRAAEIRA		
203	QLIRAAEIRASANLA		
204	AEIRASANLAATKMS		
205	SANLAATKMSECVLG		
206	ATKMSECVLGQSKRV		
207	ECVLGQSKRVDFCGK		
208	QSKRVDFCGKGYHLM		
209	DFCGKGYHLMSFPQS		
210	GYHLMSFPQSAPHGV		
211	SFPQSAPHGVVFLHV		
212	APHGVVFLHVTYVPA		
213	VFLHVTYVPAQEKNF		
214	TYVPAQEKNFTTAPA		
215	QEKNFTTAPAICHDG		
216	TTAPAICHDGKAHFP		11

Peptide number	Peptide sequence	Subunit	Pool
217	ICHDGKAHFPREGVF		
218	KAHFPREGVFVSNGT		
219	REGVFVSNGTHWFVT		
220	VSNGTHWFVTQRNFY		
221	HWFVTQRNFYEPQII		
222	QRNFYEPQIITTDNT		
223	EPQIITTDNTFVSGN		
224	TTDNTFVSGNCDVVI		
225	FVSGNCDVVIGIVNN		
226	CDVVIGIVNNTVYDP		
227	GIVNNTVYDPLQPEL		
228	TVYDPLQPELDSFKE		
229	LQPELDSFKEELDKY		
230	DSFKEELDKYFKNHT		
231	ELDKYFKNHTSPDVD		
232	FKNHTSPDVDLGDIS		
233	SPDVDLGDISGINAS		
234	LGDISGINASVVNIQ		
235	GINASVVNIQKEIDR		
236	VVNIQKEIDRLNEVA		12
237	KEIDRLNEVAKNLNE		
238	LNEVAKNLNESLIDL		
239	KNLNESLIDLQELGK		
240	SLIDLQELGKYEQYI		
241	QELGKYEQYIKWPWY		
242	YEQYIKWPWYIWLGF		
243	KWPWYIWLGFIAGLI		
244	IWLGFIAGLIAIVMV		
245	IAGLIAIVMVTIMLC		
246	AIVMVTIMLCCMTSC		
247	TIMLCCMTSCCSCLK		
248	CMTSCCSCLKGCCSC		
249	CSCLKGCCSCGSCCK		
250	GCCSCGSCCKFDEDD		
251	GSCCKFDEDDSEPVL		
252	FDEDDSEPVLKGVKL		
253	DDSEPVLKGVKLHYT		
254	MDAMKRGLCCVLLLC	tPA	tPA
255	RGLCCVLLLCGAVFV		
256	VLLLCGAVFVSASQE		

Peptide number	Peptide sequence	Subunit	Pool
257	GAVFVSASQEIHARF		
258	SASQEIHARFRRIHS		

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