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Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against *Plasmodium falciparum* infection in Kenyan adults

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Abstract

Protective immunity to the liver stage of the malaria parasite can be conferred by vaccine-induced T cells, but no subunit vaccination approach based on cellular immunity has shown efficacy in field studies. We randomly allocated 121 healthy adult male volunteers in Kilifi, Kenya, to

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⁹See supplementary materials.

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SUPPLEMENTARY MATERIALS

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Competing interests: A.V.S.H., A.N., and S.G. are listed as inventors on patent filings related to heterologous prime-boost immunization and specific malaria vaccines. E.B.I. was an employee of EVI at the time of this study, which supports the development and testing of malaria vaccines. N.K.V. is an employee of EVI, and O.L. is executive director of EVI. A.N. is an employee of Okairos and consults for GlaxoSmithKline. The other authors declare no competing interests.

vaccination with the recombinant viral vectors chimpanzee adenovirus 63 (ChAd63) and modified vaccinia Ankara (MVA), both encoding the malaria peptide sequence ME-TRAP (the multiple epitope string and thrombospondin-related adhesion protein), or to vaccination with rabies vaccine as a control. We gave antimalarials to clear parasitemia and conducted PCR (polymerase chain reaction) analysis on blood samples three times a week to identify infection with the malaria parasite *Plasmodium falciparum*. On Cox regression, vaccination reduced the risk of infection by 67% [95% confidence interval (CI), 33 to 83%; $P = 0.002$] during 8 weeks of monitoring. T cell responses to TRAP peptides 21 to 30 were significantly associated with protection (hazard ratio, 0.24; 95% CI, 0.08 to 0.75; $P = 0.016$).

INTRODUCTION

Substantial gains have been made in reducing malaria transmission in some parts of Africa but not in others (1). An effective malaria vaccine would offer an important further strategy to control malaria. RTS,S/AS01 is the most advanced malaria vaccine in development and induces high-titer antibody responses to the circumsporozoite protein (2). It confers 30 to 50% protection against clinical malaria (3).

An alternative, or perhaps complementary, vaccination strategy is to use viral vectors in heterologous prime-boost regimens to induce T cell responses. This strategy is more successful when T cells are induced to the pre-erythrocytic antigen construct comprising the thrombospondin-related adhesion protein coupled to a multiepitope string (ME-TRAP) rather than the circumsporozoite protein (4, 5). Previous regimens with viral vectors conferred partial protection against controlled human malaria infection in malaria-naïve volunteers (6) but did not confer demonstrable efficacy in field studies (7, 8). Prior malaria exposure may suppress T cell responses to vaccination, explaining the lack of efficacy in the field (9). A recent development has been to deliver ME-TRAP by priming with chimpanzee adenovirus 63 (ChAd63) before boosting with modified vaccinia virus Ankara (MVA). This approach induced the highest T cell responses seen thus far after vaccination of malaria-naïve (10) and malaria-exposed adults (11, 12), and was more protective than previous T cell-inducing vaccines in controlled human malaria infection studies (13).

We now present the safety, immunogenicity, and efficacy results of a phase 2b single-blind randomized controlled field trial of the ChAd63-MVA ME-TRAP vaccine in malaria-exposed adult male volunteers in Kenya. The end point for efficacy was infection with *P. falciparum* diagnosed by polymerase chain reaction (PCR). Antimalarials were used to clear parasites after vaccination but before monitoring by PCR began (14).

RESULTS

We conducted a randomized controlled single-blind study. Our objective was to determine the safety, immunogenicity, and efficacy of vaccination with ChAd63 ME-TRAP and MVA ME-TRAP compared with a rabies control vaccine. We randomized 121 male participants, losing 3 to follow-up because of migration (fig. S1). There were no differences between randomization groups for age of participants, bed net use, location of residence, or parasitemia upon enrollment (Table 1).

Safety and adverse events

No serious adverse events were identified. The most common local adverse event associated with both ChAd63 ME-TRAP and MVA ME-TRAP was mild to moderate pain, lasting for a few hours to 3 days (table S1). Various systemic adverse events were reported of mild to moderate intensity lasting between a few hours to 5 days (table S1). Other adverse events were reported within 30 days after vaccination but were not thought to be vaccine-related (table S2). Minor laboratory abnormalities were observed in the full blood count and in creatinine and alanine transaminase after immunization (see tables S3 to S5 for frequencies, median values, and a line-by-line listing of abnormalities).

Immunogenicity

Peak immunogenicity was observed on day 63, 7 days after vaccination with MVA ME-TRAP and 63 days after vaccination with ChAd63 ME-TRAP. The vaccine induced antibody and T cell responses (Fig. 1 and table S6). Enzyme-linked immunospot (ELISPOT) results were quantified according to the number of interferon- γ (IFN- γ)-producing cells (visualized as spots) measured by the assay. According to the ELISPOT assay, geometric mean T cell responses were below 200 spots per million peripheral blood mononuclear cells (PBMCs) in control vaccinees throughout the study. However, those receiving the ChAd63-MVA ME-TRAP vaccine showed a geometric mean of 1451 spots per million PBMCs [95% confidence interval (CI), 1220 to 1727] for homologous (T9/96) TRAP peptides and a geometric mean of 1224 spots per million PBMCs (95% CI, 1224 to 1454) for heterologous 3D7 TRAP peptides. The geometric mean total T cell response to the vaccine construct (TRAP T9/96 plus multiple epitope peptides) was 1694 spots per million PBMCs (95% CI, 1437 to 1997). These T cell responses were all significantly greater than those seen among control vaccinees ($P < 0.0001$).

The T cell responses were somewhat biased toward one peptide pool (TRAP peptides 21 to 30; Fig. 1). The T cell responses were biased toward CD8⁺ T cells on intracellular cytokine staining (ICS): CD8⁺ IFN- γ ⁺ interleukin-2 (IL-2)⁺ tumor necrosis factor (TNF)⁺ T cells; CD8⁺ IFN- γ ⁺ IL-2⁺ TNF⁺ T cells; and IFN- γ ⁺ IL-2⁺ TNF⁺ CD4⁺ T cells. T cell responses to the multiple epitope peptides (114 spots per million PBMCs) were higher than responses to TRAP peptide pools 31 to 40 and 41 to 50, but lower than the responses to the other three TRAP peptide pools. T cell responses waned fourfold over the 105 days after vaccination, but were still higher than responses in participants receiving the control rabies vaccine (Fig. 1). At peak immunogenicity (that is, day 63), based on a Poisson distribution analysis of spot numbers, positive responses to TRAP peptides were seen for 55 of 55 vaccinees receiving the ChAd63-MVA ME-TRAP vaccine and 33 of 40 vaccinees receiving the control rabies vaccine (positive responses among the latter group likely reflected natural exposure to *P. falciparum*).

Genotyping

Malaria transmission was unexpectedly low in the study area during the period of PCR monitoring for *P. falciparum* infection (fig. S2), and no clinical episodes of malaria were recorded during the follow-up period of 8 weeks. The incidence of PCR positivity for malaria infection was low and the parasitemias were low. We therefore delayed unblinding

of the study and conducted genotyping to determine whether the PCR-positive samples were new infections acquired during the monitoring period or residual/recrudescence infections that were present at enrollment and incompletely cleared by the antimalarials given after vaccination and before PCR monitoring for efficacy began. We compared pairs of samples taken from the same volunteer where both samples were taken before antimalarial treatment. Four of 14 paired samples (29%) were identical, and 2 of 14 paired samples (14%) varied at only one single-nucleotide polymorphism (SNP) position. The mean number of SNP differences was 1.3 (95% CI, 0.6 to 2.7). We then compared pairs of samples from the same volunteer where one sample was taken before antimalarial treatment and the other after antimalarial treatment. Only 1 of 19 paired samples (5%) was identical, and none varied at only one SNP position. The mean number of SNP differences was 2.7 (95% CI, 1.8 to 3.8). This was significantly different from the 1.3 SNP differences seen between sample pairs before antimalarial treatment ($P = 0.027$ using Student's t test). We concluded that most of the infections that were identified after antimalarial treatment were new infections rather than partially cleared infections acquired before antimalarials were given.

Efficacy

Kaplan-Meier plots of the distribution of time to PCR positivity are shown in Fig. 2 and are compared with the time course of interventions and the pattern of rainfall (Fig. 2A). Eleven of 61 (18%) individuals vaccinated with ChAd63-MVA ME-TRAP became PCR-positive during the 56 days of monitoring, compared with 28 of 60 (47%) control vaccinees (Fig. 2B). On Cox regression analysis, vaccine efficacy was 67% (95% CI, 33 to 83%; $P = 0.002$). Efficacy was 82% (95% CI, 46 to 94%; $P = 0.002$) for the secondary end point of a parasitemia >10 parasites/ml (Fig. 2C). Efficacy was 67% (95% CI, 7 to 88%; $P = 0.035$) for malaria infection that was a new genotype not present in samples taken before antimalarial treatment (Fig. 2D). In exploratory analysis, we required all three of the triplicate PCR runs to be positive to define the end point of infection. This end point was reached for 5 of 61 (8%) ME-TRAP vaccinees compared with 16 of 60 (27%) control vaccinees, giving a vaccine efficacy of 72% (95% CI, 23 to 89%; $P = 0.014$).

The effect of vaccination remained significant after adjusting for covariates (Table 2). The hazard ratios (HRs) for covariates on the primary end point of infection by PCR were as follows: insecticide-treated net (ITN) use, 1.31 (95% CI, 0.57 to 3.01; $P = 0.5$); location, 0.78 (95% CI, 0.41 to 1.49; $P = 0.5$); age in years, 0.95 (95% CI, 0.91 to 0.99; $P = 0.02$); and parasitemia before antimalarial treatment, 7.0 (95% CI, 2.7 to 18.5; $P < 0.0001$). Parasitemia at screening was not independently predictive of risk (HR, 2.5; 95% CI, 0.5 to 13; $P = 0.25$). We noted a higher prevalence of *P. falciparum* infection among vaccinees at day 63. This was not associated with enhanced immunogenicity (summed ELISPOT results were 5.5% lower among this group; 95% CI, -28 to +17%) and would have biased results against vaccine efficacy given that reinfection was more likely in this group (HR, 7.0; 95% CI, 2.7 to 18.5; $P < 0.0001$). Nevertheless, vaccine efficacy was significant with and without adjusting for *P. falciparum* infection at day 63, as shown in Table 2.

More new infections occurred in the first 4 weeks of follow-up than in the latter 4 weeks (that is, 33 primary end points of infection by PCR occurred in the first 4 weeks versus 6 in

the latter 4 weeks), in keeping with the brief period of high rainfall just before the final vaccination (Fig. 2A). Hence, there was limited power to identify waning efficacy ($P = 0.43$ by Schoenfeld residuals).

T cell responses to TRAP peptides 21 to 30 from parasite clone T9/96, the peptide pool to which the strongest T cell responses were induced and the allele encoded by the vaccine, were significantly associated with protection among ME-TRAP vaccinees ($P = 0.016$, Table 3 and Fig. 3A); T cell responses for the same peptide pool from the clone 3D7 showed a marginal association with protection ($P = 0.054$, Table 3 and Fig. 3B). Summed T cell responses to T9/96 peptides were not associated with protection (Fig. 3C), and neither were antibody responses by ELISA (Fig. 3D).

DISCUSSION

We demonstrate some protective efficacy against malaria infection with a T cell-inducing vaccination strategy among adults living in a malaria-endemic area in Kenya. Previous T cell-inducing vaccines have been partially effective in controlled human malaria infection studies (6) but have been ineffective in field studies (7, 8), possibly because immunogenicity is reduced in participants previously exposed to malaria (9). The use of ChAd63 for priming in the current study resulted in a sixfold improvement in immunogenicity compared to priming with DNA and a fourfold improvement compared to priming with the attenuated fowlpox strain FP9 (6–9).

Protective efficacy may vary according to transmission intensity, as has been described for other vaccines (15). In our trial, the transmission intensity was low. To achieve high protective efficacy in higher transmission settings, combination vaccine regimens that would boost cellular and humoral immunity to different antigens would most likely be needed. Synergistic protection has been seen with combined antibody and cellular responses to a vaccine (16, 17). Further vaccine development will necessitate testing in children, where the potential for immunosuppression of T cell responses after malaria infection may attenuate vaccine efficacy (9).

We identified “blips” of low-level parasitemia during monitoring, which were not detectable by microscopy. Previous field studies have identified similar “blips” (14), which are thought to represent parasites emerging from the liver with subsequent suppression of growth by either host immunity (18) or antimalarials (14). After boosting with the MVA ME-TRAP vaccine and before monitoring, we gave a combination of antimalarials to clear early and late blood-stage asexual parasites, gametocytes, and liver-stage infections. Critically, the atovaquone component of this combination may have a prolonged biological effect (19), explaining in part the low parasite densities that we observed.

We have previously reported the lower limit of quantification of this PCR assay to be 20 parasites/ml (20). However, this does not mean that low-level positive results are unreliable. Rather, whereas negative results do not rule out very low parasite densities, positive PCR results have been shown to be highly specific for *P. falciparum* infection (21).

Genotyping was conducted using a previously published method to distinguish failure to clear parasites before monitoring from reinfection during the monitoring period (22). Genotyping data suggested that most of the parasites seen after drug treatment were new infections rather than recrudescence infections, consistent with the high efficacy of the antimalarial drug regimen chosen (23).

Most malaria infections were acquired in the first 2 weeks of follow-up after vaccination with MVA ME-TRAP. This 2-week period followed antimalarial treatment (given 1 week after the second boost vaccination) to clear previously acquired infections. This also, by chance, happened to coincide with a spike in rainfall, likely explaining the short 2-week period of acquisition of malaria infections (Fig. 2A). We were therefore unable to assess the protective efficacy of our vaccination regimen beyond this 2-week period.

Vaccine-induced T cell responses, measured in an ex vivo ELISPOT assay, waned over 105 days of follow-up, as has been reported previously in studies of malaria-naïve volunteers. Immunogenicity was still detectable at day 161 by ELISPOT ($P = 0.002$), albeit at one quarter the magnitude detected at day 63. In other studies, efficacy has been observed with experimental challenge 6 months after vaccination despite waning immunogenicity (13). The central memory T cell response has been reported to be more sustained than the ex vivo response and may be associated with efficacy (24).

In our study, efficacy was partial. Breakthrough infections might occur because of genetic variation (that is, protection operates against parasites of similar genotype to the strain used to develop the vaccine) or for other reasons (for example, some parasite inocula might be large and thus would overwhelm host immunity). Given that the quantity of DNA extracted from malaria parasites from vaccinee blood was insufficient for sequencing the *TRAP* gene, we were unable to formally test whether breakthrough infections were associated with particular *TRAP* genotypes.

Previous work suggests that T cells rather than antibody are the protective mechanism induced by the immunization regimen that we used here (5). We found that T cells to the TRAP peptide pool 21 to 30 from strain T9/96 correlated with efficacy. Given the multiple comparisons (for which a Bonferroni correction to $P < 0.0016$ might be applied), this may simply be a chance association. On the other hand, this was the peptide pool that elicited the highest T cell response, and *TRAP* was the gene encoded in the vaccine, suggesting that this TRAP peptide pool is the most likely candidate correlate of protection a priori. No correlations with protection were identified with the low T cell responses seen among the control vaccinees.

The efficacy observed here was higher than the rate of sterile efficacy in controlled human malaria infection with the same vaccination regimen (21%; 95% CI, 5 to 51%) (13). This may reflect a lower sporozoite inoculum in the field (five infectious mosquito bites are used in controlled human malaria infection compared to one infectious bite in the field). The frequency of partial efficacy (that is, a delay in the time taken to develop a positive blood film relative to control vaccinees) plus sterile efficacy (that is, those whose blood films never became positive) was 57% (95% CI, 29 to 82%) in controlled human malaria infection

studies, which was not significantly different from the estimate in this Kenyan trial. T cell-inducing vaccines have been calculated to provide a greater than 90% reduction in parasite burden at the pre-erythrocytic stage in controlled human malaria infection studies (25). This reduction may result in partial protection (that is, a delay to parasitemia) when the infectious challenge is heavy and in sterile or complete protection when infectious challenge is light.

Previous proof-of-concept vaccine trials have been conducted in adults in endemic areas using microscopic detection of malaria parasites as the end point (8, 26). Efficacy measured using PCR detection may not be comparable with these previous trials, but the sensitivity of PCR allows for a smaller sample size with shorter follow-up before proceeding to trials with clinical end points in children.

It has been difficult to induce protective cellular immunity in humans in the absence of neutralizing antibodies. There was no evidence that TRAP antibodies were associated with protection in our trial (Table 3 and Fig. 3D), in keeping with preclinical studies showing that TRAP antibodies have little protective efficacy in vivo (27). The lack of correlation between vaccine efficacy and TRAP antibodies in this field trial, the induction of cellular immunogenicity, and the finding of higher ELISPOT responses to specific peptides suggest a cell-mediated mechanism of efficacy.

This study demonstrates protective efficacy over a 2-week period after vaccination in a single population in rural Kenya. The unexpectedly brief malaria transmission season precluded measuring efficacy over longer periods. Furthermore, we cannot readily generalize our results to settings with more intense malaria transmission or to other end points such as clinical malaria. We did not describe multiple episodes of malaria infection per individual because asymptomatic infections may have persisted, and so a second positive PCR result was more likely to be the result of continued infection rather than a discrete second episode of infection. The quantities of parasite DNA isolated precluded detailed genetic analysis to examine the cause of breakthrough infections.

We conclude that PCR monitoring for malaria infection is a sensitive and specific means of identifying end points with which to study vaccine efficacy using modest sample sizes. The ChAd63-MVA ME-TRAP vaccination strategy is now being examined for protective efficacy against clinical malaria in pediatric populations, and for confirmation of this efficacy signal in adult populations. We also are planning assessment of the ChAd63-MVA ME-TRAP vaccine in combination with other promising and potentially synergistic vaccination strategies (16).

MATERIALS AND METHODS

Study design

We conducted a randomized, controlled single-blind study. The end point was *P. falciparum* infection determined by PCR. We predicted that 70% of adults would be PCR-positive during the 8-week follow-up period, thus providing 80% power to detect an efficacy of 40% with 100 participants. We allowed for 20 participants to be lost to follow-up with $n = 120$. Our objective was to determine the safety, immunogenicity, and efficacy of vaccination with

ChAd63 ME-TRAP and MVA ME-TRAP compared to vaccination with a control rabies vaccine. Study participants, field workers, and the laboratory were blind to vaccination, but the study clinician and vaccination team were not blinded. The study was approved by the Kenyan Medical Research Institute National Ethics Committee, the Pharmacy and Poisons Board, Kenya (the national drug regulatory authority), and the Oxford Tropical Research Ethics Committee, and overseen by an independent data and safety monitoring board (DSMB). A local safety monitor was appointed to advise investigators and/or the DSMB on request. The study was conducted in accordance with the Helsinki Declaration of 1964 (revised in 1996) and in accordance with Good Clinical Practice (Directive 2001/83/EC, amended 2003/63/EC). The study sponsor was the University of Oxford, and funding was from the European and Developing Countries Clinical Trials Partnership (EDCTP). The trial was registered on the Pan-African Clinical Trial Registry (Ref: PACTR 201202000356208); www.pactr.org/ and ClinicalTrials.gov (Ref: NCT01666925); <http://clinicaltrials.gov>.

Study setting

The trial was conducted in Junju sublocation in Kilifi County, Kenya. Kilifi has two seasons of high transmission of *P. falciparum* malaria coinciding with the long monsoon rains (April to June) and the short rains (October to December). The entomological inoculation rate in the study area was measured at 21.7 infective bites per year in 2010 (28), but the rains failed in 2012, when the study was conducted, and clinical malaria rates were low (fig. S1).

Participants

We recruited 18- to 50-year-old adult males without evidence of any acute or chronic illness on medical and laboratory screening and without prior receipt of an experimental malaria vaccine or recent or planned use of any investigational drug.

Randomization

Participants were randomized in a 1:1 ratio. The randomization sequence was generated by an independent statistician using STATA version 12 (StataCorp). Treatment allocations were kept in sealed opaque envelopes stored in a locked cabinet by the data manager. Study clinicians opened the envelopes after confirming eligibility criteria and when ready to vaccinate the participant. Participants and field workers remained blinded until the end of the study, and the laboratory remained blinded until the PCR and immunology databases were cleaned and locked.

Interventions

The active treatment was 5×10^{10} viral particles of intramuscular ChAd63 ME-TRAP in 0.35 ml followed by 2×10^8 plaque-forming units of intramuscular MVA ME-TRAP in 0.27 ml 56 days later as previously described (11). The control treatment was 0.5 ml of intramuscular Rabies Vaccine BP (Sanofi Pasteur) at the same time points.

Safety assessment

Participants were observed for 30 min after each immunization, visited at home by a field worker the next day, and assessed in the clinic 14 and 7 days after the first and second vaccinations, respectively (details of safety assessments are given in tables S7 and S8).

Immunology

A cohort of 30 participants were randomly selected for cellular immunology studies at baseline (day 0), 2 weeks after the first vaccination (day 14), and 105 days after the second vaccination (day 161). All participants were sampled for cellular immunology on day 63 (that is, 1 week after the second vaccination). PBMCs were separated using Lymphoprep (Axis-Shield Diagnostics Ltd.) for cellular immunology, and a serum sample was saved for serology. ELISPOT assays, ICS, and ELISAs were conducted as previously described (13), using peptide pools for TRAP as described in table S9.

Antimalarial treatment before PCR monitoring

A 3-day course of directly observed atovaquone/proguanil (250 mg/100 mg) with artesunate (250 mg) was started 7 days after the final vaccination (that is, on days 63 to 65) to clear parasitemia before PCR monitoring for new *P. falciparum* infections.

PCR monitoring

Before the period of monitoring for efficacy, PCR assays were done on day 0 and on day 63 to provide baseline data for adjusting subsequent risk of infection. After vaccination, PCR assays were conducted three times per week from day 70 up to day 95 and once per week from day 98 to day 119. Blood (0.5 ml) was filtered to remove white cells (29) before DNA extraction into 50 µl of eluate, from which 15 µl (that is, 5 µl in triplicate) was amplified by quantitative PCR using a TaqMan probe (5'-FAM-AACAATTGGAGGGCAAG-NFQ-MGB-3') for the multicopy 18S ribosomal RNA genes as previously described (30). We used an Applied Biosystems 7500 PCR System with quantification by Applied Biosystems 7500 software. Quality control checks were additionally performed using an Applied Biosystems Step One Plus PCR system and software. Three negative control wells and seven serial dilutions of plasmid DNA as standards were included on each plate.

Genotyping

Parasite DNA was typed using the Sequenom assay as previously described (22) to type 200 SNPs (the SNPs used for typing are shown in table S10).

Statistical analysis

An analysis plan was agreed by the DSMB, sponsor, and investigators before unblinding. The primary efficacy analysis was the HR for vaccination as determined by Cox regression for the first PCR-positive blood sample during monitoring after antimalarials were given to clear preexisting malaria infection, and visualized using Kaplan-Meier plots, with log-rank testing for significance. Secondary analyses were hazards of (i) the first PCR-positive blood sample above a threshold of 10 parasites/ml and (ii) the first PCR-positive blood sample where genotyping data were available and indicating that the genotype was different from

the genotype present before antimalarials were given. The time at risk was considered to begin 1 week after the final vaccination with MVA ME-TRAP. Cox regression was adjusted by age (continuous variable), bed net use (intact or treated nets versus no nets or untreated nets with more than three holes), malaria parasitemia on enrollment, and location of residence. Safety data are presented for the intention to treat cohort (that is, all randomized participants), and immunogenicity and efficacy data are presented according to protocol (that is, excluding two subjects who did not complete their vaccination course). The sample size was based on a power calculation (see Supplementary Methods).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES AND NOTES

- Noor AM, Kinyoki DK, Mundia CW, Kabaria CW, Mutua JW, Alegana VA, Fall IS, Snow RW. The changing risk of *Plasmodium falciparum* malaria infection in Africa: 2000–10: A spatial and temporal analysis of transmission intensity. *Lancet*. 2014; 383:1739–1747. [PubMed: 24559537]
- Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, Schwenk R, Nielsen RA, Debebe Z, Pinelis E, Juompan L, Williams J, Dowler M, Stewart VA, Wirtz RA, Dubois MC, Lievens M, Cohen J, Ballou WR, Heppner DG Jr. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naïve adults: Safety, efficacy, and immunologic associates of protection. *J. Infect. Dis.* 2009; 200:337–346. [PubMed: 19569965]
- Agnandji ST, Lell B, Fernandes JF, Abossolo BP, Methogo BG, Kabwende AL, Adegnikaa AA, Mordmuller B, Issifou S, Kremsner PG, Sacarlal J, Aide P, Lanaspá M, Aponte JJ, Machevo S, Acacio S, Buló H, Sigauque B, Macete E, Alonso P, Abdulla S, Salim N, Minja R, Mpina M, Ahmed S, Ali AM, Mtoro AT, Hamad AS, Mutani P, Tanner M, Tinto H, D'Alessandro U, Sorgho H, Valea I, Bihoun B, Guiraud I, Kabore B, Sombie O, Guiguemde RT, Ouedraogo JB, Hamel MJ, Kariuki S, Onoko M, Odero C, Otieno K, Awino N, McMorro M, Muturi-Kioi V, Laserson KF, Slutsker L, Otieno W, Otieno L, Otsyula N, Gondi S, Otieno A, Owira V, Oguk E, Odongo G, Woods JB, Ogutu B, Njuguna P, Chilengi R, Akoo P, Kerubo C, Maingi C, Lang T, Olotu A, Bejon P, Marsh K, Mwambingu G, Owusu-Agyei S, Asante KP, Osei-Kwakye K, Boahen O, Dosoo D, Asante I, Adjei G, Kwara E, Chandramohan D, Greenwood B, Lusingu J, Gesase S, Malabeja A, Abdul O, Mahende C, Liheluka E, Malle L, Lemnge M, Theander TG, Drakeley C, Ansong D, Agbenyega T, Adjei S, Boateng HO, Rettig T, Bawa J, Sylverken J, Sambian D, Sarfo A, Agyekum A, Martinson F, Hoffman I, Mvalo T, Kamthunzi P, Nkomo R, Tembo T, Tegha G, Tsidya M, Kilembe J, Chawinga C, Ballou WR, Cohen J, Guerra Y, Jongert E, Lapierre D, Leach A, Lievens M, Ofori-Anyinam O, Olivier A, Vekemans J, Carter T, Kaslow D, Lebouilleux D, Loucq C, Radford A, Savarese B, Schellenberg D, Sillman M, Vansadia P. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N. Engl. J. Med.* 2012; 367:2284–2295. [PubMed: 23136909]
- Dunachie SJ, Walther M, Epstein JE, Keating S, Berthoud T, Andrews L, Andersen RF, Bejon P, Goonetiilleke N, Poulton I, Webster DP, Butcher G, Watkins K, Sinden RE, Levine GL, Richie TL, Schneider J, Kaslow D, Gilbert SC, Carucci DJ, Hill AV. A DNA prime-modified vaccinia virus Ankara boost vaccine encoding thrombospondin-related adhesion protein but not circumsporozoite protein partially protects healthy malaria-naïve adults against *Plasmodium falciparum* sporozoite challenge. *Infect. Immun.* 2006; 74:5933–5942. [PubMed: 16988273]

5. Hill AV. Vaccines against malaria. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2011; 366:2806–2814. [PubMed: 21893544]
6. Webster DP, Dunachie S, Vuola JM, Berthoud T, Keating S, Laidlaw SM, McConkey SJ, Poulton I, Andrews L, Andersen RF, Bejon P, Butcher G, Sinden R, Skinner MA, Gilbert SC, Hill AV. Enhanced T cell-mediated protection against malaria in human challenges by using the recombinant poxviruses FP9 and modified vaccinia virus Ankara. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:4836–4841. [PubMed: 15781866]
7. Bejon P, Mwacharo J, Kai O, Mwangi T, Milligan P, Todryk S, Keating S, Lang T, Lowe B, Gikonyo C, Molyneux C, Fegan G, Gilbert SC, Peshu N, Marsh K, Hill AV. A phase 2b randomised trial of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP among children in Kenya. *PLOS Clin. Trials.* 2006; 1:e29. [PubMed: 17053830]
8. Moorthy VS, Imoukhuede EB, Milligan P, Bojang K, Keating S, Kaye P, Pinder M, Gilbert SC, Walraven G, Greenwood BM, Hill AS. A randomised, double-blind, controlled vaccine efficacy trial of DNA/MVA ME-TRAP against malaria infection in Gambian adults. *PLOS Med.* 2004; 1:e33. [PubMed: 15526058]
9. Bejon P, Mwacharo J, Kai O, Todryk S, Keating S, Lowe B, Lang T, Mwangi TW, Gilbert SC, Peshu N, Marsh K, Hill AV. The induction and persistence of T cell IFN- γ responses after vaccination or natural exposure is suppressed by *Plasmodium falciparum*. *J. Immunol.* 2007; 179:4193–4201. [PubMed: 17785859]
10. O'Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, Goodman AL, Edwards NJ, Reyes-Sandoval A, Bird P, Rowland R, Sheehy SH, Poulton ID, Hutchings C, Todryk S, Andrews L, Folgori A, Berrie E, Moyle S, Nicosia A, Colloca S, Cortese R, Siani L, Lawrie AM, Gilbert SC, Hill AV. Clinical assessment of a recombinant simian adenovirus ChAd63: A potent new vaccine vector. *J. Infect. Dis.* 2012; 205:772–781. [PubMed: 22275401]
11. Ogwang C, Afolabi M, Kimani D, Jagne YJ, Sheehy SH, Bliss CM, Duncan CJ, Collins KA, Garcia Knight MA, Kimani E, Anagnostou NA, Berrie E, Moyle S, Gilbert SC, Spencer AJ, Soipei P, Mueller J, Okebe J, Colloca S, Cortese R, Viebig NK, Roberts R, Gantlett K, Lawrie AM, Nicosia A, Imoukhuede EB, Bejon P, Urban BC, Flanagan KL, Ewer KJ, Chilengi R, Hill AV, Bojang K. Safety and immunogenicity of heterologous prime-boost immunisation with *Plasmodium falciparum* malaria candidate vaccines, ChAd63 ME-TRAP and MVA ME-TRAP, in healthy Gambian and Kenyan adults. *PLOS One.* 2013; 8:e57726. [PubMed: 23526949]
12. Kimani D, Jagne YJ, Cox M, Kimani E, Bliss CM, Gitau E, Ogwang C, Afolabi MO, Bowyer G, Collins KA, Edwards N, Hodgson SH, Duncan CJ, Spencer AJ, Knight MG, Drammeh A, Anagnostou NA, Berrie E, Moyle S, Gilbert SC, Soipei P, Okebe J, Colloca S, Cortese R, Viebig NK, Roberts R, Lawrie AM, Nicosia A, Imoukhuede EB, Bejon P, Chilengi R, Bojang K, Flanagan KL, Hill AV, Urban BC, Ewer KJ. Translating the immunogenicity of prime-boost immunization with ChAd63 and MVA ME-TRAP from malaria naive to malaria-endemic populations. *Mol. Ther.* 2014; 22:1992–2003. [PubMed: 24930599]
13. Ewer KJ, O'Hara GA, Duncan CJ, Collins KA, Sheehy SH, Reyes-Sandoval A, Goodman AL, Edwards NJ, Elias SC, Halstead FD, Longley RJ, Rowland R, Poulton ID, Draper SJ, Blagborough AM, Berrie E, Moyle S, Williams N, Siani L, Folgori A, Colloca S, Sinden RE, Lawrie AM, Cortese R, Gilbert SC, Nicosia A, Hill AV. Protective CD8⁺ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat. Commun.* 2013; 4:2836. [PubMed: 24284865]
14. Imoukhuede EB, Andrews L, Milligan P, Berthoud T, Bojang K, Nwakanma D, Ismaili J, Buckee C, Njie F, Keita S, Sowe M, Lang T, Gilbert SC, Greenwood BM, Hill AV. Low-level malaria infections detected by a sensitive polymerase chain reaction assay and use of this technique in the evaluation of malaria vaccines in an endemic area. *Am. J. Trop. Med. Hyg.* 2007; 76:486–493. [PubMed: 17360872]
15. Bejon P, White MT, Olotu A, Bojang K, Lusingu JP, Salim N, Otsyula NN, Agnandji ST, Asante KP, Owusu-Agyei S, Abdulla S, Ghani AC. Efficacy of RTS,S malaria vaccines: Individual-participant pooled analysis of phase 2 data. *Lancet Infect. Dis.* 2013; 13:319–327. [PubMed: 23454164]
16. Ndungu FM, Mwacharo J, Kimani D, Kai O, Moris P, Jongert E, Vekemans J, Olotu A, Bejon P. A statistical interaction between circumsporozoite protein-specific T cell and antibody responses and

- risk of clinical malaria episodes following vaccination with RTS,S/AS01E. *PLOS One*. 2012; 7:e52870. [PubMed: 23300801]
17. Hutchings CL, Birkett AJ, Moore AC, Hill AV. Combination of protein and viral vaccines induces potent cellular and humoral immune responses and enhanced protection from murine malaria challenge. *Infect. Immun.* 2007; 75:5819–5826. [PubMed: 17908809]
 18. Douglas AD, Andrews L, Draper SJ, Bojang K, Milligan P, Gilbert SC, Imoukhuede EB, Hill AV. Substantially reduced pre-patent parasite multiplication rates are associated with naturally acquired immunity to *Plasmodium falciparum*. *J. Infect. Dis.* 2011; 203:1337–1340. [PubMed: 21459819]
 19. Butcher GA, Sinden RE. Persistence of atovaquone in human sera following treatment: Inhibition of *Plasmodium falciparum* development in vivo and in vitro. *Am. J. Trop. Med. Hyg.* 2003; 68:111–114. [PubMed: 12556158]
 20. Douglas AD, Edwards NJ, Duncan CJ, Thompson FM, Sheehy SH, O'Hara GA, Anagnostou N, Walther M, Webster DP, Dunachie SJ, Porter DW, Andrews L, Gilbert SC, Draper SJ, Hill AV, Bejon P. Comparison of modeling methods to determine liver-to-blood inocula and parasite multiplication rates during controlled human malaria infection. *J. Infect. Dis.* 2013; 208:340–345. [PubMed: 23570846]
 21. Murphy SC, Hermesen CC, Douglas AD, Edwards NJ, Petersen I, Fahle GA, Adams M, Berry AA, Billman ZP, Gilbert SC, Laurens MB, Leroy O, Lyke KE, Plowe CV, Seilie AM, Strauss KA, Teelen K, Hill AV, Sauerwein RW. External quality assurance of malaria nucleic acid testing for clinical trials and eradication surveillance. *PLOS One*. 2014; 9:e97398. [PubMed: 24838112]
 22. Manske M, Miotto O, Campino S, Auburn S, Almagro-Garcia J, Maslen G, O'Brien J, Djimde A, Doumbo O, Zongo I, Ouedraogo JB, Michon P, Mueller I, Siba P, Nzila A, Borrmann S, Kiara SM, Marsh K, Jiang H, Su XZ, Amaratunga C, Fairhurst R, Socheat D, Nosten F, Imwong M, White NJ, Sanders M, Anastasi E, Alcock D, Drury E, Oyola S, Quail MA, Turner DJ, Ruano-Rubio V, Jyothi D, Amenga-Etego L, Hubbart C, Jeffreys A, Rowlands K, Sutherland C, Roper C, Mangano V, Modiano D, Tan JC, Ferdig MT, Amambua-Ngwa A, Conway DJ, Takala-Harrison S, Plowe CV, Rayner JC, Rockett KA, Clark TG, Newbold CI, Berriman M, MacInnis B, Kwiatkowski DP. Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature*. 2012; 487:375–379. [PubMed: 22722859]
 23. Looareesuwan S, Chulay JD, Canfield CJ, Hutchinson DB. Malarone (atovaquone and proguanil hydrochloride): A review of its clinical development for treatment of malaria. Malarone Clinical Trials Study Group. *Am. J. Trop. Med. Hyg.* 1999; 60:533–541. [PubMed: 10348225]
 24. Keating SM, Bejon P, Berthoud T, Vuola JM, Todryk S, Webster DP, Dunachie SJ, Moorthy VS, McConkey SJ, Gilbert SC, Hill AV. Durable human memory T cells quantifiable by cultured enzyme-linked immunospot assays are induced by heterologous prime boost immunization and correlate with protection against malaria. *J. Immunol.* 2005; 175:5675–5680. [PubMed: 16237057]
 25. Bejon P, Andrews L, Andersen RF, Dunachie S, Webster D, Walther M, Gilbert SC, Peto T, Hill AV. Calculation of liver-to-blood inocula, parasite growth rates, and preerythrocytic vaccine efficacy, from serial quantitative polymerase chain reaction studies of volunteers challenged with malaria sporozoites. *J. Infect. Dis.* 2005; 191:619–626. [PubMed: 15655787]
 26. Bojang KA, Milligan PJ, Pinder M, Vigneron L, Allouche A, Kester KE, Ballou WR, Conway DJ, Reece WH, Gothard P, Yamuah L, Delchambre M, Voss G, Greenwood BM, Hill A, McAdam KP, Tornieporth N, Cohen JD, Doherty T. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: A randomised trial. *Lancet*. 2001; 358:1927–1934. [PubMed: 11747915]
 27. Gantt S, Persson C, Rose K, Birkett AJ, Abagyan R, Nussenzweig V. Antibodies against thrombospondin-related anonymous protein do not inhibit *Plasmodium* sporozoite infectivity in vivo. *Infect. Immun.* 2000; 68:3667–3673. [PubMed: 10816526]
 28. Midega JT, Smith DL, Olotu A, Mwangangi JM, Nzovu JG, Wambua J, Nyangweso G, Mbogo CM, Christophides GK, Marsh K, Bejon P. Wind direction and proximity to larval sites determines malaria risk in Kilifi District in Kenya. *Nat. Commun.* 2012; 3:674. [PubMed: 22334077]
 29. Andrews L, Andersen RF, Webster D, Dunachie S, Walther RM, Bejon P, Hunt-Cooke A, Bergson G, Sanderson F, Hill AV, Gilbert SC. Quantitative real-time polymerase chain reaction for malaria diagnosis and its use in malaria vaccine clinical trials. *Am. J. Trop. Med. Hyg.* 2005; 73:191–198. [PubMed: 16014857]

30. Sheehy SH, Duncan CJ, Elias SC, Choudhary P, Biswas S, Halstead FD, Collins KA, Edwards NJ, Douglas AD, Anagnostou NA, Ewer KJ, Havelock T, Mahungu T, Bliss CM, Miura K, Poulton ID, Lillie PJ, Antrobus RD, Berrie E, Moyle S, Gantlett K, Colloca S, Cortese R, Long CA, Sinden RE, Gilbert SC, Lawrie AM, Doherty T, Faust SN, Nicosia A, Hill AV, Draper SJ. ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: Assessment of efficacy against mosquito bite challenge in humans. *Mol. Ther.* 2012; 20:2355–2368. [PubMed: 23089736]

Fig. 1. Vaccine immunogenicity

ELISPOT and ICS assays were used to determine the numbers of cells producing IFN- γ (identified as spots on the ELISPOT assay) and percentages of cells on flow cytometry, respectively. Shown are geometric mean T cell and antibody responses with 95% CIs by time point and by vaccination group. Units are IFN- γ -producing cells (spots) per million PBMCs for the ELISPOT assay, %CD4/CD8 T cells for the ICS assay, and arbitrary units for the enzyme-linked immunosorbent assay (ELISA) detecting antibody. $P < 0.05$ is shown by a black circle for vaccinees receiving the ChAd63-MVA ME-TRAP vaccine versus control vaccinees receiving a rabies vaccine. Results are shown at screening (day 0), 14 days after priming with ChAd63 ME-TRAP (day 14), 7 days after boosting with MVA ME-TRAP (peak immunogenicity was at day 63), and after waning of the immune response (day 161). G, IFN- γ ; L, IL-2; T, TNF- α .

A Rainfall (mm/wk) and timing of interventions

B Any PCR positive *

C >10 parasites/ml *

D PCR positive and new genotype *

— Control — ME-TRAP

Fig. 2. Kaplan-Meier plots of first episodes of PCR positivity after vaccination

(A to D) The figure shows (A) rainfall data (in millimeters per week) and (B to D) Kaplan-Meier plots of (B) first episode of PCR positivity at any threshold ($P = 0.0006$ by log-rank), (C) first episode of PCR positivity at >10 parasites/ml ($P = 0.0004$ by log-rank), and (D) first episode of PCR positivity where the parasite genotype was different from a parasite genotype seen in samples collected before the start of monitoring ($P = 0.023$ by log-rank). Analysis is shown beginning 1 week after the last vaccination when antimalarial drugs were given. *, significant differences ($P < 0.05$).

Fig. 3. Kaplan-Meier plot of first episodes of PCR positivity after immune response

(A to D) We split immunological responses according to tertile among the ChAd63-MVA ME-TRAP vaccinees and plotted survival functions for time to malaria infection by PCR according to tertile. Shown are (A) T cell responses to the TRAP peptide pool 21 to 30 from T9/96 parasites; (B) T cell responses to the TRAP peptide pool 21 to 30 from 3D7 parasites; (C) summed T cell responses to all TRAP peptide pools from T9/96 parasites; (D) ELISA results showing antibody responses to TRAP. *, significant differences ($P < 0.05$). Correlations are shown between peak immune response as measured on day 63 (1 week after boost) and subsequent infection.

Table 1
Baseline characteristics of trial participants

Characteristics are given for the 121 enrolled participants. Northern versus Southern refers to the division between Chodari/Mapawa/Kolewa in the north of Junju sublocation versus Gongoni/Mwembe Tsungu/Bomani in the south of Junju sublocation. IQR, interquartile range; freq, frequency.

Parameter or category	ME-TRAP group (<i>n</i> = 61)		Control group (<i>n</i> = 60)	
	Mean/freq	% or IQR	Mean/freq	% or IQR
Mean age (years)	29.3	22.7–35.3	29.7	22.6–37.0
Bed net use				
No bed net	45	73.8	43	71.7
Impregnated bed net without holes	6	9.8	7	11.7
Impregnated bed net with holes	3	4.9	2	3.3
Untreated bed net without holes	4	6.6	1	1.7
Untreated bed net with holes	3	4.9	7	11.7
Location of residence				
Northern	32	52.5	26	43.3
Southern	27	44.2	33	55
Unknown	2	3.2	1	1.7
PCR for <i>P. falciparum</i> parasitemia at baseline				
Positive	23	38.3	10	27.7
Negative	37	61.7	49	72.2
PCR for <i>P. falciparum</i> parasitemia at day 63				
Positive	30	49.2	22	36.7
Negative	30	49.2	37	61.7
Unknown	1	1.6	1	1.6

Table 2
Vaccine efficacy by Cox regression

N, number of participants; *n*, number of end points identified. Efficacy figures are estimated from Cox regression, where efficacy = $(1 - \text{HR}) \times 100\%$.

	<u>ME-TRAP</u>		<u>Control</u>		<u>Unadjusted efficacy</u>		<u>Adjusted efficacy</u>	
	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	Efficacy (95% CI)	<i>P</i>	Efficacy (95% CI)	<i>P</i>
Any PCR positivity	61	11	60	28	67% (33–83%)	0.002	66% (31–83%)	0.003
>10 parasites/ml	61	4	60	19	82% (46–94%)	0.002	81% (42–94%)	0.03
New genotype	61	5	60	14	67% (7–88%)	0.035	65% (2–87%)	0.046

Table 3
Associations between vaccine efficacy and immunological responses by Cox regression

Correlations are shown between peak immune responses as measured on day 63 (that is, 1 week after boost) and subsequent infection. ME, multiple epitope. G, IFN- γ ; L, IL-2; T, TNF- α .

Assay	Rabies				ME-TRAP			
	HR	5%	95%	P	HR	5%	95%	P
ELISPOT: 3D7 peps 1–10	1.39	0.85	2.27	0.19	1.25	0.58	2.69	0.57
ELISPOT: 3D7 peps 11–20	0.91	0.52	1.59	0.74	1.1	0.52	2.33	0.81
ELISPOT: 3D7 peps 21–30	1.19	0.76	1.88	0.44	0.5	0.25	1.02	0.056
ELISPOT: 3D7 peps 31–40	1.01	0.57	1.8	0.97	0.88	0.37	2.09	0.77
ELISPOT: 3D7 peps 41–50	1.24	0.68	2.25	0.48	0.9	0.43	1.89	0.78
ELISPOT: T9/96 peps 1–10	0.81	0.29	2.3	0.69	1.21	0.52	2.81	0.65
ELISPOT: T9/96 peps 11–20	1.14	0.62	2.09	0.67	1.47	0.58	3.73	0.42
ELISPOT: T9/96 peps 21–30	1.36	0.87	2.13	0.18	0.24	0.08	0.75	0.014*
ELISPOT: T9/96 peps 31–40	0.9	0.48	1.69	0.74	1.02	0.5	2.07	0.95
ELISPOT: T9/96 peps 41–50	0.99	0.51	1.93	0.98	0.97	0.5	1.89	0.93
ELISPOT: T9/96 peps 51–57	0.89	0.52	1.52	0.67	0.79	0.36	1.73	0.55
ELISPOT: T9/96 + ME total	1.08	0.6	1.93	0.8	0.46	0.08	2.59	0.38
ELISPOT: 3D7 total	1.12	0.67	1.86	0.67	0.93	0.32	2.73	0.9
ELISPOT: ME	0.7	0.38	1.27	0.24	1.06	0.49	2.3	0.88
ELISPOT: T9/96 total	1.12	0.65	1.95	0.68	0.54	0.11	2.58	0.44
ICS, single: CD4G	1.22	0.61	2.47	0.57	1.04	0.38	2.85	0.94
ICS, single: CD4L	0.69	0.35	1.35	0.28	1.18	0.46	3.02	0.73
ICS, single: CD4T	0.75	0.4	1.4	0.37	1.21	0.46	3.18	0.7
ICS, single: CD8G	1.05	0.5	2.24	0.89	0.84	0.37	1.94	0.69
ICS, single: CD8L	0.95	0.49	1.82	0.87	1.08	0.39	3.03	0.88
ICS, single: CD8T	0.69	0.34	1.4	0.31	0.77	0.33	1.81	0.55
ICS: CD4 G ⁺ L ⁺ T ⁺	0.97	0.37	2.58	0.96	1.23	0.45	3.38	0.69
ICS: CD4 G ⁺ L ⁺ T ⁻	2.94	0.46	18.82	0.26	0.29	0.03	2.92	0.29
ICS: CD4 G ⁺ L ⁻ T ⁺	1.05	0.27	4.06	0.95	1.11	0.32	3.84	0.87
ICS: CD4 G ⁺ L ⁻ T ⁻	1.71	0.62	4.66	0.3	0.2	0.02	2.45	0.21
ICS: CD4 G ⁻ L ⁺ T ⁺	0.76	0.36	1.61	0.48	0.71	0.25	2.04	0.53
ICS: CD4 G ⁻ L ⁺ T ⁻	0.53	0.19	1.46	0.22	1.34	0.45	4.03	0.6
ICS: CD4 G ⁻ L ⁻ T ⁺	0.77	0.37	1.59	0.48	1.45	0.6	3.49	0.41
ICS: CD8 G ⁺ L ⁺ T ⁺	1.23	0.41	3.72	0.72	0.78	0.19	3.15	0.72
ELISA	2.4	0.8	7.16	0.12	1.25	0.26	6.05	0.78