Prime-boost vectored malaria vaccines
Progress and prospects

Adrian V.S. Hill,[1,]* Arturo Reyes-Sandoval,[1] Geraldine O'Hara,[1] Katie Ewer,[1] Alison Lawrie,[1] Anna Goodman,[1] Alfredo Nicosia,[2] Antonella Folgori,[2] Stefano Colloca,[2] Riccardo Cortese,[2] Sarah C. Gilbert[1] and Simon J. Draper[1]

[1]The Jenner Institute; University of Oxford; Oxford, UK; [2]Okairos S.R.L.; Pomezia, Rome, Italy

The difficulty of inducing protective immunity through antibodies against sporozoites led to efforts to assess vectored vaccines as a means of inducing protective T-cell immunity against the malaria liver-stage parasite. Although DNA vectored vaccines used alone were poorly immunogenic and not protective, high levels of parasite clearance in the liver has been achieved with viral vectored vaccines used in heterologous prime-boost regimes. Such vectored vaccination regimes represent one of only two approaches that have induced repeatable partial efficacy in human *P. falciparum* subunit vaccine trials. Interestingly, vectors expressing the TRAP antigen have been consistently been more immunogenic and protective than vectors expressing the circumsporozoite protein in human trials. However, sterile protection requires induction of very potent T-cell responses that are currently only achievable with heterologous prime-boost regimes. Recently, simian adenoviruses have been assessed as priming agents in Adenovirus-MVA regimes in both phase I and phase IIa trials in the UK, based on very promising pre-clinical results showing better immunogenicity and efficacy than previous prime-boost regimes. The same vectors are also being assessed clinically expressing blood-stage antigens, attempting to induce both protective antibodies and T cells as recently demonstrated in murine efficacy studies. These viral vectors now provide a major option for inclusion in a high efficacy multi-stage malaria vaccine that should achieve deployable levels of efficacy in endemic settings.

Introduction

The identification of a highly effective malaria vaccine that prevents disease in the great majority of vaccinees remains an elusive goal. However, recent data indicate that this major contribution to global public health should be achievable with the further development of approaches that are already in clinical assessment. Of the many candidate vaccines that have entered clinical efficacy testing for *P. falciparum* malaria, at least 25 in total, only two approaches have provided unequivocal evidence of some protective efficacy in humans. One approach, targeting antibodies against the central repeat of the circumsporozoite protein has led

to the development of RTS,S/AS01,[1,2] which is now in a phase III clinical trial, aiming for partial efficacy of the order of 30–50% with durability of perhaps a year. The other approach is the subject of this review, the induction of protection using viral vector vaccines. Importantly, although viral vectors have been shown to induce significant protection by targeting the TRAP antigen expressed during the liver-stages there is now considerable interest in the possibility that vectors could induce significant protection by targeting blood-stage antigens as well.[3,4]

The first evidence that vectors could provide protection in humans came from a phase IIa challenge study using the orthopox vector NYVAC that encoded seven malaria antigens, including TRAP and CSP.[5] Although antibody immunogenicity was poor, detectable lytic T-cell responses were found to TRAP, CSP and LSA-1 peptides, and 1 out of 35 challenged volunteers was sterilely protected. Use of TRAP with a polyepitope string, the ME.TRAP insert, was then assessed in DNA and MVA vectors both alone and in heterologous prime boost regimes. Significant reductions in parasite burden in the liver, estimated as approximately an 80% reduction,[6] were measured in vaccinees receiving DNA-MVA regimes but not repeated doses of the single vectors.[7,8] Using ex vivo ELISPOT assays the mean response measured after two DNA priming immunisations and an MVA boost was about 450 SFU per million. In that study one out of eight vaccinees was sterilely protected[8] and in another, using related DNA-MVA regimes, there was a significant delay in time to patency.[7] Subsequent evaluation of a fowlpox strain, FP9, as an alternative priming agent to DNA showed that two out of sixteen vaccinees were sterilely protected with an estimated 90% reduction in parasite burden in the liver.[6,9] Field testing of the potential these regimes,[10,11] particularly the latter was impeded by an unexpected drop in the potency of the vaccines when administered to 1–6 year old children amongst whom about 70% showed patent parasitaemia, a factor associated with reduced responses.[11,12] Mean T-cell responses were of the order of 400–500 SFU/million in the phase IIa studies in the UK but four times lower in a Kenyan phase IIb trial in children where no efficacy was observed.[13] Importantly in the phase IIa sporozoite challenge studies a clear correlation of T-cell responses measured by either ex vivo[9] or cultured[14] ELISPOT assays was observed with protection, the latter measured as either delay in time to patency or reduction in liver-stage parasite burden. Estimates of the magnitude of ex vivo ELISPOT responses required for sterile protection from this analysis indicated that responses exceeding a thousand were likely required for sterile protection.[9,15] This target

was sobering in that very few vaccinees administered any type of vaccination regime in malaria or any other disease,[16] reached this threshold at the time.

This led to renewed interest in using potentially more potent adenoviral vectors to try to prime more potent T-cell responses than DNA or Fowlpox. Initial prime-boost studies with adenovirus-MVA regimes in mice had shown promise in the *P. berghei* model[17] and adenovirus used alone had shown good protection against *P. yoelii* liver-stages.[18] Use of adenovirus vectors also offered a potential improvement in two other aspects of the immune responses induced by the first generation (DNA-MVA) and second generation (FP9-MVA) regimes. One of these is a greater ability of adenoviral vectors to induce antibody responses: DNA and poxvirus vectors induced only weak antibody responses even in heterologous prime boost regimes.[7] The other, of greater relevance to protective liver-stage immunity was the greater capacity of adenoviral vectors to induce CD8± rather than CD4± T-cell responses. DNA-MVA regimes in the clinic induced predominantly CD4± T-cells[7] and although FP9-MVA was better[19] the predominant response was still clearly CD4 biased.

Simian Adenoviral Vectors as Malaria Vaccines

A major concern with the use of human adenoviral vectors for malaria in African was the recognition that levels of anti-vector immunity were particularly high in African populations. For example the prevalence of antibodies to the most widely used and very potent vector Ad5 was reported as 80% and 85% in Southern and west African populations, respectively.[20]

Also, a non-significant trend towards negative efficacy in the HIV vaccine STEP trial[21] also suggested that there might be some safety concern with the widespread used of Ad5 vectors in Africa where HIV is prevalent. Reassuringly, however, further follow up of the STEP trial cohort and detailed immunological efforts[22,23] all now support the safety of Ad5 vectors. Yet the potentially impeding effect of anti-vector immunity on vaccine potency remains an issue for malaria, particularly if high level potency is required for efficacy as suggested by the TRAP studies. One option is to use less prevalent adenoviral vectors such as Ad35, and this option is being pursued by a Crucell-NIH collaboration, but Ad35 vectors appear substantially less potent than Ad5, at least in pre-clinical studies.[24] Therefore, the option of using adenoviral vectors of simian origin (**Fig. 1**) to which humans have much lower levels of anti-vector immunity appeared particularly attractive for malaria.

For clinical use a viral vector must display not just good potency, but also genetic stability in repeated passage and a high viral yield in a usable GMP-approved cell line to allow large scale manufacture. Extensive studies by groups in the US[25] and in Italy[26] have led to the identification of several potentially suitable adenoviral vectors of chimpanzee origin. Our group in Oxford has collaborated with both the University of Pennsylvania and Okairos (Rome, Italy) groups to assess several simian vectors with malaria inserts. Phylogenetic analysis of the hexons of simian and human adenoviruses (**Fig. 1**) shows substantial overlap indicating that there is no clear sequence feature that distinguished a simian from a human adenovirus: in fact these sequences suggest one

large family of higher primate adenoviruses.[25] Three vectors, C6, C7 and C9 (also called AdC68) from the Wilson lab were assessed initially followed by AdCh63 and AdCh3 from Okairos. Using these vectors we made several observations.[27,28] The safety of these vectors in mice, macaques and, recently in humans has been very similar to that of human adenovirus vectors suggesting that these might be suitable for widespread use. The T-cell immunogenicity of some of these vectors matched or even exceeded the immunogenicity of the standard Ad5 vector used as a comparator. For example in the *P. berghei* model immunogenicity of simian adenoviral vectors AdC7, AdC9 (also called AdC68)[28] and AdCh63 (unpublished) was as great as that of Ad5 and considerably stronger than with the poxviruses FP9 and MVA. For the first time it was possible to induce single dose high level protective efficacy against sporozoite challenge in the *P. berghei* model by using simian adenoviral vectors. Moreover, boosting these responses with MVA led to greater and more durable immunogenicity and also more durable efficacy against sporozoite challenge.[50] The latter may relate to a more polyfunctional phenotype of the CD8± T-cell responses post-boosting with a large proportion of IL2 positive cells observed post- but not pre-boosting with MVA.

The vector chosen for clinical assessment in malaria was the AdCh63 vector, one of the species E adenoviruses. This was found to grow well in HEK293 cells and recombinants have been stable on repeated passage. The prevalence of antibodies to AdCh63 was assessed in Kenyan children using a neutralisation assay. It was found that 23% of the children (aged 1–6 years) had high-titer neutralizing antibodies to AdHu5, but only 4% had high-titer neutralizing antibodies to AdCh63.[29] An increasing number of simian adenoviruses are now being reported of several different species suggesting that there will be sufficient different vector backbones available to allow different ones to be used for a variety of different diseases. Recently, progress in the clinical development of the simian AdCh3 vector as a vaccine against hepatitis C has been reported.[30]

Vectors for Blood-Stage Malaria

Early evidence that cellular immunity was relevant to protection in some murine malaria models was followed by the demonstration that repeated low dose inoculations of blood-stage parasites appeared to induce protection in human volunteers in the absence of detectable antibodies.[31] Very recently the induction of some cellular immunity to blood-stage antigens by inducing strong immunity with viable sporozoites administered with chloroquine in human volunteers, also supported the potential importance of T cells at the blood-stage in *P. falciparum* malaria.[32] This sort of evidence led to exploration of the capacity of viral vectored vaccines to produce immunity to blood-stage malaria in rodent models. Recently Draper et al.[3] identified a powerfully protective regime for *P. yoelii* using an adenovirus prime followed by an MVA boost (Ad-M) using the 42Kd C-terminal portion of the classic blood-stage antigen MSP1. This is of course an excellent regime for inducing powerful cellular immunity but the protection observed against blood-stage parasite challenge was entirely antibody-mediated. Strong antibody responses were indeed induced by this

Figure 1. Phylogenetic tree of hexon amino acid sequences of various human and chimpanzee adenoviruses. For clarity all human strains are prefixed "AdHu", thus Ad5 = AdHu5, and all chimpanzee vectors prefixed AdCh6, thus AdC6 = AdCh6. (Gilbert SC, unpublished).

regime and they could be amplified further and rendered more protective by the addition at the C-terminus of a core domain of the complement protein C4bp[33] as a fusion to the MSP1 sequence. This ability to induce powerful protective antibodies with vectored vaccines is of interest beyond malaria and potentially considerably broadens the potential applications of this technology.

The T cells recognizing MSP1 induced by this Ad-M regime were of protective value against the liver-stage parasite as manifest both by reduced liver parasite burden and a greater efficacy of the regime against sporozoite than blood-stage parasite challenge.[34] Interestingly, part of the latter effect appeared attributable to a different cytokine profile induced at the blood-stage in vaccinated mice challenged with sporozoites rather than blood-stage parasites. Analysis of the protective effects of prime-boost vectored vaccines in the rhesus *P. knowlesi* challenge model led Weiss et al. to conclude that significant protection was being induced by the blood-stage antigens MSP1 and AMA1.[4]

Goodman et al. (submitted 2009) have recently taken this approach forward to blood-stage *P. falciparum* antigens and have generated a novel MSP1 insert comprising two allelic 42Kd fragments placed C-terminal to four relatively conserved domains of this large molecule, with the aim of inducing protective antibodies

to the C-terminal fragments and T cells to conserved domains. It was found that a variety of simian adenoviral vectors could act as good priming agents and heterologous adenoviral vector strains were able to boost antibodies as well as recombinant MVA. Strong cross-strain growth inhibitory activity was found associated with the high antibodies induced and this activity was against strains expressing both alleles of the highly polymorphic 42Kd fragment. Similar work with a biallelic AMA1 insert is in progress, also with promising results (Biswas et al. unpublished).

These exciting preclinical data led to generation of biallelic MSP1 and AMA1 constructs in the AdCh63 and MVA vectors and all four vaccine vectors have now completed GMP manufacture prior to planned clinical trials. In view of the added protection observed in pre-clinical studies with sporozoite compared to blood-stage parasite challenge, the first phase IIa efficacy trials of these new vectors will involve a sporozoite rather than a blood-stage parasite challenge protocol.

Clinical Development

The vectored vaccine programme at Oxford has continued to focus primarily on the TRAP antigens for T-cell-inducing

vaccines targeting the liver-stage. This is supported by data from both DNA-MVA and FP9-MVA trials that found significant efficacy with a TRAP insert but no efficacy with CSP as an insert.[8,9,35] This contrasts with the success of RTS,S as a protective CSP-based antibody inducing vaccine. However, although CSP is the major coat protein on the sporozoite, recent evidence suggests that TRAP may be a better target of protective T cells, consistent with the vectored vaccine clinical trial data. Firstly, analysis of the time course of expression of TRAP and CSP by *P. falciparum* in cultured human hepatocytes found more durable expression of the former, while CSP disappeared rapidly.[36] Secondly, population genetic analysis searching for evidence of selection in the patterns of polymorphism observed in these two genes found stronger evidence that coding variation in TRAP reflected selection, consistent with immune attack from T cells.[37]

Several challenges were faced in translating the promising pre-clinical data with simian adenoviruses to phase I/II trials. A set of simian vectors that showed promise in malaria and were owned by the University of Pennsylvania were in-licensed by GSK Biologicals and became unavailable to the Oxford malaria programme. Fortunately, a more promising vector was available from Okairos (Rome, Italy), AdCh63, and ME-TRAP recombinants showed not only excellent genetic stability but high yields in the HEK293 cell line without generating any replication competent adenovirus. Studies of the prevalence of neutralizing antibodies to this chimpanzee virus in the UK (unpublished) and also at a malaria vaccine trial site in Kilifi, Kenya, found low anti-vector prevalences, well below the rates for Ad5 antibodies.[29] This AdCh63 vector encoding ME-TRAP was then manufactured to GMP standard at the University of Oxford's Clinical Biomanufacturing Facility and approved for clinical testing in 2007 by UK regulatory and ethical review committees, the first clinical trial of any simian adenoviral vector. During the phase I trial data emerged on a possible, albeit non-statistically significant, increased incidence of HIV infections in as subset of vaccines administered an HIV vaccine comprising three different Ad5 vectors encoding HIV antigens.[21] This had no impact on the continuation of the AdCh63 malaria trial and recent follow-up data support the view that the slightly increased infection rate in the HIV STEP trial was likely a chance occurrence.

A dose escalation phase I study and phase IIa sporozoite challenge study of AdCh63-MVA vaccinees have now been undertaken with very encouraging results. In particular T-cell immunogenicity with AdCh63-MVA regimes is very substantially higher than with any previously assessed prime-boost regimes and many-fold greater that immunogenicities reported for adenovirus vectors used alone, or even DNA prime-adenovirus boost regimes,[38] in HIV vaccine trials (O'Hara G et al., unpublished).

Encouraged by these safety, immunogenicity and efficacy findings with ME-TRAP as an insert both MSP1 and AMA1 inserts have been successfully manufactured to GMP standard (Draper S et al., unpublished) in the same simian adenoviral and MVA vectors and will soon enter clinical testing. If either or both of these new blood-stage vectored vaccine regimes show efficacy in phase IIa testing the objective would be to develop a combination

product with the ME-TRAP vectors. Adenoviral vectors have been used as mixtures in several previous HIV vaccine trials[21] without evidence of interference or antigenic competition, and MVA has the capacity to express multiple antigens from the same recombinant virus.

In parallel, two groups have been assessing human adenoviral vectors expressing CSP and/or AMA1 in US-based clinical trials. The US Navy-Genvec program using Ad5 vectors is described elsewhere in this volume and an NIH-Crucell collaboration has initiated a phase I clinical trial of an Ad35 vector expressing CSP.[39]

The Oxford-Okairos program aims to initiate the first phase Ib trials of the simian adenovirus-MVA approach in a malaria endemic area of Africa in late 2009 with support from the European and Developing Countries Clinical Trials Partnership and other funders.

Towards Higher Efficacy: Combination Approaches

Although significant efficacy in clinical trials has now been achieved with several vectored vaccine regimes encoding ME-TRAP the levels attained are probably still insufficient for deployment of this single antigen vaccine on its own.[40] But upcoming trials of blood-stage vectors may well lead to further efficacy and a combination of TRAP-expressing vectors and a blood-stage antigen vector may be more efficacious as suggested by several preclinical studies.

Another approach would be to assess the utility of combining the two existing subunit vaccines that show significant efficacy in human, RTS,S in adjuvant and TRAP-expressing vectors. RTS,S in the adjuvants AS01 and AS02 generates sterile protective immunity in some 32–50% of vaccines in sporozoite challenge trials[2] and similar levels of field efficacy in some[41] phase IIb studies in Africa.[42,43] Immunological analysis indicates strongly that antibodies are the likely main protective mechanism as T-cell responses induced are very modest.[44,45] Hence, this is an anti-sporozoite vaccine whereas vectored TRAP-based vaccines are anti-liver stage. We recently assessed in a pre-clinical *P. berghei* model in mice whether combined use of a protein-adjuvant based anti-sporozoite vaccine could add to the partial efficacy of a vectored vaccine against the liver-stage parasite.[46]

The results were striking (**Fig. 2**). Each vaccine individually afforded 33–35% sterile efficacy but the combination showed 90% sterile efficacy against sporozoite challenge. Immunological analysis showed that the combination induced both the antibody response induced by the protein-adjuvant vaccine plus the T-cell response induced by the vectors.[46] Hence it appears that there can be synergistic efficacy with this approach, probably resulting from a substantial reduction in sporozoite number effected by the antibodies making it much easier for the T cells to clear a far smaller number of infected liver cells. Data of this type make a compelling case for assessing RTS,S and TRAP vectors together in humans. Interestingly, the MVA vector itself has been found to have significant adjuvant activity when it is used in a mixture with a co-administered protein, at least in pre-clinical studies, and if this adjuvantation is also found in humans it may facilitate further combination vaccine strategies.[47]
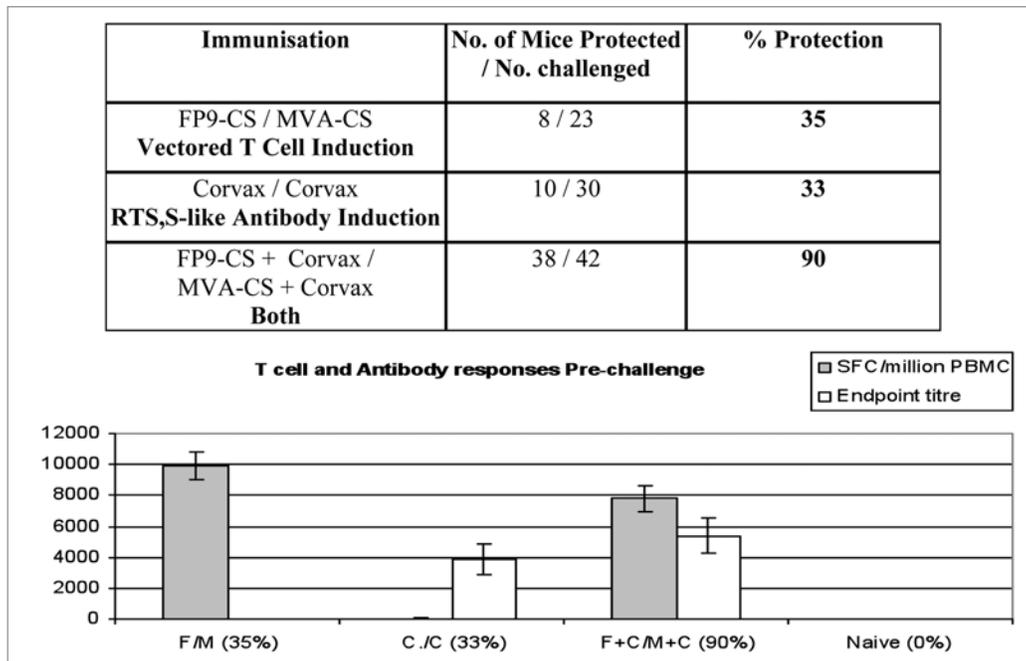
Immunisation	No. of Mice Protected / No. challenged	% Protection
FP9-CS / MVA-CS **Vectored T Cell Induction**	8 / 23	**35**
Corvax / Corvax **RTS,S-like Antibody Induction**	10 / 30	**33**
FP9-CS + Corvax / MVA-CS + Corvax **Both**	38 / 42	**90**

Figure 2. Analysis of the immunogenicity and protective efficacy of vectored vaccines and protein/adjuvant vaccines targeting the sporozoite and liver-stage of malaria, used singly and as a combination. The liver-stage vaccine is a fowlpox-MVA prime-boost regime encoding the circumsporozoite protein of *P. berghei*; the sporozoite vaccine is a recombinant hepatitis B core particle expressing the central repeats of the same protein. Sterile efficacy (protection) against sporozoite challenge is shown in the upper panel table. Immunogenicity results for T-cell responses and antibody as measured by ELISPOT and ELISA assays, respectively, are shown in the lower panel.[46]

Concluding Remarks

The progress with improved immunogenicity and efficacy of vectored vaccine regimes supports the view that these should be an important future component of highly effective deployable malaria vaccines. The immunogenicity of T-cell responses now being observed in malaria are about five to 10 fold higher than the best responses seen with earlier generation candidates and, importantly, improved immunogenicity continues to correlate with enhanced efficacy. However, the evidence that very strong immunogenicity will be required for sterile protection provides a sobering perspective on the challenges ahead.

More broadly this progress in malaria may have useful implications for other fields. In the heavily funded area of HIV vaccine development there was considerable despondency when a vaccination regime deploying only Ad5 adenovirus vectors failed to show any protective efficacy.[21] However the mean T-cell response in vaccinees to each antigen with this (non heterologous prime-boost) regime was of the order of 300 SFU/million PBMCs. This is lower that the malaria field achieved with first generation prime-boost vectors and much lower that in recent malaria trials. This provides an opportunity for the HIV field to adopt regimes that will allow the protective efficacy of broader and more abundant T cells to be assessed. Similarly, in TB and other disease areas where efficacy is more difficult and expensive to assess the differential abilities of adenovirus and MVA to prime and boost efficiently may be of value for the design of new vectored vaccination regimes. For the moment, malaria appears to provide the only clear example of a T-cell-inducing subunit vaccine providing repeatable, albeit partial, efficacy in vaccine trials, but success in other diseases should be close.

It is interesting to reflect on the comparative performance of DNA vaccines and viral vectors in human vaccine trials. Ten years ago there was enormous interest in the possibility that plasmid DNA vaccines[48] could provide an approach to inducing easily strong cellular as well as antibody responses in humans. Although those hopes have been dashed, viral vectors are now showing the performance that was anticipated by enthusiastic advocates of DNA vaccination. Equally importantly, substantial progress has been achieved in overcoming the challenge for viral vectors of genetic stability, large scale low cost manufacturing and avoiding anti-vector immunity. We are even gaining important insights from the field of innate immunity about the diverse pattern recognition receptors that underlie the remarkable potency of some vectors.[49] As reviewed recently, viral vectors now present a major option for controlling diseases ranging from HIV, TB and malaria to dengue, Japanese encephalitis, Ebola and pandemic influenza.[51]

References

1. Ballou WR. The development of the RTS,S malaria vaccine candidate: challenges and lessons. Parasite Immunol 2009; 31:492-500.

2. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy and immunologic associates of protection. J Infect Dis 2009; 200:337-46.

3. Draper SJ, Moore AC, Goodman AL, Long CA, Holder AA, Gilbert SC, et al. Effective induction of high-titer antibodies by viral vector vaccines. Nat Med 2008; 14:819-21.

4. Weiss WR, Kumar A, Jiang G, Williams J, Bostick A, Conteh S, et al. Protection of rhesus monkeys by a DNA prime/poxvirus boost malaria vaccine depends on optimal DNA priming and inclusion of blood stage antigens. PLoS One 2007; 2:1063.

5. Ockenhouse CF, Sun PF, Lanar DE, Wellde BT, Hall BT, Kester K, et al. Phase I/IIa safety, immunogenicity and efficacy trial of NYVAC-Pf7, a pox-vectored, multiantigen, multistage vaccine candidate for Plasmodium falciparum malaria. J Infect Dis 1998; 177:1664-73.

6. Bejon P, Andrews L, Andersen RF, Dunachie S, Webster D, Walther M, et al. 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; 191:619-26.

7. McConkey SJ, Reece WH, Moorthy VS, Webster D, Dunachie S, Butcher G, et al. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. Nat Med 2003; 9:729-35.

8. Dunachie SJ, Walther M, Epstein JE, Keating S, Berthoud T, Andrews L, et al. A DNA prime-modified vaccinia virus ankara boost vaccine encoding thrombospondin-related adhesion protein but not circumsporozoite protein partially protects healthy malaria-naive adults against Plasmodium falciparum sporozoite challenge. Infect Immun 2006; 74:5933-42.

9. Webster DP, Dunachie S, Vuola JM, Berthoud T, Keating S, Laidlaw SM, et al. 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 USA 2005; 102:4836-41.

10. Moorthy VS, Imoukhuede EB, Milligan P, Bojang K, Keating S, Kaye P, et al. A Randomised, Double-Blind, Controlled Vaccine Efficacy Trial of DNA/MVA ME-TRAP Against Malaria Infection in Gambian Adults. PLoS Med 2004; 1:33.

11. Bejon P, Mwacharo J, Kai O, Mwangi T, Milligan P, Todryk S, et al. 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; 1:29.

12. Bejon P, Mwacharo J, Kai O, Todryk S, Keating S, Lowe B, et al. The induction and persistence of T cell IFNgamma responses after vaccination or natural exposure is suppressed by Plasmodium falciparum. J Immunol 2007; 179:4193-201.

13. Bejon P, Ogada E, Mwangi T, Milligan P, Lang T, Fegan G, et al. Extended follow-up following a phase 2b randomized trial of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP among children in Kenya. PLoS ONE 2007; 2:707.

14. Keating SM, Bejon P, Berthoud T, Vuola JM, Todryk S, Webster DP, et al. 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; 175:5675-80.

15. Hill AV. Pre-erythrocytic malaria vaccines: towards greater efficacy. Nat Rev Immunol 2006; 6:21-325.

16. McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat Med 2004; 10:1240-4.

17. Gilbert SC, Schneider J, Hannan CM, Hu JT, Plebanski M, Sinden R, et al. Enhanced CD8 T cell immunogenicity and protective efficacy in a mouse malaria model using a recombinant adenoviral vaccine in heterologous prime-boost immunisation regimes. Vaccine 2002; 20:1039-45.

18. Rodrigues EG, Claassen J, Lee S, Wilson JM, Nussenzweig RS, Tsuji M. Interferon-gamma-independent CD8⁺ T cell-mediated protective anti-malaria immunity elicited by recombinant adenovirus. Parasite Immunol 2000; 22:157-60.

19. Vuola JM, Keating S, Webster DP, Berthoud T, Dunachie S, Gilbert SC, et al. Differential immunogenicity of various heterologous prime-boost vaccine regimens using DNA and viral vectors in healthy volunteers. J Immunol 2005; 174:449-55.

20. Nwanegbo E, Vardas E, Gao W, Whittle H, Sun H, Rowe D, et al. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa and the United States. Clin Diagn Lab Immunol 2004; 11:351-7.

21. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008; 372:1881-93.

22. Hutnick NA, Carnathan DG, Dubey SA, Cox KS, Kierstead L, Ratcliffe SJ, et al. Baseline Ad5 serostatus does not predict Ad5 HIV vaccine-induced expansion of adenovirus-specific CD4⁺ T cells. Nat Med 2009; 15:876-8.

23. O'Brien KL, Liu J, King SL, Sun YH, Schmitz JE, Lifton MA, et al. Adenovirus-specific immunity after immunization with an Ad5 HIV-1 vaccine candidate in humans. Nat Med 2009; 15:873-5.

24. Rodriguez A, Mintardjo R, Tax D, Gillissen G, Custers J, Pau MG, et al. Evaluation of a prime-boost vaccine schedule with distinct adenovirus vectors against malaria in rhesus monkeys. Vaccine 2009.

25. Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, et al. Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog 2009; 5:1000503.

26. Peruzzi D, Dharmapuri S, Cirillo A, Bruni BE, Nicosia A, Cortese R, et al. A novel chimpanzee serotype-based adenoviral vector as delivery tool for cancer vaccines. Vaccine 2009; 27:1293-300.

27. Sridhar S, Reyes-Sandoval A, Draper SJ, Moore AC, Gilbert SC, Gao GP, et al. Single-dose protection against Plasmodium berghei by a simian adenovirus vector using a human cytomegalovirus promoter containing intron A. J Virol 2008; 82:3822-33.

28. Reyes-Sandoval A, Sridhar S, Berthoud T, Moore AC, Harty JT, Gilbert SC, et al. Single-dose immunogenicity and protective efficacy of simian adenoviral vectors against Plasmodium berghei. Eur J Immunol 2008; 38:732-41.

29. Dudareva M, Andrews L, Gilbert SC, Bejon P, Marsh K, Mwacharo J, et al. Prevalence of serum neutralizing antibodies against chimpanzee adenovirus 63 and human adenovirus 5 in Kenyan children, in the context of vaccine vector efficacy. Vaccine 2009; 27:3501-4.

30. Okairos. www.okairos.it.

31. Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk C, Bryden J, Cloonan N, et al. Immunity to malaria after administration of ultra-low doses of red cells infected with Plasmodium falciparum. Lancet 2002; 360:610-7.

32. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, et al. Protection against a malaria challenge by sporozoite inoculation. N Engl J Med 2009; 361:468-77.

33. Ogun SA, Dumon-Seignovert L, Marchand JB, Holder AA, Hill F. The oligomerization domain of C4-binding protein (C4bp) acts as an adjuvant, and the fusion protein comprised of the 19-kilodalton merozoite surface protein 1 fused with the murine C4bp domain protects mice against malaria. Infect Immun 2008; 76:3817-23.

34. Draper SJ, Goodman AL, Biswas S, Forbes EK, Moore AC, Gilbert SC, et al. Recombinant viral vaccines expressing merozoite surface protein-1 induce antibody- and T cell-mediated multistage protection against malaria. Cell Host Microbe 2009; 5:95-105.

35. Walther M, Thompson FM, Dunachie S, Keating S, Todryk S, Berthoud T, et al. Safety, immunogenicity, and efficacy of prime-boost immunization with recombinant poxvirus FP9 and modified vaccinia virus Ankara encoding the full-length Plasmodium falciparum circumsporozoite protein. Infect Immun 2006; 74:2706-16.

36. Bodescot M, Silvie O, Siau A, Refour P, Pino P, Franetich JF, et al. Transcription status of vaccine candidate genes of Plasmodium falciparum during the hepatic phase of its life cycle. Parasitol Res 2004; 92:449-52.

37. Weedall GD, Preston BM, Thomas AW, Sutherland CJ, Conway DJ. Differential evidence of natural selection on two leading sporozoite stage malaria vaccine candidate antigens. Int J Parasitol 2007; 37:77-85.

38. Cox KS, Clair JH, Prokop MT, Sykes KJ, Dubey SA, Shiver JW, et al. DNA gag/adenovirus type 5 (Ad5) gag and Ad5 gag/Ad5 gag vaccines induce distinct T-cell response profiles. J Virol 2008; 82:8161-71.

39. Limbach KJ, Richie TL. Viral vectors in malaria vaccine development. Parasite Immunol 2009; 31:501-19.

40. Greenwood B, Targett G. Do we still need a malaria vaccine? Parasite Immunol 2009; 31:582-6.

41. Guinovart C, Aponte JJ, Sacarlal J, Aide P, Leach A, Bassat Q, et al. Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a phase IIb trial in Mozambican children. PLoS One 2009; 4:5165.

42. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomised controlled trial. Lancet 2004; 364:1411-20.

43. Bejon P, Lusingu J, Olotu A, Leach A, Lievens M, Vekemans J, et al. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. N Engl J Med 2008; 359:2521-32.

44. Pinder M, Reece WH, Plebanski M, Akinwunmi P, Flanagan KL, Lee EA, et al. Cellular immunity induced by the recombinant Plasmodium falciparum malaria vaccine, RTS,S/AS02, in semi-immune adults in The Gambia. Clin Exp Immunol 2004; 135:286-93.

45. Barbosa A, Naniche D, Aponte JJ, Manaca MN, Mandomando I, Aide P, et al. Plasmodium falciparum-specific cellular immune responses after immunization with the RTS,S/AS02D candidate malaria vaccine in infants living in an area of high endemicity in Mozambique. Infect Immun 2009; 77:4502-9.

46. 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; 75:5819-26.

47. Hutchings CL, Gilbert SC, Hill AV, Moore AC. Novel protein and poxvirus-based vaccine combinations for simultaneous induction of humoral and cell-mediated immunity. J Immunol 2005; 175:599-606.

48. Wang R, Doolan DL, Le TP, Hedstrom RC, Coonan KM, Charoenvit Y, et al. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. Science 1998; 282:476-80.

49. Delaloye J, Roger T, Steiner-Tardivel QG, Le Roy D, Knaup Reymond M, Akira S, et al. Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. PLoS Pathog 2009; 5:1000480.

50. Reyes-Sandoval A, Berthoud T, Alder N, Siani L, Gilbert SC, Nicosia A, Colloca S, Cortese R, et al Prime-boost immunization with adenoviral and modified vaccinia virus ankara vectors enhances the durability and polyfunctionality of protective malaria CD8+ T-cell responses. Infect Immun 2010; 78:145-53.

51. Draper SJ, Heeney JL. Viruses as vaccine vectors for infectious diseases and cancer. Nat Rev Microbiol 2010; 8:62-73.