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A single dose of ChAdOx1 Chik vaccine induces neutralising antibodies against four chikungunya virus lineages in a phase 1 clinical trial.

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ABSTRACT

Chikungunya virus (CHIKV) is a reemerging mosquito-borne virus that causes swift outbreaks. Major concerns are the persistent and disabling polyarthralgia in infected individuals. Here we present the results from a first-in-human trial of the candidate simian adenovirus vectored vaccine ChAdOx1 Chik, expressing the CHIKV full-length structural polyprotein (Capsid, E3, E2, 6k and E1).

24 adult healthy volunteers aged 18-50 years, were recruited in a dose escalation, open-label, non-randomised and uncontrolled phase 1 trial (registry NCT03590392). Participants received a single intramuscular injection of ChAdOx1 Chik at one of the three pre-established dosages and were followed-up for 6 months. The primary objective was to assess safety and tolerability of ChAdOx1 Chik. The secondary objective was to assess the humoral and cellular immunogenicity.

ChAdOx1 Chik was safe at all doses tested with no serious adverse reactions reported. The vast majority of solicited adverse events were mild or moderate, and self-limiting in nature. A single dose induced IgG and T-cell responses against the CHIKV structural antigens. Broadly neutralising antibodies against the four CHIKV lineages were found in all participants and as early as 2 weeks after vaccination. In summary, ChAdOx1 Chik showed excellent safety, tolerability and 100% $PRNT_{50}$ seroconversion after a single dose.

INTRODUCTION

Since its emergence in Tanzania in 1952[1], and subsequent reemergence in a series of outbreaks in Kenya, the Indian Ocean (2004-2006)[2] and the Americas (2013-2017)[3], Chikungunya virus (CHIKV) has become a major international health concern, with both acute and long-term impacts on public health. CHIKV has been identified in over 100 countries across Africa, Asia, Europe and the Americas[4].

CHIKV is an RNA alphavirus of the *Togaviridae* family that is transmitted to humans in urban settings by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Both mosquito species have dispersed to all continents, with *Ae. aegypti* present mainly in tropical and sub-tropical regions and *Ae. albopictus* expanding through temperate regions[5]. Their rapid global

expansion accounts for the possibility of an even greater burden of chikungunya fever (CHIKF) beyond tropical regions.

Swift CHIKV outbreaks have recently taken place in Europe, where the East-Central-South African (ECSA) CHIKV lineage has been transmitted by the local *Ae. albopictus* vector[6]. Examples of outbreaks occur in either, low and middle income (LMIC) or high income (HIC) countries. For instance, one of the largest recorded outbreaks occurred during 2004-2007 in islands of the Indian Ocean and India[2]. During this outbreak, 5,202 new CHIKV cases were reported in one month, between February and March 2005. Nevertheless, seroepidemiology studies indicated that nearly 215,000 people were actually infected within one month, corresponding to 63% of the total Grande Comore Island population, leaving 79% of the cases hospitalised or staying at home[7]. Nine months later, in December 2005 the outbreak had extended to the neighbouring region of Reunion Island resulting in approximately 255,000 cases or 33% of the total population and an estimate of 225 deaths constituting a case-fatality rate for CHIKF of 1/1000 cases[8]. In Italy, an outbreak occurred in 2007, affecting 205 individuals in only two months[9]. Autochthonous cases of CHIKF have also been recorded in France in 2010, 2014 and 2017[10–12], and have spread across local populations in 1-3 months after the identification of the index case. This demonstrates the need for effective actions to control outbreaks, and highlights the impact that CHIKV preventative vaccines would have if they are able to induce effective immunity rapidly after a single vaccine dose.

CHIKV infections result in a wide spectrum of clinical presentations, spanning from asymptomatic to chronic, severe and even disabling arthritis[13]. Infections are of major concern and have a significant economic impact. Studies have estimated 151,031 CHIKV-related chronic inflammatory rheumatism DALYs (Disability Adjusted Life Years) after the 2014 outbreak in the Americas, roughly twice as many as the 69,000 dengue DALYs calculated in 2004 for the same region[14].

We have developed a replication-deficient simian adenoviral vector from chimpanzee origin expressing the entire structural cassette polyprotein of CHIKV. ChAdOx1 Chik is a chimpanzee adenoviral vector vaccine expressing the CHIKV structural proteins: Capsid, E3, E2, 6K and E1. We have previously shown, by transmission electron microscopy, that expression of the CHIKV structural cassette in mammalian cells leads to the formation of virus-like particles (VLPs) that resemble wild-type CHIKV particles[15]. This suggests that vaccination with ChAdOx1 Chik can induce the formation of CHIKV VLPs, which mimic the tridimensional antigen structure of CHIKV particles released during CHIKV infections.

In pre-clinical mouse models, high levels of neutralizing antibodies have been induced upon a single, unadjuvanted ChAdOx1 Chik dose[15,16], eliciting complete protection against a lethal CHIKV challenge[16]. ChAdOx1 vectored vaccines are currently in various stages of clinical development and have been assessed in more than 18,000 volunteers across 18 clinical trials spanning 10 diseases, including Zika (NCT04015648), Chikungunya (NCT03590392), MERS (NCT04170829, NCT04170829) and COVID-19 (NCT04324606, NCT04400838, NCT04444674, ISRCTN89951424). A consistent safe and immunogenic profile has been observed following vaccination with these ChAdOx1 vectored vaccines. Here we report safety and immunogenicity data from a first-in-human trial of the ChAdOx1 Chik candidate CHIKV vaccine.

RESULTS

Study Population.

Between 18 July 2018 and 18 October 2019, 24 twenty-four healthy adult subjects received a single dose of ChAdOx1 Chik at $5x10^9$, $2.5x10^{10}$ or $5x10^{10}$ vp (Fig. 1). Baseline characteristics are summarised in Table 1.

Vaccine Safety.

ChAdOx1 Chik was safe at doses up to 5 x 10^{1}□ vp with no serious adverse reactions reported. A total of 112 local and systemic solicited adverse events (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 72h 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. 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 Table S1. Only one serious adverse event was reported but was deemed not related with ChAdOx1 Chik.

Four participants reported a short-lived temperature above 37.5°C within the first 72h post vaccination (2 in the intermediate dose group and 2 in the high dose group). The highest temperature recorded was 38°C (classed as mild). All febrile episodes resolved within 24h. The proportion of moderate and severe AEs was significantly higher in group 3 compared to group 2 (relative risk 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 S3). Unsolicited AEs of note include: Shivering/Chills (1 severe at D0 and D1, resolved by D2; 1 moderate at D0, resolved by D1 and 1 mild at D1, resolved by D2; all in Group 3); Insomnia (1 severe at D2, resolved by D3 - Group 3) and Lower Back Pain (1 severe at D0, resolved by D2 - Group 2) Laboratory AEs considered at least possibly related with the study intervention were self-limiting and predominantly mild in severity (Table S3).

Humoral immunogenicity.

Neutralising antibody titers by $PRNT_{50}$ were blindly measured from all 24 participants. All doses were highly immunogenic upon a single immunisation, reaching a 100% seroconversion rate at 14 days against representative isolates from three CHIKV lineages: Indian Ocean Lineage (IOL), West African Lineage (WAf) and Asian Lineage (As). $PRNT_{50}$ titers to La Réunion (IOL), 37997 (WAf) and SV-0444 (As), were significantly increased from baseline and were maintained throughout the 182 days follow-up period. $PRNT_{50}$ to YO111213, from the Asian-American Lineage (AsAm), demonstrated a 100% seroconversion rate on day 28 but slightly lower seroconversion rates on days 14, 56 and 182 (91.6%, 91.6% and 83.3%, respectively). By day 14, Geometric Mean Titers (GMT) between 40 and 226.3 were measured across the four lineages. 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 titers peaked at day 56 for As (75.3, 95% CI 48.0 - 118.3) (Table 3 and Fig. 2a). An analysis per dose group (Fig. 2b) indicated that all doses are effective at inducing broadly neutralising antibodies against all CHIKV isolates tested, with $PRNT_{50}$ GMT significantly higher than baseline at almost every time point. The best neutralisation 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; 320, 640 and 320 for AsAm (Fig. S2).

Broad cross-neutralising and protective IgG antibodies which recognise epitopes on the CHIKV E2 protein have been found in convalescent individuals and in animals, shortly after infection[17–21]. A single ChAdOx1 Chik dose induced high antibody titers 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) and 169.70 (95% CI 71.94 - 400.3) for the low, intermediate and high dose, respectively (Table 4). 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 4). Compared to day 0, anti-E2 IgG antibody levels started to increase by day 14 (P= 0.089), were significantly higher at day 28 (P= 0.0003) and reached maximum levels at between day 56 and 182 (P= <0.0001) following vaccination (Fig. 3a). 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 (Fig. 3b). 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, we decided to further validate the seronegativity of these individuals with two commercially available ELISA kits. Both anti-chikungunya virus IgG ELISA kits, from Abcam and Euroimmune, confirmed that none of the participants that had high background in our in-house ELISA were seropositive for CHIKV at baseline (data not shown).

$PRNT_{50}$ against CHIKV IOL showed a significant positive correlation with the measured ELISA units from all dose groups, being the intermediate dose group the most positively correlated (Spearman's Rho=0.699 [95%CI 0.5047-0.8269]; P<0.0001) (Fig. 3c). Within the intermediate dose group, a maximum correlation between $PRNT_{50}$ and ELISA units was reached at day 180 (Spearman's Rho=0.939 [95%CI 0.5047-0.8269]; P<0.0003) (Fig. 3d).

Cellular immunogenicity.

Cellular immunogenicity was measured from fresh peripheral blood mononuclear cells (PBMC) by an *ex vivo* IFN-γ ELISpot assay, using pools of peptides spanning the structural CHIKV proteins (Capsid, E3, E2, 6k and E1) as stimuli. Total responses were quantified at days 0 (baseline), 14, 28, 56 and 182 after vaccination. At baseline, the geometric mean of IFN-γ spot forming cells (SFC) per million PBMC was 180.1 (IQR 149.9 - 216.4), across all dosage groups. ChAdOx1 Chik significantly increased the number of IFN-γ SFC, peaking at

day 14 post vaccination (1031, IQR 748.9 – 1420) and remained significantly higher than baseline throughout days 28 (541.1, IQR 411.6 - 711.13); 56 (398.2, IQR 298.6 -530.9) and 182 (352.8, IQR 270.1 - 460.8) (Fig. 4a).

Breadth of responses against each of the five CHIKV structural antigens was measured by an *ex vivo* IFN-γ ELISpot assay using pools of overlapping peptides. Responses to all structural proteins, except 6K, peaked at day 14 after vaccination (Fig. 4b). We observed the largest proportion of responses against E1 and E2, with E2 responses remaining high for a longer period as compared with the other proteins. However, the size of the proteins must be taken into consideration. While E1 and E2 have a similar size and were divided into a similar number of peptides (45 and 42, respectively), the other structural proteins are significantly smaller and had a smaller number of peptides. Capsid was the third largest protein with 26 peptides, whereas E3 and 6K both had only 6 peptides each.

Intracellular Cytokine Staining (ICS) by flow cytometry was carried out at baseline and at day 28 after vaccination. PBMC were stimulated with pools of peptides covering the complete CHIKV structural polypeptide, and analysed for production of IFN-γ, IL-2 or TNF-α. Analysis by the individual cytokines, demonstrated that only IFN-γ producing $CD4^+$ T-cells were significantly increased from baseline. An increment on TNF-α^+ and IL-2^+ producing $CD4^+$ T cells was observed in a proportion of the participants but did not reach significance. $CD8^+$ T-cell responses did not show significant differences for any of the 3 cytokines between baseline and day 28 (Fig. 4c).

DISCUSSION

We have shown safety and an excellent immunogenicity profile by a novel CHIKV vaccine using the replication-deficient chimpanzee adenoviral vector ChAdOx1 expressing the structural proteins capsid, E3, E2, 6k and E1 from CHIKV. Our findings demonstrate that the candidate ChAdOx1 Chik vaccine given as a single dose was safe and well tolerated across all doses tested, including 5×10^9, 2.5×10^{10} and 5×10^{10} vp. Higher reactogenicity was observed at the highest dose of 5×10^{10} vp. No serious adverse reactions to ChAdOx1 Chik occurred. The majority of AEs reported were mild or moderate in severity, and all were self-limiting. We observed transient cases of leucopenia, neutropenia and lymphopenia in 5, 5 and 1 volunteer, respectively. 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 adverse events reported here is similar to the reported for other ChAdOx1 vectored vaccines expressing different antigens[22,23].

CHIKV causes swift outbreaks, affecting large populations and spreading to neighbouring regions rapidly. This highlights the requirement for vaccines with capacity to rapidly stimulate immunity within days after 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[18,24–26], primarily directed against structural proteins[17–19]. A virus-like particle (VLP) vaccine has reported to induce neutralising antibodies 8 weeks following a homologous prime-boost vaccination[27]. Similarly, a measles vectored vaccine (MV-CHIK) which also requires prime boost regimens at 4- and 28-week intervals, demonstrated induction of immune responses after 8 and 32 weeks, respectively[28]. Recently, a live attenuated vaccine (LAV) CHIKV vaccine based on an ECSA lineage has been reported to use a single dose to induce 100% homologous seroconversion at day 14 after a single administration[29]. Our data demonstrates that the ChAdOx1 Chik platform achieves equal levels of 100% seroconversion by $PRNT_{50}$ in only 14 days after a single administration, with evidence of cross protective functional antibodies against 4 distinctive CHIKV lineages. To our knowledge, only one additional vaccine candidate in clinical trials has reported broad cross-neutralising responses against isolates from the 3 CHIKV genotypes (ECSA, Asian and West African) and the Indian Ocean sub-lineage[30].

The role of T-cell immunity in clearance of CHIKV is not well understood and controversial. Although activation of CHIKV specific $CD4^+$ and/or $CD8^+$ T-cells has been observed upon vaccination and natural infection[31–35], some publications have rejected that they might have a protective role[18,24,36]. Moreover, presence of reactive cytokine producing $CD4^+$ T-cells in the joints appear to exacerbate disease and lead into the development of arthritogenic disease[37,38]. IFN responses are likely not pathogenic, whereas TNF-α and Th2 cytokines might be[39,40]. ChAdOx1 induced a $CD4^+$ IFN-γ^+ biased cellular response towards CHIKV E1 and E2 proteins but had no significant effect on $CD8^+$ T-cells. $CD8^+$ T-cells appear to have no protective role[37] and are of limited value in normal settings; only when antibodies are missing can a protective role be seen[41].

Limitations of this study include the relatively short follow-up period of six months, small sample size and an open-labelled, non-randomised, 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 chronic arthritis following CHIKV infection.

In conclusion, ChAdOx1 Chik was safe and well tolerated at all tested doses. A single dose showed compelling evidence of rapid stimulation of cellular responses and induction of high titers of functional antibodies with capacity to neutralise multiple CHIKV lineages. Since our platform does not require an adjuvant and shows to be immunogenic even at a low dose, ChAdOx1 Chik constitutes an attractive product for manufacturers and an affordable preventive vaccine for low-income countries. The ability to induce robust cellular and humoral immunity upon a single administration portraits ChAdOx1 Chik as a suitable candidate to limit swift 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.

METHODS

Trial objectives, participants and oversight.

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. The sample size was selected based on other previous phase I trials using the same vector. This sample size was able to detect significant differences in immune responses from baseline while exposing a limited number of people to an investigational medicinal product that was being used for the first time.

The primary objective was to assess safety and tolerability of ChAdOx1 Chik 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 CHIKV structural antigen-specific humoral and cellular immune responses induced by ChAdOx1 Chik as measured by enzyme-linked immunosorbent assay (ELISA), plaque reduction neutralisation test (PRNT) and *ex vivo* interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot).

Eligible volunteers were recruited at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, United Kingdom (CONSORT diagram, Fig. 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. Screening for previous CHIKV exposure was conducted on participants with significant travel history to CHIKV endemic areas, using a commercial ELISA kit (Anti-Chikungunya Virus IgG Human ELISA Kit, Abcam ab177835) and were excluded if positive. Full details of the eligibility criteria are described in the trial protocol provided in the Supplementary Materials.

The corresponding author had full access to all the data in the trial and had final responsibility for the decision to submit the manuscript for publication. All the trial data were available to all the authors.

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 Good Clinical Practice (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 of the Oxford University Hospitals National Health Service Trust (GMSC reference number GM462.18.102). The trial is 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.

Trial procedures.

ChAdOx1 Chik was administered as a single intramuscular injection into the deltoid at a low dose of $5x10^9$ vp (group 1), intermediate dose of $2.5x10^{10}$ 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 conducted prior to dose escalation (details provided in study protocol).

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. 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.

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 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 definitively 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. An independent Local Safety Monitor (LSM) provided safety oversight. The relevant clinical data was recorded in a study database using OpenClinica (Enterprise Edition) v3.13.

ChAdOx1 Chik Vaccine.

ChAdOx1 Chik uses the replication-deficient adenovirus vector derived from the E1 E3-defficient ChAdY25[42] and is currently a leading vaccine platform against COVID-19[43]. 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 an analysis of full-length structural polyprotein sequences from multiple CHIKV lineages. Sequences were collected from the NCBI database and aligned using Clustal Omega and a neighbour-joining tree (Juke-Cantor, 100 bootstraps). Intra- and inter-clade conservation was calculated using a sliding window approach with a sequence weighting method to enable equal representation of all lineages and variants. A synthetic gene cassette was produced by GeneArt® (ThermoFisher Scientific), which was subsequently cloned into a pMono plasmid to be driven by a CMV promoter expression[15]. The vaccine was manufactured to current Good Manufacturing Practice (cGMP) by the Clinical Biomanufacturing Facility (University of Oxford, Oxford, UK) in a HEK 293 cell line. The

vectored vaccine was purified and sterile filtered to generate a clinical lot at a concentration of $1.57x10^{11}$ viral particles per mL.

ELISA.

Total anti-CHIKV IgG was measured using a standardised in-house indirect ELISA[44]. To this end, 1 µg/ml of CHIKV E2 recombinant protein in phosphate buffered saline (PBS) were used to coat Nunc-immuno 96 well plates. Plates were incubated at 4°C for 18 h overnight[15,45]. 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 4 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 three 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 seronegativity of serum samples that had a higher background in our in-house E2 ELISA at baseline. The anti-chikungunya virus IgG ELISA kit from Abcam (ab177835) and the anti-chikungunya virus IgG ELISA kit from Euroimmune (EI 293a-9601G) were both performed according to manufacturer's instructions.

Plaque Reduction Neutralisation Tests (PRNT).

Induction of serum neutralising antibodies was evaluated with a plaque reduction neutralisation tests (PRNT) on monolayers of Vero-cells (Vero ATCC CCL-81) cultured in 12-well plates using standard methods[46,47]. Neutralising antibody titers 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 Center for Emerging Viruses and Arboviruses at UTMB[46]. Titers were quantified as the highest serum dilution that inhibited plaque formation in 50% ($PRNT_{50}$). Seroconversion was considered positive in samples with reciprocal titers of $PRNT_{50} \geq 10$ [48]. Limits of detection were between 10 and 1280, and any samples without a detectable titer were listed as either 5 or 2560.

ELISpot.

Cellular immune responses were quantified at the selected timepoints using an ex-vivo Enzyme-linked immunospot (ELISpot) for IFN-γ[43,44]. PBMC were stimulated with 125 synthetic peptides (20mers overlapping by ten 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). Peptide sequences and pooling are summarised in supplementary Table S4. Data were analysed according to a quality control standard operational procedure. The lower limit of detection for the assay was 4 spot-forming cells (SFCs) for summed responses to the 13 CHIKV structural antigen peptide pools. The following antibodies were used for ELISpot assay: anti-human IFN-y capture IgG1 mouse monoclonal Ab (dil 1:100) and anti-human IFN-y biotinylated , detection mouse IgG1 monoclonal Ab (dil 1:1000).

Flow cytometry.

Intracellular cytokine staining for flow cytometry (ICS) was performed to quantify $CD4^+$ and $CD8^+$ T-cell responses to the vaccine[49]. Five peptide pools from the structural CHIKV cassette were used as stimuli (Table S4). Representative gating strategy is shown in Fig S1. For the stimulation and staining, the following antibodies were used: anti-human CD14 eFluor450 (dil 1:100), anti-human CD19 eFluor450 (dil 1:100), anti-human CD3 AF700 (dil 1:50), anti-human CD4 APC (dil 1:25), anti-human CD8a APC eFluor780 (dil 1:10), anti-human IFN-y FITC (dil 1:250), anti-human TNF-a PE-Cy7 (dil 1:500), anti-human IL-2 PE

(dil 1:50), anti-human CD28 (1 μg/mL) and anti-human CD49d (Integrin alpha 4) (1 μg/mL). Samples were ran in a LSRFortessa (Becton Dickinson); FACSDiva v 8.02 (BD Biosciences) and FlowJo v10.6.2 (BD Biosciences) were used for data recording and analysis, respectively.

Statistics.

Safety endpoints are described as frequencies with their respective percentages alongside 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). A Kruskal-Wallis test with Dunn's correction for multiple tests was used to assess the CHIKV *ex vivo* ELISpot IFN-γ responses, whereas a two-tailed Mann-Whitney test was used for ICS data. ELISA and PRNT data were analysed by either Kruskal-Wallis test or Friedman test with Dunn's correction for multiple parameters, as appropriate. A *P* value <0.05 was considered significant. Statistical analysis of safety and immunogenicity data was conducted using GraphPad Prism version 9.1 (GraphPad Software Inc., California, USA).

DATA AVAILABILITY

There is a restriction on the availability of the data presented on this manuscript due to the data being used to feed a patent application and because data will be linked to an ongoing Phase 1b blinded study funded by a different research award. Anonymised participant data may be available upon requests directed to the corresponding author (arturo.reyes@ndm.ox.ac.uk). Proposals will be reviewed and approved by the sponsor (CTRG- https://researchsupport.admin.ox.ac.uk/ctrg#/), principal investigator, and collaborators on the basis of scientific merit. If approved and upon signature of a data access agreement, data can be shared through a secure online platform. Data sharing may take a period of up to 6 weeks from receiving the request. All data will be made available for a minimum of 5 years from the end of the trial. The study protocol is available with this publication as part of the supplementary material.

REFERENCES

1. Robinson, M. C. An epidemic of virus disease in Southern Province, Tanganyika

territory, in 1952–1953. *Trans. R. Soc. Trop. Med. Hyg.* **49**, 28–32 (1955).

2. Kariuki Njenga, M. *et al.* Tracking epidemic Chikungunya virus into the Indian Ocean from East Africa. *J. Gen. Virol.* **89**, 2754–2760 (2008).

3. Yactayo, S., Staples, J. E., Millot, V., Cibrelus, L. & Ramon-Pardo, P. Epidemiology of Chikungunya in the Americas. *J. Infect. Dis.* **214**, S441–S445 (2016).

4. Suhrbier, A. Rheumatic manifestations of chikungunya: emerging concepts and interventions. *Nat. Rev. Rheumatol.* **15**, 597–611 (2019).

5. Kraemer, M. U. *et al.* The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. *Elife* **4**, (2015).

6. Amraoui, F. & Failloux, A.-B. Chikungunya: an unexpected emergence in Europe. *Curr. Opin. Virol.* **21**, 146–150 (2016).

7. Sergon, K. *et al.* Seroprevalence of Chikungunya virus infection on Grande Comore Island, union of the Comoros, 2005. *Am. J. Trop. Med. Hyg.* **76**, 1189–93 (2007).

8. Josseran, L. *et al.* Chikungunya Disease Outbreak, Reunion Island. *Emerg. Infect. Dis.* **12**, 1994–1995 (2006).

9. Rezza, G. *et al.* Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* **370**, 1840–1846 (2007).

10. Calba, C. *et al.* Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. *Eurosurveillance* **22**, (2017).

11. Delisle, E. *et al.* Chikungunya outbreak in Montpellier, France, September to October 2014. *Eurosurveillance* **20**, (2015).

12. Grandadam, M. *et al.* Chikungunya Virus, Southeastern France. *Emerg. Infect. Dis.* **17**, 910–913 (2011).

13. Cunha, R. V. da & Trinta, K. S. Chikungunya virus: clinical aspects and treatment - A Review. *Mem. Inst. Oswaldo Cruz* **112**, 523–531 (2017).

14. Cardona-Ospina, J. A., Diaz-Quijano, F. A. & Rodríguez-Morales, A. J. Burden of chikungunya in Latin American countries: estimates of disability-adjusted life-years (DALY) lost in the 2014 epidemic. *Int. J. Infect. Dis.* **38**, 60–61 (2015).

15. López-Camacho, C. *et al.* Assessment of Immunogenicity and Neutralisation Efficacy of Viral-Vectored Vaccines Against Chikungunya Virus. *Viruses* **11**, 322 (2019).

16. Kroon Campos, R. *et al.* A Single and Un-Adjuvanted Dose of a Chimpanzee Adenovirus-Vectored Vaccine against Chikungunya Virus Fully Protects Mice from Lethal Disease. *Pathogens* **8**, 231 (2019).

17. Kam, Y. *et al.* Early neutralizing IgG response to Chikungunya virus in infected

patients targets a dominant linear epitope on the E2 glycoprotein. *EMBO Mol. Med.* **4**, 330–343 (2012).

18. Lum, F.-M. *et al.* An Essential Role of Antibodies in the Control of Chikungunya Virus Infection. *J. Immunol.* **190**, 6295–6302 (2013).

19. Quiroz, J. A. *et al.* Human monoclonal antibodies against chikungunya virus target multiple distinct epitopes in the E1 and E2 glycoproteins. *PLOS Pathog.* **15**, e1008061 (2019).

20. Kam, Y.-W. *et al.* Unique Epitopes Recognized by Antibodies Induced in Chikungunya Virus-Infected Non-Human Primates: Implications for the Study of Immunopathology and Vaccine Development. *PLoS One* **9**, e95647 (2014).

21. Fox, J. M. *et al.* Broadly Neutralizing Alphavirus Antibodies Bind an Epitope on E2 and Inhibit Entry and Egress. *Cell* **163**, 1095–1107 (2015).

22. Rodríguez-Morales, A. J., Cardona-Ospina, J. A., Fernanda Urbano-Garzón, S. & Sebastian Hurtado-Zapata, J. Prevalence of Post-Chikungunya Infection Chronic Inflammatory Arthritis: A Systematic Review and Meta-Analysis. *Arthritis Care Res. (Hoboken).* **68**, 1849–1858 (2016).

23. Handler, M. Z., Handler, N. S., Stephany, M. P., Handler, G. A. & Schwartz, R. A. Chikungunya fever: an emerging viral infection threatening North America and Europe. *Int. J. Dermatol.* **56**, e19–e25 (2017).

24. Chu, H. *et al.* Deciphering the protective role of adaptive immunity to CHIKV/IRES a novel candidate vaccine against Chikungunya in the A129 mouse model. *Vaccine* **31**, 3353–3360 (2013).

25. Yoon, I.-K. *et al.* High Rate of Subclinical Chikungunya Virus Infection and Association of Neutralizing Antibody with Protection in a Prospective Cohort in The Philippines. *PLoS Negl. Trop. Dis.* **9**, e0003764 (2015).

26. Plante, K. *et al.* Novel Chikungunya Vaccine Candidate with an IRES-Based Attenuation and Host Range Alteration Mechanism. *PLoS Pathog.* **7**, e1002142 (2011).

27. Chen, G. L. *et al.* Effect of a Chikungunya Virus–Like Particle Vaccine on Safety and Tolerability Outcomes. *JAMA* **323**, 1369 (2020).

28. Reisinger, E. C. *et al.* Immunogenicity, safety, and tolerability of the measles-vectored chikungunya virus vaccine MV-CHIK: a double-blind, randomised, placebo-controlled and active-controlled phase 2 trial. *Lancet* **392**, 2718–2727 (2018).

29. Wressnigg, N. *et al.* Single-shot live-attenuated chikungunya vaccine in healthy adults:

529 a phase 1, randomised controlled trial. *Lancet Infect. Dis.* **20**, 1193–1203 (2020).
530 30. Goo, L. *et al.* A Virus-Like Particle Vaccine Elicits Broad Neutralizing Antibody
531 Responses in Humans to All Chikungunya Virus Genotypes. *J. Infect. Dis.* **214**, 1487–
532 1491 (2016).
533 31. Hoarau, J.-J. *et al.* Identical Strength of the T Cell Responses against E2, nsP1 and
534 Capsid CHIKV Proteins in Recovered and Chronic Patients after the Epidemics of
535 2005-2006 in La Reunion Island. *PLoS One* **8**, e84695 (2013).
536 32. Messaoudi, I. *et al.* Chikungunya Virus Infection Results in Higher and Persistent
537 Viral Replication in Aged Rhesus Macaques Due to Defects in Anti-Viral Immunity.
538 *PLoS Negl. Trop. Dis.* **7**, e2343 (2013).
539 33. Wauquier, N. *et al.* The Acute Phase of Chikungunya Virus Infection in Humans Is
540 Associated With Strong Innate Immunity and T CD8 Cell Activation. *J. Infect. Dis.*
541 **204**, 115–123 (2011).
542 34. Roques, P. *et al.* Attenuated and vectored vaccines protect nonhuman primates against
543 Chikungunya virus. *JCI Insight* **2**, (2017).
544 35. Dias, C. N. de S. *et al.* Human CD8 T-cell activation in acute and chronic chikungunya
545 infection. *Immunology* **155**, 499–504 (2018).
546 36. Hoarau, J.-J. *et al.* Persistent Chronic Inflammation and Infection by Chikungunya
547 Arthritogenic Alphavirus in Spite of a Robust Host Immune Response. *J. Immunol.*
548 **184**, 5914–5927 (2010).
549 37. Teo, T.-H. *et al.* A Pathogenic Role for CD4 + T Cells during Chikungunya Virus
550 Infection in Mice. *J. Immunol.* **190**, 259–269 (2013).
551 38. Miner, J. J. *et al.* Therapy with CTLA4-Ig and an antiviral monoclonal antibody
552 controls chikungunya virus arthritis. *Sci. Transl. Med.* **9**, eaah3438 (2017).
553 39. Venugopalan, A., Ghorpade, R. P. & Chopra, A. Cytokines in Acute Chikungunya.
554 *PLoS One* **9**, e111305 (2014).
555 40. Carissimo, G. *et al.* Viperin controls chikungunya virus–specific pathogenic T cell
556 IFNγ Th1 stimulation in mice. *Life Sci. Alliance* **2**, e201900298 (2019).
557 41. Burrack, K. S., Montgomery, S. A., Homann, D. & Morrison, T. E. CD8 + T Cells
558 Control Ross River Virus Infection in Musculoskeletal Tissues of Infected Mice. *J.*
559 *Immunol.* **194**, 678–689 (2015).
560 42. Dicks, M. D. J. *et al.* A Novel Chimpanzee Adenovirus Vector with Low Human
561 Seroprevalence: Improved Systems for Vector Derivation and Comparative
562 Immunogenicity. *PLoS One* **7**, e40385 (2012).

43. 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. *Lancet* **396**, 467–478 (2020).
44. 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. *Lancet Infect. Dis.* **20**, 816–826 (2020).
45. Kim, Y. C. *et al.* Development of an E2 ELISA Methodology to Assess Chikungunya Seroprevalence in Patients from an Endemic Region of Mexico. *Viruses* **11**, 407 (2019).
46. Rossi, S. L. *et al.* Immunogenicity and Efficacy of a Measles Virus-Vectored Chikungunya Vaccine in Nonhuman Primates. *J. Infect. Dis.* **220**, 735–742 (2019).
47. Langsjoen, R. M. *et al.* Chikungunya Virus Strains Show Lineage-Specific Variations in Virulence and Cross-Protective Ability in Murine and Nonhuman Primate Models. *MBio* **9**, e02449-17 (2018).
48. Ramsauer, K. *et al.* Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. *Lancet Infect. Dis.* **15**, 519–527 (2015).
49. Ewer, K. *et al.* A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. *N. Engl. J. Med.* **374**, 1635–1646 (2016).

AUTHOR CONTRIBUTIONS

PMF, AH, and ARS designed the study. PMF, FRL, DJ, IP and RR collected study data and oversaw participant visits. AL provided regulatory oversight, and MT, NT and RR provided project management. Immunogenicity testing was done and interpreted by KH, MB, LPL, AF, DB, RM, JS, KE and ARS. YCK produced purified protein for ELISA and performed immunopotency assay of the GMP batch. The analysis of samples by PRNT assays was designed, done and interpreted by SRA, RKC and SLR. Clinical trial data management was done by PMF and IP. Safety data analysis and interpretation were done by PMF. PMF, KH, LPL and ARS wrote the manuscript. AH was the chief investigator. EB was responsible for vaccine manufacture. ARS applied and obtained funding to support this project. All authors contributed to the reviewing and editing of the report and approved the final version.

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COMPETING INTERESTS

Pedro M. Folegatti is a consultant to Vaccitech, which is developing adenoviral vectored vaccines. Adrian Hill is a co-founder of and consultant to Vaccitech Ltd and is named as an inventor on a patent covering design and use of ChAdOx1-vectored vaccines. All other authors declare no competing interests.

FIGURE LEGENDS

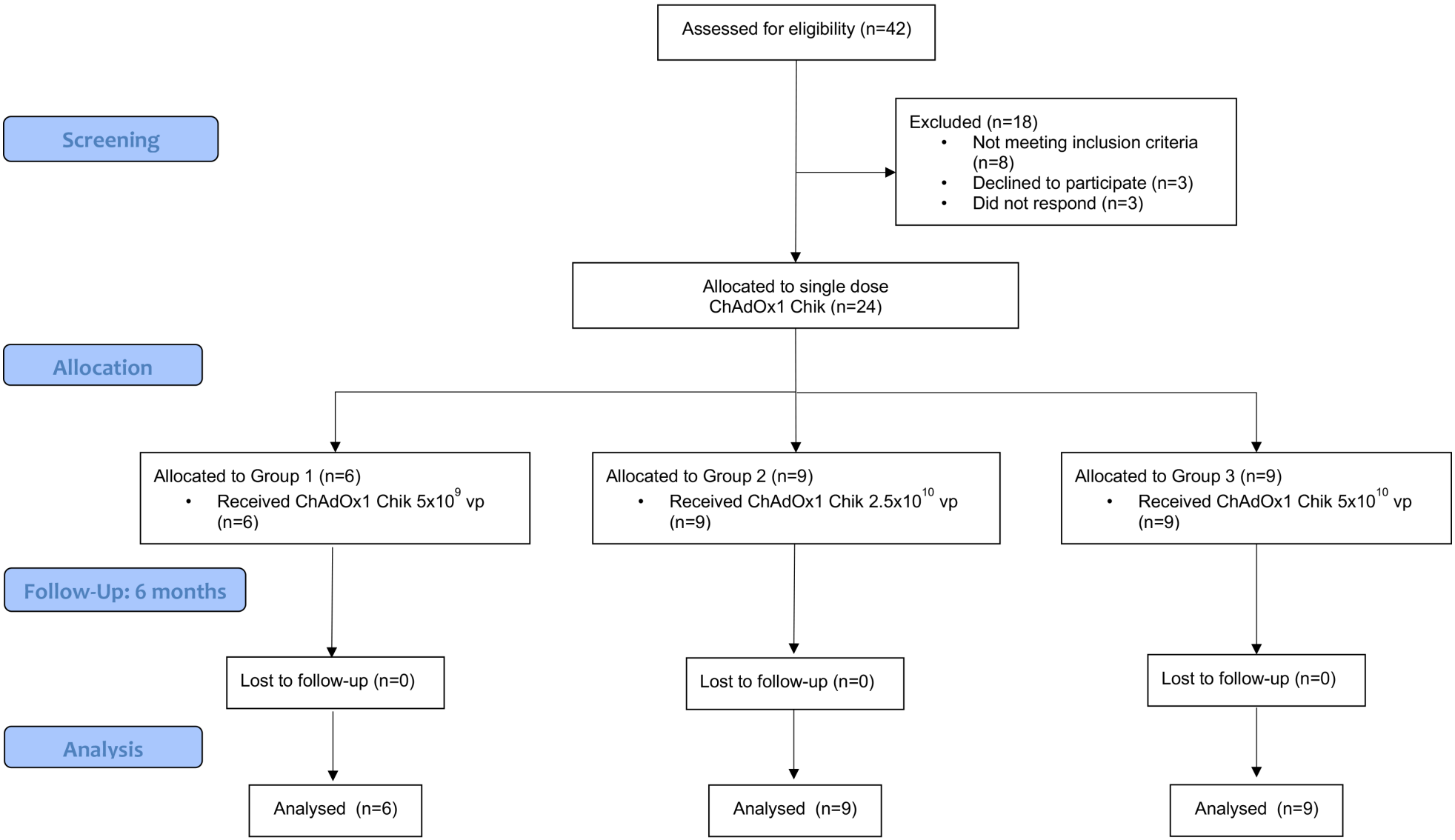
Figure 1. Trial profile. Study profile showing allocation of participants to 3 dosage groups: Group 1 low dose at $5x10^9$ vp, Group 2 intermediate dose at $2.5x10^{10}$ vp and Group 3 high dose at $5x10^{10}$ vp. None of the 24 recruited participants were lost in follow-up.

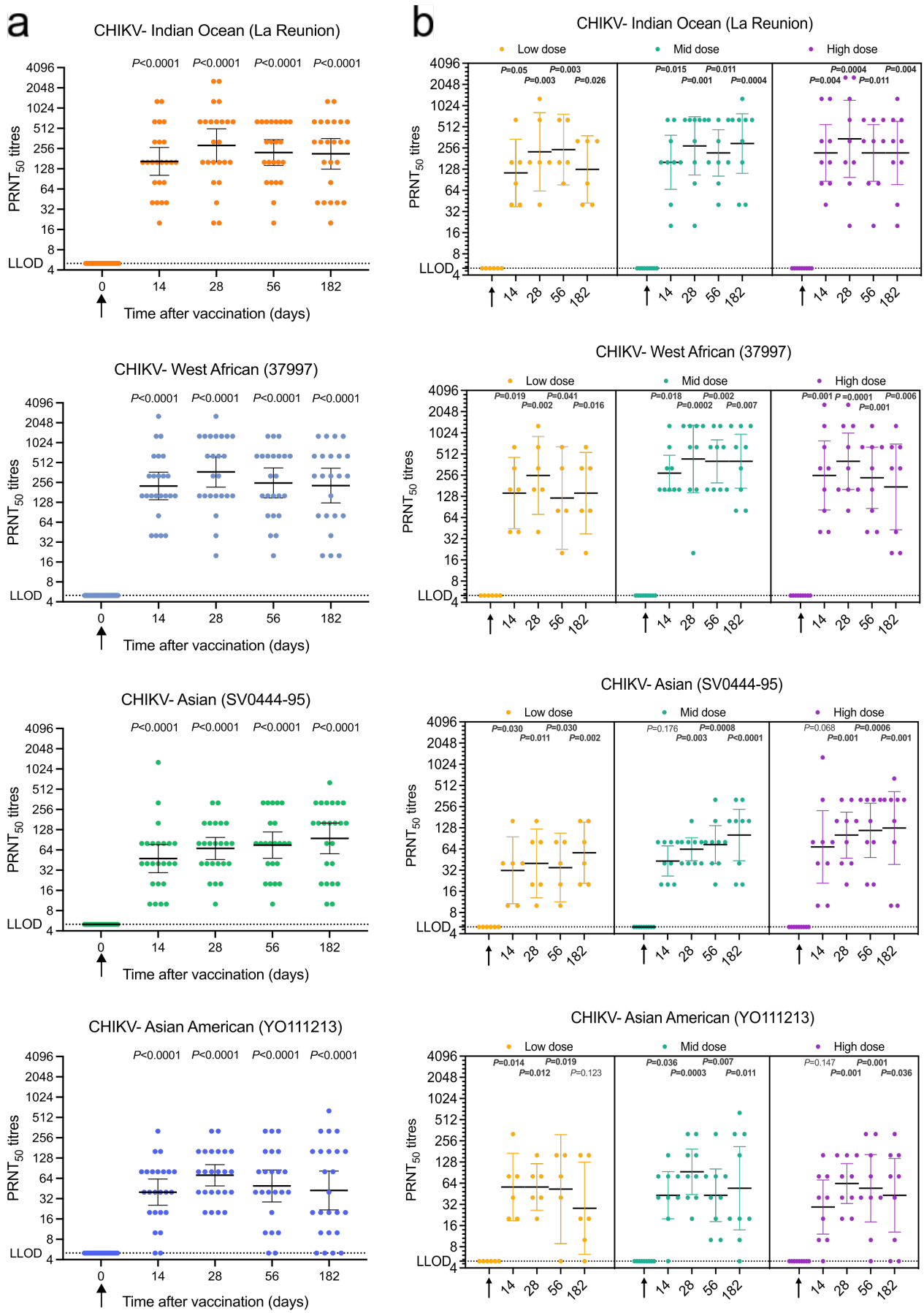
Figure 2. $PRNT_{50}$ values over time. $PRNT_{50}$ reciprocal titres are shown for each participant. Arrows indicate when ChAdOx1 Chik was administered. Lower limit of detection (LLOD) is 5. **a)** Overall responses by timepoint in all trial volunteers (n = 24). Geometric means and 95% CI; Kruskal-Wallis test with Dunn's correction. **b)** Same data as a) but analysed by dosage groups: low dose was $5x10^9$ vp (n = 6), intermediate (mid) dose was $2.5x10^{10}$ vp (n =

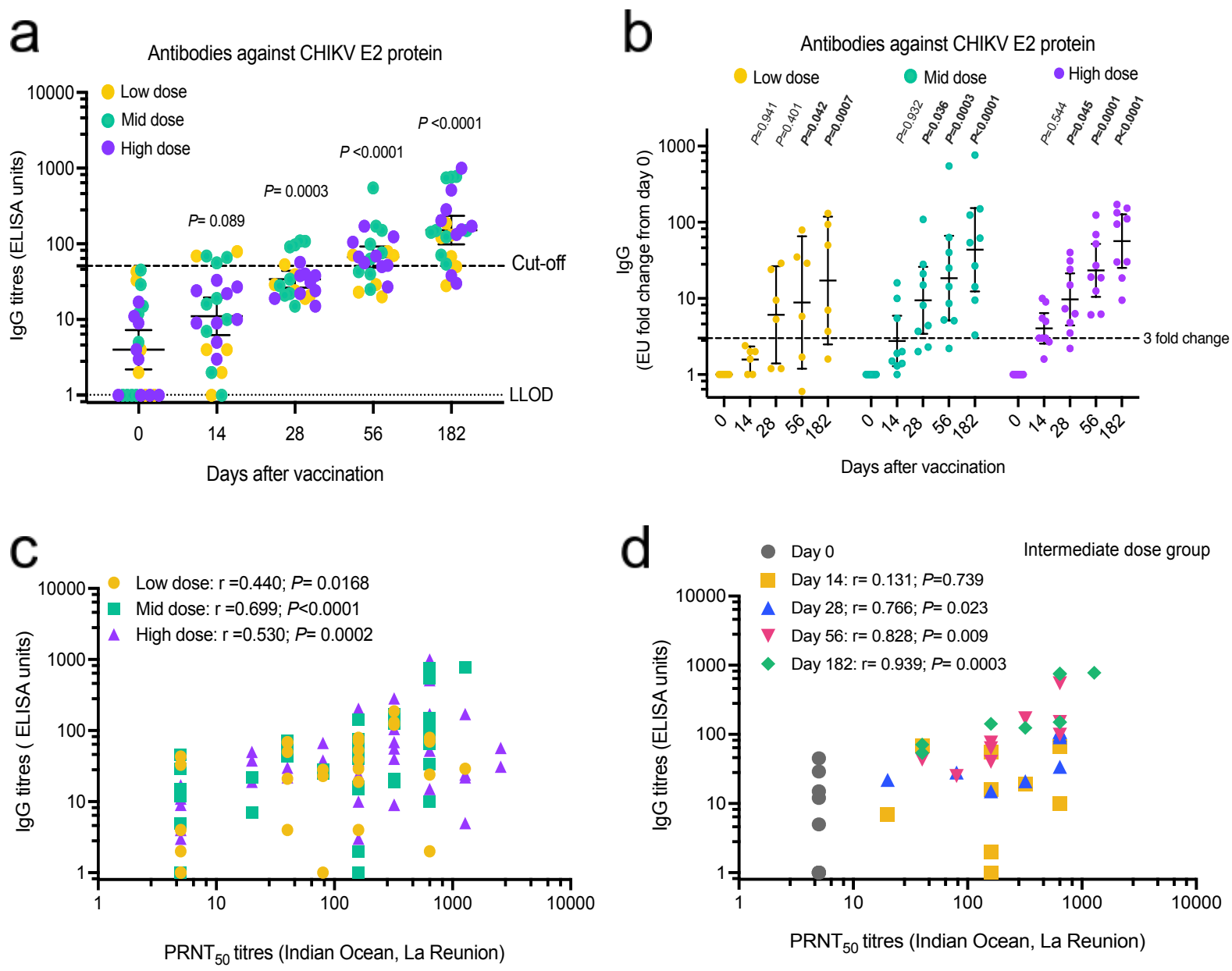
9) and high dose was $5x10^{10}$ vp (n = 9). Geometric means and 95% CI; Kruskal-Wallis test with Dunn's correction. One day 56 sample from the low dose group was excluded from analysis due to QC failure.

Figure 3. ELISA titers over time. CHIKV IgG response by standardised ELISA to E2 protein in 120 serum samples of trial participants. **a)** Individual IgG titers over time (n =24). The dashed line represents the cut-off value for seropositivity. Geometric means and 95% CI; Friedman test with Dunn's correction. **b)** Same data as a) but represented as fold-change from baseline (day 0) and analysed by dosage group: low dose was $5x10^9$ vp (n = 6), intermediate (mid) dose was $2.5x10^{10}$ vp (n = 9) and high dose was $5x10^{10}$ vp (n = 9). Geometric means and 95% CI; Friedman test with Dunn's correction. **c)** Correlation of $PRNT_{50}$ and IgG ELISA by dosage group at 5 time points. Low dose, 6 participants, n = 29 (one day 56 sample was excluded due to QC failure); intermediate (mid) dose, 9 participants (n = 45) and high dose, 9 participants (n = 45). Spearman correlation, two-tailed. **d)** Same data as c) but correlation is only shown for the 9 participants vaccinated at the intermediate (mid) dose (n = 9 per timepoint). Spearman correlation, two-tailed.

Figure 4. T cell responses over time. **a)** *Ex-vivo* Enzyme-linked immunospot (ELISpot) for IFN-γ to CHIKV structural antigens measured as total responses to CHIKV peptides (sum of 13 pools spanning C, E3, E2, 6K, E1). SFC per million PBMC during a 6-month follow-up period (n = 24). Median and IQR; Kruskal-Wallis test with Dunn's correction. **b)** Proportion of spots contributed by C, E3, E2, 6K and E1 over time. **c)** Intracellular Cytokine Staining (ICS) by flow cytometry to assess $CD4^+$ and $CD8^+$ T-cell functionality. Percentage of cytokine producing $CD4^+$ and $CD8^+$ T-cells (n = 24). Median and IQR; Mann-Whitney test two-tailed.


Assessed for eligibility (n=42)
Screening
Excluded (n=18)
• Not meeting inclusion criteria (n=8)
• Declined to participate (n=3)
• Did not respond (n=3)
Allocated to single dose ChAdOx1 Chik (n=24)
Allocation
Allocated to Group 1 (n=6)
• Received ChAdOx1 Chik 5x10^9 vp (n=6)
Allocated to Group 2 (n=9)
• Received ChAdOx1 Chik 2.5x10^10 vp (n=9)
Allocated to Group 3 (n=9)
• Received ChAdOx1 Chik 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)


a
CHIKV- Indian Ocean (La Reunion)
CHIKV- West African (37997)
CHIKV- Asian (SV0444-95)
CHIKV- Asian American (YO111213)
PRNT50 titres
LLOD
Time after vaccination (days)
b
Low dose
Mid dose
High dose


a
Antibodies against CHIKV E2 protein
Low dose
Mid dose
High dose
IgG titres (ELISA units)
10000
1000
100
10
1
P= 0.089
P= 0.0003
P <0.0001
P <0.0001
Cut-off
LLOD
0
14
28
56
182
Days after vaccination
b
Antibodies against CHIKV E2 protein
Low dose
Mid dose
High dose
IgG
(EU fold change from day 0)
P=0.941
P=0.401
P=0.042
P=0.0007
P=0.932
P=0.036
P=0.0003
P<0.0001
P=0.544
P=0.045
P=0.0001
P<0.0001
3 fold change
Days after vaccination
c
Low dose: r =0.440; P= 0.0168
Mid dose: r =0.699; P<0.0001
High dose: r =0.530; P= 0.0002
IgG titres (ELISA units)
PRNT50 titres (Indian Ocean, La Reunion)
d
Intermediate dose group
Day 0
Day 14: r= 0.131; P=0.739
Day 28; r= 0.766; P= 0.023
Day 56: r= 0.828; P= 0.009
Day 182: r= 0.939; P= 0.0003
IgG titres (ELISA units)
PRNT50 titres (Indian Ocean, La Reunion)

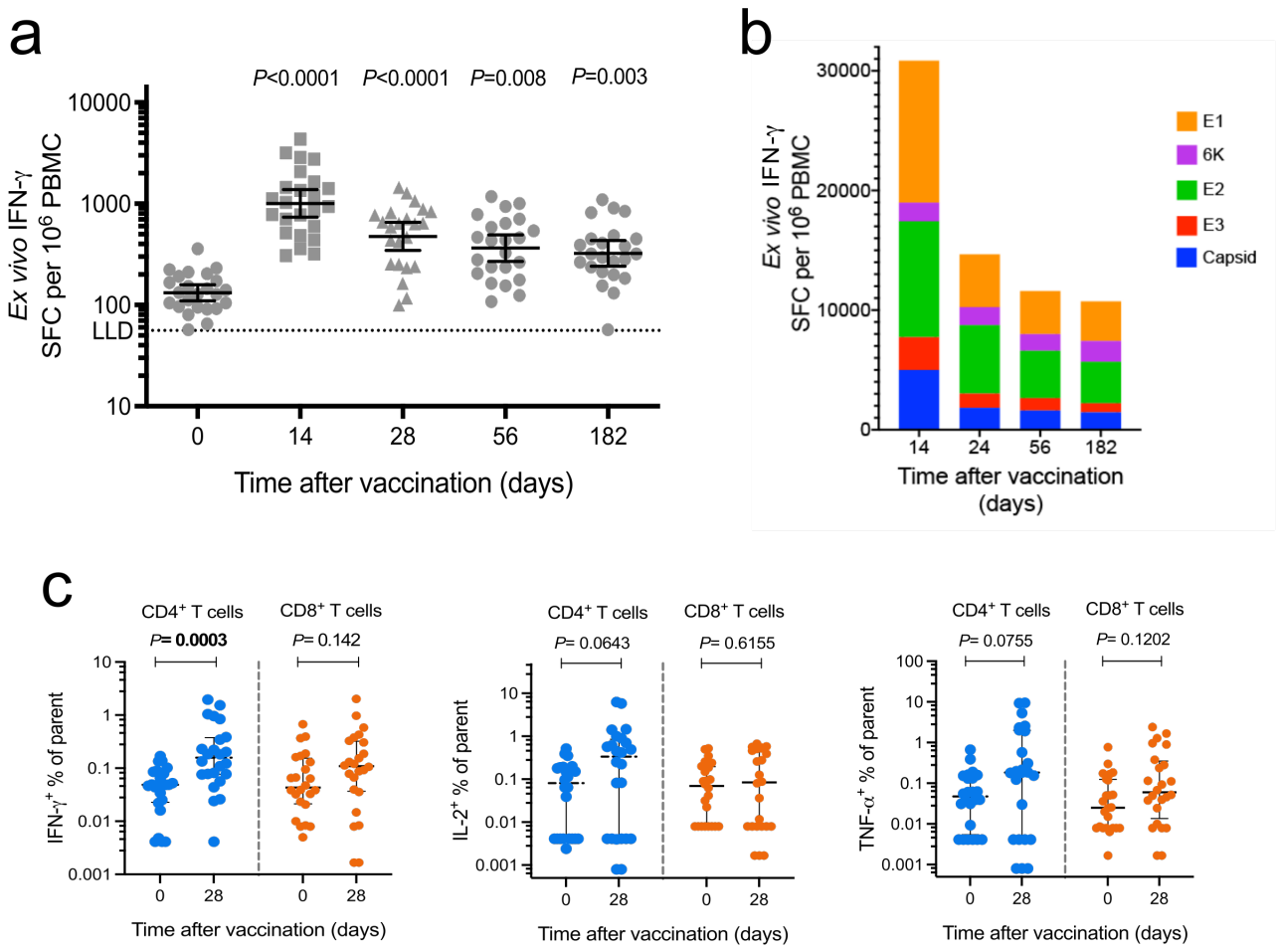

a
P<0.0001
P<0.0001
P=0.008
P=0.003
Ex vivo IFN-γ SFC per 10^6 PBMC
10000
1000
100
LLD
10
0
14
28
56
182
Time after vaccination (days)
b
Ex vivo IFN-γ SFC per 10^6 PBMC
30000
20000
10000
0
E1
6K
E2
E3
Capsid
14
24
56
182
Time after vaccination (days)
c
CD4+ T cells
CD8+ T cells
P= 0.0003
P= 0.142
IFN-γ+ % of parent
Time after vaccination (days)
P= 0.0643
P= 0.6155
IL-2+ % of parent
Time after vaccination (days)
P= 0.0755
P= 0.1202
TNF-α+ % of parent
Time after vaccination (days)

Table 1. Summary of participants' baseline characteristics.

*Mixed: White and Asian.

Variable	Group 1 Low dose (*n* = 6)	Group 2 Intermediate dose (*n* = 9)	Group 3 High dose (*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* – *n* (%)	-	1 (11·11)	-	1 (4·17)
Latin American – *n* (%)	-	3 (33·33)	1 (11·11)	4 (16·67)

Table 2. 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 3. $PRNT_{50}$ against CHIKV lineages.

$PRNT_{50}$ = serum dilution required to reduce viral plaques by 50% of the control value. Seroconversion was measured as $PRNT_{50}$ values of 10 or greater.

*One day 56 sample, from the low dose group, was excluded from analysis due to QC failure.

$PRNT_{50}$								
	Indian Ocean (La Reunion)		West African (37997)		Asian (SV044-95)		Asian/American (YO-111213)	
	% of seroconversion	Geometric mean titres (95% CI)	% of seroconversion	Geometric mean titres (95% CI)	% of seroconversion	Geometric mean titres (95% CI)	% of seroconversion	Geometric mean titres (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 56*	100%	222.9 (143.3 - 346.6)	100%	251.4 (148.9 - 424.6)	100%	75.3 (48.0 - 118.3)	91.6%	49.4 (28.6 - 85.2)
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 4. CHIKV IgG response by standardised ELISA to E2 protein.

Data are geometric mean with 95% CI. Analysis was made using Friedman with Dunn's. Cut-off = average of days 0 + 3 SDEV.

ELISA						
	Low dose (n = 6)		Intermediate dose (n = 9)		High dose (n = 9)	
	Seropositivity n (%)	Geometric mean titres (95% CI)	Seropositivity n (%)	Geometric mean titres (95% CI)	Seropositivity n (%)	Geometric mean titres (95% CI)
Day 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)
Day 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)
Day 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)
Day 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)
Day 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)