A Polyprotein Multi-Stage Malaria Vaccine: Clinical Trials

Application to the European Malaria Vaccine Initiative

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by

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B3 Objectives and Expected Achievements

The development of an effective malaria vaccine is one of the most important challenges for public health. Recent advances in measuring the cellular immune response have facilitated the identification of more powerful immunisation strategies for inducing protective immunity mediated by T lymphocytes. The most promising strategy for inducing protective T cells against the liver-stage malaria parasite is the use of heterologous prime-boost immunisation regimes that have demonstrated efficacy in both animal models and in human phase IIa trials (Gilbert et al., 1997; Schneider et al., 1998; McConkey et al. 2002). We propose to test this novel approach in phase I / IIa clinical trials using an attenuated fowlpox (FP9) and modified vaccinia virus Ankara (MVA) vectors encoding the full sequences of many of the most promising malaria vaccine candidate antigens.

A large polyprotein has been constructed consisting of six *P. falciparum* antigens: LSA3/D260, STARP, Exp1, Pfs16, TRAP, LSA1 (Figure 1). All are expressed at the pre-erythrocytic stage of infection and three are expressed by asexual blood stage parasites and three by gametocytes. This insert has been expressed in both the FP9 strain of fowlpox and recombinant modified vaccinia Ankara vectors and been found to be immunogenic for effector T cell induction.

In 2001 EMVI funded the University of Oxford to complete the pre-clinical development of prime-boost vaccines encoding this insert to the stage of GMP manufacture and regulatory filing. The GMP manufacture is undertaken in Germany by Impfstoffwerke Dessau-Tornau. The work content of this award was modified by mutual agreement in early 2002 and the duration of the award extended to two years or allow completion of the manufacture of the fowlpox polyprotein construct.

It is proposed now to undertake a phase I / IIa clinical trial to assess the immunogenicity and protective efficacy of these vaccines in a heterologous prime-boost combination.

Thus the specific objectives will be:-

1. To assess the safety and immunogenicity of the polyprotein FP9 vaccine in healthy volunteers.

2. To assess the safety and immunogenicity of the polyprotein MVA vaccine in healthy volunteers.

3. To assess the safety and immunogenicity of the two vaccines used in prime-boost combinations in healthy volunteers.

4. To assess the protective efficacy of these vaccines using challenge with *P. falciparum* sporozoites delivered by mosquito bites.
B5 Innovation Aspects
With over a million deaths a year from *P. falciparum* malaria and increasing drug resistance, a malaria vaccine is a major global public health priority at the beginning of the 21st Century. This proposal aims to develop a novel polyprotein approach combined with some of the most immunogenic vaccine delivery systems available for inducing strong CD8 and CD4 T cell responses.

The initial rationale for the design of this polyprotein vaccine was the known protective efficacy of strong T cell responses against the liver-stage plasmodial parasite. Thus the selected antigens were chosen in 1999 on the basis that they were amongst the only characterised antigens known then to be expressed by pre-erythrocytic parasites. The main target of the T cells to be induced by this construct is the liver-stage parasite. Liver stage vaccines are attractive for several reasons. 1. A successful vaccine would be of value both to traveller and in endemic areas. Just as bed nets can reduce overall malarial mortality while being only partially effective, so also a liver stage vaccine, even if incompletely effective would be of great value in reducing global malaria mortality. 2. A valuable model of pre-erythrocytic protective immunity is available: the irradiated sporozoite vaccine (Nussenzweig *et al.*, 1967). Analysis of this model has revealed that such protection is mainly mediated by T cells against pre-erythrocytic stage antigens, and in most systems CD8 T cells are of most protective importance (Schofield *et al.*, 1987; Weiss *et al.*, 1988). However, CD4 T cells can clearly also be protective (Wang *et al.* 1996) in some animal models. 3. An effective and ethical challenge system for assessing the efficacy of malarial vaccines is available (Church *et al.*, 1997). Although originally used in the United States this challenge system in now in regular use in Europe and over 100 volunteers have been challenged with *P. falciparum* sporozoites.

A research programme based in Oxford identified target antigens and epitopes for CD8 T cells in naturally exposed populations (Hill *et al.*, 1992; Aidoo *et al.*, 1995; Plebanski *et al.*, 1997) and more recently focused on identifying the most immunogenic approach for inducing CD8 T cells by vaccination. It was been found that numerous pre-erythrocytic antigens are targeted by CD8 T cells and six of these are contained in the vaccines to be tested. Importantly a highly immunogenic prime-boost regime has been described that induced five to ten times higher levels of CD8 T cells than all other systems tested (Schneider *et al.*, 1998). This entails priming of the immune response by one delivery system, e.g. plasmid DNA or a fowlpox vector, and then boosting the primed immune response with a different non-replicating recombinant poxvirus encoding the same insert. The most suitable poxvirus vector is the modified virus Ankara strain of vaccinia virus, originally isolated by Mayr in Munich and used in the smallpox eradication campaign (Meyer *et al.*, 1991). A DNA-MVA prime-boost regime was the first subunit vaccine approach to induce CD8 T cell-mediated complete protection against sporozoite challenge in rodent malaria. The reverse order of immunisation was ineffective. Similar results were later obtained by US investigators using the NYVAC strain of vaccinia as a boosting agent in the *P. yoelii* rodent malaria model (Sedegah *et al.* 1998). Comparable very high levels of immunogenicity for CD8 T cells in primates were observed in extensive studies of DNA-MVA SIV vaccines by the groups of McMichael and Robinson (Hanke *et al.*, 1998; Hanke *et al.*, 1999, Amara *et al.* 2001) and in more limited studies of chimpanzees with malaria constructs (Schneider *et al.*, 2001). We and others have found that fowlpox and NYVAC poxvirus recombinants can also be effectively used as boosting agents in both mice and macaques (Kent *et al.*, 1998; Robinson *et al.*, 1999) but the greatest amount of experience has been with the MVA strain virus. DNA-MVA immunisation strategies are now being assessed clinically in several diseases (malaria, hepatitis, melanoma, HIV, tuberculosis), but the most extensive data are available for malaria and are summarised briefly below.

A frequently contentious issue is the choice of antigen to be included in malaria vaccines. There are now more than half a dozen well-characterised pre-erythrocytic antigens...
all of which are suitable for further development as vaccine candidates. However many more antigens are becoming available from the malaria parasite genome project and the entire sequence of the 3D7 strain of *P. falciparum* has just been published (Gardner et al. 2002). Accompanying papers described the expression stage of many of the encoded proteins using sensitive proteomic and mass spectrometry techniques (Lasonder et al. 2002; Florens et al. 2002). This has provided a much fuller picture than previously available of the stage-specificity of expression of many candidate vaccine antigens. The constructs to be tested encode almost all of the unique sequence of six *P. falciparum* antigens in a single polyprotein construct. The encoded insert of over 3200 amino acids contains the genes for: LSA3, STARP, Exp1, PfS16, TRAP and LSA1. After much consideration, we decide to omit the CS antigen as it is highly polymorphic and has variable epitopes shown to act as altered peptide ligand antagonists of cellular immune responses (Gilbert *et al.*, 1998). This affects both primary (Plebanski *et al.*, 1999b) and secondary CD8 T cell responses and also the TH1-TH2 profile of CD4 T cells, with some variants inducing a switch from interferon gamma to IL-10 secretion (Plebanski *et al.*, 1999a). However vectors expressing this CS antigen alone have recently been made to GMP under separate funding and ultimately these different vaccines could be used as mixtures if necessary.

LSA-1 is the only antigen believed to be expressed only at the liver stage of infection (Guerin Marchand *et al.*, 1987; Zhu & Hollingdale, 1991) and was implicated in protective immunity by our analysis of the mechanism of a protective HLA-B*53 association in Gambian children (Hill *et al.*, 1992). More recent epidemiological studies also support a protective role for immune responses against this antigen (Kurtis *et al.* 2001). TRAP (Robson *et al.*, 1988), also known as PfSSP2 (Rogers *et al.*, 1992), is the homologue of protective antigens in both *P. yoelii* (Khushmith *et al.*, 1994) and *P. berghei* (Schneider *et al.*, 1998) and numerous CD8 and CD4 T cell epitopes are defined. It was a component of the NYVAC-Pf7 vaccine (Ockenhouse *et al.*, 1998) and T cell responses against this antigen are observed in volunteers immunised with irradiated sporozoites (Wizel *et al.* 1995). PfExp1 (Simmons *et al.*, 1987) is the homologue of a protective antigen in *P. yoelii* (Doolan *et al.*, 1996), that is expressed by both the late liver-stage and blood stage parasite, and CTL epitopes have been identified (Doolan *et al.*, 1997; Aidoo *et al.* 2000). LSA3 (Marchand & Druilhe, 1990; BenMohamed *et al.*, 1997), also known as D260 (Barnes *et al.*, 1995) and RESA-H3 (Gardner *et al.*, 1998), was described by Druilhe and colleagues as a pre-erythrocytic antigen but is also expressed by the blood-stage parasites (Barnes *et al.*, 1995; Florens *et al.* 2002; Lasonder *et al.* 2002). Recent studies demonstrating protective efficacy against *P. falciparum* sporozoite challenge (Daubersies *et al.* 2000) in chimpanzees support its evaluation in phase I studies. STARP is another pre-erythrocytic antigen that appears to manifest relatively limited polymorphism, is the target of CTL in Africans and is expressed by sporozoites and possible early blood-stage parasites (Fidock *et al.*, 1994 a and b). Finally, the small antigen, PfS16, is expressed by sexual stage parasites and probably by sporozoites too (Moelans *et al.*, 1991a and b; Moelans *et al.*, 1995). New information from proteomic studies have recently shown that the stage-specificity of expression of some of these antigens may be wider than originally estimated. Thus Lasonder *et al.* (2000) find that STARP and Exp1, in addition to PfS16, are expressed by gametocytes, and in addition to blood-stage expression of LSA-3 and PfExp 1, PfS16 was also found in trophozoites.

Thus there is evidence that all six antigens are expressed by pre-erythrocytic stage parasites, at least three by asexual blood stage parasites and three by gametocytes. Although the DNA-poxvirus prime boost strategy is strikingly immunogenic for CD8 T cell responses (Schneider *et al.*, 1998) it also manifests strong immunogenicity for gamma-interferon secreting CD4 T cell responses in humans and animals (McShane *et al.* 2001; McConkey *et al.* 2002) and for antibody responses (Sedegah *et al.*, 1998) in animal models. Therefore this construct should generate a much broader from of protective immunity than polyepitope strings encoding multiple CTL epitopes (Gilbert *et al.*, 1997).
The potential importance of the inclusion of blood-stage antigens in prime-boost vaccine vectors has been emphasized by recent studies. Firstly, in clinical trials of FP9-MVA and DNA-MVA vaccination using the ME-TRAP malaria insert remarkably high levels of T cell responses have been induced and over half of the T cells responding are of the CD4 phenotype (McConkey et al. 2002). Thus this immunisation strategy has induced the highest levels of effector CD4 T cell responses recorded in any clinical vaccination trial (McConkey et al 2002). Secondly, recent studies from Michael Good’s group in Australia have provided compelling evidence that T cell responses against blood stage antigens can protect against malaria independently of antibody. In murine studies CD4 T cells against blood-stage antigens have been protective in adoptive transfer studies (Wipasa et al 2002) and in humans low dose blood-stage parasite immunization has induce protective immunity to blood-stage malaria in the absence of detectable antibody (Pombo et al. 2002). These recent studies indicate that T cell induction against the polyprotein antigens expressed by blood stage parasites could provide an important degree of protection supplementary to that provided by T cell responses induced against antigens expressed by the liver-stage parasite. Thus, in effect, the polyprotein vaccination strategy proposed offers the prospect of a multistage multi-antigen malaria vaccine.

The six antigen polyprotein construct is illustrated in the appended figure. This insert is about 10 Kb in size and expresses a polyprotein of 3,240 amino acids. To our knowledge this will be by far the largest inserted gene product to enter clinical vaccine trials. This construct has been made by E Prieur in Oxford while a Marie Curie EC-funded fellow. There is a short flexible linker, of amino acid sequence glycine-glycine-glycine-proline-glycine-glycine-glycine, sequence between each pair of antigens. Transfection studies, immunostaining and western blot analysis have confirmed the expression of each of the six antigens in individual plasmid DNA constructs and in the six antigen polyprotein construct (Prieur et al. 2002). The same 3240 amino acid-encoding insert has also been expressed in the MVA and FP9 viruses. FP9 is an attenuated strain of fowlpox virus and unpublished data from this lab show that, at least in mice, FP9 is more immunogenic for CD8 T cell induction than other fowlpox strains (Anderson et al. unpublished). Also FP9-MVA immunisation was more immunogenic and more protective in the mouse P. berghei model than DNA-MVA immunisation. Vaccinia transcription termination sequences were removed from the insert sequences prior to ligation of the polyprotein gene components. The immunogenicity of plasmid DNA, MVA and FP9 polyprotein constructs has been assessed in immunogenicity studies in mice using ELISPOT, tetramer and lysis assays with excellent immunogenicity using the virus-virus heterologous prime-boost regimes. However, weaker immunogenicity of the DNA plasmid construct was found leading to the decision to progress the MVA and FP9 but not the DNA vector to clinical studies. Pre-clinical data with these vectors is summarised in the appended manuscript (Prieur et al. 2002).

As well as containing multiple potentially protective antigens there are additional reasons for using a polyprotein construct as a malaria vaccine. The immunogenetic diversity of human populations implies that different antigens and epitopes may be of most protective relevance in different populations. Furthermore, the extensive polymorphism of many malarial antigens implies that a reserve of other epitopes for a particular HLA type needs to be included in any construct lest an individual fail to recognise particular epitopes due to sequence polymorphism in a particular parasite epitope or antigen. Similarly a multiplicity of antigens in a vaccine should slow the emergence of vaccine escape mutations in a successfully immunised population.
Recent and Ongoing Work

Construct Development

The polyproteins insert has been expressed in the highly immunogenic and attenuated strain of fowlpox, FP9, that was recently used in clinical trial of another malaria insert (ME-TRAP) in Oxford. This recombinant showed substantial immunogenicity in mice particularly when used in prime-boost regimes with MVA (Prieur et al 2002). This recombinant was made with GFP as a selectable marker and a recombinant without this marker is currently been constructed. The polyprotein insert has also been expressed in the ALVAC (canarypox) avipox vector and this virus will be used along with overlapping peptides for immunogenicity assays in the proposed clinical trial.

Manufacture

The MVA polyprotein construct has been bulked up in Oxford and sent to the contract manufacturers IDT where a master seed virus (MSV) and working virus bank were constructed. A manufacturing batch was made in 2001-2002 and most of the planned QA/QC tests are now completed with satisfactory results. This included several test at the manufacturers including sterility, endotoxin content, tests for extraneous agents in chickens and guinea pigs and cell lines, and also virus titre. Small animal toxicology and biodistribution studies have been completed to GLP standard at Huntingdon Life Sciences in the UK and at the University of Oxford. Potency and purity assays have been completed at Oxford. Sequencing of the insert is underway and should be completed shortly. It is planned to submit a DDX application for a clinical trial to the Medicine Control Agency in London in early 2003.

The FP9 construct will follow similar manufacturing protocols and QA/QC tests. IDT have experience of manufacturing a previous FP9 construct and we anticipate no difficulty with this manufacture. It is aimed to have all toxicology and QC tests on the clinical grade FP9-polyprotein completed by the end of the current funding period, September 2003.

Ethical Approval

Ethical permission for the clinical trial will be sought from the Oxford Clinical Research Ethics Committee. This committee has now assessed numerous malaria vaccine trial protocols including studies of FP9-MVA prime-boost immunisation using the ME-TRAP insert. We anticipate no particular difficulty in obtaining ethical approval for the proposed trials.

Update on Recent and Ongoing Prime-Boost Immunisation Studies

Since 1999 a programme of phase I / IIa studies of DNA and viral vector candidate malaria vaccines studies has been in progress at Oxford funded mainly by the Wellcome Trust with more recent support from the Malaria Vaccine Initiative at PATH. In 2000 phase I trials of the same vaccines started in The Gambia. In most trials the insert in the vectors has been a multi-epitope string (Gilbert et al.1997) fused to the T9/96 strain of TRAP (denoted ME-TRAP) but recently circumsporozoite protein inserts have also been assessed. These studies have, firstly, demonstrated the safety of DNA, MVA and FP9 as vaccine vectors in, respectively, 83, 160 and 60 (UK or Gambian) volunteers. Findings to date also support the view that these non-replicating viral vectors could be widely and safely used in Africa. Notably, MVA and other non-replicating poxvirus vectors have now been used by others as potential therapeutic vaccines in several studies in HIV-infected individuals with good safety findings. This supports evidence from studies of MVA in severely immunosuppressed macaques (Stittelaar et al. 2001) indicating that these non-replicating viral vectors should be safe to use in HIV positive individuals. In these clinical studies T cell immunogenicity has been impressive with average immune responses exceeding 500-1000 specific cells per million peripheral blood mononuclear cells with some prime-boost immunisation regimes.
(McConkey et al. 2002). These are the highest levels of T cell immunogenicity ever reported after subunit vaccination in humans. Higher doses of DNA and the viral vectors have been more immunogenic than lower doses with 1-1.5 \times 10^8 pfu a highly immunogenic dose of both MVA and FP9. Sporozoite challenge studies with a heterologous 3D7 strain of \textit{P. falciparum} has shown highly statistically significant, but generally partial, protection with both FP9-MVA and DNA-MVA immunisation regimes. More volunteers have been fully protected with the former (FP9 priming) regimes but overall reductions in estimated liver-stage parasite burdens have been comparable: of the order of an 80% reduction in liver parasite burden. Because the dose of parasite used in the sporozoite challenge model is about 10 times that of a typical field bite we would anticipate observing higher levels of overall protection in the field. Three phase I studies have also been undertaken with DNA, FP9 and MVA in semi-immune adults in The Gambia and also with MVA in young children - with good safety findings and similar immunogenicity to that observed in non-immunes (Moorthy et al unpublished). Responses induced by prime-boost immunisation to TRAP are ten times greater that those observed in healthy east or west African adults who have had lifelong malaria exposure. A phase IIb trial of prime-boost immunisation with ME-TRAP vaccines has recently begun in 372 Gambians and efficacy data will be available in early 2003.

Thus although significant but partial efficacy is achievable with ME-TRAP as an insert we anticipate that better efficacy may be obtainable with the polyprotein insert.

B6 Project Work plan

Clinical Trial

We propose to undertake the phase I and I / Ia trials (WP1 and WP2) in Oxford, building on our experience of such trials in recent years. We shall use the facilities of the new phase I vaccine testing unit in Oxford in the new Centre for Clinical Vaccinology and Tropical Medicine. For sporozoite challenge studies we shall continue to collaborate with R Sinden and G Butcher to undertake the actual challenges at Imperial College London. To date just over a hundred Oxford volunteers have been challenged in vaccine studies as part of this collaboration.

Although the primary aim is to study prime-boost combinations, for safety reasons each vaccine will first be tested individually (WP1). Four volunteers will be immunised with 5 \times 10^7 pfu of the MVA vaccine on two occasions and, if there are no safety concerns, later four further volunteers will be vaccinated with 1 \times 10^8 pfu of MVA on two occasions. Inter-vaccination intervals will be of 3 – 4 weeks. MVA will be administered intradermally as in all malaria, HIV, tuberculosis, melanoma and hepatitis trials undertaken to date.

Based on these phase I studies we shall select a dose of each vaccine to use in the phase I / Ia prime-boost study (WP2). This will use heterologous prime-boost immunisation regimes as we have established that such immunisation regimes are far more immunogenic for T cell responses than homologous immunisation in humans as well as animals (McConkey et al. 2002). We have the capacity to challenge up to 24 volunteers in one study and six of these should be non-vaccinated controls to allow comparison with the vaccines. We shall therefore have the capacity to challenge 18 vaccinees; this number will allow comparison of two immunisation regimes with nine volunteers receiving each. These regimes will be two immunisations with the FP9 vaccines followed by a single booster immunisation with MVA compared to the converse. The converse is two immunisations with the MVA vaccine followed by a single booster immunisation with the FP9 vaccine. This proposed immunisation regime is based on experience with ME-TRAP vaccines where we have found that two priming immunisations are better than one, but little different to three; and a single booster immunisation appears to be adequate for high level immunogenicity. In preclinical studies with these vaccines there has been limited evidence that the regime with FP9
administered second may be slightly better (Prieur et al. 2002) but this requires assessment in humans.

For all groups safety and reactogenicity will be assessed using standard vaccination clinical protocols. Haematology and biochemical assays will performed at intervals through the study. A sample clinical trial protocol used for a recent study is available on request.

**Immunogenicity Measurements**

Immunogenicity will be monitored using a variety of assays as follows. The primary objective is to induce a strong protective effector T cell response so T cell assays are of most interest.

1. ELISPOT assays to determine the numbers of both CD4 and CD8 gamma-interferon producing cells specific to the polyprotein (e.g. Lalvani et al., 1997). We have found that ex-vivo ELISPOT assays are the most sensitive means of quantifying vaccine-induced CD4 and CD8 T cell responses and this phenotype is likely to be of direct protective relevance (unpublished data). We shall use overlapping peptides to measure the malaria specific responses. This will allow the overall T cell response to each of the six malaria antigens to be quantified. CD4 and CD8 T cell responses will be distinguished by negative selection of the respective T cells using magnetic beads.

3. Chromium release cytotoxicity assays will be performed using both selected peptides when CD8 responses are detected by ELISPOT. Tetramers (Altman et al., 1996; Ogg & McMichael, 1998) may also be used for selected epitopes.

4. Antibody levels to sporozoites, blood-stage parasites and individual proteins will be measured by ELISA.

**Challenge Study**

In the challenge studies undertaken to date we have followed closely the protocol used over several years mainly by north American investigators (Church et al., 1997), but we have introduced a few modifications that we see as improvements. The protocol entails challenge with five infectious mosquito bites. 18 vaccinees and six non-immunised controls will be studied. The 3D7 strain of *P. falciparum* provided by D. Walliker is used. Thus, as with the ME-TRAP vaccine studies we shall be undertaking a heterologous strain challenge as the vaccine antigens used are cloned from the T9/96 strain of *P. falciparum*. Anopheles stephensi mosquitoes originally from India are used and infected by an established and standardised protocol. Volunteers are monitored twice daily from day 6.5 after challenge for blood-stage parasites. If any parasite is detected on blood film examination the volunteers will be treated immediately.

Unlike those in north American studies, our challenged volunteers are allowed home each night but are always contactable by mobile phone. In addition we use a very sensitive PCR technique twice a day during the study to provide real time monitoring of parasite density. This 18S RNA gene PCR method is based one developed at Nijmegen (Hermsen et al. 2001). Delay in time to parasitaemia will be compared in vaccines and controls by Kaplan Meier analysis.

**Competing Approaches**

There are various other types of pre-erythrocytic vaccine that we are aware of in clinical trials. DNA vaccines alone are being developed by the US Navy but to date have shown only modest immunogenicity and no protective efficacy against sporozoite challenge (Wang et al., 1998).

A multiple antigen peptide vaccine with the NANP of the CS protein fused to a T cell epitope is being developed by E Nardin and colleagues (Nardin et al., 1995). This was immunogenic for antibody responses only in individuals of specific HLA type (Nardin et al. 2000).
A long peptide CS vaccine is being developed by G Corradin et al. in Switzerland; some immunogenicity data have been reported but no sporozoite challenge studies (Lopez et al 2001).

The pre-erythrocytic vaccine that has progressed furthest, to a phase IIb trial in The Gambia, is the RTS,S vaccine which is a HBsAg-CS fusion protein in a proprietary adjuvant. Although significant partial efficacy is achieved against homologous sporozoite challenge, protection is short lived both in sporozoite challenge studies and in the field (Stoute et al., 1997; Stoute et al., 1998; Bojang et al 2001). However, a large scale phase IIb efficacy trial is in progress in children in Mozambique.

**Timetable**

The details of the timetable for each part of the study are shown in the workpackages. The time scale outlined is realistic based on previous experience of trials in Oxford with other vaccine constructs.

**State of Development of the Delivery Systems to be Used**

MVA, modified vaccinia virus Ankara, is one of a large class of poxviruses that do not replicate in mammalian cells and that are now seen to have considerable potential for human immunisation particularly as part of heterologous prime-boost immunisation regimes. This class includes the avipoxviruses, ALVAC (canarypox) and fowlpox, and other replication-impaired vaccinia strains such as NYVAC, which was used in a phase I malaria vaccine trial in the USA (Ockenhouse et al., 1998). The FP9 strain of fowlpox in an attenuated virus that has been used as a vaccine against fowlpox in fowl. Like all avipoxviruses it cannot replicate in any mammalian cell (Somogyi et al. 1993). There have been numerous human trials of ALVAC recombinants (Belshe et al., 1998) and a smaller number of NYVAC recombinants. Recently MVA has been in clinical trials as a potential vaccine for several diseases – malaria, hepatitis B, tuberculosis, HIV and melanoma. MVA has been found to be non-pathogenic in severely immunosuppressed macaques (Stittelaar et al. 2001) and has been safely administered to some HIV positive individuals. It was widely used in Germany in the 1970s as a smallpox vaccine. This extensive experience has established the safety of these recombinants for human use and useful GMP manufacturing experience has been gained at IDT. Satisfactory GMP manufacturing protocols have been developed to grow virus in chick embryo fibroblasts, which are also used for manufacture of many common viral vaccines such as rubella. A poxvirus purification procedure to produce MVA or FP9 in batches of the order of $10^{11}$ pfu is established. This allows phase I / II studies to be undertaken but further process development will be required for large phase II studies. However, poxviruses can be produced at low cost and distributed easily after lyophilisation. The success of the smallpox campaign demonstrated this superbly. Thus poxvirus recombinants, if effective, would be particularly suitable for malaria vaccination in low-income countries.

**Ethical and Environmental Considerations**

There are several ethical considerations in the proposed study. The first is the safety of the vaccine constructs to be administered

The recombinant MVA virus to be used is one of a large class of poxviruses of which there is now extensive experience in vaccine studies. The vaccinia virus that was used extensively to immunise against smallpox, is a replicating virus and had a well defined but imperfect safety profile. As noted above, MVA was widely used in >100,000 people as a smallpox vaccine (Mahnel & Mayr 1994) and recent safety data in humans using MVA recombinants over the last three years have been reassuring with no vaccine-induced serious adverse events (unpublished data). All available evidence suggests to us that MVA as well as FP9 will also be safe to use in HIV positive vaccines. However, we shall not be immunising any HIV infected or otherwise immunocompromised individuals in the proposed trial.
Different and further ethical considerations apply in the proposed challenge studies. Volunteers will be recruited explicitly for an immunogenicity or an immunogenicity plus challenge study (i.e. individuals showing high immunogenicity in the former will not be requested later to undergo challenge). They will be fully informed of the likely symptomatology of a malaria attack (Church et al., 1997). Vaccinees and controls will be made aware of their high likelihoods of experiencing significant symptoms. The experience with challenge studies in the USA has been reviewed recently and the challenge protocol is well established and reproducible (Hoffman, 1997) and our own experience supports this assessment. In contrast to the use of challenge studies to assess blood–stage vaccine there is general agreement in the field concerning the acceptability of sporozoite challenge to assess pre-erythrocytic vaccines. Although these vaccine constructs may induce useful blood-stage immunity we shall not attempt to measure this directly in the challenge studies.

There are also some environmental issues to be considered. The use of recombinant MVA and FP9 is regulated by the legislation on the use of genetically modified organisms. This trial will be conducted on a contained-release, as distinct from a deliberate release, basis and we are familiar with the procedures required by the UK Health and Safety Executive to avoid or minimise potential environmental release. These will be observed. Finally, detailed discussions with the relevant safety offices ensured that infectious mosquitoes are handled routinely with minimal risk to the investigators, volunteers and the environment.

**Indemnity**
The proposed clinical trials will be undertaken by University investigators who will be insured under the University of Oxford’s clinical trials insurance scheme with an insurance company (currently Sun Alliance).

**Further Development Plan**
If these vaccines are found to be safe and immunogenic and induce efficacy comparable to or better than the most protective vaccines that have been assessed at the time the challenge study ends, we shall discuss a plan for further development of these vaccines with EMVI. This could include early field studies of this approach in Africa and rapid progression to a phase IIb efficacy study. Further studies in Europe on the duration of vaccine induced immunity and some regime optimization studies may also be required.

**European Dimension**
The polyprotein is very much a European vaccine. Two of the key contributors to its generation have been French and German Marie-Curie fellows. Three of the six antigens included were discovered in France, two in the UK and one in the Netherlands. FP9 and MVA originated in Germany; the prime-boost approach with FP9 and MVA came from the UK and prime-boost immunisation with MVA boosting was first used in primates in the Netherlands. The Oxford laboratory has strong collaborative interactions with numerous other malaria groups in Europe, through several EU-supported programmes, and these will all contribute directly or indirectly to the execution of the proposed trial.
### Budget

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<td>- 18 phase I/IIa volunteers</td>
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<td>- 6 challenge controls</td>
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<td>(34 vaccinees @ 1.125K euros each; 24 challengees @ 3.75K euros each)</td>
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<td>Sporozoite costs =</td>
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<td>Total: 357,000 Euros + 20% overheads = 428,400 Euros</td>
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#### Budget Justification

The budget request includes only marginal costs required for the undertaking the clinical trial. Support is not requested for the principal investigator’s salary, nor for the costs of PCR monitoring of the trial nor for statistical, project management nor nursing support. These will be provided by ongoing programme support to the Oxford research group by the Wellcome Trust.

**Salaries** These are quoted at UK university national rates. A clinician is required to write protocols undertake review committee submissions and undertake the clinical studies. A post-doctoral scientist will undertake the immunoassays and laboratory work including the vaccine potency and stability studies.

**Consumables** These consumables cost cover the reagents required for immunological assays performed on samples from the volunteers. This includes a very large number of peptides, ELISPOT kits and generation of some viral reagents for immunoassays.

**Volunteer Clinical costs**. Currently costs for vaccinees (n= 34 in the proposed work) are about 1,125 Euros per volunteer and for challengees about 3,750 Euros (n= 24 in the proposed work) This covers the costs of volunteer remuneration and expenses, recruitment costs including advertising, a facility fee for the use of the Oxford Vaccine Centre, safety and screening bloods and the transport costs of the volunteers during the challenge study. Safety bloods include the costs of the blood assays (haematology, biochemistry, virology etc) required for each volunteer, typically nine sets of assays per volunteer in an immunogenicity study. An estimated 1500 such assays will be required for the 34 selected volunteers.

**Sporozoite Costs**. This sum represents a contribution to the ongoing insectary costs of the Imperial College research group (R Sinden and G Butcher) supplying the *P. falciparum* sporozoite-infected mosquitoes suitable for human challenge studies.
WP1. Phase I Safety and Immunogenicity Study of the Polyprotein Vaccines

Objectives
1. To assess the safety and immunogenicity in human volunteers of a new polyprotein recombinant MVA vaccine
2. To assess the safety and immunogenicity in human volunteers of a new polyprotein recombinant FP9 vaccine
3. To compare the safety and immunogenicity of two different doses of each of these vaccines.

Description of Work
MVA polyprotein study. Four volunteers will be immunised twice at three weekly intervals intradermally with $5 \times 10^7$ pfu of the MVA polyprotein. Blood samples will be taken for safety and immunogenicity assays at weeks 0, 1, 3, 4, 6 and 12 after immunisation. If safety data are satisfactory a further four volunteers will be immunised as before but with a higher dose of $1 \times 10^8$ pfu MVA.

FP9 polyprotein study. Four volunteers will be immunised twice at three weekly intervals intradermally with $5 \times 10^7$ pfu of the FP9 polyprotein. Blood samples will be taken for safety and immunogenicity assays at weeks 0, 1, 3, 4, 6 and 12 after immunisation. If safety data are satisfactory a further four volunteers will be immunised as before but with a higher dose of $1 \times 10^8$ pfu FP9.

It may be possible to conduct the FP9 and MVA phase I studies in parallel subject to a sufficient number of volunteers being available at the time.

Immunological evaluation will be primarily by the use of pools of overlapping peptides from each antigens using ELISPOT assays for gamma-interferon release. These will be conducted both \textit{ex vivo} and with a 10 day restimulation; the former measures circulating activated T cells, the latter assays the memory T cell population. CD4 and CD8 response will be distinguished by magnetic bead depletion and in the case of strong CD8 T cell responses, lysis assays performed. If strong CD8 T cell responses are observed to particular epitopes we shall make tetramer peptide-MHC reagents for these and characterise the induced cells more fully. Antibodies will be measured by ELISA assays and immunofluorescence assays on sporozoites and blood-stage parasites.

Deliverables


Timetable and Milestones
1-2 months: volunteer recruitment.

3-6 months: safety and immunogenicity studies of both vaccines

6-9 months: data analysis
WP2. Phase IIa Clinical Trial of a Prime-Boost Immunisation Strategy with Sporozoite Challenge

Objective

1. To assess the safety and immunogenicity of polyprotein FP9-MVA and MVA-FP9 heterologous prime-boost immunisation regimes.
2. To assess vaccine-induced protection against challenge with *P. falciparum* sporozoites in selected volunteers using a well-established and safe challenge protocol with sensitive PCR monitoring.
2. To identify any immunological correlates of protection in the vaccinees.

Description of Work

Volunteers will be immunised with the following regimes:

Group A: Two FP9 polyprotein immunisations intradermally at three week intervals followed by a single MVA intradermal immunisation after a further three weeks.

Group B: Two MVA polyprotein immunisations intradermally at three week intervals followed by a single MVA intradermal immunisation after a further three weeks.

There will be nine volunteers per group and the vaccine dose will be chosen based on the phase I study data, but we anticipate that this is likely to be 1 x 10^8 pfu. Blood samples will be taken for safety and immunogenicity assays at weeks 0, 1, 3, 4, 6, 7 after immunisation and also on the day of challenge. Sporozoite challenge will take place at week 8, i.e. two weeks after the last immunisation. Use of this timepoint allows comparison with earlier data on many different vaccination approaches using this time point for challenge.

We now have extensive experience with a sporozoite challenge protocol that is based on the Walter Reed protocol used in the USA and we shall use this again in this workpackage. The 18 vaccinees and 6 controls will be challenged two weeks after the last immunisation with five bites from *Anopheles* mosquitoes carrying sporozoites of the chloroquine-sensitive 3D7 strain of *P. falciparum*. These mosquitoes will be provided by collaborators, G Butcher and R Sinden at Imperial College London, where the actual challenge study is performed. Monitoring of malaria infection will be by twice daily blood film and PCR analysis for blood-stage parasites. Treatment will be instituted as soon as any microscopic evidence (but not PCR) of blood-stage infection is found. Efficacy will be compared both by chi-squared analysis of the numbers of infected and non-infected vaccinees and controls and by a Kaplan-Meier analysis of the time to parasitaemia in the two groups.

Finally, assuming at least partial efficacy of the immunisation approach, correlates of protection will be sought with immunological variables, particularly those measured on the day of challenge.

Deliverables

Ethical approval of the challenge protocol by the Oxford Research Ethics Committee.

Safety and immunogenicity data on the polyproteins vaccines used in heterologous prime-boost immunisation regimes

Assessment of the protective efficacy of these vaccines in human malaria-naïve volunteers against *P. falciparum* sporozoite challenge.

An indicator of the likely efficacy of this immunisation regime against field challenge with malaria.
A correlate of protective immunity to malaria induced by this immunisation approach

**Timetable and Milestones**

6-9 months: volunteer recruitment

9-15 months: conduct of the phase IIa study

15-18 months: volunteer follow-up and data analysis
REFERENCES


containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations. Nat Med, 5, 526-34.


responses against two overlapping epitopes of the *Plasmodium falciparum* sporozoite surface protein.


Appendix: Structure of the Polyprotein Insert

Legend: Arrangement of the antigens in the polyprotein. Antigens are labelled with their standard abbreviations – see text. The length of the encoded protein is shown in amino acids. The seven amino acid flexible linker is shown between LSA3 and STARP; the same linker exists between each other pair of antigen genes. Repeat sequences are show with cross-hatching.