Safety and Immunogenicity of a candidate MERS-CoV Viral Vectored Vaccine: a phase I, open-labelled, first-in-human, dose-escalation trial.

Pedro M. Folegatti[1], Mustapha Bittaye[1], Amy Flaxman[1], Fernando Ramos Lopez[1], Duncan Bellamy[1], Alexandra Kupke[2,3], Catherine Mair[1], Rebecca Makinson[1], Jonathan Sheridan[1], Cornelius Rohde[2,3], Sandro Halwe[2,3], Yuji Jeong[4], Young-Shin Park[4], Jae-Ouk Kim[4], Manki Song[4], Amy Boyd[1], Nguyen Tran[1], Daniel Silman[1], Ian Poulton[1], Mehreen Datoo[1], Julia Marshal[1], Yrene Themistocleous[1], Alison Lawrie[1], Rachel Roberts[1], Eleanor Berrie[1], Stephan Becker[2,3], Teresa Lambe[1], Adrian Hill[1], Katie Ewer[1] and Sarah Gilbert[1]

Affiliation:

[1] The Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK

[2] Institute of Virology, Philipps University of Marburg, Marburg, Germany

[3] German Center for Infection Research, TTU Emerging Infections, Marburg, Germany

[4] International Vaccine Institute, Science Unit, Seoul, 08826, Republic of Korea

Summary

Background: Middle East Respiratory Syndrome Coronavirus (MERS-CoV) cases continue to arise in the Arabian Peninsula seven years after it was first described in Saudi Arabia. MERS-CoV poses a significant risk to public health security due to lack of currently available effective countermeasures. Here we present the safety and immunogenicity results of a first-in-human trial of the candidate simian adenovirus vectored vaccine expressing the full-length Spike surface glycoprotein, ChAdOx1 MERS.

Methods: 24 healthy adult volunteers aged 18-50 years received a single intra muscular injection of ChAdOx1 MERS as part of a phase I, dose escalation, open-label, non-randomised and uncontrolled clinical study at 3 different doses ($5x10^9$, $2{\cdot}5x10^{10}$ and $5x10^{10}$ viral particles). The primary objective was to assess safety and tolerability of ChAdOx1 MERS, measured by the occurrence of solicited,

unsolicited and serious adverse events (AEs) post vaccination. The secondary objective was to assess the cellular and humoral immunogenicity of ChAdOx1 MERS, measured by interferon gamma ELISpot, ELISA and virus neutralising assays post vaccination. Participants were followed for up to 12 months. This study is registered with Clinicaltrials.gov. Registration number NCT03399578.

Findings: A single dose of ChAdOx1 MERS was safe at doses up to 5 x 10^{10} vp with no serious adverse reactions reported. The vast majority of solicited AEs were mild (92/124; 74%, 95%CI 66-81) or moderate (31/124; 25%, 95%CI 18-33) and all were self-limiting in nature. A significant increase from baseline in T-cell and IgG responses to the MERS-CoV Spike antigen was observed at all doses. Neutralising antibodies against live MERS-CoV were observed in four of nine (44%, 95%CI 19-73) participants who received the 5x10^{10} vp dose at 4 weeks post vaccination, and 19 of 24 (79%, 95% CI 58-93) participants had antibodies capable of neutralisation in a pseudotyped virus neutralisation assay.

Interpretation: 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 field Phase Ib and II trials.

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INTRODUCTION

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes an emerging zoonotic viral respiratory disease that was first described in 2012 and is now endemic in Saudi Arabia.[1] Clinical presentation of MERS-CoV infections varies from asymptomatic to severe acute respiratory distress and death. MERS-CoV has since spread to different countries in the Middle East and other regions with 2494 laboratory confirmed cases of MERS-CoV infection including 858 deaths in 27 countries reported, as of November 2019.[2] 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 research and development by the World Health Organization (WHO) and other health agencies around the globe. Dromedary camels are now a recognised source of zoonotic infections and occupational exposure has been associated with seroconversion,[3] although only 40% of primary cases have been associated with direct camel exposure.[4] Human to human transmission, especially in hospital environments, has been responsible for the majority of cases seen in recent outbreaks.[5] However, no sustained human to human transmission has been recorded to date (overall $R_0<1$) and the role of asymptomatic or mild cases in transmission patterns remain 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 whilst the S protein is involved in receptor binding, mediating virus entry into host cells during CoVs infection via different receptors.[6] 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.[7] 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 novel coronavirus 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.[8]

The spike protein is a type I, trimeric, transmembrane glycoprotein located at the surface of the viral envelope of CoVs, which can be divided into two functional subunits: the N-terminal S1 and the C-terminal S2. S1 and S2 are responsible for cellular receptor DPP4 binding (MERS-CoV) via the receptor binding domain (RBD) and fusion of virus and cell membranes respectively, thereby mediating the entry of MERS-CoV into target cells.[6] 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.[9] 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.[10]

The simian adenovirus vectored vaccine ChAdOx1 MERS expresses a codon-optimised coding sequence for the full-length Spike protein from MERS-CoV. 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).[11] A single dose of ChAdOx1 MERS also conferred protective efficacy in transgenic human DPP4 mice against lethal MERS-CoV challenge, which supported progression into clinical development.[12] Immunogenicity and partial protective efficacy in a natural transmission model in dromedary camels has also been reported.[13] Here we report up to 12 months' follow-up safety and immunogenicity results of a first-in-human trial of the ChAdOx1 MERS candidate MERS-CoV vaccine.

METHODS

ChAdOx1 MERS Vaccine

ChAdOx1 MERS consists of the replication-deficient simian adenovirus vector ChAdOx1, which has been described elsewhere,[14] expressing a codon-optimised coding sequence for the full-length structural surface glycoprotein (Spike protein – S1 and S2 subunits) of the MERS-CoV isolate Camel/Qatar_2_2014 (GenBank: KJ650098.1), including a 32 amino acid N-terminal tPA (tissue plasminogen activator) leader sequence. The recombinant adenovirus was produced as previously described.[11] The vaccine was manufactured to current Good Manufacturing Practice (cGMP) by the Clinical Biomanufacturing Facility (University of Oxford, Oxford, UK) in a Tet repressed HEK 293 cell line. The vectored vaccine was purified and sterile filtered to generate a clinical lot at a concentration of $1{\cdot}74 \times 10^{11}$ viral particles per mL.

Study Design and Participants

This is a first-in-human, dose escalation, open-label, non-randomised and uncontrolled clinical study of 24 healthy male and female subjects aged 18–50 years old. Eligible volunteers were recruited at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, United Kingdom (CONSORT diagram: Figure 1). 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. Full details of the eligibility criteria are described in the trial protocol provided in the Supplementary Materials.

Written informed consent was obtained in all cases, and the trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP). This study was approved within the UK by the Medicines and Healthcare Products Regulatory Agency (MHRA reference 21584/0381/001-0001) and the South Central - Oxford A Research Ethics Committee (REC reference 17/SC/0552). Vaccine use was authorized by the Genetically Modified Organisms Safety Committee of

the Oxford University Hospitals National Health Service Trust (GMSC reference number GM462.18.101). An independent Local Safety Monitor (LSM) provided safety oversight. The trial is registered at www.clinicaltrials.gov (identifier: NCT03399578).

The primary objective was to assess safety and tolerability of ChAdOx1 MERS in healthy volunteers, measured as: a) occurrence of solicited local reactogenicity signs and symptoms for 7 days following vaccination; b) occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following vaccination; c) occurrence of unsolicited adverse events (AEs) for 28 days following vaccination; d) change from baseline for safety laboratory measures and; e) occurrence of serious adverse events (SAEs) during the whole study duration.

The secondary objective was to assess cellular and humoral immunogenicity of ChAdOx1 MERS as measured by interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot), enzyme-linked immunosorbent assay (ELISA), and virus neutralising antibody (NAb) assays.

Study Procedures

ChAdOx1 MERS was administered as a single intramuscular injection into the deltoid at $5x10^9$ vp (group 1), $2{\cdot}5x10^{10}$ vp (group 2) and 5×10^{10} vp (group 3). A staggered-enrolment approach was used for the first 3 participants in each group and interim safety reviews conducted prior to dose escalation (details provided in study supplementary materials).

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, 182 and 364 days following enrolment. Participants were observed in the clinic for one hour after the vaccination procedure and were asked to record any AEs using electronic diaries during the 28-day follow-up period. Swelling at the injection site was objectively assessed by a member of the study team during the study visits.

Solicited 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 SAEs were recorded throughout the follow-up period.

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); and (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 definitely related with the study vaccine were reported. Laboratory AEs were graded using site specific toxicity tables which were adapted from the US Food and Drug Administration toxicity grading scale.

Humoral Immunity: ELISA and MERS-CoV Neutralising Antibody Assays

MERS Spike-specific Total IgG ELISA

Total anti-MERS spike IgG was measured using a standardised in-house indirect ELISA. Nunc-immuno 96 well plates 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, Australia) in phosphate buffered saline (PBS) and incubated at 4°C for 18 h overnight. The coated plates were washed six times with PBS-Tween and then blocked with casein for 1 h at room temperature (RT). 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 was followed by incubation of the plates at RT for 2 h and washing of the plates as initially described. The plates were then incubated at RT for 1 h with a secondary antibody, alkaline phosphatase conjugated goat anti-human IgG (gamma-chain specific). After a final wash, plates were developed by adding 4-nitrophenyl phosphate in diethanolamine substrate buffer (Thermo Scientific). The standard curve used on each plate was derived from a pool of volunteers' sera containing high titre anti-MERS IgG. End point titre ELISA was used to identify the volunteer samples with the highest anti-MERS IgG titres post vaccination. A 1:100 dilution of the standard pool was used in a 2-fold serial dilution to produce 10 standard points that were assigned arbitrary ELISA units (EUs). The optical density (OD) values of the standard points were

fitted to a 4-parameter hyperbolic curve against the arbitrary EUs using GEN5 software (version 3.04) and the parameters estimated from the standard curve were used to convert absorbance values of individual test samples into EUs. Each ELISA plate consists of the samples and internal positive control (1:800 dilution of the standard pool, corresponding to standard 4) in triplicates, 10 standard points in duplicates and 4 blank wells. The OD reading of the plates at 405 nm was performed using an ELx808 microplate reader (BioTek).

Neutralising Antibody Assay

Neutralising antibodies against live MERS-CoV were measured from 22 subjects (5 participants in G1, 8 participants in G2 and 9 participants in G3) using paired serum samples obtained at day 0 and day 28. Induction of virus-neutralising antibodies was confirmed according to previously published protocols.[15] 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 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) served as a neutralization control. Full neutralization was observed at a dilution of 1:512 (+/- 1 dilution step acceptable as inter-assay variation) which corresponds to a concentration of 0.2 µg/ml.[16] Neutralization titres were calculated as reciprocal values of geometric mean titres of four replicates. A titre of 8 was considered positive.

Pseudovirus neutralization assay

MERS-CoV EMC/2012, KOR/KNIH/002 and England-1 (ΔER, aa 1-1338) - spike pseudotyped lentiviral particles were generated and titrated using Lentivirus-Associated p24 ELISA Kit (Cell Biolabs).[17] Two-fold diluted serum was incubated with $2x10^6$ vp of pseudotyped virus for 60 minutes at 37°C and added to 786O cells, incubated for 6 hrs, replaced with fresh medium, and luciferase-reporter activity was measured three days later. A commercially available neutralizing monoclonal antibody (Sino Biological

Inc. #40069-R723) was used as positive control. All the samples were analysed in duplicates. Data was expressed as mean ± SD.

Cellular Immunity

ELISpot assays were performed using fresh peripheral blood mononuclear cells (PBMCs) to determine responses to the MERS spike vaccine antigen. Methodology was as described previously with the following exceptions.[18] PBMCs were separated from whole blood within 4 hours of venepuncture. A total of 275 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 13 pools for the MERS spike protein containing 18 or 21 peptides, plus a single pool of 5 peptides for the tPA leader. Peptide sequences and pooling are summarised in Supplementary Table 4. Data were analysed according to a quality control (QC) standard operational procedure and data from 6 out of 139 assays (4·3%) were removed from the final data set due to negative controls being above the pre-defined QC parameters.

Responses to the negative control (PBMC with no stimulation) were low, with a median of 17 SFC (IQR 9·3-22·7). The lower limit of detection for the assay was 56 SFC for summed responses to the 13 MERS spike peptide pools.

Statistical Analysis

Safety endpoints are described as frequencies with their respective percentages alongside their 95% confidence intervals (CI). The association between the frequency of moderate or severe solicited AEs and group allocation (groups 2 and 3) is reported as relative risk with the respective 95% CI and *p* value (Fisher's exact test). Immunology data were tested for normal distribution using the D'Agostino-Pearson omnibus normality test. Data were analysed with non-parametric measures if data was not normally distributed or the sample size was small. Kruskal-Wallis with Dunn's multiple comparison post-test was used to compare across timepoints or vaccine groups. P values <0·05 were considered

significant. For ELISpot data, values are SFC per million PBMCs. Statistical analysis of safety and immunogenicity data was conducted using GraphPad Prism version 8·01 for Windows (GraphPad Software Inc., California, USA).

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Study Population

Twenty-four healthy adult subjects received a single dose of ChAdOx1 MERS at $5x10^9$, $2{\cdot}5x10^{10}$ and $5x10^{10}$ vp (CONSORT diagram) and their baseline characteristics are summarised in table 1.

Vaccine Safety

ChAdOx1 MERS was safe at doses up to 5×10^{10} vp with no serious adverse reactions reported. A total of 124 local and systemic solicited AEs were reported. The vast majority of solicited AEs were mild (92 of 124; 74%, 95%CI 66-81) or moderate (31 of 124; 25%, 95%CI 18-33) and self-limiting in nature. All solicited AEs were completely resolved within 6 days and 97% of them had their onset within the first 72h post vaccination (54% at D0, 39% at D1 and 4% at D2). Injection site pain was the most common local AE, reported by 18 of 24 (75%) participants and was predominantly mild in severity. Fatigue was the most common systemic AE followed by headache and malaise. Frequencies of local and systemic solicited AEs reported during the first 7 days are summarised in Table 2. Median duration of solicited AEs is summarised in Supplementary Table 1. Only one serious adverse event has been reported which was deemed not related with ChAdOx1 MERS.

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 (relative risk 5·83, 95%CI 2·11 –17·42, p< 0·001), but there were no safety concerns despite 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 (Supplementary Table 2). Laboratory AEs considered at least possibly related with the study intervention were self-limiting and predominantly mild in severity (Supplementary Table 3).

Humoral Immunogenicity

A single dose of the vaccine induced a strong antibody response, which persisted for one year post-vaccination. Antibody responses increased rapidly after vaccination peaking 28 days post vaccination for all the dose groups combined (Figure 2A). Seroconversion occurred in 18/24 volunteers (75%) at two weeks post vaccination, increasing to 92% (22 volunteers) up to 56 days post vaccination. Seropositivity was maintained in 13/19 volunteers (68%) up to a year post vaccination (Geometric Mean (GM) 381·1, 95% CI 251·2-578·3, p=0·0043, D0 vs D364. Antibody responses peaked at D28 with the high dose group producing the highest antibody response (Figure 2B). IgG titres increased significantly at D28 and D56 post vaccination with increasing ChAdOx1 MERS dose compared to the baseline (Figure 2B and Supplementary Figure 1A) and no significant differences in anti-MERS IgG was found between the dose groups at these time points (data not shown). Detectable anti-MERS IgG titres were observed in 4/24 volunteers 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 volunteers at D28 and D56 post vaccination (Supplementary Figure 1B).

Virus neutralising antibody titres were measured at D0 and were negative, and at D28. Neutralising antibodies were detected only in the low (1/5 volunteers) and high (4/9 volunteers) dose groups, with only the high dose group producing a significant increase in neutralising antibody titres compared to the baseline (p<0·0001, Kruskall-Wallis with Dunn's multiple comparisons test) in an assay testing

neutralisation of live MERS-CoV heterologous to the vaccine derived strain. A further investigation of the relationship between the total IgG and neutralising antibody titres for the high dose group at the peak antibody response showed a moderate positive correlation between the total IgG and neutralising antibody titres (Supplementary Figure 1C).

Neutralisation tests of three pseudotyped lentiviruses expressing Spike protein from three different MERS-CoV strains were negative at D0 but 17/24 samples (71%, 95% CI 49-87) were positive at D28 when tested against EMC/2012 and KOR/KNIH/002, and 19/24 (79%, 95% CI 58-93) were positive when tested against England1 (Figure 3). The neutralising antibody titres measured against the live MERS virus for the high dose group correlated significantly with those measured using the three different pseudotyped lentiviral particles (Supplementary Figure 1D).

Cellular Immunogenicity

Cellular immunogenicity to ChAdOx1 MERS was assessed by *ex vivo* IFN-γ ELISpot. As expected, no response to the exogenous tPA leader sequence was detected (median 5 SFC, IQR 4-12 SFC).

High frequencies of IFN-γ-secreting T cells recognising MERS spike peptides were elicited (Figure 4). Responses peaked at 14 days post vaccination for all three dose groups (Figure 4A, Group 1: median 1617, IQR 1227-3833, Group 2: median 2631, IQR 1373-4966, Group 3: median 4019, IQR 2349- 5013). No significant effect of vaccine dose on magnitude of response was detected at any time point, therefore, data were pooled for subsequent analyses. We observed a significant increase in T cell response compared to baseline at all time points (Figure 4B). Of note, this increase persisted to one year post vaccination at four fold higher than baseline (GM 695·6, 95% CI 537·5-900·2, p = 0·0029 D364 vs D0). Individual T-cell responses per dose group are shown in Supplementary Figure 2.

We investigated the relative contribution of individual peptide pools at the peak time point (D14) to look for regions of immunodominance within the antigen (Figure 4C). Pools 3, 5, 7 and 11 were the most frequently recognised. Responses to S1 were higher than for S2 at D14 for most vaccinees

(Supplementary Figure 3A). T cell responses to both the receptor binding domain (within S1) and S2 persisted to D364 post vaccination (Supplementary Figure 3B).

We detected responses to MERS spike peptides prior to vaccination (Figure 4B and Supplementary Figure 2 GM 208·9, 95% CI 164·9-264·6) in some study participants. These were mainly towards the receptor binding domain peptides (pools 4-6, Supplementary Figure 3B). There was no correlation between the magnitude of pre-existing and post vaccination responses at D182 (Figure 4D, Spearman's r=0·28, p=0·175). No association between D0 responses and HLA type was detected (data not shown), nor was there an association between the magnitude of pre-existing T cell immunity and humoral responses measured at D0. There was no association between the magnitude of T cell and antibody responses to ChAdOx1 MERS at D28 (Supplementary Figure 4, Spearman's r = -0·05, p = 0·82).

DISCUSSION

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 seven years after it was first described.[2] The nonspecific clinical features of MERS-CoV 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.[19] 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.

In this study we have shown that 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 $5x10^{10}$ vp dose, with five out of nine participants in that group reporting short-lived fever above 37·5°C. No serious adverse reactions occurred. 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 and other closely related simian adenoviruses, such as ChAdOx2, ChAd3 and ChAd63 vectored vaccines expressing different antigens.[18,20-23]

Safety concerns around the use of full length coronavirus Spike glycoproteins as a vaccine antigen have been raised following historical reports of immunopathology and antibody dependant enhancement (ADE) reported *in vitro* and post SARS-CoV challenge in mice, ferrets and non-human primates immunised with whole SARS-CoV inactivated or full-length S protein based vaccines.[24-26] To date, there has been one report of lung immunopathology following MERS-CoV challenge in mice immunised with an inactivated MERS-CoV candidate vaccine.[27] However, in preclinical studies of ChAdOx1 immunisation and MERS-CoV challenge, no ADE was observed in hDPP4 transgenic mice, dromedary camels or non-human primates (van Doremalen et al, manuscript submitted).[12,13]

The vaccine was immunogenic at all doses, inducing seroconversion in the majority of 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 peaking 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 is likely to 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.[8] 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 age 10, and it is likely that the rate is similar in the UK[28]. With the emergence of SARS-CoV2, 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.

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.[29] Here we demonstrated that 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. Despite differences in methodology, there was a strong positive correlation between NAbs from pseudotyped viruses and NAbs obtained from the live virus assay in the high dose group (Supplementary Figure 1D). Strategies to increase NAbs seroconversion include a 2-dose regimen and investigations for this hypothesis are underway. Importantly, in MERS survivors CD8+ T cell responses were found to correlate with less severe disease and lower virus shedding.[30] 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 four fold higher than baseline. Adenoviral vectored vaccines are potent inducers of CD8+ T cell responses and the phenotype of T cells induced by vaccination will be determined in further studies.

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.[13] 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 a ChAdOx1 vectored vaccine

against influenza has been demonstrated in older adults.[20] For use in outbreaks, rapid onset of immunity after a single dose as demonstrated here is highly desirable.

The Coalition for Epidemic Preparedness (CEPI) is supporting the clinical development of four 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. [15,31,32] 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.

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 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.

CONTRIBUTORS

The study was designed by SG, AH, PF and KE. PF, FRL, DS, IP, JS, MD, JM, YT collected study data and oversaw participant visits. AL provided regulatory oversight and RR, AB and NT provided project management. Immunogenicity testing was done by MB, DB, AF, CM, AK, CR, SH, RM, JS, and was interpreted by KE, MB, AF and SB. The analysis of samples by pseudotyped neutralising assay was

designed by JK, and MS. The assay was done by YJ, YP, and was interpreted by JK and MS. Safety data analysis and interpretation was done by PF. Clinical trial data management was done by PF and IP. PF, AF, MB and SG wrote the manuscript. All authors contributed to the reviewing and editing of the report and approved the final version

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DECLARATION OF INTERESTS

S.C.G. and A.V.S.H. are co-founders of, consultants to and shareholders in Vaccitech plc which is developing adenoviral vectored vaccines. TL and PF are consultants to Vaccitech.

DATA SHARING STATEMENT

The study protocol is available with this publication as part of the supplementary material. Individual participant data may be available upon request directed to the corresponding author and after approval of a proposal may be shared through a secure online platform.

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Research in context

Evidence before this study

There are currently no licensed vaccines to prevent Middle East respiratory syndrome (MERS) or specific therapeutics to treat it. ChAdOx1 MERS has been previously reported to be immunogenic and protective in mice in a challenge model, and immunogenic and partially protective in dromedary camels in a natural transmission model. On 20th November 2019 we searched PubMed for publications (no language or date restrictions) using various combinations of the terms "MERS", MERS-CoV", "Middle East Respiratory Syndrome", "anti-Middle East respiratory syndrome", "vaccine", "phase" and "clinical trial". One clinical trial has been published, describing a phase I study of a DNA vaccine against MERS, using a three dose vaccination regimen of intramuscular injection followed by co-localised intramuscular electroporation at weeks 0, 4 and 12. The study was conducted in the US.

Added value of this study

This study is the first clinical study of ChAdOx1 MERS. At all dose levels tested ($5x10^9$, $2{\cdot}5x10^{10}$ and $5x10^{10}$ viral particles) the vaccine was safe and well tolerated. In the majority of participants humoral and cellular MERS CoV-specific immune responses were induced, and maintained at levels above the pre-vaccination response during the 1 year follow-up period. The study was conducted in the UK.

Implications of all the available evidence

A vaccine against MERS-CoV could be used to prevent zoonotic transmission, especially in those frequently exposed to camels in the Middle East, to immunise healthcare workers in regions where hospital outbreaks have occurred or to respond to an outbreak in a healthcare setting or community. The immune correlates of protection against MERS-CoV have not yet been determined in any species. Immunisation with ChAdOx1 MERS results in rapid induction of immune responses against MERS-CoV, which are maintained for at least 1 year, and may therefore have value in preventing or limiting

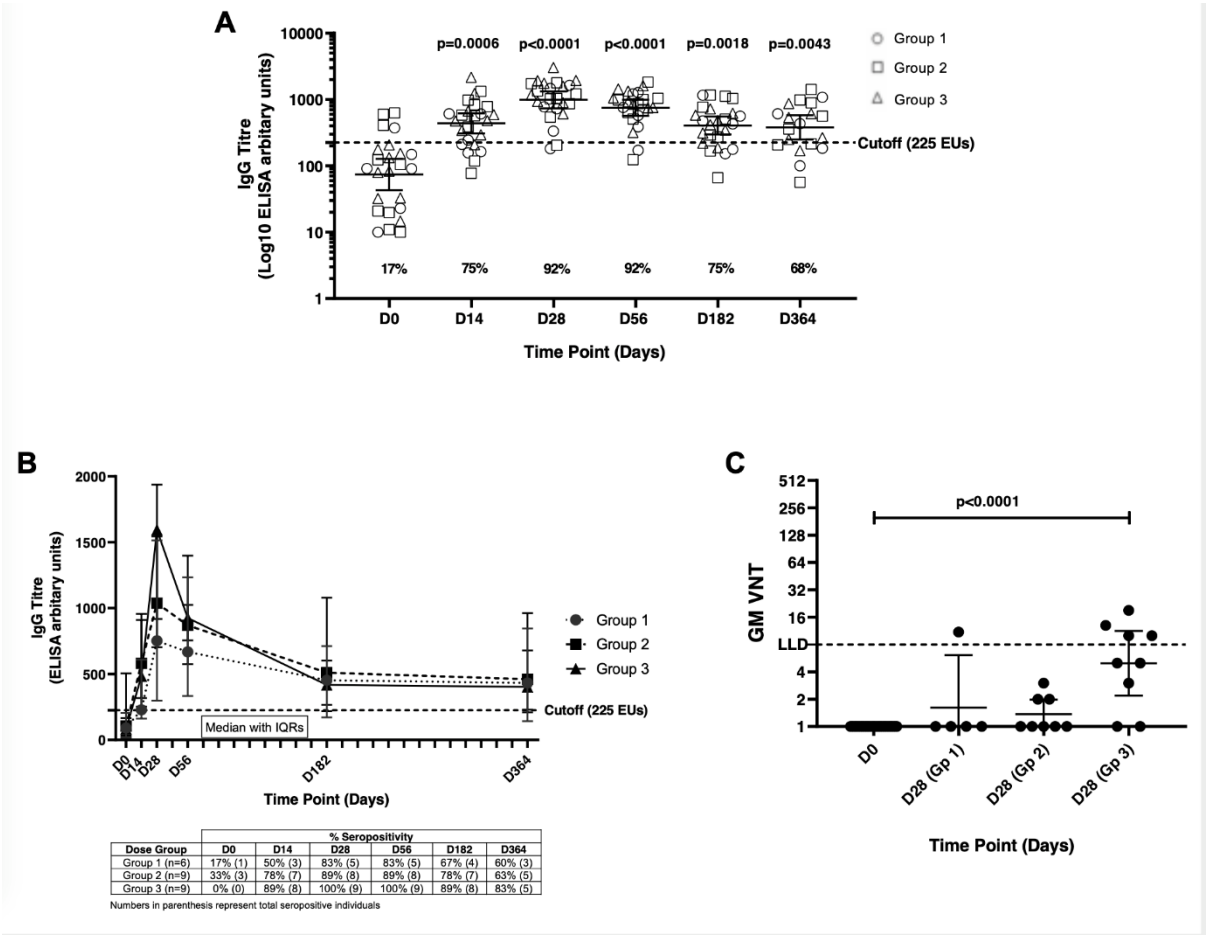
outbreaks in endemic regions. Further clinical studies, especially in endemic regions, should be conducted with this vaccine.

Table 2. 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

Figure 2. Humoral responses to ChAdOx1 MERS vaccine. (A) Individual IgG titres at different dose groups. Bars represent geometric means with 95% CI. (B) Mean and IQR for IgG titres in each dose group. (C) Geometric Mean of Virus Neutralising Titres (GMVNT). P values calculated by Kruskal-Wallis with Dunn's multiple comparison post-test.



	% Seropositivity					
Dose Group	D0	D14	D28	D56	D182	D364
Group 1 (n=6)	17% (1)	50% (3)	83% (5)	83% (5)	67% (4)	60% (3)
Group 2 (n=9)	33% (3)	78% (7)	89% (8)	89% (8)	78% (7)	63% (5)
Group 3 (n=9)	0% (0)	89% (8)	100% (9)	100% (9)	89% (8)	83% (5)

Numbers in parenthesis represent total seropositive individuals

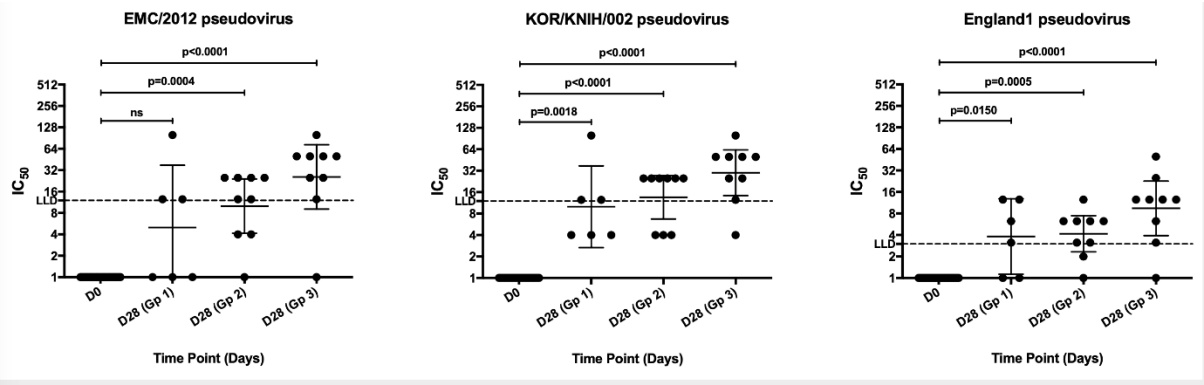


Figure 3. MERS-CoV spike-pseudotyped neutralization. Neutralising antibody titres to MERS-CoV spike-pseudotyped virus at day 0 and day 28 were analysed. A set of 2-fold serial dilutions with a total of 5 concentrations of each serum was tested (2-fold dilutions from 1/12·5 or 1/25 for EMC/2012 and KOR/KNIH/002, and from 1/3·125 or 1/6·25 for England1 depending on S1-binding IgG level). The last dilution showing greater 50% neutralization was expressed as IC_{50}. P values were calculated using Kruskall-Wallis with Dunn's multiple comparison post-test (ns – not significant). The dotted lines represent lower-limits of detection (LLD) under our experimental condition. Bars represent geometric means with 95% CI.

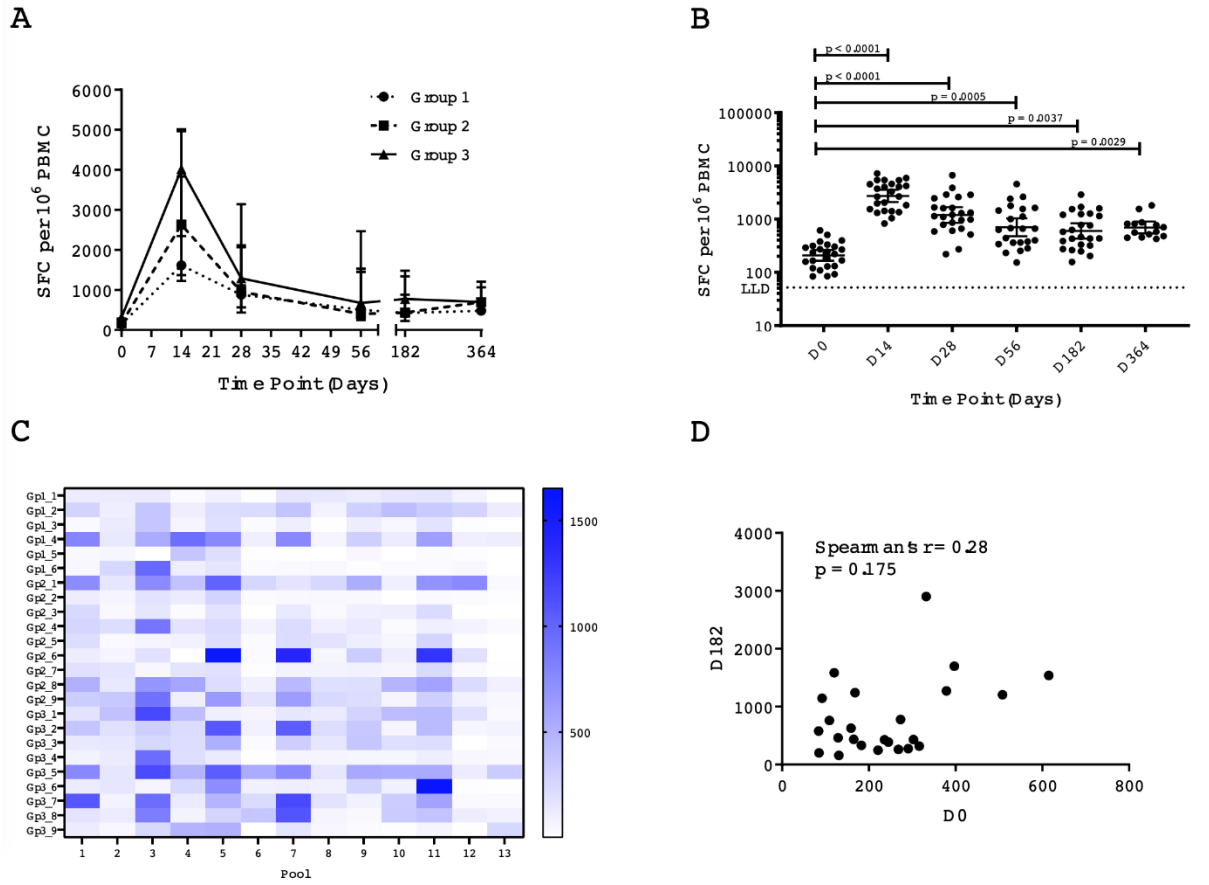


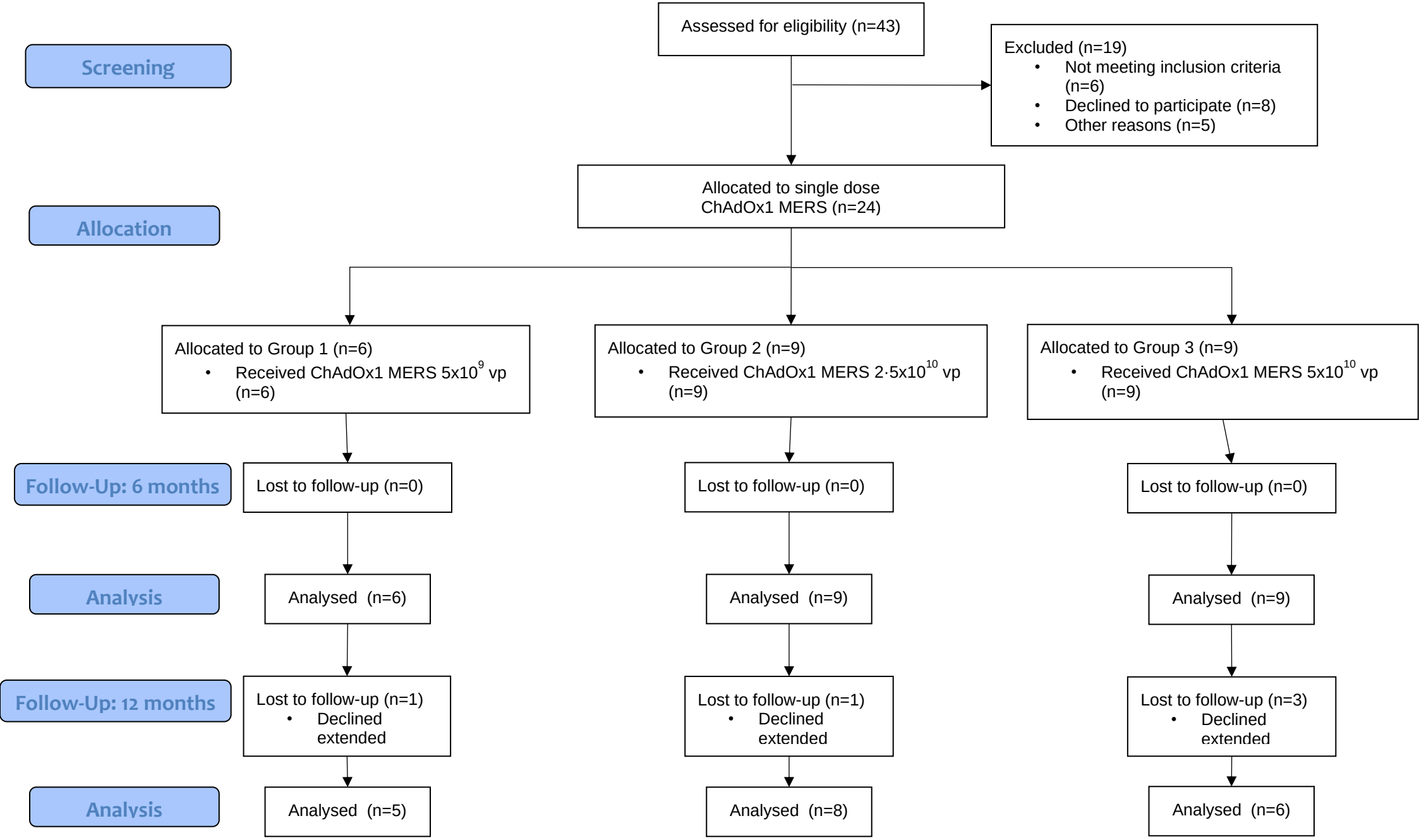
Figure 4. T cell responses to ChAdOx1 MERS. Ex vivo IFN-γ ELISpot responses to MERS spike protein. (A) Median time course by dose group, median with IQR shown (B) Responses for individual vaccinees (groups combined). Geometric mean with 95% CI, Kruskal-Wallis with Dunn's Multiple Comparison test. (C) Heat map of responses to each peptide pool for each participant, arranged by ascending dose group. Higher intensity blue shade indicates higher SFC per million PBMCs. (D) Correlation between baseline (D0) and 6 month (D182) responses.

In (A), (B) and (D) the total response to MERS spike peptides (sum of 13 pools) is shown. In (C) the responses to the 13 individual pools are shown.

Group 1: N=6 volunteers for all timepoints except D364 where N=3

Group 2: N=9 volunteers for all timepoints except D56 (N=8) and D364 (N=6)

Group 3: N=9 Volunteers for all timepoints except D364 where N=5


Screening
Assessed for eligibility (n=43)
Excluded (n=19)
• Not meeting inclusion criteria (n=6)
• Declined to participate (n=8)
• Other reasons (n=5)
Allocation
Allocated to single dose ChAdOx1 MERS (n=24)
Allocated to Group 1 (n=6)
• Received ChAdOx1 MERS 5x10^9 vp (n=6)
Allocated to Group 2 (n=9)
• Received ChAdOx1 MERS 2·5x10^10 vp (n=9)
Allocated to Group 3 (n=9)
• Received ChAdOx1 MERS 5x10^10 vp (n=9)
Follow-Up: 6 months
Lost to follow-up (n=0)
Lost to follow-up (n=0)
Lost to follow-up (n=0)
Analysis
Analysed (n=6)
Analysed (n=9)
Analysed (n=9)
Follow-Up: 12 months
Lost to follow-up (n=1)
• Declined extended
Lost to follow-up (n=1)
• Declined extended
Lost to follow-up (n=3)
• Declined extended
Analysis
Analysed (n=5)
Analysed (n=8)
Analysed (n=6)