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RESEARCH ARTICLE

Potent immunogenicity and protective efficacy of a multi-pathogen vaccination targeting Ebola, Sudan, Marburg and Lassa virus

Amy Flaxman[1], Sarah Sebastian[1☯], Sofia Appelberg[2☯], Kuan M. Cha[1], Marta Ulaszewska[1], Jyothi Purushotham[1], Ciaran Gilbride[1], Hannah Sharpe[1], Alexandra J. Spencer[1], Sagida Bibi[3], Daniel Wright[3], Isabel Schmidt[3], Stuart Dowall[4], Linda Easterbrook[4], Stephen Findlay-Wilson[4], Sarah Gilbert[1], Ali Mirazimi[2], Teresa Lambe[1,3,5]*

1 Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, **2** Public Health Agency of Sweden, Solna, Sweden, **3** Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, United Kingdom, **4** UK Health Security Agency, Porton Down, Salisbury, Wiltshire, United Kingdom, **5** Chinese Academy of Medical Science (CAMS) Oxford Institute, University of Oxford, Oxford, United Kingdom

☯ These authors contributed equally to this work.
* teresa.lambe@paediatrics.ox.ac.uk

Abstract

Viral haemorrhagic fevers (VHF) pose a significant threat to human health. In recent years, VHF outbreaks caused by Ebola, Marburg and Lassa viruses have caused substantial morbidity and mortality in West and Central Africa. In 2022, an Ebola disease outbreak in Uganda caused by Sudan virus resulted in 164 cases with 55 deaths. In 2023, a Marburg disease outbreak was confirmed in Equatorial Guinea and Tanzania resulting in over 49 confirmed or suspected cases; 41 of which were fatal. There are no clearly defined correlates of protection against these VHF, impeding targeted vaccine development. Any vaccine developed should therefore induce strong and preferably long-lasting humoral and cellular immunity against these viruses. Ideally this immunity should also cross-protect against viral variants, which are known to circulate in animal reservoirs and cause human disease. We have utilized two viral vectored vaccine platforms, an adenovirus (ChAdOx1) and Modified Vaccinia Ankara (MVA), to develop a multi-pathogen vaccine regime against three filoviruses (Ebola virus, Sudan virus, Marburg virus) and an arenavirus (Lassa virus). These platform technologies have consistently demonstrated the capability to induce robust cellular and humoral antigen-specific immunity in humans, most recently in the rollout of the licensed ChAdOx1-nCoV19/AZD1222. Here, we show that our multi-pathogen vaccines elicit strong cellular and humoral immunity, induce a diverse range of chemokines and cytokines, and most importantly, confers protection after lethal Ebola virus, Sudan virus and Marburg virus challenges in a small animal model.

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Competing interests: TL reports consulting fees from Vaccitech on an unrelated project, an honorarium from Seqirus, grant support from the DHSC for this work, and is named as an inventor on a patent application for a vaccine against SARS-CoV-2. SG is named as an inventor on patents covering use of ChAdOx1-vectored vaccines and a vaccine against SARS-CoV-2.

Author summary

Outbreaks caused by ebolaviruses and Lassa virus have made headlines worldwide in recent years. Most recently, in 2023 a Marburg virus outbreak resulted in 49 confirmed or probable cases, 41 of which were fatal. Apart from vaccines against Ebola virus, no licensed vaccine exists to protect against viral haemorrhagic fevers caused by filoviruses. An ideal vaccine against viral haemorrhagic fevers would induce long-lasting immunity to, and protection from, viruses causing disease and concomitant fatalities. We developed vaccines which can target multiple orthoebolaviruses, the closely related Marburg virus and Lassa virus. The geographical ranges of these viruses overlap in West and Central Africa. We used viral vector platform technologies to generate these vaccines; ChAdOx1 has now been administered worldwide as part of COVID-19 vaccine rollouts, and MVA has been used in numerous clinical trials thus far. We found that both long lasting, antigen specific T cell and antibody responses were induced after vaccination. Lastly, we demonstrated that these vaccines could protect small animals against challenge with Ebola virus, Sudan virus and Marburg virus.

Introduction

Lassa fever (LF) is a viral haemorrhagic fever (VHF) whose aetiologic agent, Lassa virus (LASV), is a member of the *Arenaviridae* family. Each year, LASV is estimated to infect 100,000–300,000 humans with an estimated 5,000 deaths [1]. It is transmitted via contact with rodents and is endemic throughout most of West Africa. This geographical area overlaps with filovirus outbreaks, including Ebola virus (EBOV) and Sudan virus (SUDV) outbreaks, as well as the fruit bat reservoir of Marburg virus (MARV) [2]. Whilst VHFs caused by ebolaviruses and MARV are associated with high levels of morbidity and mortality, LF has a much lower-case fatality rate but causes a significant burden of disease in West Africa. The need to develop therapeutic and prophylactic treatments against re-emerging and outbreak pathogens became starkly apparent during both the 2013–2016 West Africa EBOV epidemic [3] [4] and the 2020–2022 COVID-19 pandemic. Indeed, in recent years there have been numerous further outbreaks, including very large LF outbreaks in Nigeria in 2018 and 2019, with high case fatality rates (approximately 25%), the second largest outbreak of Ebola virus disease (EVD) in the Democratic Republic of the Congo in 2020, a SUDV outbreak in Uganda in 2022 and a MARV outbreak in Equatorial Guinea in 2023. All of these further underpin the critical need for preventive measures against VHF [5] [6].

Antibodies against viral surface proteins can prevent viral attachment and subsequent infection while a cellular immune response to the surface protein (as well as to internal viral proteins) can reduce disease severity and transmission in a number of infectious disease settings. As a result, viral surface proteins are the main antigenic targets in licensed vaccines against viral diseases today. The recombinant, replication competent vesicular stomatitis virus-based vaccine (rVSV-ZEBOV), licensed in 2019, encodes the single EBOV surface glycoprotein (GP). This vaccine offers substantial protection against disease, shown in trials during the 2013–2016 and 2018 outbreaks [7] with other VSV vaccine candidates in development against SUDV and MARV [8, 9]. A VSV-based LF vaccine candidate encoding the GP of LASV demonstrated protective efficacy in a non-human primate challenge against both a homologous and heterologous virus [10, 11], suggesting that LASV GP antigen is a tenable vaccine target. Adenovirus-vectored vaccines have demonstrated the ability to induce both a

strong humoral and cellular immune response against viral antigens after a single dose, while also having the cargo capacity to encode more than one antigen [12].

A multivalent vaccine approach, which is the WHO's preferred target product profile, will ideally prevent disease caused by multiple filoviruses and other haemorrhagic fever viruses. Working towards this goal, we sought to develop multi-pathogen vaccines that can provide effective and long-lived protection against VHF caused by filoviruses and LASV. We demonstrate that our multi-pathogen adenoviral vectored vaccine confers protection in both a lethal EBOV challenge in guinea pigs and in lethal EBOV, SUDV and MARV challenges in a type-I interferon receptor (IFNAR) knockout mouse model. No evidence of immune competition between vaccine-encoded antigens is found after administration of our adenoviral-vectored multi-pathogen vaccine. The adaptive immune response after adenovirus immunisation can be further enhanced by a boost vaccination with a multi-pathogen Modified Vaccinia Ankara (MVA) vaccine, resulting in a strong adaptive immune response and a diverse chemokine and cytokine profile.

Methods

Ethics statement

Mouse immunogenicity studies carried out under UK Home Office Project licences 30/2889 and P9804B4F1, were approved by the University of Oxford Animal Care and Ethical Review Committee and were carried out at the University of Oxford, Old Road Campus. Guinea pig procedures carried out under UK Home Office Project licence P82D9CB4B, were approved by the Public Health England (PHE) (now UKHSA) Animal Welfare Ethics Review Board (AWERB) and were carried out at PHE (now UKHSA), Porton Down, Salisbury. Animal work performed in the UK was carried out in accordance with the UK Home Office Animal Testing and Research Guidance as per the Animals (Scientific Procedures) Act 1986.

Mouse challenge studies were carried out under the Public Health Agency of Sweden's, ethical licence 16676–2020, which were approved by Stockholm ethical committee for animal research. The study was carried out at the Karolinska Institute and at the Public Health Agency of Sweden. The mice were housed according to the Karolinska Institutes rules regarding animal care and observed at least once a day.

Antigens

Amino acid sequences for antigens used in vector construction were as follows: EBOV-Makona-Kissidougou-C15 GP (GenBank: KJ660346.1), SUDV-Boniface GP (UniProtKB Q66814.1), MARV- Angola/2005 GP (UniProtKB Q1PD50.1), and LASV-Mouse/Sierra Leone/Josiah/1976 GP precursor (GPC) (NCBI Reference Sequence: NP_694870.1). Antigen sequences were codon-optimised for *H. sapiens* and synthesised by Geneart (ThermoFisher, Germany).

Vector construction

The derivation of the ChAdOx1 vector has been described before [13]. To generate recombinant vectors, antigen cassettes consisting of a TetR-repressible CMV promoter, antigen coding sequence, and polyA sequence were inserted into the viral backbone using the Gateway recombination system (Life Technologies). Briefly, antigen cassettes were cloned into an ENTRY plasmid, sequence-verified, and recombined *in vitro* with ChAdOx1-DEST or ChAdOx1-biDEST (containing a Gateway destination cassette in the E1 locus, or in both the E1 and the E4 loci, respectively). E1 and E4 expression cassettes both contained the TetR-repressible CMV

promoter; E1 cassettes contained the BGH polyA sequence, while E4 expression cassettes contained the SV40 polyA sequence. E1 insertion occurs at the deleted E1 locus, while the E4 insertion site is located upstream of the intact E4 region. Monovalent control vectors encode the vaccine antigen at the E1 locus.

Construction of the recombinant MVA vector was performed using BAC recombineering methods as described previously [14]. Briefly, EBOV and SUDV GP coding sequences (under the control of the short synthetic promoter (SSP) and the modified H5 (mH5) promoter, respectively) were inserted at the F11 locus, and MARV and LASV GP coding sequences (under the control of the short synthetic promoter (SSP) and the modified H5 (mH5) promoter, respectively) were inserted at the B8 locus.

Virus production

Viral vectors were produced at the Viral Vector Core Facility at the Jenner Institute using standard methods [15] [13]. All adenovirus vectors were produced in the T-REx-293 cell line (Thermo Fisher Scientific), which allows for transcriptional repression of the vaccine antigens during vector production. Vectors underwent quality control (including titration, identity PCR and sterility testing) before being used in *in vitro* and *in vivo* studies.

Expression testing

Expression of vaccine antigens from monovalent, bivalent and tetravalent viral vectors was assessed by western blot according to standard methods. Briefly, HEK293 cells were infected with vectors (MOI = 1), harvested after 24 hours and lysed in RIPA buffer. Reduced and denatured lysates were resolved by 4–12% SDS-PAGE and transferred onto nitrocellulose. GPs were detected using mouse antiserum from mice previously vaccinated with monovalent vectors (ChAdOx1-EBOV, ChAdOx1-SUDV) or commercial anti-MARV GP antibody (ab190459, abcam) or anti-LASV GP2 antibody (ab190655, abcam), HRP-conjugated secondary antibody was added followed by chemiluminescence imaging (ChemiDoc, BioRad).

Mouse immunogenicity studies

At least 6 week old female BALB/c mice (Envigo, UK) or CD-1 mice (Charles River, UK) were randomly distributed into individually ventilated cages on arrival, housed in groups of 3, 4, 5 or 6 under specific pathogen free conditions, fed and watered *ad libitum* with a 12:12 light-dark cycle.

In most cases, ChAdOx1-biEBOV and ChAdOx1-biLAMA were co-administered; mice received 50μl of ChAdOx1-biEBOV in the left rear leg and 50μl of ChAdOx1-biLAMA in the right rear leg. Monovalent mix refers to a mix of monovalent ChAdOx1-EBOV and ChAdOx1-SUDV administered in the left rear leg and a mix of monovalent ChAdOx1-MARV and ChAdOx1-LASV administered in the right rear leg. Blood samples (from the tail vein) were taken at various time points post vaccination. In prime-boost experiments, second vaccinations of 10^6 PFU MVA expressing filovirus and LASV GPs ('tetraMVA') were administered intramuscularly after the relevant time interval. Mice were culled humanely at the endpoint of the experiment via an approved Schedule 1 method; blood and spleens were harvested for further immunological analysis.

ELISpot

Murine IFN-γ producing splenocytes were assessed by ELISpot assay after vaccination with filovirus viral vectors as previously described, with a few exceptions [16]. Briefly, MAIP

ELISPOT plates (Millipore, UK Ltd) were coated with anti-mouse-IFN-γ mAb (Mabtech, UK) overnight. After blocking, splenocytes were added to ELISpot plates at concentrations ranging from 1.25 x 10^5 to 5 x 10^5 cells/well and stimulated with pools of peptides at a final concentration of 1μg/mL per peptide for 18 hours at 37˚C. Peptide pools consisted of 15-mer peptides overlapping by 11 amino acids, spanning EBOV GP, SUDV GP, MARV GP or LASV GP. Plates were developed as described [16]. For graphical presentation, the number of IFNγ producing cells were calculated as the number of spot forming cells in the presence of peptides minus the number of spot forming cells without peptides and reported per million splenocytes.

ELISA

Antibody responses were measured against trimerised EBOV GP (strain H.sapiens-wt/GIN/2014/Kissidougou-C15, amino-acids 1–649 of GenBank protein AHX24649.1). Protein was produced in-house as described previously [12]. Antibody responses against monomeric SUDV GP (produced in-house as above, UniProtKB: Q66814.1, strain Boniface-76) and recombinant MARV-Angola GP (Alpha Diagnostic International) were also measured. To test cross-reactivity against strains/species not in our vaccines, antibody responses were also measured against EBOV-Mayinga GP (Sino Biologicals), EBOV-Kikwit GP (Native Antigen Company), SUDV-Gulu GP (Sino Biologicals) and Bundibugyo (BDBV) GP (IBT Bioservices). Methods were as described previously [17] except that 2μg/ml of GP antigen was used to coat plates, blocking was performed with PBS/T containing 10% skimmed milk. Antibody responses against LASV were measured using pre-coated plates obtained from ReLASV Pan-Lassa IgG/IgM ELISA Test Kit (GP) manufactured by Zalgen Labs, and all steps after blocking carried out as above. To test for ELISA-reactivity we generated four reference pools of mouse serum which were reactive toward either EBOV-Makona-Kissidougou GP, SUDV-Boniface GP, MARV-Angola/2005 GP, or LASV-Josiah GP. The reactive sera were used to form a standard curve for each of the ELISAs. The relevant pool was added at an initial dilution of 1:250 (EBOV, MARV and LASV) or 1:125 (SUDV) in PBS/T and underwent ten 2-fold dilutions. An arbitrary number of ELISA units (AU) were assigned to the reference pool (62.5 AU for EBOV, MARV and LASV; 125AU for SUDV), and OD450 values of each dilution were fitted to a 4-parameter logistic curve using SOFTmax PRO software. ELISA units were calculated for each sample using the OD values of the sample and the parameters of the standard curve. All ELISA data presented are in AU, with the exception of IgG1/IgG2a ratios, which were calculated using OD.

Intracellular cytokine staining (ICS)

Splenocytes were prepared as described above, plated in 96-well round bottom plates and stimulated using peptide pools for EBOV GP, SUDV GP, MARV GP or LASV GP (as described above) at a final concentration of 5μg/mL or media only. Stimulation and staining was then performed as described previously [18] except that the following antibodies were used: anti-CD4-Qdot605, anti-CD127-APCef780 (Invitrogen), anti-CD62L-PeCy7 anti-CD8-PerCP/Cy5.5 antibodies (eBioscience), LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific), anti-TNF-Alexa488, anti-IL-2-PE and anti-IFN-γ-e450 antibodies (eBioscience). Antigen-specific cells were identified by gating based on doublet negative, size, live cells and either $CD4^+$ or $CD8^+$ surface expression. Background responses in unstimulated control samples were subtracted from responses of peptide stimulated T cells.

Measurement of cytokines and chemokines

Supernatants from ELISpot assays (as described above) were harvested after an 18-hour incubation and stored at -20ºC. These samples were assayed using MSD Technology V-PLEX Mouse Cytokine 29-Plex kit according to the manufacturer's instructions. Data analysis was performed using MSD Discovery Workbench 4.0. 18 of the 29 cytokines measured had detectable levels suitable for further analysis, which was performed using SPSS Statistics 25 (IBM) Correlations and heat maps were calculated and plots produced using RStudio (Version 0.99.903, packages corrplot (0.77) and package pheatmap).

Viral RNA detection

The presence of viral RNA was assessed in the serum and livers of mice at the time of culling. To inactivate virus in serum, Trizol was added to the samples at a ratio of 1:3. For liver samples, PBS was added to give a final concentration of 1g/mL to crush samples. Thereafter, liver samples were centrifuged (5 min at 5000 x g) and 100 μl of each suspension was added to Trizol (1:3). RNA was extracted using the Direct-zol RNA Miniprep kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. Viral RNA was measured by quantitative reverse-transcription polymerase chain reaction (RT-qPCR) using a StepOnePlus Real Time PCR system (Thermo Fisher Scientific). For the liver samples, mouse ACTB mix (Thermo Fisher Scientific) was used as an endogenous control.

Guinea pig challenge experiment design

Groups of female Hartley strain guinea pigs (n = 6/group) were intramuscularly vaccinated with 5 x 10^8 IU of ChAdOx1-biEBOV or a mix of monovalent ChAdOx1 controls (ChAdOx1-EBOV and ChAdOx1-SUDV) or a negative control (ChAdOx1 with irrelevant antigen). 28 days after immunisation, the vaccinated animals were challenged subcutaneously with a lethal dose (10^3 $TCID_{50}$) of guinea pig-adapted EBOV (EBOV Yambuku-Ecran strain) [19]. The EBOV was passaged five times in guinea pigs to achieve lethality, as previously described [20]. Virus was titrated by 50% tissue culture infective dose (TCID50) assay in VeroE6 cells (European Collection of Cell Cultures, UK). Animals were assessed daily with respect to temperature and weight loss throughout the experiment. Clinical signs were monitored at least twice daily, and the following numerical score was assigned for analysis: 0 (normal); 2 (ruffled fur); 3 (lethargy, hunched and wasp waisted); 5 (rapid breathing); 10 (immobile, neurological). To prevent unnecessary suffering to animals, humane endpoints were used where animals would be culled upon reaching 10% weight loss and a moderate clinical sign or any of the following: 20% weight loss; immobility; paralysis; or neurological signs.

$IFNAR^{-/-}$ mouse challenge experiment design

Female $IFNAR^{-/-}$ mice (A129, Marshalls BioResources, UK or #010830, Jackson Laboratory, USA) were immunised at the age of 6–8 weeks with 2 x 10^8 infectious units (IU) of adenovirus vaccine in 50 μl via intramuscular injection in the right hind limb under anesthesia. Three weeks later mice were challenged with 3000 focus-forming units (ffu) or plaque-forming units (pfu), depending on virus, in 100 ul via intraperitoneal injection under anesthesia. Four experiments were performed to assess efficacy of a single dose of ChAdOx1-biEBOV and ChAdOX1-biLAMA vaccine co-administered upon subsequent challenge with either of EBOV-Guinea Kissidougou, SUDV -Boniface, MARV-Musoke and LASV-Josiah. Within each experiment, controls receiving either an irrelevant ChAdOx1 (GFP) vector or the relevant monovalent control (ChAdOx1-EBOV, ChAdOx1-SUDV, ChAdOx1-MARV or

ChAdOx1-LASV) was included. Six mice were vaccinated and challenged within each group, with the exception of ChAdOx1-GFP mice challenged with MARV Musoke, for which there were only 5 mice. Mice challenged with EBOV, SUDV or MARV were assessed for up to 12 days after infection for signs of disease and weight loss and culled if reaching the humane end point. The experimental design for LASV challenge was slightly different. Based on previous studies of LASV infection of IFNAR$^{-/-}$ mice demonstrated that this model is not uniformly lethal therefore 2 mice from each group (ChAdOx1 biEBOV+biLAMA, ChAdOx1-LASV or ChAdOx1 GFP) which were challenged with LASV were culled on day 4, 8 and 12 post-infection (end of study). However, on post-infection day 8, all mice in the control group (ChAdOx1 GFP) had to be euthanized due to reaching the humane endpoint. The remaining mice in the two vaccine groups (ChAdOx1 biEBOV+biLAMA, ChAdOx1-LASV) which had been challenged with LASV did not reach humane endpoints and were culled at the end of the study (day 12).

Statistics

Statistical analyses were carried out using GraphPad Prism version 7.01, unless otherwise **stated**. Grouped data are presented as means with SEM, unless otherwise indicated. Statistical significance of variations in continuous variables by group was analysed by Mann-Whitney or Kruskal-Wallis tests (for skewed data) or t-tests or ANOVA (for non-paired normally distributed data) as stated in results. For comparisons across multiple groups, Dunn's multiple comparisons test was used for skewed data and Holm-Sidak multiple comparisons test was used for normally distributed data.

Results

Dual-antigen vaccine design

Two recombinant, dual-antigen ChAdOx1 vectors were constructed, one encoding SUDV GP at the E1 locus and EBOV GP at the E4 insertion site (ChAdOx1-biEBOV), and the other encoding LASV GP at the E1 locus and MARV GP at the E4 insertion site (ChAdOx1-biLAMA) (Fig 1A). Western blot analysis was performed to assess expression of the four antigens in infected cells. Cells infected with vectors expressing a single antigen only were used as positive controls (termed monovalent control vectors). Proteins of the correct size for all four inserts were expressed from ChAdOx1-biEBOV and -biLAMA (Fig 1B). We also generated a

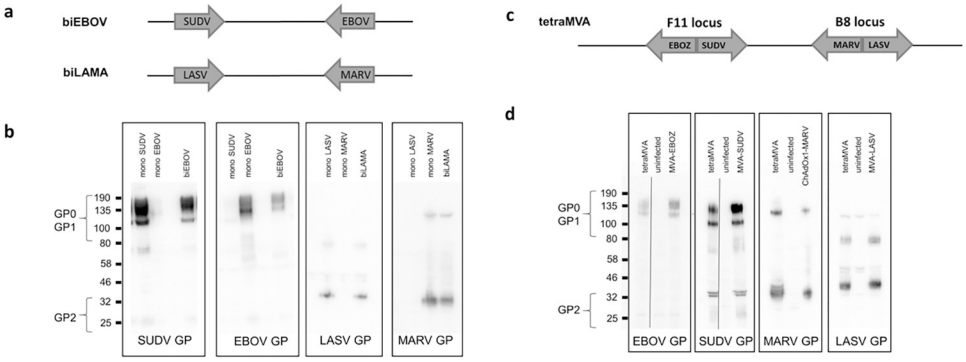

Fig 1. ChAdOx1 bivalent constructs (a) Schematic to show design of bivalent vectors. To test expression, vaccines were added to HEK293 cells at 10 MOI, cells were lysed after 24 h and lysate loaded for analysis by Western blot. (b) Expression of antigens in the bivalent constructs compared to monovalent controls (c) Schematic to show design of tetraMVA construct. (d) Expression of antigen in tetraMVA compared to monovalent controls.

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tetravalent MVA vector (tetraMVA) expressing all four GP antigens (Fig 1C) to test in a heterologous prime-boost regimen, as this was previously shown to elicit greater immunogenicity compared with homologous prime-boost or prime only, with either of the viral vector platforms [12] [18]. Western blot analysis of cells infected with the tetraMVA vector showed expression of all four antigens at levels equivalent to those of relevant monovalent controls (Fig 1D).

Optimisation of administration regimen

We and others have previously observed differences in cellular immunogenicity when two monovalent vaccines are administered compared to a single bivalent vaccine that encodes the same two antigens [21]. We investigated the difference in immunogenicity between administration of two separate vaccine constructs encoding monovalent antigens with a single construct that encodes both (ChAdOx1-biEBOV versus monovalent mix and the ChAdOx1-biLAMA versus monovalent mix). Study regimens and bleed schedules are illustrated in S1 Fig. Very minimal differences in cellular immunogenicity to either antigen was observed whether two monovalent vaccines, or a single bivalent vaccine, was administered (S1 Table).

However, when combining both bivalent vaccines (ChAdOx1 -biEBOV and -biLAMA) into a single shot and comparing it to a mix of all four monovalent vaccines in a single shot, lower T cell responses were measured against the EBOV antigen (S1 Table–regimen 3). We have previously found that the administration of the two competing vectors into different muscles alleviated the cellular immune competition and can improve immunogenicity [21]. Therefore, we administered ChAdOx1-biEBOV and -biLAMA to the left and right rear leg, respectively and compared this to a mix of monovalent EBOV and SUDV vaccines (left leg) and a mix of monovalent LASV and MARV vaccines (right leg). This improved the response of the bivalent, in comparison to monovalent, regimens (S1 Table—regimen 4). Co-administration was, therefore, considered the optimal regimen going forward and was used unless otherwise stated.

Humoral immunogenicity

Next, we assessed the humoral immune response after co-administration of our bivalent vaccines; individual dual-antigen vaccines were delivered to a separate hind leg of a mouse (e.g. ChAdOx1-biEBOV to the right hind limb and ChAdOx1-biLAMA to the left hind limb). For comparison, the monovalent controls were mixed as follows; ChAdOx1-EBOV and -SUDV were mixed and administered to one leg, and ChAdOx1-MARV and -LASV were mixed and administered to the other leg. 6 weeks post vaccination, humoral immune responses were assessed by ELISA against each of the four antigens in the outbred CD-1 mouse strain. Across all four antigens, there were no significant differences (Mann-Whitney test) in the total IgG titres elicited by the dual-antigen vaccines compared to the mixed monovalent controls (Fig 2A).

To augment humoral immunity, BALB/c mice that had been primed with the dual-antigen ChAdOx1 vaccines were boosted with the tetraMVA vector expressing all four antigens. The humoral immune response to the four antigens was increased at two weeks post-boost (6 weeks post-prime) compared to pre-boost levels to varying degrees (Fig 2B).

To further delineate antibody responses, we performed isotype-specific ELISAs. Initially, this was performed in the inbred BALB/c strain and the relative Th1/Th2 contributions were evaluated as the ratio of IgG1/IgG2a (S2 Fig). After prime vaccination with bivalent vaccines, the antibody responses were approximately Th1-Th2 balanced for all 4 antigens. After boost

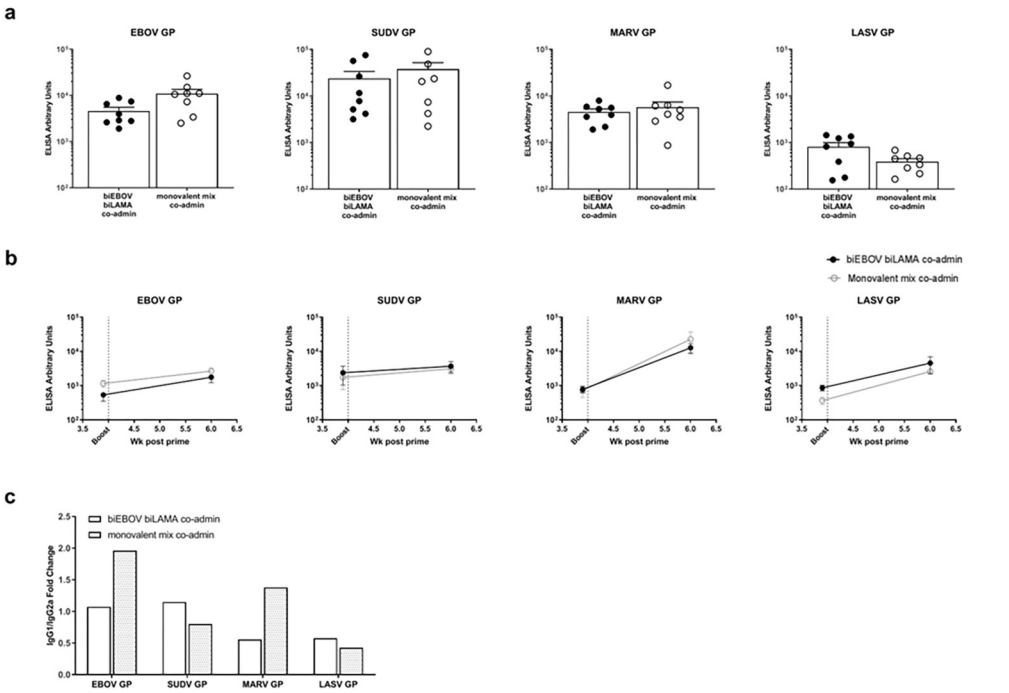

Fig 2. Total antigen-specific IgG levels induced by ChAdOx1 dual antigen prime, and following tetraMVA boost. (a) CD-1 mice were immunised with either ChAdOX1-biEBOV and -biLAMA co-administered or a mix of monovalent controls. Total IgG levels were measured 6 weeks later. (b) BALB/C mice were primed with either ChAdOx1-biEBOV and -biLAMA co-administered or a mix of monovalent controls, 4 weeks later all mice were boosted with tetraMVA. Total IgG levels were measured pre-boost and at 2 weeks post boost vaccination. (c) IgG1 and Ig2a isotype levels were measured in CD-1 mice 8 weeks after prime vaccination, and are expressed as a ratio.

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vaccination with tetraMVA, the antibody response against MARV GP in mice primed with bivalent vaccines was Th2-biased while responses remained balanced against the other three antigens. In mice primed with monovalent vaccine the Th1/Th2 was balanced post-prime and post-boost (S2 Fig). To account for potential bias introduced by a fixed haplotype in the BALB/c model, we also measured IgG1 and IgG2a in CD-1 mice. We detected similar levels for IgG1 and IgG2a in CD-1 mice vaccinated with our bivalent vectors and a mix of monovalent controls (no significant differences, Mann-Whitney test). In CD-1 mice vaccinated with bivalent vaccines, the IgG1/IgG2a ratios against MARV GP and LASV GP antigens suggest skewing towards Th1, whereas the ratios for EBOV GP and SUDV GP were Th1-Th2 balanced (Fig 2C). CD-1 responses after monovalent regimes were variable; EBOV skewed towards Th2, LASV skewed towards Th1 while SUDV and MARV were balanced (Fig 2C). These combined results demonstrate that the humoral immune response (Th1 or Th2) after vaccination with our multi-antigen vectors differ between mouse strains, can vary after prime and prime-boost vaccination and may be antigen- rather than vector- dependent. The levels of other isotypes (IgG2b, IgG3, IgM and IgA) were assessed, but were below the limit of detection in our assay.

It will be important for new vaccines against VHF to be protective across viruses. To measure humoral reactivity to different filoviruses, CD-1 mice were primed with co-administered bivalent ChAdOx1 vectors or a mix of monovalent controls. Cross-strain humoral immunity was measured against the following filovirus GPs: EBOV-Mayinga GP (AAC54887.1), EBOV--Kikwit GP (AQ55048.1), SUDV-Gulu GP (YP_138523.1) and Bundibugyo GP (BDBV) (ACI28624.1). Total IgG levels 6 weeks after vaccination demonstrated that antibody responses against all proteins could be detected (S3A Fig). Booster vaccination with MVA has previously

been shown to not only increase but also broaden antigen-specific immune responses [22]. To assess if the cross-reactive immune responses could be augmented, bivalent-primed BALB/c mice were boosted with tetraMVA 4 weeks post-prime. At 8 weeks post-boost, total IgG antibody titres increased (by at least 2-fold) against all filoviruses tested except for SUDV Gulu (S3B Fig). This includes cross-reactive responses observed against BDBV which was not present in the vaccine construct. In all cases, responses to the bivalent vaccines were comparable to the monovalent control mix. Naïve mouse serum did not react with the antigens in the assays.

Cellular immunogenicity

Immunogenicity can vary in different mouse strains so both CD-1 and BALB/c mice were immunised with ChAdOX1-biEBOV and -biLAMA vaccines to assess the cellular immunogenicity of all four VHF antigens. The bivalent vaccines were co-administered, and immunogenicity was assessed by ELISpot 2 weeks post vaccination (Fig 3A and 3B). In CD-1 mice (Fig 3A), the magnitudes of ELISpot responses were comparable between the co-administered dual-antigen ChAdOx1 vectors and the monovalent controls for EBOV GP and MARV GP. However, for SUDV GP and LASV GP, the ELISpot responses were significantly higher in the dual-antigen regimen ($p = 0.013$ and $p = 0.007$ respectively, Mann-Whitney test). In BALB/c mice (Fig 4B), there were no differences in the ELISpot responses between the two regimens for SUDV GP, MARV GP and LASV GP, but the response to EBOV GP was significantly higher following vaccination with co-administered dual-antigen vaccines than for the monovalent controls ($p = 0.0014$, Mann-Whitney test).

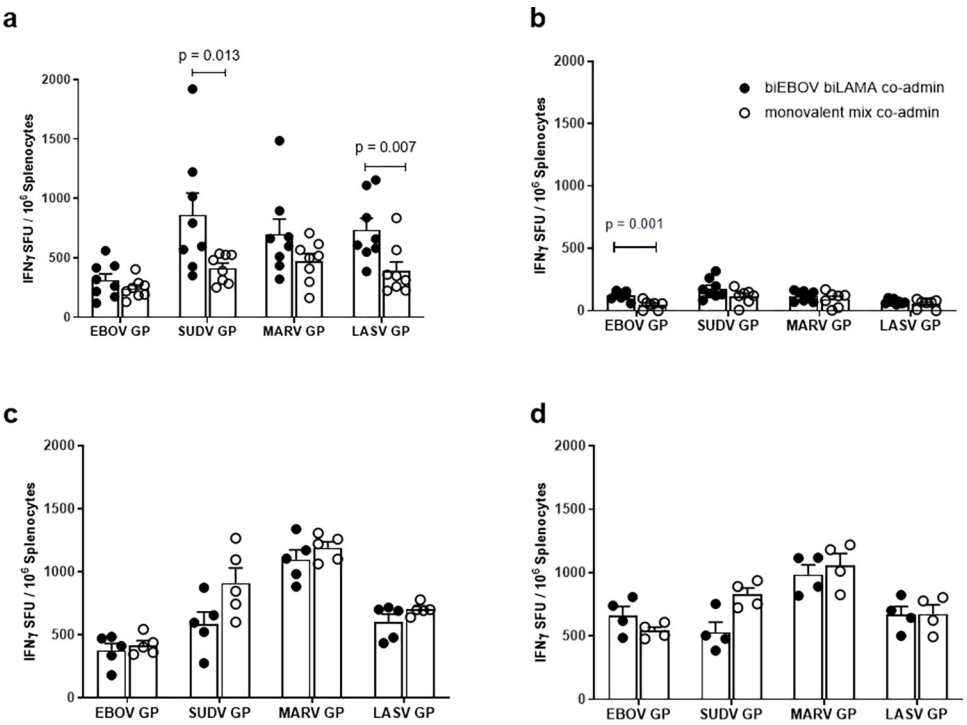

Fig 3. T cell immunogenicity of ChAdOx1 dual antigen prime and prime-boost (ChAdOx1 and MVA) approaches. In all panels mice received either ChAdOx1-biEBOV and -biLAMA co-administered or a mix of monovalent controls. IFNγ ELISpot in CD-1 mice 8 weeks post vaccination (a) and in BALB/C mice 2 weeks post vaccination (b). In experiments shown in (c) and (d), BALB/C mice were primed as described above and 4 weeks later boosted with tetraMVA. IFNγ ELISpot 2 weeks post boost vaccination (c) and 8 weeks post boost vaccination (d), *p* values determined by Mann-Whitney tests with only those achieving statistical significance annotated on the graph. X-axis labels show peptide pools used for splenocyte stimulation.

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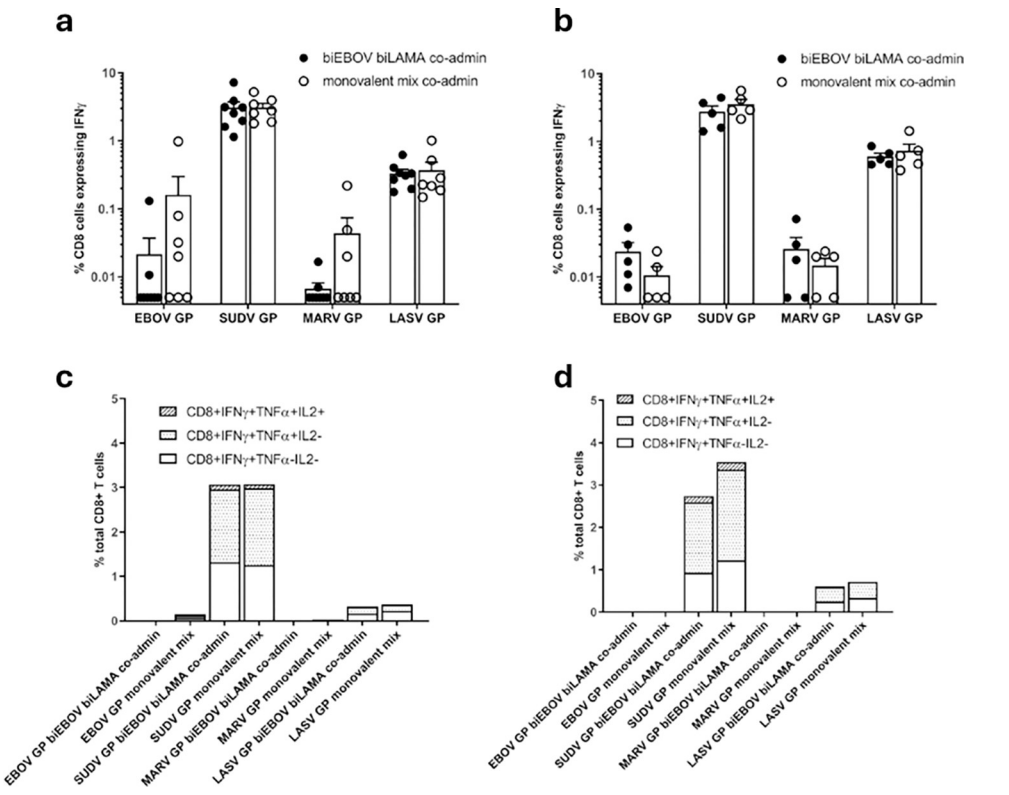

Fig 4. CD8+ T cell immunogenicity of prime only and prime-boost vaccination determined by ICS. BALB/c mice were primed with either ChAdOx1-biEBOV and -biLAMA co-administered or a mix of monovalent controls. (a) % of CD8+ T cells expressing IFNγ after stimulation with each of the peptide pools (a) 2 weeks post-prime and (b) 2 weeks post-boost with tetraMVA. $CD8^+$ T cells expressing IFNγ, TNFα and IL-2 determined by ICS (c) 2 weeks post-prime and (d) 2 weeks post-boost with tetraMVA.

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Cellular immunogenicity of a prime-boost regimen was assessed in BALB/c mice at 2 weeks and 8 weeks after a tetraMVA boost (Fig 3C and 3D). No statistically significant differences were observed between the co-administered dual-antigen ChAdOx1 vectors and monovalent mix control for the four antigens, although a trend toward lower responses in the dual-antigen group was observed for SUDV GP. ELISpot responses in both groups were substantially boosted 2 weeks after tetraMVA administration (Fig 3B and 3C). Notably, SUDV, MARV, and LASV GP ELISpot responses did not wane from week 2 to 8, and EBOV GP-specific responses further increased during this period (Fig 3C and 3D).

Intracellular cytokine staining was performed on splenocyte samples harvested 2 weeks after prime vaccination (Fig 4A), and 2 weeks after tetraMVA boost (Fig 4B). Antigen-specific responses primarily consisted of $IFN\gamma^+$ $CD8^+$ T cells, the majority of which also secreted TNFα; a small proportion of these cells additionally produced IL-2 (Fig 4C and 4D). The highest percentage of cytokine positive $CD8^+$ T cells were detected against SUDV GP, followed by LASV GP. Meanwhile, the lowest responses were measured against EBOV GP. For $CD4^+$ T cells, MARV GP-specific responses demonstrated the highest percentage of cytokine positive $CD4^+$ T cells and the lowest responses were measured against EBOV GP (Fig 5A and 5B). Of the vaccine-specific $CD4^+$ responses post-prime and post-boost, there was a greater percentage of triple cytokine positive cells detected compared to cells that were double positive for IFNγ and TNFα against EBOV GP, SUDV GP, MARV GP and LASV GP (Fig 5C and 5D). These

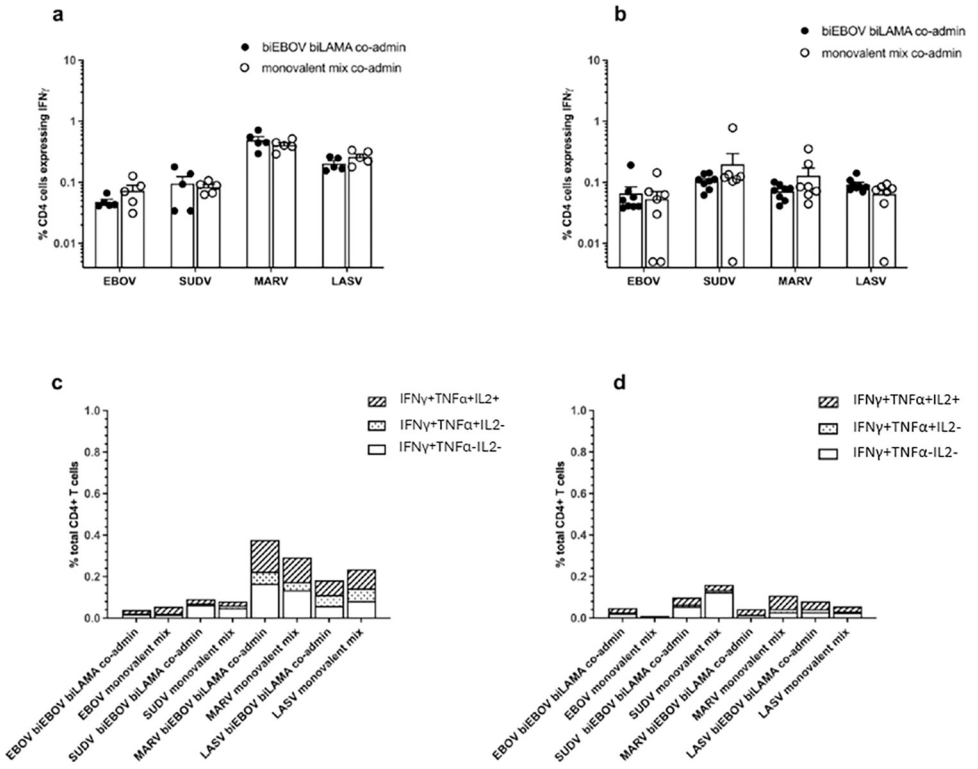

Fig 5. CD4+ T cell immunogenicity of prime only and prime-boost vaccination determined by ICS. BALB/c mice were primed with either ChAdOx1-biEBOV and -biLAMA co-administered or a mix of monovalent controls. (a) % of CD4+ T cells expressing IFNγ after stimulation with each of the peptide pools (a) 2 weeks post-prime and (b) 2 weeks post-boost with tetraMVA. $CD4^+$ T cells expressing IFNγ, TNFα and IL-2 determined by ICS (c) 2 weeks post-prime and (d) 2 weeks post-boost with tetraMVA.

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data imply that the hierarchy of immune dominance of VHF antigens responses may differ depending on the response ($CD8^+$ and $CD4^+$) being measured.

A multiplex mouse cytokine kit was used to measure antigen-specific vaccine-induced expression of 28 different cytokine/chemokines from splenocyte samples 2 weeks after boost vaccination (sample sets were as analysed in Fig 4B). Advantageously, the multiplex assay can measure cytokines not routinely measured by ELISpot or ICS and can generate a more complete picture of the immune profile post-vaccination. Only those cytokines and chemokines that could be reliably measured post vaccination were tested for clustering—18 different cytokines were included (S4 Fig). The data indicate that groups of certain cytokines are more likely to be concurrently detected e.g. IL-1β, TNFα, IP-10, IFNγ, IL-2, IL-5, IL-4, IL-10 and IL-6 were clustered together and the induction of these cytokines following stimulation with filovirus peptides is to be expected [23]. Cytokine fold change (when compared to unstimulated cells) was calculated for each of the four antigens tested (S2 Table) and interactions between cytokines across samples were depicted in a heatmap (Fig 6). Across all four antigens, IFNγ was the most highly expressed cytokine, followed by MIP-1α, IL-17 and MIP-2. IL-6, IL-4, IL-2, IL-10 and IL-5 were also highly expressed in supernatant from MARV GP-stimulated cells. Supernatant from SUDV GP-stimulated cells displayed a different pattern of cytokine expression. A number of cytokines were produced at lower levels by SUDV GP stimulated cells compared to unstimulated. However, no significant differences in fold change between the monovalent mix and dual-antigen vaccination regimens were observed when stimulated with EBOV, SUDV or MARV GPs (S2 Table).

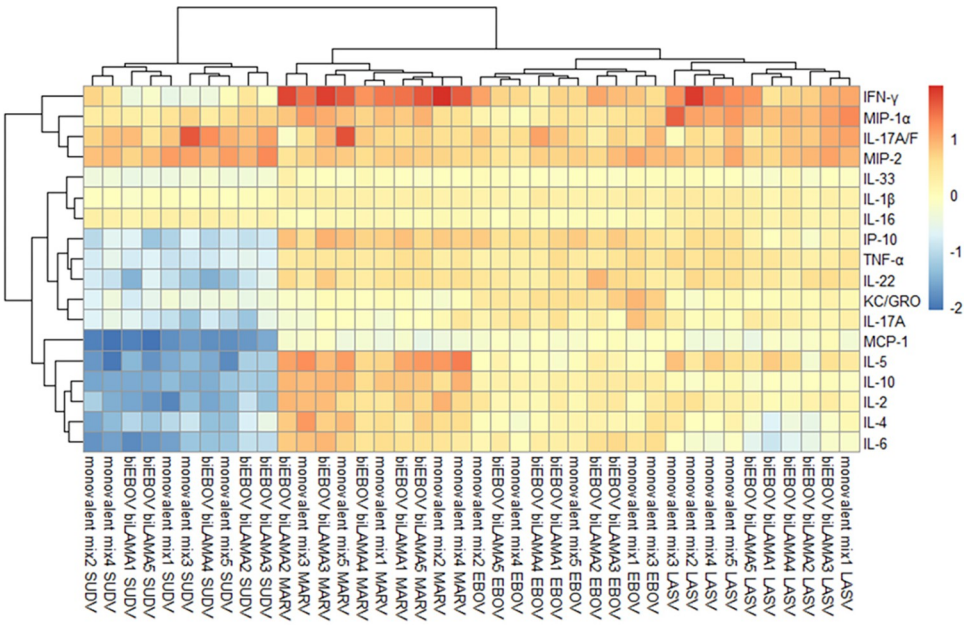

Fig 6. Cytokine and chemokine analysis after prime-boost vaccination. BALB/c mice were primed with either ChAdOx1-biEBOV and -biLAMA co-administered (n = 5) or a mix of monovalent controls (n = 5), 4 weeks later all mice were boosted with tetraMVA. Cytokine levels (in supernatant from splenocytes stimulated with the peptide pools for EBOV GP, SUDV GP, MARV GP and LASV GP) determined by MSD technology platform 2 weeks post boost. Heat map shows fold change in peptide stimulated vs unstimulated supernatant across peptide pools (each stimulation for splenocytes from each mouse is labelled 1–5) and vaccine regimen. Red indicates positive fold change, blue indicates negative fold change.

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Overall, humoral and cell-mediated (ELISpot, flow cytometry and cytokine) immunogenicity data demonstrated that the dual-antigen vaccines can induce immune responses, and that these responses are diverse, polyfunctional and relatively sustained for the 12-week duration of the study.

Lethal challenge to assess protection

We next assessed the efficacy of our biEBOV vaccine in a lethal guinea pig EBOV challenge model (using a guinea pig-adapted EBOV based on the EBOV- Yambuku-Ecran strain, GenBank: AF086833.2) (S5A Fig). Guinea pigs vaccinated with ChAdOx1-biEBOV or a mix of ChAdOx1-EBOV and -SUDV survived the duration of the challenge, whereas all control animals succumbed to infection and reached the humane end point by day 7–9 post-infection (S5B Fig). Animals vaccinated with the control ChAdOx1 GFP vaccine lost weight from day 5 onwards, and exhibited an increase in temperature (S5C and S5D Fig). Animals vaccinated with ChAdOx1-biEBOV or a mix of monovalent control vaccines continued to gain weight post challenge with no significant temperature fluctuations (S5C and S5D Fig). During the study, guinea pigs were clinically assessed; clinical signs were first observed in the ChAdOx1 GFP negative-control group on day 5 post challenge (S5E Fig). No animal vaccinated with ChAdOx1-biEBOV or a mix of monovalent controls exhibited any clinical signs (S5E Fig).

We used IFNAR$^{-/-}$ mice to further assess the efficacy of ChAdOx1-biEBOV and -biLAMA due to their increased susceptibility to these viruses. Mice were vaccinated with either a co-administration of ChAdOx1- biEBOV and -biLAMA, the relevant monovalent controls or a negative control and subsequently challenged with the specific virus as described (Fig 7A). Induction of binding IgG towards EBOV GP, SUDV GP, and MARV GP prior to challenge

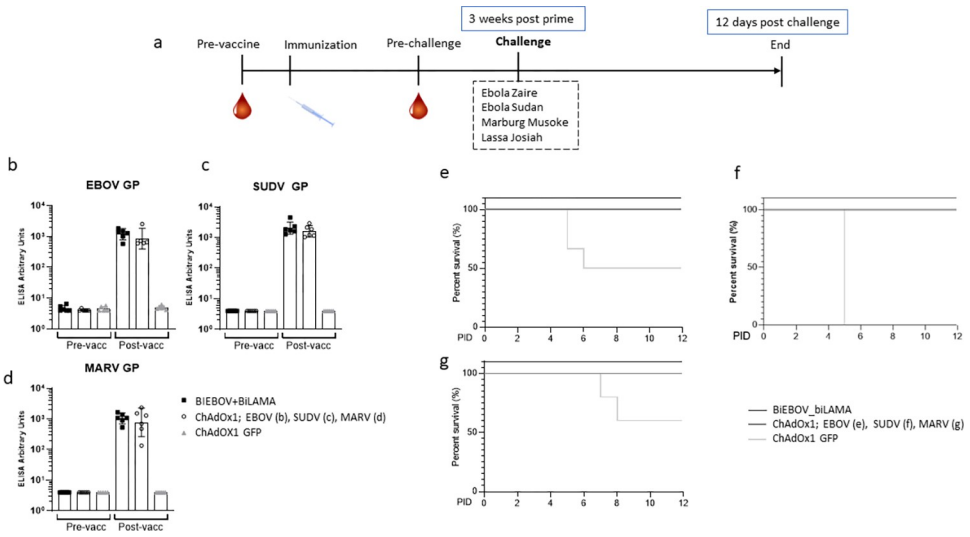

Fig 7. Efficacy of ChAdOX1-biEBOV and -biLAMA against EBOV, SUDV, and MARV infection in mice. Groups of 6 IFNAR$^{-/-}$ mice received either ChAdOx1-biEBOV and -biLAMA, relevant monovalent control or irrelevant control immunizations and were challenged 3 weeks later via i.p injection of specific virus. Animals were culled upon reaching the humane end point or the end of the study (12 days post-infection) (a) Experimental Design. Total IgG binding titres in towards (b) EBOV GP, (c) SUDV GP and (d) MARV GP in mice later challenged with their respective viruses. Survival analysis for each of the challenge viruses: (e) Ebola Zaire Guinea Kissidoguo, (f) Sudan virus Boniface, (g) MARV Musoke. p values determined by Log rank (Mantel-Cox) test. Survival analysis for LASV challenge not included due to pre-determined culls throughout study.

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was demonstrated by ELISA (Fig 7B, 7C and 7D). A single dose of ChAdOx1-biEBOV and -biLAMA co-administered resulted in protection from EBOV (Fig 7B and 7E) or SUDV (Fig 7C and 7F) infection ($p = 0.028$ and $p<0.001$ respectively). Similar results were observed in mice infected with MARV Musoke, although this did not reach significance as the challenge was not uniformly lethal ($p = 0.068$) (Fig 7D and 7G). In the control groups (vaccinated with ChAdOx1 GFP) infected with EBOV or SUDV, 50% and 100% of the mice succumbed to infection, respectively (Fig 7B, 7E and 7F). The surviving EBOV infected control mice all showed signs of disease (weight loss, less active, piloerection etc.) but recovered (S6A–S6C Fig).

Previous studies have demonstrated non-uniform lethality in the IFNAR$^{-/-}$ model following LASV challenge [24,25]. Therefore, we designed the LASV challenge to assess viraemia by euthanizing two randomly selected mice from each group after days 4, 8 and 12 post-infection. At 4 days post-infection, 5/6 mice in the control group (ChAdOx1 GFP), and 0/6 in either ChAdOx1 LASV and ChAdOx1 biEBOV/biLAMA vaccinated group, had lost weight (S6D Fig). The 4 mice left in the control group were euthanized on day 8 post-infection due to reaching the humane endpoint. By day 8 post-infection, weight loss was also observed in the ChAdOx1 LASV (1/6) and ChAdOx1 biEBOV/biLAMA (5/6) mice but none had reached their humane endpoint by end of study. No LASV RNA was detected in serum from either ChAdOx1-biEBOV -biLAMA or ChAdOx1 LASV vaccinated mice at any of the time points (S7A Fig). Viraemia was detected in 1/2 and 4/4 control mice on days 4 and 8 post-infection, respectively. Liver samples also showed higher levels of viral RNA in control mice compared to ChAdOx1-biEBOV -biLAMA or ChAdOx1 LASV vaccinated mice at days 4 and 8 post-infection (S7B Fig). Due to the absence of control mice at day 12 post-infection, viraemia in the liver is not included at that timepoint.

Discussion

This work aims to address the global need for vaccines in preparedness for future outbreaks of haemorrhagic fevers caused by EBOV, SUDV, MARV and LASV. The unprecedented ease of EVD spread in West and Central Africa in the preceding 20 years along with the recent MARV and SUDV outbreaks in Tanzania, Equatorial Guinea, and Uganda, exemplify the consequences of having no deployable prophylactic intervention against a high-impact outbreak pathogen. Indeed, the geographical overlap of *Filoviridae* and LASV continues to expand. This expanding overlap is partially due to better detection methods, including the isolation of genetically diverse filoviruses from flying fruit bats (e.g. *Rousettus aegyptiacus*) and the identification of new hosts for LASV [26–28]. The large geographical areas habituated by these natural hosts, which can travel vast distances, indicate that there is an increased risk of outbreaks in previously unaffected areas and likely an increased incidence of undocumented subclinical infection [26–30].

During the 2013–2016 West African EBOV outbreak, six of seven vaccine candidates tested in clinical trials employed viral vector technologies, including three adenoviral and two MVA vectored vaccines. Results from the Phase III rVSV-ZEBOV clinical trial run during the 2013–2016 EBOV outbreak demonstrated that a single-dose regimen of a viral vectored vaccine (rVSV-ZEBOV) is both safe and efficacious in preventing EVD [7]. While correlates of protection are not clearly defined for all VHF, a protective role for antibodies has been demonstrated in EBOV [31–33]. Comparison of the protective neutralising antibody responses achieved after rVSV-ZEBOV immunisation with those elicited by an adenoviral vectored EBOV vaccine candidate showed that the latter induced equivalent antibody levels [12], providing encouraging evidence to support further clinical development of adenoviral vaccine vectors against viral haemorrhagic fevers. In mice, neutralising antibodies are typically IgG1, while IgG2a antibodies are associated with induction of stronger ADCC and complement activation [34]. Overall, our data indicate that the bivalent vaccines induce a mixed humoral response inducing both IgG1 and IgG2a post-vaccination. The levels of other isotypes (IgG2b, IgG3, IgM and IgA) were assessed but were below the limit of detection in our assay. We also demonstrate that immunisation with bivalent vaccines induces antibodies which recognise proteins from different filoviruses including Bundibugyo with further work to be performed to characterise the breadth of this heterosubtypic immunity.

The adenoviral vectored vaccines tested here also induce high levels of T cell immunity which have been demonstrated to play an important role during against VHF infections caused by EBOV and LASV in particular (reviewed [35]). Ebolavirus challenge studies in recombinant adenovirus–vaccinated macaques have demonstrated that protection is associated with robust dual-cytokine secreting (IFNγ and TNFα) $CD8^+$ T cells [36] [37]. Depleting these macaques of $CD8^+$ T cells before challenge abolished vaccine-mediated protection [38], suggesting that $CD8^+$ T cells, and/or $CD8^+$ NK cells, are necessary for virus clearance in this model system. In mice, the predominant cellular immune response observed following vaccination with our bivalent vaccines was $IFN\gamma^+$ $CD8^+$ T, with a large proportion also being $TNF\alpha^+$. Our multiplex cytokine data showed an array of cytokines induced by antigenic peptides after filovirus and arenavirus vaccination and adds breadth to the customarily reported IFNγ, TNFα and IL-2 expression in T cells. We observed some differences in the cytokine profile, notably reduced IFNγ expression, of cells stimulated with SUDV peptides compared to the other three antigens. In contrast, ELISpot and flow cytometry data showed that SUDV peptide stimulation elicited higher numbers of IFNγ producing cells. However, the differential stimulation times with SUDV peptides may exhaust the cells or be cytotoxic, resulting in lower levels of IFNγ being detected in supernatant and measured in our multiplex cytokine assay.

We have demonstrated here that a single dose of our ChAdOx1-biEBOV and -biLAMA vaccines co-administered can confer protective efficacy in a lethal, small animal challenge model for three of our viruses of interest, EBOV, SUDV, and MARV. While the EBOV challenge in Guinea pigs and mice used a heterologous strain to that encoded by the vaccine; SUDV, MARV and LASV challenge strains were homologous to their vaccine insert, and therefore protection against heterologous strains is not yet determined. The delay in weight loss and survival of animals vaccinated with ChAdOx1-biEBOV/biLAMA following LASV challenge suggest that the vaccine may be protective. However, due to the study design with animals culled at pre-determined times—no definite conclusions can be drawn. Thus, further work is needed to explore the efficacy of the ChadOx1-biEBOV and -biLAMA vaccine combination against Lassa fever. We have, however, previously demonstrated that guinea pigs immunised with a single dose of ChAdOx1-LASV were protected against LASV challenge [39].

ChAdOx1-biEBOV has progressed to a first-in-human, phase I clinical trial, primarily assessing safety, tolerability and immunogenicity. While the well-characterised inbred BALB/c mouse strain facilitates easy comparison between vaccine constructs, the broader nature of the immune response in outbred CD-1 is perhaps more relevant as a model for human responses. While mice and guinea pigs have previously been used as models of EVD, the NHP model is considered to more closely recapitulate human disease. A recently completed NHP study, using a stringent SUDV challenge demonstrated that despite a robust immune response, ChAdOx1-biEBOV failed to protect against fatal disease [40] demonstrating the value in progressing to NHP models after promising data in small animals [40, 41]. While the high infectious doses and route of viral challenge in NHP may not be fully representative of natural infection or human exposure, it is unclear if the level of immunity achieved post ChAdOx1-biEBOV in humans would be efficacious in an outbreak setting.

Heterologous prime-boost vaccine regimen could fare better and be particularly appropriate for healthcare practitioners and first-line responders, as this regimen is demonstrated here and in clinical trials to induce long-lived, high-titre responses that will be better able to protect against repeated high-risk exposure to the viral targets. Ideally, a vaccine for use in an outbreak setting would rapidly generate protective immunity. Previous experience with the ChAdOx1 vector shows rapid onset of immunity, with humoral immune responses peaking around 4 weeks post-vaccination in animal models and humans. If, however, a booster is required before protection is conferred, then a regimen with a short interval would be crucial. The COVID-19 pandemic has demonstrated the feasibility of two-dose regimens in outbreak settings, globally. Chimpanzee Adenovirus (ChAd)-based vaccines are frequently administered as the first vaccination (prime) and can be followed with a second, MVA-based vaccination (boost)–this ChAd-MVA 'prime-boost' regimen benefits from remarkable immunogenicity and long-lived immunity [4]. For example, the longevity of high titre neutralising antibodies and cellular immune responses to EBOV GP has been demonstrated following a ChAd-MVA prime-boost regimen both in man and macaques, where protective efficacy, in the latter, against EBOV challenge was significantly extended post-vaccination following a boost immunisation [36] [12] [42]. In addition to our bivalent adenoviral vectors, we therefore also developed a multi-antigen MVA vector that encodes all four VHF antigens (tetraMVA). Using tetraMVA in a prime-boost regimen together with the adenoviral vectors induced a higher cellular and humoral response and a diverse chemokine/cytokine profile and could be progressed for further preclinical and clinical testing.

In summary, modifications of the ChAdOX1-biEBOV construct are being made to achieve single-shot efficacy for rapid protection during outbreaks. While heterologous prime-boost regimens may be required for long-lived protective efficacy in front-line workers, thus

improving the ability to curb epidemics and avoid future public health and humanitarian crises. Further development of these vectors will contribute to the common goal: deployable vaccine solutions for future VHF outbreaks.

Supporting information

S1 Fig. Schematic outlining the vaccination schedules. Immunisations of either CD1 or BALB/C mice for prime only schedules or prime boost schedules.
(TIF)

S2 Fig. Antibody isotype levels induced by ChAdOx1 dual-antigen prime and tetraMVA boost. BALB/c mice were primed with either co-administered biEBOV and biLAMA or a mix of monovalent controls and boosted with tetraMVA 4 weeks later. IgG1 and Ig2a isotype levels are expressed as a ratio at 4 weeks after prime vaccination (left panel) and 8 weeks after boost vaccination.
(TIF)

S3 Fig. Cross reactivity induced by ChAdOx1 dual antigen prime and followed by tetraMVA boost. Antibody titres against EBOV-Mayinga GP, EBOV-Kikwit GP, SUDV-Gulu GP and BDBV GP were assessed. (a) CD-1 mice were primed with either biEBOV and biLAMA co-administered or a mix of monovalent controls. Total IgG levels were measured 8 weeks later (p values determined by Mann Whitney test). (b) BALB/c mice were primed with either biEBOV and biLAMA co-administered or a mix of monovalent controls. 4 weeks later all mice were boosted with tetraMVA. Total IgG levels before boost and at 8 weeks post boost vaccination.
(TIF)

S4 Fig. Interaction of cytokines and chemokines after prime-boost vaccination. Mice were primed with either biEBOV and biLAMA co-administered or a mix of monovalent controls. 4 weeks later, all mice were boosted with tetraMVA. Cytokine levels (in the supernatant of peptide pool-stimulated splenocytes) at 2 weeks post boost were measured using a commercial assay (MSD). Correlation matrix among 18 cytokines measured, red indicates negative correlation, blue indicates positive correlation.
(TIF)

S5 Fig. Protective efficacy of ChAdOx1 bivalent vaccines against Ebola Zaire infection in Guinea Pigs. Groups of 6 Hartley Guinea Pigs received either biEBOV, mix of monovalent controls or control without antigen and were challenged 4 weeks later IP with Ebola virus. Animals were culled upon reaching humane end point or 21 days post challenge. (a). Experimental design. (b). Survival analysis. *p* value determined by Log rank (Mantel-Cox) test. (c). Weight change post challenge. (d). Temperature post challenge. (e). Clinical score post challenge.
(TIF)

S6 Fig. Weight loss after EBOV, SUDV, MARV and LASV infections in vaccinated mice. Groups of 6 IFNγ-/- mice received either biEBOV and biLAMA, relevant monovalent control or irrelevant control and were challenged 3 weeks later IP with virus. Animals were culled upon reaching humane end point. Weight loss for each of the challenge viruses: (a) EBOV--Guinea Kissidoguo, (b) SUDV-Boniface (c) MARV-Musoke and (d) LASV-Josiah. Mean and SD shown.
(TIF)

S7 Fig. Post-challenge LASV levels measured by RT-qPCR. Groups of 6 IFNγ-/- mice were culled at pre-determined timepoints post-challenge (n = 2 per group at days 4, 8 and 12) or when reaching their humane endpoint. Viraemia was measured in (a) serum and (b) liver of culled mice. Mean and SD shown.
(TIF)

S1 Table. Cellular immunogenicity in fold change of monovalent and ChAdOx1 bivalent vaccines. BALB/c mice received either bivalent vaccines or mix of monovalent controls and IFNγ ELISpot was performed at 2 weeks post vaccination. Vaccine regimens shown are: 1.biEBOV versus monovalent mix (EBOV & SUDV) 2. biLAMA versus monovalent mix (MARV & LASV) 3. biEBOV and biLAMA mixed versus monovalent mix (EBOV, SUDV, MARV, LASV) and 4. EBOV and biLAMA co-administered versus monovalent mix (EBOV, SUDV, MARV, LASV). Red indicates lowest fold change observed for regimen and green indicates highest.
(TIF)

S2 Table. Cytokine and chemokine levels after prime-boost vaccination. BALB/C Mice were primed with either biEBOV and biLAMA co-administered or a mix of monovalent controls, 4 weeks later all mice were boosted with tetraMVA. Cytokine levels (in supernatant from splenocytes stimulated with the relevant peptide pools) determined using an MSD assay 2 weeks post boost. Log_{10} fold change in cytokine/chemokine levels in unstimulated versus peptide stimulated was compared across the vaccine groups. Values given to 2 d.p. for descriptives and 3 d.p. for p values (determined by Mann-Whitney).
(TIF)

S1 Raw Data file. Contains the raw data from Figs 2B, 2C, 4C, 4D, 5C, 5D, 6, S2, S3B, S5C, S5D, S5E and S6.
(XLSX)

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Author Contributions

Conceptualization: Amy Flaxman, Teresa Lambe.

Data curation: Amy Flaxman, Sofia Appelberg, Kuan M. Cha.

Formal analysis: Amy Flaxman, Isabel Schmidt.

Funding acquisition: Teresa Lambe.

Investigation: Amy Flaxman, Sarah Sebastian, Sofia Appelberg, Kuan M. Cha, Marta Ulaszewska, Jyothi Purushotham, Ciaran Gilbride, Hannah Sharpe, Alexandra J. Spencer, Isabel Schmidt, Linda Easterbrook, Stephen Findlay-Wilson.

Methodology: Amy Flaxman, Stuart Dowall, Sarah Gilbert, Teresa Lambe.

Project administration: Teresa Lambe.

Resources: Stuart Dowall, Ali Mirazimi, Teresa Lambe.

Supervision: Stuart Dowall, Sarah Gilbert, Ali Mirazimi, Teresa Lambe.

Validation: Amy Flaxman, Kuan M. Cha.

Visualization: Amy Flaxman, Sagida Bibi, Daniel Wright.

Writing – original draft: Amy Flaxman.

Writing – review & editing: Sagida Bibi, Daniel Wright, Teresa Lambe.

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Epub 2018/03/28. https://doi.org/10.1038/s41541-018-0049-5 PMID: 29581897; PubMed Central PMCID: PMC5861057 competing financial interests.

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