HHS Public Access

Author manuscript

Lancet. Author manuscript; available in PMC 2024 January 28.

Published in final edited form as:

Lancet. 2023 January 28; 401(10373): 294–302. doi:10.1016/S0140-6736(22)02400-X.

Safety, tolerability, and immunogenicity of the chimpanzee adenovirus type 3-vectored Marburg virus (cAd3-Marburg) vaccine in healthy adults in the USA: a first-in-human, phase 1, open-label, dose-escalation trial

A full list of authors and affiliations appears at the end of the article.

Summary

Background—The World Health Organization has identified Marburg as an emerging virus requiring urgent vaccine research and development, particularly relevant due to its recent emergence in Ghana. Here we report results from a first-in-human clinical trial evaluating a replication-deficient recombinant chimpanzee adenovirus type-3 (cAd3) vectored vaccine encoding a wild-type Marburg Angola glycoprotein (cAd3-Marburg) in healthy adults.

Methods—We did a first-in-human, phase 1, open-label, dose-escalation trial of the cAd3-Marburg vaccine at the Walter Reed Army Institute of Research Clinical Trials Center in the USA. Healthy adults aged 18–50 years were assigned to receive a single intramuscular dose of cAd3-Marburg vaccine at either 1×10^{10} or 1×10^{11} particle units (pu). Primary safety endpoints included reactogenicity assessed for the first 7 days and all adverse events assessed for 28 days after vaccination. Secondary immunogenicity endpoints were assessment of binding antibody responses and T-cell responses against the Marburg virus glycoprotein insert, and assessment of neutralising antibody responses against the cAd3 vector 4 weeks after vaccination. This study is registered with ClinicalTrials.gov, NCT03475056.

Findings—Between Oct 9, 2018, and Jan 31, 2019, 40 healthy adults were enrolled and assigned to receive a single intramuscular dose of cAd3-Marburg vaccine at either 1×10^{10} pu (n=20) or 1×10^{11} pu (n=20). The cAd3-Marburg vaccine was safe, well tolerated, and immunogenic. All enrolled participants received cAd3-Marburg vaccine, with 37 (93%) participants completing follow-up visits; two (5%) participants moved from the area and one (3%) was lost to follow-

*Corresponding author: Katherine V. Houser, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA, katherine.houser@nih.gov, Phone: 301-742-3901.

Author Contributions

MJH and JAA are the principal investigators of RV 507. JAA, JGG, ABM, RAK, JRM, NJS, and JEL contributed to conception and design. CA, GY, SH, SS, SV, OT, and PM provided protocol and regulatory support and expertise. MJH, JAA, CL, AP, BA, JNH, PTS, PEW, CS, MLR, MRG, IJG, LAH, and ATW contributed to investigation and sample collection. TM, CNMD, SRN, PAS, MB, JT, MP, BF, and SO collected data. DAS, AP, RH, MJH, KVH, ARH, AMO-V, JAA, CL, PAS, MDM, KM, MH, JHC, EEC, JEL, and NJS contributed to data analysis and interpretation. KVH, ARH, AMO-V, and LS contributed to figure design. All authors contributed to manuscript writing and final approval of the manuscript. MJH, KVH, and ARH have accessed and verified the data in this manuscript.

Declaration of Interests

NJS is listed on patents involving cAd3-vectored vaccines. All other authors declare no competing interests.

up. No Serious Adverse Events (SAEs) related to vaccination occurred. Mild to moderate reactogenicity was observed following vaccination, with symptoms of injection site pain and tenderness (*n*=27/40, 68%), malaise (*n*=18/40, 45%), headache (*n*=17/40, 43%), and myalgia (*n*=14/40, 35%) most commonly reported. Glycoprotein-specific antibodies were induced in 38 (95%) of 40 participants 4 weeks after vaccination, with geometric mean titres of 421 [95% CI 209–846] in the 1×10^{10} pu group and 545 [276–1078] in the 1×10^{11} pu group, and remained significantly elevated at 48 weeks compared with baseline titres (39 [95% CI 13–119] in the 1×10^{10} pu group and 27 [95–156] in the 1×10^{11} pu group; both $p<0{\cdot}0001$). T-cell responses to the GP insert and neutralizing responses against the cAd3 vector were also increased at four weeks after vaccination.

Interpretation—This first-in-human trial demonstrated this novel cAd3-Marburg vaccine is safe and immunogenic, with a safety profile similar to previously tested cAd3-vectored filovirus vaccines. Ninety-five percent of participants produced a GP-specific antibody response after a single vaccination, that remained in 70% of participants at 48 weeks. These findings represent a critical step in development of a vaccine for emergency deployment against a re-emerging pathogen that has recently expanded its reach to new regions.

Funding—National Institutes of Health funded the study via an interagency agreement with WRAIR.

Introduction

In 2018, the World Health Organization identified Marburg virus (MARV) as a priority pathogen requiring urgent vaccine research and development. [1] MARV is estimated to threaten a population of 105 million, compared to the 22 million at risk for Ebola virus. [2,3] The African fruit bat, *Rousettus aegyptiacus*, is a known viral reservoir. The bats' wide distribution across Africa creates potential for outbreaks in many areas beyond those with previously recorded outbreaks. [2] MARV outbreaks in the Democratic Republic of Congo from 1998–2000 and Angola from 2004–2005 claimed hundreds of lives with reported case fatality rates of 83% and 90%, respectively. [4,5] Uganda has also suffered from recurrent MARV outbreaks since 2007, with the most recent outbreak of four cases ending in December 2017 with a case fatality rate of 75%.[6] In July of 2022, Ghana confirmed the first outbreak of this disease in the country, with a 75% mortality rate among four individuals, [7] further demonstrating the urgent need for an effective vaccine capable of providing rapid protection.

Marburgvirus is one of three genera in the family Filoviridae, along with *Ebolavirus* and *Cuevavirus*. Most viruses of the family can cause viral hemorrhagic fever. [8] The single virus of the *Marburgvirus* genus, MARV, is classified as a Category A pathogen and select agent. [9] MARV has a 19 kb negative-strand RNA genome that encodes seven viral proteins. [5] Viral entry into host cells is facilitated by the surface glycoprotein (GP). [10] The viral GP has been the primary target for vaccine development, as antibodies specific for GP are correlated with protective immunity in guinea pig and nonhuman primate (NHP) models. [11,12]

A variety of candidate Marburg vaccine platforms have been evaluated preclinically, including vesicular stomatitis virus (VSV) or adenoviral vectors, virus-like particles, DNA,

inactivated viruses, virus-like replicons, and combinatorial modalities. [9,13] Replication-deficient adenoviral vectors encoding the Ebola virus GP were the first filovirus vaccines to demonstrate 100% protective efficacy in an NHP Ebola virus challenge model, [14,15] and this successful approach translated to MARV. [12,16,17] Among the adenoviruses evaluated as vectors for filovirus vaccines, chimpanzee adenovirus 3 (cAd3) has emerged as a promising candidate for clinical development due to a combination of minimal prior human immunity against the vector, [18,19] strong elicited protective immunity against the vaccine inserts in preclinical models, [20,21] and an excellent safety profile in Ebola vaccine clinical trials. [22–26] Monovalent and bivalent cAd3-vectored Ebola GP vaccines that demonstrated protection in NHPs against lethal Ebola infection five weeks after a single shot, [20] were proven safe and immunogenic in numerous phase 1 and phase 2 trials (phase 2 trials:: 22, 24, 27, 28). Based on the safety and immunogenicity of the cAd3 Ebola virus vaccines, a cAd3-Marburg vaccine was developed by the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) and tested preclinically in NHPs. The data showed that a 10^{10} viral particle dose of cAd3-Marburg provided 100% protection from viremia and death after MARV challenge one month later. [12] Importantly, protection was observed as early as one week after vaccination and as long as one year after vaccination. The rapid onset and long duration of immunity indicate that the cAd3-Marburg vaccine could be efficacious for both ring vaccination strategies and protection of residents and healthcare workers in Marburg-endemic regions.

Responding to the urgent need for an effective Marburg vaccine and following the promising preclinical results, we assessed the cAd3-Marburg vaccine in a phase 1 trial. In this trial we evaluated the safety, tolerability, and vaccine-induced immune responses of cAd3-Marburg in healthy adults (NCT03475056). Since this was the first-in-human evaluation of this vaccine, we examined two doses which have previously proven safe for the cAd3 vaccine platform in a dose-escalation trial. [23] We administered a single vaccination of either 1×10^{10} or 1×10^{11} particle units (PU) of cAd3-Marburg to 40 participants (20 per group) and followed them for up to 48 weeks after vaccination.

Methods

Study Design and Participants

This study was a phase 1, dose-escalation, open-label clinical trial to determine the safety, tolerability, and immunogenicity of an investigational recombinant cAd3-Marburg virus vaccine. The VRC at NIAID of the NIH developed the vaccine and sponsored the trial conducted by Walter Reed Army Institute of Research (WRAIR) investigators at the WRAIR Clinical Trials Center in Silver Spring, MD. Study participants were recruited from the Baltimore-Washington, D.C. metropolitan area using Institutional Review Board (IRB)-approved written and electronic media. Eligible participants were 18- to 50-year-old adults in good general health by physical exam and laboratory assessments, without previous receipt of an investigational Ebola, Marburg, or cAd3-vectored vaccine. Women who were pregnant, breast-feeding, or planning to become pregnant during the first 24 weeks of the trial were excluded from enrollment, and pregnancy was evaluated during screening and on the day of enrollment with a β-HCG pregnancy test. Full inclusion and exclusion criteria

are detailed in the trial protocol (supplementary appendix). The study was reviewed and approved by the WRAIR IRB. All participants provided written informed consent prior to study enrollment. A test of understanding was performed during consent, and study participants had to score 90% or greater by the third attempt of the test to enroll.

Vaccine

The recombinant cAd3-Marburg vaccine is composed of a replication-deficient cAd3 vector modified by an E1 region deletion and insertion of a codon-optimized Marburg Angola GP sequence. The drug substance was manufactured at Advent (Pomezia, Italy), a subsidiary of Okairos (now GlaxoSmithKline). The drug product (VRC-MARADC087-00-VP), and diluent (VRC-DILADC065-00-VP) were manufactured according to cGMP regulations at the VRC Pilot Plant (VPP), operated by the Vaccine Clinical Materials Program, Leidos Biomedical Research, Inc., Frederick, MD. The drug product was a sterile, aqueous, buffered solution composed of cAd3-Marburg drug substance filled into single dose vials at 1×10^{11} PU/mL. This monovalent vaccine was evaluated at two doses in this trial: 1×10^{10} (henceforth 10^{10}) PU, and 1×10^{11} (henceforth 10^{11}) PU. Diluent was added to prepare the 10^{10} PU dose on the day of vaccine administration. The diluent consisted of 10 mM Tris, 10 mM histidine, 5% sucrose (w/v), 75 mM sodium chloride, 1 mM magnesium chloride, 0.02% polysorbate 80 (w/v), 0.1 mM EDTA, and 0.5% (v/v) ethanol.

Study Procedures

Two groups of 20 participants sequentially enrolled according to a dose-escalation plan. Study enrollments for the 10^{10} PU vaccine dose were limited to one participant per day for the first three participants. Enrollment for the remaining participants in the 10^{10} PU dose group occurred following a minimum of seven days follow-up and safety review by the protocol safety review team (PSRT). Enrollment of participants into the 10^{11} PU dose group began in the same manner following a minimum of seven days follow-up on the last 10^{10} PU dose participant and a dose escalation safety review approval by the PSRT.

All vaccinations were given intramuscularly into a deltoid muscle in a 1 mL volume by needle and syringe. Safety monitoring included a 30 minute post-vaccination observation period, telephone follow-up the next day, and clinical and laboratory evaluations conducted at eight follow-up visits over the 48 weeks of the study. Safety and tolerability of the cAd3-Marburg vaccine were defined by the occurrence of solicited local and systemic reactogenicity signs and symptoms for seven days following vaccination, change from baseline for safety laboratory measures, occurrence of adverse events (AEs) for 28 days after vaccination, and the occurrence of Serious Adverse Events (SAEs) and new chronic medical conditions through the last study visit. Participants self-reported local and systemic symptoms and the use of concomitant medications for seven days following vaccination. AEs were recorded for 28 days following vaccination and were graded according to the FDA Guidance for Industry: “Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials” as well as additional grading parameters for absolute neutrophil counts and arthralgias (supplementary appendix).

Anti-Marburg GP IgG ELISA

Methods for the GP IgG ELISA have been described previously[23,29] with minor modifications: Nunc-Immuno MaxiSorp™ plates (Nunc, Rochester, NY) were coated with 10 μg/ml of lectin *Galanthus nivalis* (Sigma-Aldrich, St Louis, MO) at 4°C overnight and then blocked with 10% fetal calf serum at 4°C overnight followed by six washes with PBS containing 0.2% Tween 20 (Sigma-Aldrich). Prepared lectin plates were then incubated at 4°C overnight with a transmembrane-deleted form of the MARV GP Popp strain (Lake Victoria MARV, strain Popp 1967, UniProtKB - P35254), washed, and incubated with serial dilutions of 1:50–1:50,000 of participant sera in triplicate. Bound IgG was detected using goat anti-human IgG (Southern Biotech, Birmingham, AL) conjugated to horseradish peroxidase. Optical density readings were performed at 450 nm using a Victor X3 plate reader (Perkin Elmer, Waltham, MA). Results are expressed as EC_{90} (90% effective concentration) titers, the reciprocal serum dilution at which there is a 90% decrease in antigen binding, and all post-vaccination titers were baseline-subtracted from the matched pre-vaccination titer.

T cell intracellular cytokine staining

Peripheral blood mononuclear cells (PBMC) were isolated from participants' blood samples at baseline and week four post-vaccination using Ficoll-Hypaque density centrifugation, cryopreserved and stored at −150°C. T cell intracellular staining (ICS) assays were performed as described previously. [30] Briefly, PBMCs were thawed, rested overnight and stimulated for 6 hours using a pool of peptides from Marburg Angola GP. [29] Cells were stained for viability using LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit (Invitrogen), surface stained using antibodies against CD3, CD4, CD8, CD28, CD45RA, CCR7 and intracellularly using antibodies against IFN-γ, IL-2 and TNF-α (all from BD Biosciences). Following staining, samples were collected on a BD FACSymphony A5 flow cytometer, and all samples were analyzed using FlowJo 10.6.2. FlowAI was used to identify "good" events which were used for all downstream analyses. [31] Total T cell CD4 and CD8 populations were used to determine the percentage of participants with positive T-cell responses, and the frequency of Marburg GP-reactive T cells producing IFN-γ, IL-2 or TNF-α upon stimulation was assessed within the non-naïve CD4 and CD8 populations, defined by CD45RA and CCR7 expression. For each subject, GP-specific total cytokine response was determined using Boolean gating for T cells producing any combination of IFN-γ, IL-2 or TNF-α following background subtraction of negative control sample (dimethyl sulfoxide-stimulated).

cAd3 serologic assessment

An adenovirus neutralization assay was used to assess the baseline and week four post-vaccination neutralizing antibody titers specific for the cAd3 vector. The assay was performed as previously described, [23] and reciprocal antibody titers are reported as the 90% inhibitory concentration (IC_{90}: the titer at which 90% of infectivity is inhibited).

Outcomes

The primary study endpoints were safety and tolerability of the cAd3-Marburg vaccine. The secondary endpoints included evaluation of antibody responses to the Marburg GP insert by ELISA, T cell responses by ICS, and neutralizing antibody titers against the cAd3 vector at four weeks after vaccination. In addition, an exploratory endpoint assessed longer-term vaccine-induced antibody durability through 48 weeks after vaccination.

Statistical analysis

Participants who completed their vaccination schedules were analyzed for safety, reactogenicity, and vaccine-induced immune responses. Sample size calculations for safety were expressed in terms of the ability to detect SAEs. Sample sizes were chosen so that within a group there was a 90% chance to observe at least one SAE if the true rate was no less than 0.109, and a 90% chance of observing no event if the true rate was less than or equal to 0.005. Further, with group sizes of 20 participants, the study had 80% power to detect changes in laboratory measures from baseline of 0.66 standard deviations or higher, and 82% power to detect a between-group difference in the proportion of participants experiencing an adverse event of 40%.

Positive antibody responses were determined by a finding of significance ($p < 0.05$) from a within-participant *t*-test comparing the ELISA readings at week 4 with those at baseline for each participant. In a post-hoc analysis, positive antibody responses were alternatively determined by an EC_{90} titre cutoff at 30 or higher as previously conducted in trials on DNA-vectored Ebola virus and Marburg virus. [29,32] GMTs and 95% CIs of background-subtracted Marburg GP-specific ELISA antibody titers were calculated at each visit by group. Group ELISA antibody titers for each dose were compared via Welch's two-sample *t*-test at each time post-infection.

An ICS response was defined as positive if the result of a one-sided Fisher's exact test for the 2 × 2 table, consisting of positive and negative cells by peptide and negative control, had a *p* value < 0.01, and the background subtracted % positive cells exceeded the following: as a percentage of CD4 T cells, IFN-γ^+: 0.071%, TNF-α^+: 0.028%, and IL-2^+: 0.028%; as a percentage of CD8 T cells, IFN-γ^+: 0.322%, TNF-α^+: 0.094% and IL-2^+: 0.015%. These baseline data-defined thresholds were determined such that only 1% of the participants had cytokine responses positive for each cytokine (per T-cell subset) at baseline. The proportion of participants with positive T-cell responses by cytokine and overall was calculated along with 95% Clopper-Pearson CI. The medians and interquartile ranges of the background-subtracted CD4 and CD8 non-naïve T-cell responses were calculated based on the percentage of the T-cell subset responding with any cytokine. Comparisons of the percent of participants with positive T-cell responses between groups were performed using two-sided Barnard's tests. For each dose, comparisons of baseline-subtracted T-cell non-naïve cytokine responses between week four and baseline were performed using paired *t*-tests. This comparison was also performed using Wilcoxon signed rank tests in a post-hoc analysis.

Between-group comparisons of baseline log_{10}-transformed cAd3 neutralizing antibody titers were performed via two sample *t*-tests. Spearman's correlations were calculated between baseline log_{10}-transformed cAd3 neutralizing antibody titers and week four Marburg GP-specific ELISA antibody titers, and week four CD4 and CD8 non-naïve T cell responses. Neutralization assay results below the lower limit of detection (LOD, ≤12) are imputed using ½ LOD. These immunogenicity results are descriptive since no adjustments for multiple comparisons were performed per the protocol. All analyses were performed in R version 4.0.4, with DescTools and Barnard packages.

Role of the Funding Source

NIAID funded the study via an interagency agreement with WRAIR and approved the study design. VRC sponsored and designed the study and conducted the research assays, data analysis, and data interpretation. VRC and WRAIR investigators contributed to writing this report.

Results

Forty participants were enrolled in the study between 9 October 2018, and 31 January 2019 (Figure 1). Study participants averaged 34 9 years of age (range: 19–48 years) and were 63% female (*n*=25/40). Additional participant demographics are shown in Table 1. All participants (*n*=40/40, 100%) received the cAd3-Marburg vaccination and 37/40 (93%) completed all follow-up visits. Two (*n*=2/40, 5%) participants moved from the area and one (*n*=1/40, 3%) participant was lost to follow-up (Figure 1), all from the 10^{11} PU dose group.

The vaccine was safe and well tolerated in this trial (Figure 2). Most participants (*n*=27/40, 68%) had mild to moderate injection site pain or tenderness. Systemic symptoms in all participants were mainly mild to moderate, with the most common symptoms being malaise (*n*=18/40, 45%), headache (*n*=17/40, 43%), and myalgia (*n*=14/40, 35%). There was one case (*n*=1/40, 3%) of severe fever (39.1°C/ 102.4°F) reported one day after vaccination with 10^{11} PU cAd3-Marburg that resolved the following day. There were no SAEs related to the vaccine. The most common AEs attributed to vaccination were mild to moderate transient, asymptomatic decreases in white blood cell counts, which occurred in 4/40 (10%) of participants (Supplemental Tables 1 and 2). All AEs resolved without sequelae.

Marburg GP-specific antibody responses were evaluated by ELISA assay. In both groups, 95% of participants responded to the vaccine at four weeks after vaccination as determined by a significant increase in Marburg GP-specific antibody titers over baseline (Figure 3). Binding antibodies increased more than 100-fold, peaking at 4 weeks post vaccination with GMTs of 421 (95% CI 209–846; $p<0{\cdot}0001$) and 545 (276–1078; $p<0{\cdot}0001$) for the 10^{10} PU and 10^{11} PU dose groups, respectively (Figure 3A, Supplemental Table 3). We observed a rapid increase in antibody titers following vaccination, with participants approaching peak titers by two weeks (Figure 3B, Supplemental Table 3). There was no significant difference in binding antibody titers between dose groups at any point following cAd3-Marburg vaccination. Importantly, GMTs at the last study visit (48 weeks post-vaccination) remained significantly elevated over baseline titres with GMTs of 39 (95% CI 13–119; $p<0{\cdot}0001$) and 27 (5–156; $p=0{\cdot}0012$), $p < 0.0001$ for the 10^{10} PU and 10^{11} PU dose groups, respectively;

with positive responses in 70–71% of individuals (Figure 3C), demonstrating the durability of this vaccine-induced antibody response. When positivity was assessed as in previous DNA-vectored Ebola virus and Marburg virus vaccine trials using a cutoff of 30 EC_{90} [29,32] similar rates of positivity were observed (appendix p 5).

T cell responses were also evaluated at four weeks post-vaccination. cAd3-Marburg vaccination increased the frequency of participants with CD4 and/or CD8 T cells producing IFN-γ, IL-2, and TNF-α in response to Marburg GP peptides four weeks after vaccination (Supplemental Table 4). The frequency of non-naïve T cells responding with any cytokine to Marburg virus glycoprotein peptide stimulation at 4 weeks post vaccination increased significantly for CD4 T cells in both dose groups ($p<0{\cdot}0001$), and for CD8 T cells in the 10^{10} pu dose group ($p=0{\cdot}0008$; figure 4). Noting that the protocol-specified *t*-test comparison of CD8 T cell frequencies before and after vaccination resulted in a *p* value approaching significance for the 10^{11} PU dose group ($p=0.058$), a post-hoc analysis of the T cell frequencies was performed by a non-parametric Wilcoxon signed rank test. This revealed significant differences in CD8 T cells over baseline after both 10^{10} ($p=0.0015$) and 10^{11} PU ($p=0.0093$) dose groups, indicating that the lack of significance for CD8 T cells found by a *t*-test for the 10^{11} PU dose group is likely due to the spread of the data. Together, the data demonstrate that cAd3-Marburg vaccination increases both the frequency of recipients with Marburg-GP-reactive T cells and the average frequency of GP-specific non-naïve T cells per individual.

Antibody responses to the cAd3 virus vector were also assessed. As in previous adenoviral vector clinical trials, [22,23,33,34] we observed an antibody response against the vector in our trial participants. Neutralizing antibody titers specific for the cAd3 vector increased significantly from baseline to 4 weeks post vaccination regardless of dose ($p<0{\cdot}0001$) (Supplemental Figure 1). However, we found in an ad hoc analysis that the presence of pre-existing titers to the cAd3 vaccine vector had no impact on the frequency of the CD4 T cell response, and had a weak impact, if any, on the ELISA titers and CD8 T cell response (Supplemental Figure 2), a pattern seen previously with this vector. [23]

Discussion

This is the first report of a cAd3-Marburg vaccine evaluated in humans. These clinical trial results demonstrate that a single dose of cAd3-Marburg is safe, well-tolerated, and immunogenic, eliciting both humoral and cellular immune responses. The safety and reactogenicity profile of the cAd3-Marburg vaccine was similar to other cAd3-vectored vaccines evaluated in prior clinical studies. [23,35] These results support further evaluation of the cAd3-Marburg vaccine, including implementation of this platform in outbreak responses.

This study is the first Marburg vaccine clinical trial to report durable binding antibody responses in 70% of participants at 48 weeks. Only two other known Marburg virus vaccine human trials have reported Marburg virus-specific immunity,[29,32] both evaluating DNA vaccines that required three vaccinations to reach peak titres.[13] A vaccine tested by Kibuuka and colleagues[32] elicited a binding antibody response in 31% of participants, and a vaccine tested by Sarwar and colleagues[29] elicited a binding antibody response in 80%

of participants, which dropped to 11% by 24 weeks after vaccination. A positive response in those trials was based on post vaccination EC_{90} titers ≥30. In comparison, we observed binding antibodies responses in 95% of our participants following a single vaccination, based on a significant increase in a participant's EC_{90} titer over baseline. Although there was a modest decease in response rate at the individual level by the end of the trial, the dose group antibody response was maintained at a level significantly higher than baseline to 48 weeks. The assays used to evaluate the EC_{90} titres in the three trials are not identical; however, when evaluating our positive responses with the EC_{90} titre cutoff at 30 or higher, the response rates in our participants were essentially unchanged.

Binding antibody responses have been consistently associated with protection across numerous MARV NHP challenge studies in a recent systematic review, [13] and have been identified as a preliminary correlate of protection in NHPs in a recent preclinical study of this vaccine (currently in preprint). [12] In NHPs, EC_{90} titers of approximately 420 or higher at four weeks after vaccination was shown to result in near uniform (95%) protection during preclinical challenge studies. [12] In our first-in-human clinical trial, > 65% of participants in the 10^{11} PU cAd3-Marburg elicited a EC_{90} titers above 420 four weeks post vaccination. Furthermore, we observed near peak titers within two weeks after vaccination in both dose groups, indicating titers associated with rapid protection in NHP could be observed in humans with this vaccine platform. Protection against lethal challenge with MARV within a week of vaccination was observed in the NHP study preprint, reinforcing this theory. [12] While it is important to note that no correlate of protection has been determined for humans to date, these findings still indicate that this vaccine platform could potentially provide valuable rapid protection against MARV during and in anticipation of outbreaks.

The cAd3 vaccine vector is known to elicit T cell responses comparable to Ad5 and Ad6 vectors, with minimal impact from pre-existing immunity in the human population. [18] In our trial we observed Marburg GP-specific T cell responses at four weeks post vaccination similar to other preclinical and clinical studies that used cAd3-vectored vaccines.[20,36] We observed a significant increase in non-naive CD4 T cells 4 weeks after both vaccine doses. We also observed a significant increase in non-naive CD8 T cells after both doses in a post-hoc nonparametric test, indicating that the lack of significance found in the per-protocol t test for the 10^{11} pu dose group is probably due to the spread of the data. Additional studies are needed to determine the role of T cells in protective immunity to MARV following cAd3-Marburg vaccination.

There are a few limitations to this clinical trial. Since this study was a first-in-human phase 1 trial focused on the safety and tolerability of the vaccine platform, the group sizes were calculated based on the ability to detect AEs. The limited trial size hindered our ability to make strong conclusions regarding the vaccine-induced immune response and optimal vaccine dose, which will need to be examined in larger future trials. While this is standard for phase 1 trials, this limits the power of the statements we can make regarding the immunological outcomes of vaccination. The analysis is also hindered by the lack of an established correlate of protection in humans. This trial also occurred in the US to evaluate the vaccine safety and tolerability in MARV-naïve adults. Future trial locations should be selected to ascertain safety and immunogenicity in endemic settings.

We found the cAd3-Marburg vaccine safe and well tolerated in this trial, with a durable GP-specific antibody response. The Sabin Vaccine Institute is continuing the advanced development of the cAd3-Marburg vaccine.[37] Future evaluations of this vaccine include a planned outbreak response clinical protocol in Ghana, a phase 2 clinical trial in Kenya and Uganda, and a phase 1b clinical trial evaluating the 10^{11} PU dose in the United States (NCT04723602). The safety and vaccine-induced immune response observed in this trial provide knowledge critical for the implementation of this vaccine for emergency deployment to protect against ongoing and future MARV outbreaks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Melinda J. Hamer, MD[1,2], Katherine V. Houser, PhD[3,*], Amelia R. Hofstetter, PhD[3], Ana M. Ortega-Villa, PhD[4], Christine Lee, MD[1], Anne Preston, BS[1], Brooke Augustine, BS[1], Charla Andrews, MS[3], Galina V Yamshchikov, MS[3], Somia Hickman, PhD[3], Steven Schech, BS[5], Jack N. Hutter, MD[1], Paul. T Scott, MD[1], Paige E. Waterman, MD[1], Mihret F. Amare, MBA[5], Victoria Kioko, MPH[5], Casey Storme, MPH[5], Kayvon Modjarrad, MD[1], Melanie D. McCauley, MD[5], Merlin L. Robb, MD[5], Martin R. Gaudinski, MD[3], Ingelise J. Gordon, RN[3], LaSonji A. Holman, FNP[3], Alicia T. Widge, MD[3], Larisa Strom, MPH[3], Myra Happe, PhD[3], Josephine H. Cox, PhD[3], Sandra Vazquez, MS[3], Daphne A. Stanley, MS[3], Tamar Murray, BS[3], Caitlyn N. M. Dulan, MS[3], Ruth Hunegnaw, PhD[3], Sandeep R. Narpala, MS[3], Phillip A. Swanson II, PhD[3], Manjula Basappa, BS[3], Jagada Thillainathan, BS[3], Marcelino Padilla, BS[3], Britta Flach, PhD[3], Sarah O'Connell, MS[3], Olga Trofymenko, MD[3], Patricia Morgan, MS[3], Emily E. Coates, PhD[3], Jason G. Gall, PhD[3], Adrian B. McDermott, PhD[3], Richard A. Koup, MD[3], John R. Mascola, MD[3], Aurélie Ploquin, PhD[3], Nancy J. Sullivan, PhD[3], Julie A. Ake, MD[1], Julie E. Ledgerwood, DO[3], the RV 507 Study Team.

Affiliations

[1]Walter Reed Army Institute of Research, Silver Spring, MD, USA

[2]George Washington University School of Medicine and Health Sciences, Department of Emergency Medicine, Washington, DC, USA

[3]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

[4]Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

[5]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA

Acknowledgements

We thank the clinical trial participants who contributed to furthering vaccine research. We acknowledge our WRAIR colleagues Jacqueline Sievers, Yessenia Gomez and David Wallace for administrative, regulatory, programmatic and quality assurance support, and our VRC colleague Lesia K. Dropulic for her review of the manuscript. We also acknowledge the contributions of the EMMES corporation for assistance with data management and protocol development. The RV 507 Study Team at the VRC included Rebecca Lampley, Brenda Larkin, Pamela Costner, Hope Wilson, and Mike Read. The National Institutes of Health funded the study via an interagency agreement with WRAIR.

The views expressed are those of the authors and should not be construed to represent the positions of the US Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in Army Regulation 70–25.

Data Sharing Statement

Data generated in this study is available as de-identified data on ClinicalTrials.gov (NCT03475056). The study protocol, statistical analysis plan, and informed consent form are available on ClinicalTrials.gov (https://www.clinicaltrials.gov/ProvidedDocs/56/NCT03475056/Prot_SAP_ICF_000.pdf). Additional data may be made available upon reasonable request to the corresponding author.

REFERENCES

1. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. Antiviral Res 2018; 159: 63–7. [PubMed: 30261226]
2. Pigott DM, Golding N, Mylne A, et al. Mapping the zoonotic niche of Marburg virus disease in Africa. Trans R Soc Trop Med Hyg 2015; 109(6): 366–78. [PubMed: 25820266]
3. Pigott DM, Golding N, Mylne A, et al. Mapping the zoonotic niche of Ebola virus disease in Africa. Elife 2014; 3: e04395. [PubMed: 25201877]
4. Bausch DG, Nichol ST, Muyembe-Tamfum JJ, et al. Marburg hemorrhagic fever associated with multiple genetic lineages of virus. N Engl J Med 2006; 355(9): 909–19. [PubMed: 16943403]
5. Towner JS, Khristova ML, Sealy TK, et al. Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. J Virol 2006; 80(13): 6497–516. [PubMed: 16775337]
6. World Health Organization. Uganda ends Marburg Virus disease outbreak. Geneva: World Health Organization; 2017.
7. Bonney JHK, Sanders T, Adams P, et al. First-ever outbreak of Marburg virus disease declared in Ghana, 2022. 2022 September 01 ed. virological.org; 2022.
8. Burk R, Bollinger L, Johnson JC, et al. Neglected filoviruses. FEMS Microbiol Rev 2016; 40(4): 494–519. [PubMed: 27268907]
9. Reynolds P, Marzi A. Ebola and Marburg virus vaccines. Virus Genes 2017; 53(4): 501–15. [PubMed: 28447193]
10. Manicassamy B, Wang J, Rumschlag E, et al. Characterization of Marburg virus glycoprotein in viral entry. Virology 2007; 358(1): 79–88. [PubMed: 16989883]
11. Swenson DL, Warfield KL, Negley DL, Schmaljohn A, Aman MJ, Bavari S. Virus-like particles exhibit potential as a pan-filovirus vaccine for both Ebola and Marburg viral infections. Vaccine 2005; 23(23): 3033–42. [PubMed: 15811650]
12. Hunegnaw R, Honko AN, Wang L, et al. A single-shot ChAd3-MARV vaccine confers rapid and durable protection against Marburg virus in nonhuman primates. Sci Transl Med 2022; 14: eabq6364. [PubMed: 36516269]
13. Dulin N, Spanier A, Merino K, et al. Systematic review of Marburg virus vaccine nonhuman primate studies and human clinical trials. Vaccine 2021; 39(2): 202–8. [PubMed: 33309082]

14. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. Nature 2000; 408(6812): 605–9. [PubMed: 11117750]
15. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. Nature 2003; 424(6949): 681–4. [PubMed: 12904795]
16. Callendret B, Vellinga J, Wunderlich K, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. PLoS one 2018; 13(2): e0192312. [PubMed: 29462200]
17. Swenson DL, Wang D, Luo M, et al. Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections. Clin Vaccine Immunol 2008; 15(3): 460–7. [PubMed: 18216185]
18. Colloca S, Barnes E, Folgori A, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. Sci Transl Med 2012; 4(115): 115ra2.
19. Quinn KM, Da Costa A, Yamamoto A, et al. Comparative analysis of the magnitude, quality, phenotype, and protective capacity of simian immunodeficiency virus gag-specific CD8+ T cells following human-, simian-, and chimpanzee-derived recombinant adenoviral vector immunization. J Immunol 2013; 190(6): 2720–35. [PubMed: 23390298]
20. Stanley DA, Honko AN, Asiedu C, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat Med 2014; 20(10): 1126–9. [PubMed: 25194571]
21. Peruzzi D, Dharmapuri S, Cirillo A, et al. A novel chimpanzee serotype-based adenoviral vector as delivery tool for cancer vaccines. Vaccine 2009; 27(9): 1293–300. [PubMed: 19162112]
22. Tapia MD, Sow SO, Ndiaye BP, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in adults in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis 2020; 20(6): 707–18. [PubMed: 32199491]
23. Ledgerwood JE, DeZure AD, Stanley DA, et al. Chimpanzee Adenovirus Vector Ebola Vaccine. N Engl J Med 2017; 376(10): 928–38. [PubMed: 25426834]
24. De Santis O, Audran R, Pothin E, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. Lancet Infect Dis 2016; 16(3): 311–20. [PubMed: 26725450]
25. Ewer K, Rampling T, Venkatraman N, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. N Engl J Med 2016; 374(17): 1635–46. [PubMed: 25629663]
26. Venkatraman N, Ndiaye BP, Bowyer G, et al. Safety and Immunogenicity of a Heterologous Prime-Boost Ebola Virus Vaccine Regimen in Healthy Adults in the United Kingdom and Senegal. J Infect Dis 2019; 219(8): 1187–97. [PubMed: 30407513]
27. Tapia MD, Sow SO, Mbaye KD, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in children in Africa: a randomised, observer-blind, placebo controlled, phase 2 trial. Lancet Infect Dis 2020; 20: 719–30. [PubMed: 32199492]
28. Kennedy SB, Bolay F, Kieh M, et al. Phase 2 placebo-controlled trial of two vaccines to prevent Ebola in Liberia. N Engl J Med 2017; 377: 1438–47. [PubMed: 29020589]
29. Sarwar UN, Costner P, Enama ME, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. J Infect Dis 2015; 211: 549–57. [PubMed: 25225676]
30. Swanson PA 2nd, Padilla M, Hoyland W, et al. AZD1222/ChAdOx1 nCoV-19 vaccination induces a polyfunctional spike protein-specific TH1 response with a diverse TCR repertoire. Sci Transl Med 2021; 13: eabj7211. [PubMed: 34591596]
31. Monaco G, Chen H, Poidinger M, Chen J, de Magalhães JP, Larbi A. flowAI: automatic and interactive anomaly discerning tools for flow cytometry data. Bioinformatics 2016; 32: 2473–80. [PubMed: 27153628]
32. Kibuuka H, Berkowitz NM, Millard M, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo controlled clinical trial. Lancet 2015; 385: 1545–54. [PubMed: 25540891]

33. Ledgerwood JE, Costner P, Desai N, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine 2010; 29: 304–13. [PubMed: 21034824]

34. Zhu FC, Hou LH, Li JX, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet 2015; 385: 2272–79. [PubMed: 25817373]

35. Folegatti PM, Bittaye M, Flaxman A, 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 2020; 20: 816–26. [PubMed: 32325038]

36. Barnes E, Folgori A, Capone S, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med 2012; 4: 115ra1.

37. Sabin Vaccine Institute. Sabin Vaccine Institute receives additional $34.5 million from BARDA for further development of Ebola Sudan and Marburg vaccines. 2021. https://www.sabin.org/resources/sabin-vaccine-institute-receives-additional-34-5-millionfrom-barda-for-further-development-of-ebola-sudan-and-marburgvaccines-2/ (accessed Jan 9, 2023).

Research In Context

Evidence before this study

Marburg virus (MARV) is a WHO priority pathogen due to its severity, lack of approved vaccines, and threat to public health. MARV outbreaks have occurred at least once every five years since 2000, including its recent emergence in Ghana in 2022. This current outbreak is especially concerning as the virus was never previously detected in Ghana. Several candidate Marburg vaccine platforms have been evaluated preclinically, including those based on vesicular stomatitis virus (VSV) and human adenovirus (Ad5 and Ad26) vectored vaccines, DNA formulations, and others. Binding antibody titers are a correlate of protection in nonhuman primate (NHP) challenge studies. However, no Marburg vaccine to date has demonstrated a significant and durable binding antibody response in a human clinical trial. We searched PubMed from 1/1/1990 through 12/31/2019 using search terms “Marburg”, “vaccine”, “virus”, “efficacy”, and “clinical trials”. Only three human Marburg vaccine trials have been performed; of those, only two reported Marburg-specific immunogenicity. Both of those trials utilized DNA vaccines and required three shots to reach peak titers, with reported antibody response rates of 80% and 31% respectively, but these responses declined to 11% after 24 weeks in one trial and returned to baseline levels by 44 weeks in the other. Robust and long-lasting antibody responses elicited by a single vaccination have yet to be reported. In preclinical studies, chimpanzee adenoviral (cAd) vectors such as cAd3 have exhibited promise for Marburg. The cAd3 vector has been proven safe and immunogenic in phase 1 trials with Ebola glycoprotein (GP) inserts, with minimal impact from pre-existing immunity.

Added value of this study

This first-in-human Marburg cAd3-vectored vaccine clinical trial is the first study to demonstrate robust binding antibody responses in 95% of participants, which remain in 70% of participants to 48 weeks. This clinical trial provides evidence that the cAd3-Marburg vaccine is safe and well-tolerated in humans. Furthermore, this vaccine is administered in a single dose, which increases its potential utility in an outbreak scenario.

Implications of all the available evidence

The results of this clinical trial support further evaluation of the cAd3-Marburg vaccine. Future trials include a planned outbreak response clinical protocol (Ghana), a phase 2 clinical trial in Kenya and Uganda, and a phase 1b clinical trial in the United States (NCT04723602). The safety and immunogenicity profile observed in this trial signal the utility for the use of the cAd3-Marburg vaccine in Marburg outbreak responses.

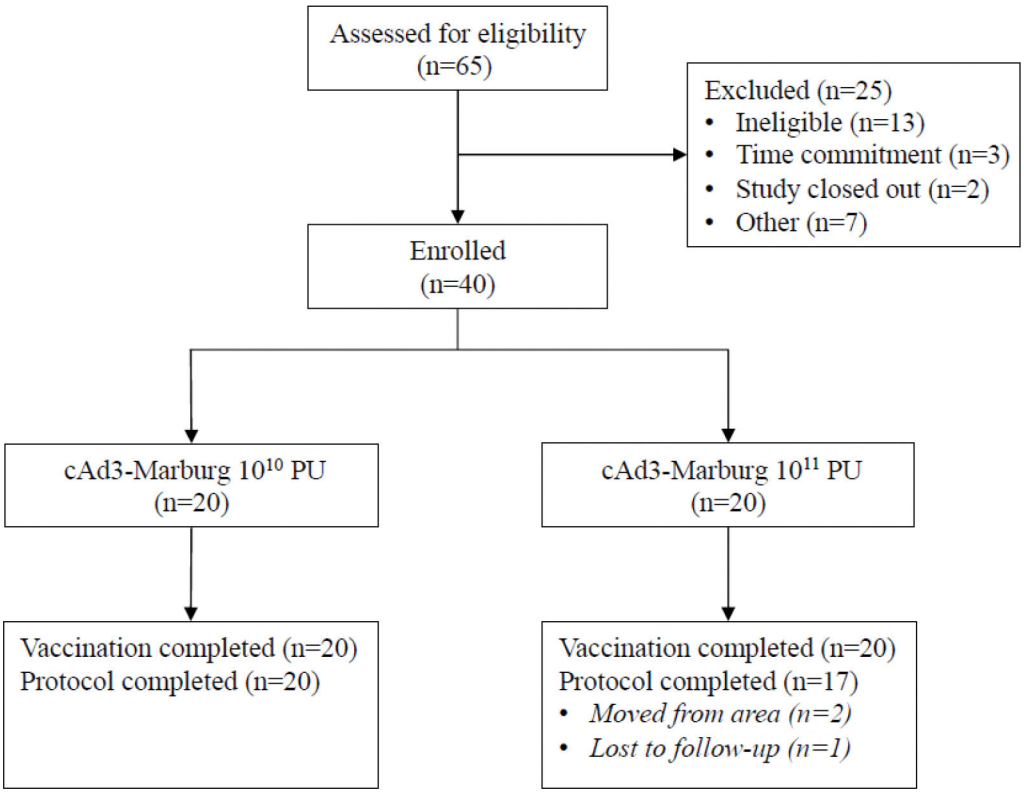


Figure 1: Study profile
pu=particle units.

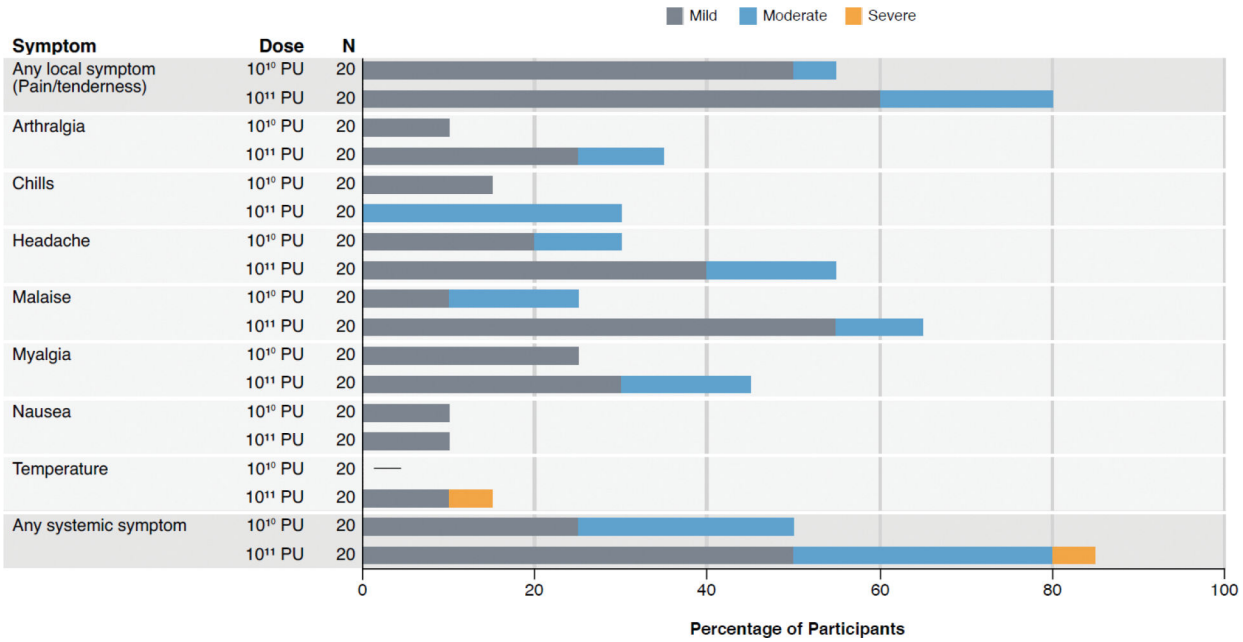
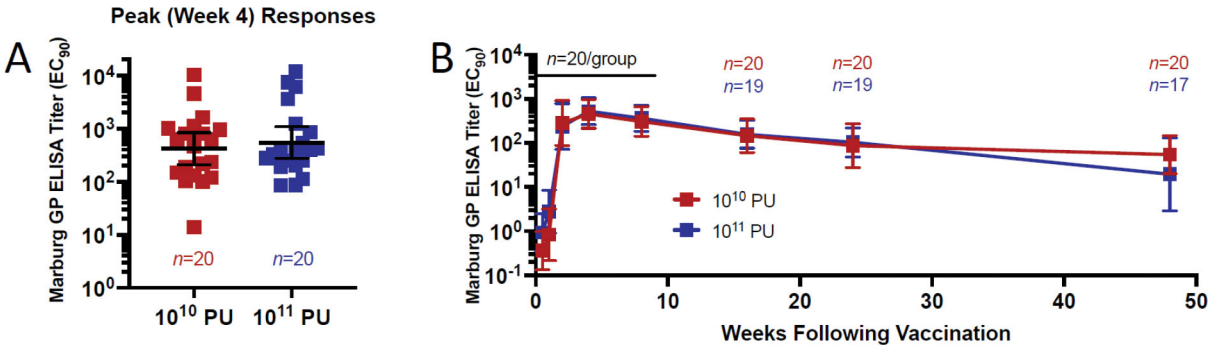


Figure 2: Maximum local and systemic solicited symptoms 7 days after vaccination with chimpanzee adenovirus type 3-vectored Marburg virus

For symptoms persisting for more than 1 day, a single count per person at the maximum severity of the symptom was used for the figure. No swelling or redness was observed at the vaccine injection site. pu=particle units.



C

Dose Group	Week 4 Positive Response *n*, % (95% CI)	Week 48 Positive Response *n*, % (95% CI)
10^{10} PU	19/20, 95% (75%-100%)	14/20, 70% (46%-88%)
10^{11} PU	19/20, 95% (75%-100%)	12/17, 71% (44%-90%)

Figure 3: Chimpanzee adenovirus type 3-vectored Marburg virus vaccine antibody titres during the 48-week follow-up

Baseline-subtracted serum Marburg glycoprotein ELISA EC_{90} titres are plotted by vaccine dose at the peak, week 4, of the response (A) and at weeks post vaccination during the 48 weeks after vaccination (B). Symbols indicate group geometric mean titres and whiskers denote 95% CIs. (C) Positive response rates at week 4 and week 48, as defined by a significant increase over baseline titres. EC_{90}=90% effective concentration. pu=particle units.

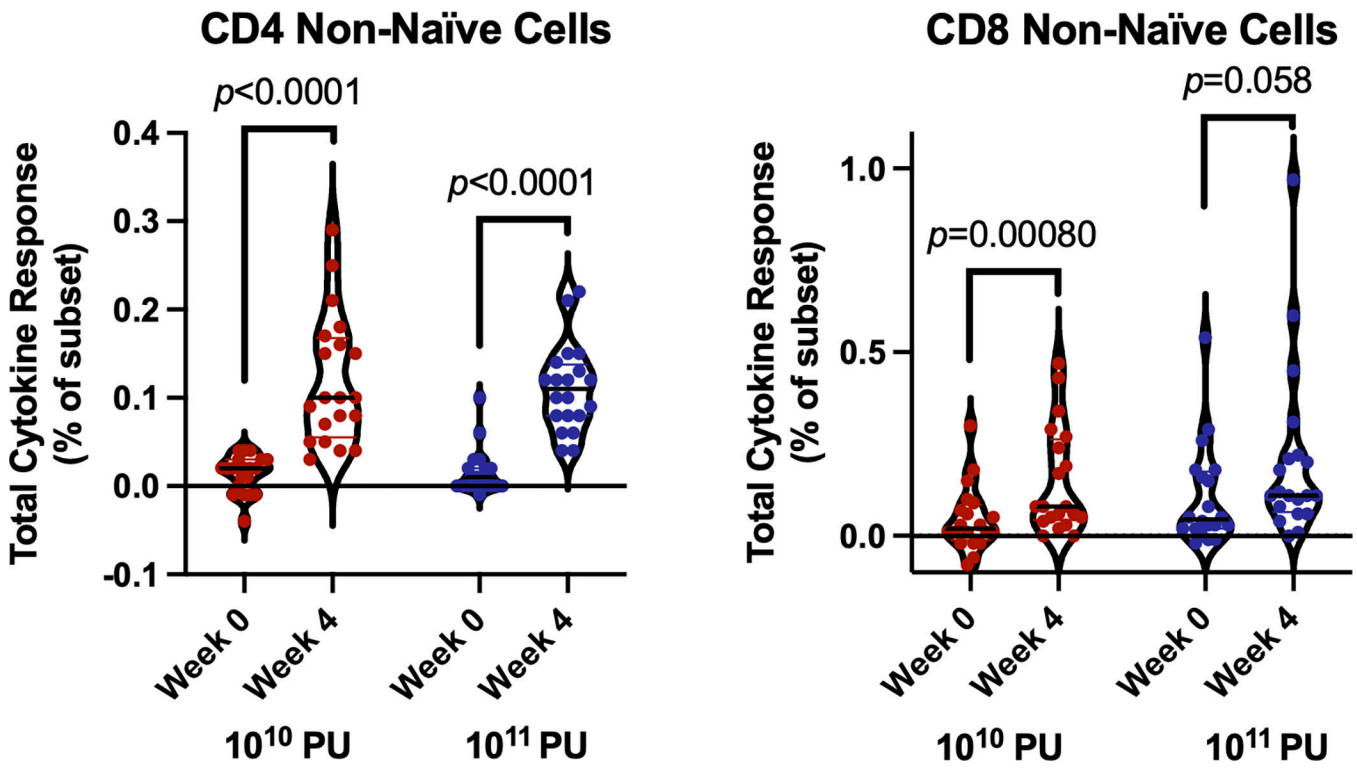


Figure 4: Frequency of Marburg glycoprotein-specific non-naive CD4 and CD8 T cells at baseline and at 4 weeks after vaccination

Percentage of background-subtracted non-naive CD4 T cells or CD8 T cells producing any one of the three tested cytokines (interferon gamma, interleukin, and tumour necrosis factor) in response to Marburg glycoprotein peptide stimulation at baseline and 4 weeks post vaccination by dose group. Within each violin plot, the black line indicates the median and the coloured lines indicate the quartiles. n=20 per group at each timepoint. pu=particle units.

Table:

Baseline characteristics

	10^{10} pu group (n=20)	10^{11} pu group (n=20)	Total (n=40)
Sex *			
Male	8 (40%)	7 (35%)	15 (38%)
Female	12 (60%)	13 (65%)	25 (63%)
Age			
Mean (SD)	35·8 (8·4)	33·9 (7·9)	34·9 (8·1)
Range	19–48	21–48	19–48
Race			
American Indian or Alaskan Native	0	1 (5%)	1 (3%)
Asian	2 (10%)	3 (15%)	5 (13%)
Black or African American	10 (50%)	4 (20%)	14 (35%)
Native Hawaiian or other Pacific Islander	1 (5%)	0	1 (3%)
While	7 (35%)	11 (55%)	18 (45%)
Multiracial	0	1 (5%)	1 (3%)
Ethnicity			
Non-Hispanic or Latino	18 (90%)	16 (80%)	34 (85%)
Hispanic or Latino	2 (10%)	4 (20%)	6 (15%)
BMI †			
Mean (SD)	27·3 (5·0)	27·9 (5·5)	27·6 (5·2)
Range	20·3–38·0	21·5–39·6	20·3–39·6

Data are n (%) unless stated otherwise. No significant differences were observed between vaccine groups for any baseline characteristics pu-particle units.

*Sex, race, and ethnic group were self reported by the participants Sex was reported based on the sex assigned at birth.

†Body mass index at enrolment, reported as weight in kg divided by the square of height in metres