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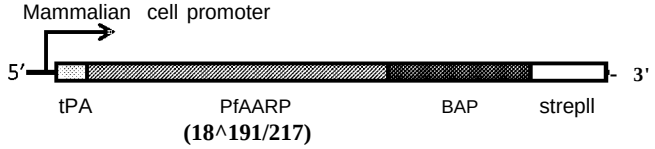
(54) Title: TREATMENT AND PREVENTION OF MALARIA

Figure 1A



(57) Abstract: The present invention relates to antigens, antibodies and vaccines for treatment or prevention of malaria.

TREATMENT AND PREVENTION OF MALARIA

The present invention relates to antigens, antibodies and vaccines for treatment or prevention of malaria.

The infection of red blood cells (RBCs) by the blood-stage form of the *Plasmodium* parasite is responsible for the clinical manifestations of malaria. Examples of *Plasmodium* parasite include the species *P. falciparum, P. vivax, P. ovale* and *P. malariae.* The parasite of particular interest is *P. falciparum,* as it is this parasite which causes the most lethal infections since it can infect RBCs of all ages and is not limited to immature RBCs. *P. falciparum* alone is responsible for around a million deaths per year, mainly in children.

Current vaccine candidates based on the RTS.S protein particle, which acts by blocking infection of *P. falciparum* in the liver, have achieved only partial efficacy. There is therefore a need for a vaccine which can emulate natural immunity by protecting against the disease-causing blood-stage *Plasmodium* parasite. Previous studies have investigated the potential for antigens to induce antibodies which are effective against blood-stage malaria parasites *in vitro,* using the standard growth inhibitory activity (GIA) assay.

One such antigen is apical membrane antigen 1 (PfAMAI). However, antibodies against PfAMAI appear only to be effective at an extremely high concentration. In addition, PfAMAI induces strain-specific antibodies which are not effective against genetically diverse strains of the *Plasmodium* parasite (A. L. Goodman, S. J. Draper, *Ann. Trop. Med. Parasitol.* **104,** 189 (2010)). In addition, vaccine development has been hampered by the requirement for potentially reactogenic chemical adjuvants in addition to the antigen to induce sufficient antibody responses in human subjects.

The applicant's own earlier published patent application WO 2012/1 14125 discloses a suitable antigen for *P. falciparum* based upon Reticulocyte-binding protein Homologue 5 (PfRH5). There is however a need for further antigens which will induce antibodies that are effective at similarly low or even lower concentrations of immunoglobulin. There is a need for further antigens which will induce antibodies that are effective against genetically diverse strains and/or other species of the *Plasmodium* parasite. There is a

need for further antigens that are effective without requiring potentially reactogenic chemical adjuvants.

The present invention addresses one or more of the above needs by providing antigens, vectors encoding the antigens, and antibodies (and antibody-like molecules including aptamers and peptides) raised against the antigen, together with the uses thereof (either alone or in combination) in the prevention or treatment of malaria. Antibodies and antibody-like molecules raised against the antigen may bind *(e.g.* specifically bind) to the antigen.

In one aspect, the invention provides a *Plasmodium falciparum* apical asparagine rich protein (PfAARP) antigen or a fragment thereof. The PfAARP antigen or fragment thereof may be present in the form of a vaccine formulation.

For the avoidance of doubt, it should be understood that reference to an "antigen" includes a fragment thereof. The term "antigen or fragment thereof" means any peptide-based sequence that can be recognised by the immune system and/or that stimulates a cell-mediated immune response and/or stimulates the generation of antibodies.

In another aspect the present invention provides a vector (such as a viral vector, RNA vaccine or DNA plasmid) that expresses PfAARP antigen or a fragment thereof. Such a vector may be present in the form of a vaccine formulation.

Plasmodium falciparum apical asparagine rich protein (PfAARP) is encoded by the *P. falciparum* clone 3D7 gene PF3D7_0423400 (previously known as MAL4P1.216 or PFD1105W).

The PfAARP antigen or fragment thereof of the present invention induces antibodies which are highly effective in the GIA assay against the blood-stage *Plasmodium* parasite. This is very surprising since earlier studies (Wickramarachchi, T., *et al.,* PLoS One, 2008. 3(3): p. e1732; and Pandey, A.K., *et al.,* Infection and immunity, 2013. 81(2): pp. 441-51; both of which are incorporated herein by reference) have suggested that vaccine-induced antibodies to PfAARP are no more potent than the merozoite antigen PfMSPI, which is known to be poorly effective *in vivo* (Sheehy, S.H., *et al.,* Mol Ther, 2012. 20(12): p. 2355-68; Lyon, J.A., *et al.,* PLoS One, 2008. 3(7): p. e2830; and Ogutu,

B.R. *et ai ,* PLoS One, 2009. 4(3): e4708; all of which are incorporated herein by reference).

The PfAARP antigen or fragment thereof of the present invention surprisingly induces antibodies which neutralise parasites more effectively than PfMSPI and remain effective at lower concentrations of immunoglobulin. The antibodies also neutralise parasites more effectively than the BG98 anti-AMA1 standard (Faber, B.W., *et ai.,* Infection and immunity , 201 3; incorporated herein by reference). In addition, the PfAARP antigen or fragment thereof induces antibodies which have also surprisingly been found to be effective against genetically diverse strains of the *Plasmodium* parasite, which is likely to be of critical importance in achieving vaccine efficacy against the variety of strains circulating in the natural environment.

The PfAARP antigen or fragment thereof preferably provides protection (such as long term protection) against disease caused by *Plasmodium* parasites.

The PfAARP antigen or fragment thereof preferably provides an antibody response (e.g. a neutralising antibody response) to *Plasmodium* parasitic infection.

The PfAARP antigen or fragment thereof may provide an antibody response, wherein said antibody or antibodies do not bind, or do not substantially bind, to a PfAARP antigen N-terminal fragment as defined herein, and/or do not bind to said PfAARP antigen N-terminal fragment sufficiently well to confer protective immunity against malaria. In this context, the term "does not bind to said PfAARP antigen N-terminal fragment sufficiently well to confer protective immunity against malaria" may be considered to mean: (i) that said antibody or antibodies do not bind to said PfAARP antigen N-terminal fragment well enough to provide a clinically useful immune response, for example to provide protective immunity against malaria; and/or (ii) that said antibody or antibodies bind to said PfAARP antigen N-terminal fragment, but that this binding does not provide a clinically useful immune response, for example to provide protective immunity against malaria. Immunity to malaria may be quantified using any appropriate technique, examples of which are known in the art.

The present inventors have also found that even greater efficacy can be achieved through combining the PfAARP antigen or fragment thereof with one or more of other *P.*

falciparum antigens. GIA assays involving such combinations have demonstrated an effect which is greater than the sum of inhibition with individual antibodies, *i.e.* a synergistic effect.

In particular, a combination of PfAARP and PfRH5 has surprisingly been found to be particularly effective. Such a combination may provide >90% GIA at a total antibody concentration of 0.625 mg/mL mouse IgG. Importantly, this combination appears to be equally effective against both the vaccine-homologous 3D7 clone and the vaccine-heterologous FVO strain.

Accordingly, the present invention provides the PfAARP antigen or a fragment thereof, and a PfRH5 antigen or a fragment thereof. The antigens or fragment(s) thereof may be present in the form of a vaccine formulation.

Thus the present invention provides a combination of the two antigens PfAARP or a fragment thereof; and PfRH5, or a fragment thereof. The combination of the invention may be present in a single vaccine product capable of inducing antibodies against both antigens. Alternatively the combination of the invention can be effected by mixing two separate recombinant protein vaccines (Pichyangkul, S., *et ai.,* Vaccine , 2009. 28(2): p. 452-62; and Ellis, R.D., *et ai,* PLoS One , 2012. 7(10): p. e46094; both of which are incorporated herein by reference), or by co-delivering the antigens or fragments thereof using vaccine platforms such as particle-based protein vaccine delivery (Bachmann, M.F., *et ai,* Nat Rev Immunol , 2010. 10(1 1): p. 787-96; incorporated herein by reference), or virus-like particles (VLP), or by fusing or conjugating the antigens or fragments thereof to a construct or constructs that allow for particle formation and/or enhanced immunogenicity (Spencer, A.J., *et ai.,* PLoS One , 2012. 7(3): p. e33555; and Wu, Y., *et ai.,* Proc Natl Acad Sci U S A , 2006. 103(48): p. 18243-8; both of which are incorporated herein by reference). In one embodiment, the antigens or fragments thereof may be delivered as a fusion protein (Biswas, S., *et at.,* PLoS One , 201 1. 6(6): p. e20977; incorporated herein by reference). Additionally or alternatively, the antigens or fragments thereof may be delivered using a mixture of viral vectors expressing the individual antigens (Forbes, E.K., *et ai,* J Immunol , 201 1. 187(7): p. 3738-50; and Sheehy, S.H., *et ai.,* Mol Then 2012. 20(12): p. 2355-68; both of which are incorporated herein by reference), or viral vectors co-expressing both antigens or fragments thereof. Where the antigens or fragments thereof are co-expressed, this may be in the form of a

fusion protein (Porter, D.W., *et ai,* Vaccine , 201 1. 29(43): p. 7514-22; incorporated herein by reference), or two antigens expressed as separate transcripts under the control of separate promoters (Bruder, J.T., *et ai,* Vaccine , 2010. 28(18): p. 3201-10; and Tine, J.A., *et ai* , Infect Immun , 1996. 64(9): p. 3833-44; both of which are incorporated herein by reference), or two proteins translated as a single polypeptide which undergoes cleavage to yield two separate antigens (Ibrahimi, A., *et al.,* Hum Gene Ther , 2009. 20(8): p. 845-60; incorporated herein by reference).

The PfAARP antigen of the invention, or fragment thereof, may be used in combination with one or more additional malarial antigen(s), including malarial antigens already known in the art. In one embodiment, the present invention relates to the combination of the PfAARP antigen or fragment thereof with the PfRH5 antigen or fragment thereof (defined herein). One or more additional malarial antigen(s) can be used in combination with the PfAARP (or fragment) and PfRH5 (or fragment) combination.

In one embodiment, the present invention excludes antigen combinations comprising or consisting of PfAARP, PfRH5 and PfRH1 , PfAARP, PfRH5 and PfRH2, PfAARP, PfRH5 and PfRH4, PfAARP, PfRH5 and PfF2, PfAARP, PfRH5 and MSP1 , and/or PfAARP, PfRH5 and MSP1i_9-MSP3 or combinations comprising fragments of said antigens. For example, the present invention may exclude antigen combinations comprising or consisting of a PfAARP N-terminal fragment, PfRH5 and PfRH1 ; a PfAARP N-terminal fragment, PfRH5 and PfRH2; a PfAARP N-terminal fragment, PfRH5 and PfRH4; a PfAARP N-terminal fragment, PfRH5 and PfF2; a PfAARP N-terminal fragment, PfRH5 and MSP1 ; and/or a PfAARP N-terminal fragment, PfRH5 and MSP1i_9-MSP3.

The present invention accordingly provides a vector that expresses PfAARP antigen or a fragment thereof, and a PfRH5 antigen or a fragment thereof. In another aspect, the present invention provides a vector that expresses PfAARP antigen or a fragment thereof, together with a further vector that expresses a PfRH5 antigen or a fragment thereof. The vector or vectors may be present in the form of a vaccine formulation.

The vector may be a viral vector. The viral vector may be an adenovirus (of a human serotype such as AdHu5, a simian serotype such as ChAd63, or another form) or poxvirus vector (such as a modified vaccinia Ankara (MVA)).

Viral vectors are usually non-replicating or replication-impaired vectors, which means that the viral vector cannot replicate to any significant extent in normal cells *(e.g.* normal human cells), as measured by conventional means - *e.g.* via measuring DNA synthesis and/or viral titre. Non-replicating or replication impaired vectors may have become so naturally *(i.e.* they have been isolated as such from nature) or artificially (e.g. by breeding *in vitro* or by genetic manipulation). There will generally be at least one cell-type in which the replication-impaired viral vector can be grown - for example, modified vaccinia Ankara (MVA) can be grown in CEF cells.

Typically, the viral vector is incapable of causing a significant infection in an animal subject, typically in a mammalian subject such as a human or other primate.

The vector may be selected from a human or simian adenovirus or a poxvirus vector.

The vector may be a DNA vector, such as a plasmid-based DNA vaccine. The DNA vector may be capable of expression and secretion in a mammalian cell expression system, such as an immunised cell.

The vector may be a RNA vector, such as a self-amplifying RNA vaccine (Geall, A.J. *et al.*, Proc Natl Acad Sci USA 2012; 109(36) pp. 14604-9; incorporated herein by reference).

PfAARP is predicted to be 217 amino-acids (aa) long, whereby the first 17 aa encoded in PfAARP are predicted to form a signal peptide (Petersen, T.N., *et ai,* Nat Methods, 201 1. 8(10): pp. 785-6; incorporated herein by reference), which is likely cleaved from the final protein in *P. falciparum.* The full-length sequence, including the *Plasmodium* signal peptide, is provided herein as SEQ ID NO: 1.

PfAARP has also previously been described as containing a transmembrane region (Wickramarachchi, T., *et al.,* PLoS One, 2008. 3(3): p. e1732; incorporated herein by reference), with the last ectodomain residue being P191, numbered relative to SEQ ID NO: 1.

The PfAARP sequence contains five putative mammalian N-linked glycosylation sites, which are N24, N76, N107, N168 and N169, numbered relative to SEQ ID NO: 1 (Zhang, M., *et al.,* Glycobiology , 2004. 14(12): pp. 1229-46; incorporated herein by reference).

The PfAARP antigen of the invention may be that defined by SEQ ID NO: 2. This antigen lacks the 17 amino acid signal peptide and runs from amino acids K18 to P217, numbered relative to SEQ ID NO: 1.

The present invention embraces fragments thereof, which comprise or consist of at least 80 or at least 90 consecutive amino acid residues of SEQ ID NO: 2 *{e.g.* at least 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, or 190 consecutive amino acid residues in length). One particular fragment is defined by SEQ ID NO: 3. This fragment lacks the transmembrane domain of PfAARP and accordingly runs from K18 to P191 , numbered relative to SEQ ID NO: 1 *(i.e.* lacking amino acids T192 to P217, numbered relative to SEQ ID NO: 1).

In one embodiment, the present invention embraces fragments of the PfAARP antigen which exclude the N-terminal region of PfAARP. The N-terminal region of PfAARP may be defined as the region consisting of amino acid residues M1 to D107 of SEQ ID NO: 1, or as the region consisting of amino acid residues K1 to D90 of SEQ ID NO: 2 (these two definitions of the PfAARP N-terminal region are identical, except that the N-terminal region defined in relation to SEQ ID NO: 1 also comprises the 17 amino acid signal peptide of M1 to G17 of SEQ ID NO: 1). The N-terminal region of PfAARP may be defined as the region consisting of the amino acid residues of the previously described 'ICGEB' fragment of SEQ ID NO:4. Fragments of the PfAARP antigen which exclude the N-terminal region of PfAARP as defined herein may be referred to as C-terminal fragments or regions of the PfAARP antigen.

Thus, in one embodiment, the present invention embraces C-terminal fragments of the PfAARP antigen. Such C-terminal fragments may comprise or consist of at least 80 or at least 90 consecutive amino acid residues from amino acid residues N108 to P217 of SEQ ID NO: 1, at least 80 or at least 90 consecutive amino acid residues from amino acid residues N91 to P200 of SEQ ID NO: 2, or at least 80 consecutive amino acid residues from amino acid residues N91 to P174 of SEQ ID NO: 3 *(e.g.* at least 80, 90,

100, 110, 120, 130, 140, 150, 160, 170, 180, or 190 consecutive amino acid residues in length).

The PfAARP fragments of the invention have a common antigenic cross-reactivity with the PfAARP antigen of the invention.

The PfRH5 fragments have a common antigenic cross-reactivity with the PfRH5 antigen, which may be the antigen defined by SEQ ID NO: 5. PfRH5 fragments may comprise or consist of at least 170 consecutive amino acid residues of the PfRH5 antigen *(e.g.* at least 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490 or 500 consecutive amino acid residues in length); PfRH5 fragments of at least 380 consecutive amino acid residues in length may be of particular interest.

The above-mentioned antigen or fragment thereof embraces functional *(i.e.* antigenic) variants exhibiting at least 90% *(e.g.* 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% 98% or 99%) identity with the antigen defined by SEQ ID NO: 1, or a fragment thereof; or SEQ ID NO: 2, or a fragment thereof; or SEQ ID NO: 3, or a fragment thereof; or (for PfRH5) SEQ ID NO: 5, or a fragment thereof.

The above-mentioned antigen or fragment thereof may additionally or alternatively include amino acid sequences with one or more amino acid substitutions, deletions or insertions. Substitutions are particularly envisaged and include conservative substitutions.

Conventional methods for determining amino acid sequence identity are discussed in more detail later in the specification.

The PfAARP antigen or fragment thereof may have substitutions at one or more of the N-glycosylation sequons, identified by the amino acid sequence N-X-S/T (where X = any amino acid except proline). In PfAARP these are identified by amino acid residues N24, N76, N107, N168 and/or N169 (numbered relative to SEQ ID NO: 1), wherein the amino acid N, S or T is replaced by another amino acid.

The invention also provides a vector which expresses PfAARP defined by SEQ ID NO: 2 (or a fragment thereof as defined above), or SEQ ID NO: 3 (or a fragment thereof as defined above), or a PfAARP C-terminal fragment (as defined above). The PfAARP antigen, or fragment thereof, may be fused to the mammalian signal peptide from tissue plasminogen activator (Sridhar, S., **et** a/., Journal of Virology, 2008. 82(8): p. 3822-33; and Biswas, S., *et al.,* PLoS One, 201 1. 6(6): p. e20977; both of which are incorporated herein by reference), in order to allow secretion, for example into a culture supernatant, or (for a vaccine vector) to allow antigen secretion from an immunised cell. This replaces the native parasite leader sequence. Where the vector is for production of the antigen (e.g. for manufacture of a protein-based vaccine), the PfAARP antigen or fragment thereof may also be C-terminally tagged *{e.g.* with a 15 amino acid (aa) biotin acceptor peptide, optionally followed by an 8 aa Strepl l tag), to allow purification.

PfAARP is a component of the mechanism by which the *Plasmodium* parasite invades RBCs. It is apically located and is thought to be involved in the binding of the *Plasmodium* parasite to RBCs via the N-terminal of PfAARP.

Compounds that bind to PfAARP inhibit this process and prevent the invasion of RBCs.

Accordingly, the present invention also provides binding compounds to PfAARP antigen or a fragment thereof.

The present invention also provides binding compounds to PfAARP antigen or a fragment thereof, in combination with binding compounds to PfRH5 antigen, or fragments thereof.

The binding compound may be an antibody, such as a monoclonal antibody or polyclonal antibody. The binding compound may be an antigen-binding fragment of a monoclonal or polyclonal antibody, or a peptide which binds *(e.g.* specifically binds) to PfAARP (or a fragment thereof). The antibody may be a Fab, F(ab')2, Fv, scFv, Fd or dAb.

The binding compound may be an oligonucleotide aptamer. The aptamer may bind to PfAARP or a fragment thereof. The aptamer may bind to PfAARP or a fragment thereof. Such aptamers can be found by known methods (e.g. as set out in D. H. J. Bunka, P. G.

Stockley, *Nature Reviews Microbiology* 4, 588 (2006); incorporated herein by reference). The aptamer may be optimised to render it suitable for therapeutic use, *e.g.* it may be conjugated to a monoclonal antibody to modify its pharmacokinetics *{e.g.* half-life and biodistribution) and/or recruit Fc-dependent immune functions.

In one embodiment, the binding compound does not bind, or does not substantially bind, to a PfAARP antigen N-terminal fragment as defined herein, and/or does not bind to said PfAARP antigen N-terminal fragment sufficiently well to confer protective immunity against malaria. In this context, the term "does not bind to said PfAARP antigen N-terminal fragment sufficiently well to confer protective immunity against malaria" may be considered to mean: (i) that said antibody or antibodies do not bind to said PfAARP antigen N-terminal fragment well enough to provide a clinically useful immune response, for example to provide protective immunity against malaria; and/or (ii) that said antibody or antibodies bind to said PfAARP antigen N-terminal fragment, but that this binding does not provide a clinically useful immune response, for example to provide protective immunity against malaria. Immunity to malaria may be quantified using any appropriate technique, examples of which are known in the art. In one embodiment, said binding compound is an antibody or fragment thereof.

The binding compound may bind, or substantially bind, to a PfAARP antigen C-terminal fragment as defined herein, and/or bind to said PfAARP antigen C-terminal fragment sufficiently well to confer protective immunity against malaria. In this context, the term bind to said PfAARP antigen C-terminal fragment sufficiently well to confer protective immunity against malaria" may be considered to mean that said antibody or antibodies bind to said PfAARP antigen C-terminal fragment well enough to provide a clinically useful immune response, for example to provide protective immunity against malaria. Immunity to malaria may be quantified using any appropriate technique, examples of which are known in the art. In one embodiment, said binding compound is an antibody or fragment thereof, and said antibody may be raised against said PfAARP antigen C-terminal fragment.

The binding compound of the invention may be used in combination with a binding compound to one or more additional malarial antigen(s), including malarial antigens already known in the art. In a preferred embodiment, the present invention relates to the combination of a binding compound to the PfAARP antigen or fragment thereof with a

binding compound to the PfRH5 antigen or fragment thereof (defined herein). One or more binding compound(s) to one or more additional malarial antigens can be used together with the combination of a binding compound to PfAARP (or fragment) and the binding compound to PfRH5 (or fragment).

In one embodiment, the present invention excludes combinations comprising or consisting of binding compounds to PfAARP, PfRH5 and PfRH1 , PfAARP, PfRH5 and PfRH2, PfAARP, PfRH5 and PfRH4, PfAARP, PfRH5 and PfF2, PfAARP, PfRH5 and MSP1 , and/or PfAARP, PfRH5 and MSP1$_{19}$-MSP3 or combinations comprising binding compounds to fragments of said antigens. For example, in one embodiment, the present invention excludes binding compounds to antigen combinations comprising or consisting of a PfAARP N-terminal fragment, PfRH5 and PfRH1 ; a PfAARP N-terminal fragment, PfRH5 and PfRH2; a PfAARP N-terminal fragment, PfRH5 and PfRH4; a PfAARP N-terminal fragment, PfRH5 and PfF2; a PfAARP N-terminal fragment, PfRH5 and MSP1 ; and/or a PfAARP N-terminal fragment, PfRH5 and MSP1i$_{9}$-MSP3.

The present invention also provides a method of stimulating or inducing an immune response in a subject comprising administering to the subject a PfAARP antigen or fragment thereof, of the invention, or vector of the invention, or a binding compound of the invention (as described above).

The method of stimulating or inducing an immune response in a subject may comprise administering a PfAARP antigen or fragment thereof, of the invention, or a vector of the invention, or a binding compound of the invention (as described above) to a subject.

In the context of the therapeutic uses and methods, a 'subject' is any animal subject that would benefit from stimulation or induction of an immune response against *Plasmodium* parasite. Typical animal subjects are mammals, such as primates, for example, humans.

The present invention accordingly provides a method for treating or preventing malaria.

The present invention provides a PfAARP antigen or fragment thereof, for use in prevention or treatment of malaria.

In a related aspect, the present invention provides a PfAARP antigen or fragment thereof, and a PfRH5 antigen or a fragment thereof; for use in prevention or treatment of malaria.

In a further aspect, the present invention provides the above vectors for use in prevention or treatment of malaria.

In yet a further aspect, the present invention provides the above binding compounds for use in prevention or treatment of malaria.

In a further aspect, the present invention provides the use of the antigen or fragment thereof, vector, or binding compound of the invention (as described above) for use either alone or in combination in prevention or treatment of malaria.

In a related aspect, the present invention provides the use of the antigen or fragment thereof, vector, or binding compound of the invention (as described above) either alone or in combination, in the preparation of a medicament for the prevention or treatment of malaria.

Methods for treating or preventing malaria may comprise administering a therapeutically effective amount of a PfAARP antigen or fragment thereof, or binding compound, or a vector, of the invention (as described above), either alone or in combination, to a subject.

As used herein, the term "treatment" or "treating" embraces therapeutic or preventative/prophylactic measures, and includes post-infection therapy and amelioration of malaria.

As used herein, the term "preventing" includes preventing the initiation of malaria and/or reducing the severity or intensity of malaria.

A PfAARP antigen or fragment thereof (possibly in combination with a PfRH5 antigen or fragment thereof), or binding compound, or a vector, of the invention (as described above) may be administered to a subject (typically a mammalian subject such as a

human or other primate) already having malaria, or a condition or symptoms associated with malaria, to treat or prevent malaria. In one embodiment, the subject is suspected of having come in contact with *Plasmodium* parasite, or has had known contact with *Plasmodium* parasite, but is not yet showing symptoms of exposure.

When administered to a subject (e.g. a mammal such as a human or other primate) that already has malaria, or is showing symptoms associated with *Plasmodium* parasite infection, the PfAARP antigen or fragment thereof, or binding compound, or a vector, of the invention (as described above) can cure, delay, reduce the severity of, or ameliorate one or more symptoms, and/or prolong the survival of a subject beyond that expected in the absence of such treatment.

Alternatively, a PfAARP antigen or fragment thereof, or binding compound, or a vector, of the invention (as described above) may be administered to a subject *(e.g.* a mammal such as a human or other primate) who ultimately may be infected with *Plasmodium* parasite, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of malaria, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment, or to help prevent that subject from transmitting malaria.

The treatments and preventative therapies of the present invention are applicable to a variety of different subjects of different ages. In the context of humans, the therapies are applicable to children *(e.g.* infants, children under 5 years old, older children or teenagers) and adults. In the context of other animal subjects *(e.g.* mammals such as primates), the therapies are applicable to immature subjects and mature/adult subjects.

The PfAARP antigen or fragment thereof, or a vector, of the invention (as described above) can be employed as a vaccine.

In one aspect, the present invention provides a vaccine composition comprising the PfAARP antigen of the invention or a fragment thereof.

In a related aspect, the present invention provides a vaccine composition comprising PfAARP antigen or a fragment thereof, and a PfRH5 antigen or a fragment thereof.

In a further aspect the present invention provides a vaccine composition comprising PfAARP antigen, or a fragment thereof, optionally together with PfRH5 or a fragment thereof, where either or both PfAARP and/or PfRH5 may be expressed as a virus like particle. Recombinant particulate vaccines are well known in the art. They may be, for example, either fusion proteins or proteins chemically conjugated to particles. Examples of fusion proteins are hepatitis B surface antigen fusions (e.g. as in the RTS.S malaria vaccine candidate), hepatitis B core antigen fusions, or Ty-virus like particles. Examples of chemical fusion particles are the Q-beta particles under development by the biotechnology company Cytos (Zurich, Switzerland).

In a related aspect, the present invention provides a vaccine composition comprising PfAARP antigen, or a fragment thereof, optionally together with PfRH5 or a fragment thereof, where either or both PfAARP and/or PfRH5 may be expressed as a soluble recombinant protein. Recombinant protein-based vaccines are well known in the art. They may be, for example, monomeric soluble proteins or soluble fusion proteins. Such proteins are typically administered or formulated in a vaccine adjuvant. Examples of protein-based vaccines are diphtheria and tetanus toxoids, or soluble malaria protein antigens such as the AMA1 protein vaccine candidates developed for blood-stage malaria (Spring, M.D., *et al.,* PLoS ONE. 2009. 4(4): p. e5254; incorporated herein by reference).

In a further aspect, the present invention provides a vaccine composition comprising a vector that expresses PfAARP antigen or a fragment thereof.

In yet a further aspect, the present invention provides a vaccine composition comprising a vector that expresses PfAARP antigen or a fragment thereof, and a PfRH5 antigen or a fragment thereof. Alternatively, the present invention provides a vaccine composition comprising a vector that expresses PfAARP antigen or a fragment thereof, together with a vector that expresses a PfRH5 antigen or a fragment thereof.

As used herein a "vaccine" is a formulation that, when administered to an animal subject such as a mammal (e.g. a human or other primate) stimulates a protective immune response against *Plasmodium* parasitic infection. The immune response may be a humoral and/or cell-mediated immune response. A vaccine of the invention can be

used, for example, to protect a subject from the effects of *P. falciparum* infection *(i.e.* malaria).

Preferably the PfAARP antigen or fragment thereof of the invention is capable of inducing antibodies which exert similarly high levels of GIA against both the vaccine-homologous clone, 3D7, and against a vaccine-heterologous strain, FVO. The total IgG induced by the PfAARP antigen or fragment thereof of the invention preferably has an EC50 which is comparable to total IgG against PfRH5, and significantly lower than that of the anti-PfAMA1 BG98 standard (Faber, B.W., *et al.,* Infection and immunity*,* 201 3; incorporated herein by reference).

It has unexpectedly been found that the total IgG induced by the PfAARP antigen or fragment thereof of the invention has an advantageously low EC70 and EC90 value (see the Examples below). These percentage reductions in parasite multiplication rates *in vivo* have been linked to protection against uncontrolled parasitaemia and ultimate self-cure in an *in vivo* model of P. *falciparum* infection of *Aotus nancymaae* (data not shown).

Thus the vaccine of the invention can lead to improved outcomes after infection by *P. falciparum* and/or other species of the *Plasmodium* parasite. Monoclonal antibodies, DNA oligonucleotide aptamers, RNA oligonucleotide aptamers, and other engineered biopolymers against PfAARP may also be able to replicate the activity of the vaccine-induced polyclonal antibodies described here. As a vaccine, PfAARP is likely amenable to expression by recombinant viral vectored vaccines, as well as nucleic acid-based vaccines such as RNA or DNA; and recombinant protein expressed in mammalian expression systems or insect cell systems. It may also be possible to express the 'extended' or full-length PfAARP antigens in bacteria or yeast, as well as plant/algae systems.

Preferably the vaccine of the invention comprises a PfAARP antigen or fragment thereof which will result in a GIA of least 50% and preferably at least 75% against the blood-stage *Plasmodium* parasite, at an IgG concentration of 0.5 mg/ml mouse IgG. The vaccine of the invention may comprise a combination of PfAARP antigen or fragment thereof and PfRH5 antigen or fragment thereof that gives >90% GIA at a total antibody concentration of 0.625 mg/mL mouse IgG. This combination is preferably equally effective against both the vaccine-homologous 3D7 clone and the vaccine-heterologous

FVO strain. The PfAARP antigen or fragment thereof and PfRH5 antigen or fragment thereof may be combined to provide a single vaccine product (as described above) capable of inducing antibodies against both antigens, e.g. by mixing two separate recombinant protein vaccines, or by co-delivering the antigens using vaccine platforms such as particle-based protein vaccine delivery, or using a fusion of the two antigens; or by using a mixture of viral vectors expressing the individual antigens, or viral vectors co-expressing both antigens.

The lack of polymorphism at the PfAARP locus (one non-synonymous SNP across its entire length (Manske, M., *et al.,* Nature, 2012. 487(7407): pp. 375-9; incorporated herein by reference)) in circulating *P. falciparum* parasites suggest either a lack of substantial immune pressure, or a high degree of functional constraint that prevents mutations from freely occurring. This property makes it highly likely that functional antibodies raised against a single allele of PfAARP will have broadly neutralising activity.

Importantly, unlike PfRH5 which is only present in *P. falciparum* and the closely related species *P. reichenowi* from chimpanzees, PfAARP has orthologues in other *Plasmodium* spp., including the rodent and simian malarias, as well as the other human malaria species, including *P. vivax.* Thus the invention may additionally provide a PvAARP antigen, or fragment thereof, which may be in the form of a vaccine formulation. The PvAARP antigen or fragment thereof may be combined with other leading *P. vivax* invasion ligand vaccine candidates such as the full-length or region II of Pv Duffy-binding protein (PvDBP).

The term "vaccine" is herein used interchangeably with the terms "therapeutic/prophylactic composition", "formulation" or "medicament".

The vaccine of the invention (as defined above) in addition to a pharmaceutically acceptable carrier can further be combined with one or more of a salt, excipient, diluent, adjuvant, immunoregulatory agent and/or antimicrobial compound.

Pharmaceutically acceptable salts include acid addition salts formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or with organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium,

ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Administration of immunogenic compositions, therapeutic formulations, medicaments and prophylactic formulations (e.g. vaccines) is generally by conventional routes e.g. intravenous, subcutaneous, intraperitoneal, or mucosal routes. The administration may be by parenteral injection, for example, a subcutaneous, intradermal or intramuscular injection. Formulations comprising neutralizing antibodies or aptamers may be particularly suited to administration intravenously, intramuscularly, intradermal^, or subcutaneously.

Accordingly, immunogenic compositions, therapeutic formulations, medicaments and prophylactic formulations (e.g. vaccines) of the invention are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may alternatively be prepared. The preparation may also be emulsified, or the peptide encapsulated in liposomes or microcapsules.

The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

Generally, the carrier is a pharmaceutically-acceptable carrier. Non-limiting examples of pharmaceutically acceptable carriers include water, saline, and phosphate-buffered saline. In some embodiments, however, the composition is in lyophilized form, in which case it may include a stabilizer, such as BSA. In some embodiments, it may be desirable to formulate the composition with a preservative, such as thiomersal or sodium azide, to facilitate long term storage.

Examples of additional adjuvants which may be effective include but are not limited to: complete Freunds adjuvant (CFA), Incomplete Freunds adjuvant (IFA), Addavax, MF59 - a licensed vaccine adjuvant, Saponin, a purified extract fraction of Saponin such as Quil A, a derivative of Saponin such as QS-21, TLR agonists such as MPL and R837, lipid

particles based on Saponin such as ISCOM/ISCOMATIX and Matrix M, *E. cols* heat labile toxin (LT) mutants such as LTK63 and/ or LTK72, CoVaccineHT, aluminium hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1 '-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryl oxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2 % squalene/ Tween 80 emulsion, and the AS02, AS01 , AS03 and AS04 adjuvant formulations developed by GSK Biologicals (Rixensart, Belgium).

Examples of buffering agents include, but are not limited to, sodium succinate (pH 6.5), and phosphate buffered saline (PBS; pH 6.5 and 7.5).

Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations or formulations suitable for distribution as aerosols. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders.

The present invention encompasses polypeptides that are substantially homologous to polypeptides based on SEQ ID NO: 2 or SEQ ID NO: 3 identified in this application (including fragments thereof). The terms "sequence identity" and "sequence homology" are considered synonymous in this specification.

By way of example, a polypeptide of interest may comprise an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% amino acid sequence identity with SEQ ID NO: 2 or SEQ ID NO: 3 (or a respective fragment thereof).

There are many established algorithms available to align two amino acid sequences.

Typically, one sequence acts as a reference sequence, to which test sequences may be compared. The sequence comparison algorithm calculates the percentage sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Alignment of amino acid sequences for comparison may be conducted, for example, by computer implemented algorithms (eg. GAP, BESTFIT, FASTA or TFASTA), or BLAST and BLAST 2.0 algorithms.

The BLOSUM62 table shown below is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89: 10915-10919, 1992; incorporated herein by reference). Amino acids are indicated by the standard one-letter codes. The percent identity is calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps Introduced into the longer sequence in order to align the two sequences]}} \times 100$$

BLOSUM62 table

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										

L -1 -2 -3 -4 -1 -2 -3 -4 -3 2 4
K -1 2 0 -1 -3 1 1 -2 -1 -3 -2 5
M -1 -1 -2 -3 -1 0 -2 -3 -2 1 2 -1 5
F -2 -3 -3 -3 -2 -3 -3 -3 -1 0 0 -3 0 6
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4 7
S 1 -1 1 0 -1 0 0 0 -1 -2 -2 0 -1 -2 -1 4
T 0 -1 0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1 1 5
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1 1 -4 -3 -2 11
Y -2 -2 -2 -3 -2 -1 -2 -3 2 -1 -1 -2 -1 3 -3 -2 -2 2 7
V 0 -3 -3 -3 -1 -2 -2 -3 -3 3 1 -2 1 -1 -2 -2 0 -3 -1 4

In a homology comparison, the identity may exist over a region of the sequence that is at least 10 amino acid residues in length (e.g. at least 15, 20, 30, 40, 50, 75, 100, 120, 140, 150, 160, 160, 175, 180 or 200 amino acid residues in length) - *e.g.* up to the entire length of the reference sequence.

Substantially homologous polypeptides have one or more amino acid substitutions, deletions, or additions. In many embodiments, those changes are of a minor nature, for example, involving only conservative amino acid substitutions. Conservative substitutions are those made by replacing one amino acid with another amino acid within the following groups: Basic: arginine, lysine, histidine; Acidic: glutamic acid, aspartic acid; Polar: glutamine, asparagine; Hydrophobic: leucine, isoleucine, valine; Aromatic: phenylalanine, tryptophan, tyrosine; Small: glycine, alanine, serine, threonine, methionine. Substantially homologous polypeptides also encompass those comprising other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of 1 to about 30 amino acids (such as 1-10, or 1-5 amino acids); and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag.

Key to SEQ ID NOs

SEQ ID NO: 1 Full-length sequence of the PfAARP antigen

SEQ ID NO: 2 Amino acid sequence of a PfAARP antigen lacking the 17 amino acid signal polypeptide. The predicted transmembrane domain is shown in underline

SEQ ID NO: 3 Amino acid sequence of a PfAARP antigen lacking the 17 amino acid signal polypeptide and the predicted transmembrane domain

SEQ ID NO: 4 Amino acid sequence of a previously-generated £.co//-expressed PfAARP fragment ('ICGEB')

SEQ ID NO: 5 Amino acid sequence of the PfRH5 antigen used in the Examples; this is the native 3D7 sequence found on PlasmoDB, minus the first 25 residues which are predicted signal peptide. Additionally, two substitutions were made to remove N-glycosylation sites - N38Q and N214Q.

SEQ ID NO: 1

MWKFITII IFSI YYIDGKSILRNNKSHNNLPISKTNEEEEGKININNLKPIKQHDNIIEDVHIKE NKF ISI KNKDKNG SFIDLSMRYNEKE SDNDEEEEEDEE DNE DNNTNNNNNNNNNNNNDDDHHNDD DHHDNNDNHDNDNNDNNNNNNDNNNNNNDNNNNNNDNNNNS SAAFTAL PPPPPPVPPPPPPTLTP SGIVGNVLSTFVSHGLKLIGVP

SEQ ID NO: 2

KSILRNNKSHNNLPISKTNEEEEGKININNLKPIKQHDNI IEDVHIKENKFI SIKNKDK NGSFIDLSMRYNEKESDNDEEEEEDEEDNEDNNTNNNNNNNNNNNNDDDHHNDDDHHDNN DNHDNDNNDNNNNNNDNNNNNNDNNNNNNDNNNNS SAAFTAL PPPPPPVPPPPP PTLTPS GIVGNVLSTFVSHGL KL IGVP

SEQ ID NO: 3

KSI LRNNKSHNNLP I SKTNEEEEGKININNLKPIKQHDNI IEDVHIKENKFI SIKNKDK NGSFIDLSMRYNEKESDNDEEEEEDEEDNEDNNTNNNNNNNNNNNNDDDHHNDDDHHDNN DNHDNDNNDNNNNNNDNNNNNNDNNNNNNDNNNNS SAA FTA L PPPPPPVPPPPPP

SEQ ID NO: 4

ILRNNKSHNNLPI SKTNEEEEGKININNLKPIKQHDNI IEDVHIKENKFIS IKNKDK
NGSFIDLSMRYNEKESDNDEEEEEDEEDNED

SEQ ID NO: 5

ENAIKKTKNQENQLTLLPIKSTEEEKDDIKNGKDIKKEIDNDKENIKTNNAKDHSTYIKSYLNTN
VNDGLKYLFIPSHNSFIKKYSVFNQINDGMLLNEKNDVKNNEDYKNVDYKNVNFLQYHFKELSNY
NIANSIDILQEKEGHLDFVIIPHYTFLDYYKHLSYNSI YHKSSTYGKCIAVDAFIKKIQETYDKV
KSKCNDIKNDLIATIKKLEHPYDINNKNDDSYRYDISEEIDDKSEETDDETEEVEDSIQDTDSNH
TPSNKKKNDLMNRTFKKMMDEYNTKKKKLIKCIKNHENDFNKICMDMKNYGTNLFEQLSCYNNNF
CNTNGIRYHYDEYIHKLILSVKSKNLNKDLSDMTNILQQSELLLTNLNKKMGSYI YIDTIKFIHK
EMKHIFNRIEYHTKI INDKTKI IQDKIKLNIWRTFQKDELLKRILDMSNEYSLFITSDHLRQMLY
NTFYSKEKHLNNI FHHLI YVLQMKFNDVPIKMEYFQTYKKNKPLTQ

LIST OF FIGURES

Figure 1A: Genetic construct encoding two PfAARP polypeptides, with a biotin acceptor peptide (BAP) and StrepII tag for purification.

Figure 1B: ClustalW alignment comparing 'ICGEB', a previously generated PfAARP fragment (SEQ ID NO: 4), with two PfAARP peptides of the invention - 'Full' (SEQ ID NO: 2) and 'Extended' (SEQ ID NO: 3).

Figure 2: Amino acids KSIL...PPPP 'Extended' (SEQ ID NO: 3) and amino acids KSIL...IGVP 'Full' (SEQ ID NO: 2) of the 3D7 allele of PfAARP can be expressed in HEK293 cells as a secreted recombinant protein fused at the C-terminus with the BAP and StrepII tags. Figure 2A shows the purified protein stained with Coomassie blue. Figure 2B shows a Western blot of the purified protein, detected with StrepTactinTM-HRP conjugate. Minus (-) or plus (+) signs indicate pre- or post- treatment with PNGase, respectively.

Figure 3: 'Extended' PfAARP (SEQ ID NO: 3), when formulated with Addavax preclinical adjuvant, is an immunogenic composition. Shown are endpoint titres for four immunised mice, and the endpoint titre of the purified antibody sample used in Figure 4. Anti-OVA antibodies served as a negative control and were not detected above background level (dotted line).

Figure 4: Antisera were raised to the 'Extended' PfAARP (SEQ ID NO: 3) protein vaccine based on the 3D7 allele, to the 3D7 allele of PfRH5 and $PfMSP1_{19}$, and to OVA. For each immunogen, serum from four immunised mice was pooled and IgG was purified using protein G. The activity of the normalised IgG was measured in an assay of GIA against the 3D7 clone parasite from which the vaccine antigen sequences were derived (Figure 4A), and against FVO, a heterologous strain of *P. falciparum* (Figure 4B). The activity of the BG98 rabbit antibody standard (internal control) on each assay plate is stated.

Figure 5: Mixtures of antibodies against the 3D7 allele of PfRH5 and PfAARP perform at least additively in GIA assays. Rabbit antibodies against PfRH5 were fixed at 0.125 mg/mL, whilst mouse antibodies were tested at a range of concentrations. The predicted effect of an additive interaction between mixed antibodies is shown by the dashed line. Results for the vaccine-homologous 3D7 clone are shown in panel A, and for the vaccine-heterologous strain FVO are shown in panel B. Results for the BG98 rabbit anti-PfAMAI internal standard are shown for each assay.

EXAMPLES

The invention will be further clarified by the following examples, which are intended to be purely exemplary of the invention and are in no way limiting.

Unless otherwise stated, references to the 'Full' PfAARP are to the antigen defined by SEQ ID NO: 2 (KSIL ... IGVP); references to the 'Extended' PfAARP are to the antigen defined by SEQ ID NO: 3 (KSIL ... PPPP).

Example 1 - Design of two PfAARP vaccine constructs

Plasmodium falciparum apical asparagine rich protein (PfAARP) is encoded by the *P. falciparum* clone 3D7 gene PF3D7_0423400 (previously known as MAL4P1.216 or PFD1105w). The predicted polypeptide sequence encoded in the 3D7 clone of *P. falciparum* by the PfAARP gene was obtained from PlasmoDB (Aurrecoechea, C., *et al.,* Nucleic Acids Res, 2009. 37(Database issue): p. D539-43; incorporated herein by reference). It is predicted to be 217 amino acids (aa) long. Synthetic DNA constructs were designed to express portions of PfAARP as recombinant proteins in mammalian

cells (Figure 1). As the first 17 aa encoded in PfAARP are predicted to form a signal peptide (Petersen, T.N., *et al.,* Nat Methods , 201 1. 8(10): pp. 785-6; incorporated herein by reference), and are likely to be cleaved from the final protein in *P. falciparum,* they were removed from the constructs. Instead, the native parasite leader sequence was replaced at the N-terminus with the mammalian signal peptide from tissue plasminogen activator (tPA) (Sridhar, S., *et al.,* Journal of virology . 2008. 82(8): pp. 3822-33; and Biswas, S., ef *al.,* PLoS One *,* 201 1. 6(6): p. e20977; both of which are incorporated herein by reference), in order to allow secretion into the culture supernatant. The vaccine constructs were also C-terminally tagged with a 15 aa biotin acceptor peptide followed by an 8 aa Strepll tag to allow purification (Figure 1A).

PfAARP has previously been described as containing a transmembrane region, with the last ectodomain residue being P191 (Wickramarachchi, T., *et al.,* PLoS One , 2008. 3(3): p. e1732; incorporated herein by reference). However a bioinformatic search for the presence of a transmembrane region revealed conflicting results. Whereas TMHMM v2.0 (Krogh, A., *et al.,* J Mol Biol , 2001 . 305(3): p. 567-80; incorporated herein by reference) was unable to detect a transmembrane helix at this site, TopPred (Claros, M.G. and G. von Heijne, Comput Appl Biosci , 1994. 10(6): pp. 685-6; incorporated herein by reference) detected a transmembrane region running from residues P191-L21 1, numbered relative to SEQ ID NO: 1 (Figure 1B). Two constructs were accordingly designed. 'Full' (SEQ ID NO: 2 , Figure 1B) encoded all of the residues in the predicted PfAARP protein except for the predicted signal peptide, running from K18 to P217, numbered relative to SEQ ID NO: 1. 'Extended' (SEQ ID NO: 3 , Figure 1B) also began at K18 (numbered relative to SEQ ID NO: 1) but ran only as far as P191 (numbered relative to SEQ ID NO: 1), thus lacking the predicted transmembrane region.

Both of these constructs encoded more than 90 amino acids of the predicted PfAARP sequence, in contrast to a previously generated fragment (SEQ ID NO: 4 , Figure 1B) 'ICGEB' (Wickramarachchi, T., *et al.,* PLoS One , 2008. 3(3): p. e1732; incorporated herein by reference), which encoded fewer than 90 amino acids of the predicted PfAARP sequence (I20 to D108, numbered relative to SEQ ID NO: 1). It should be noted that none of the five putative mammalian N-linked glycosylation sequons in the PfAARP sequence (Zhang, M., *et al.,* Glvcobiology , 2004. 14(12): pp. 1229-46; incorporated herein by reference) were mutated in this case (typically identified by the consensus

sequence N-X-S/T, where X = any amino acid except proline). Mammalian cell expression could thus lead to the production of glycoforms.

The genes were cloned into a protein-expression plasmid that transcribed under the control of a CMV promoter (Sridhar, S., *et al.,* Journal of virology. 2008. 82(8): p. 3822-33; incorporated herein by reference). The transcribed region contained a Kozak sequence to enhance translation initiation. The recombinant protein contained an N-terminal signal peptide from human tissue plasminogen activator (Draper, S.J., *et al.,* Nat Med, 2008. 14(8): pp. 819-21; incorporated herein by reference), and C-terminal tags comprising a biotin acceptor peptide (BAP, aa sequence GLNDIFEAQKIEWHE) and a StrepII tag (aa sequence WSHPQFEK).

Example 2: Expression of two PfAARP constructs as recombinant proteins

Protein-expression plasmids encoding the two constructs ('Full' and 'Extended') described above were transiently transfected into HEK293 cells using polyethylenimine. After 4-7 days, supernatants were harvested and extensively dialysed into PBS using membranes with a 10 kDa molecular weight cut-off (Thermo Scientific, UK).The StrepII-tagged proteins were purified using streptactin® sepharoseTM resin as per manufacturer's instructions (IBA, Germany). Ovalbumin protein grade VII was purchased from Sigma, UK. Recombinant GST-tagged PfMSP1$_{19}$ protein (3D7/ETSR allele) was produced and purified as previously described (Goodman, A.L., *et al.,* Infection and immunity, 2010. 78(11): pp. 4601-12; incorporated herein by reference).

Both constructs were successfully purified from culture supernatant, although the 'Full' protein was obtained at a lower yield than 'Extended' (Figure 2A and data not shown). Both proteins migrated upon SDS-PAGE analysis as two strong bands between the 37kDa and the 50 kDa protein standards, with a third faint band just visible at the 37kDa marker (Figure 2A). This faint third band became the dominant band upon treatment with PNGase (Figure 2B), suggesting that the larger two represent at least two glycosylated forms.

SimplyBlueTMSafeStain (Life Technologies® UK) was used to detect protein in polyacrylamide gels. The ladder used was Precision-Plus unstained protein standards (Bio-Rad, USA). Blots were performed onto nitrocellulose membranes (Bio-Rad, USA)

and detected using streptactin-HRP (IBA, Germany). PNGase treatment (NEB, USA) was performed as per manufacturer's instructions.

Following purification, the 'Extended' PfAARP recombinant protein was buffer-exchanged into PBS and formulated with Addavax® preclinical adjuvant.

Example 3: GIA assay using the 'Extended' PfAARP protein

Groups of four female BALB/c mice aged 6 weeks were injected with 20 µg protein immunogen in PBS, adjuvanted with Addavax adjuvant (Invivogen, USA) as per manufacturer's instructions. Three doses were administered in total with a four-week interval between immunisations. All vaccines were given via the intra-muscular route.

Included as control groups were mice immunised with control ovalbumin (OVA) protein formulated in Addavax® and mice immunised with a heterologous prime-boost viral vectored PfRH5 regime (Douglas, A.D., *et al.,* Nat Commun , 2011. 2: p. 601; and Williams, A.R., *et al.,* PLoS pathogens , 2012. 8(11): p. e1002991 ; both of which are incorporated herein by reference).

Serum was harvested by cardiac puncture two weeks after the final boost, pooled, and the IgG was purified using protein G and normalised to 2 mg/mL. Substantial quantities of anti-PfAARP antibody were induced, as measured by an endpoint titre ELISA (Figure 3): antigen was coated onto Maxisorp plates (Nunc, Denmark) overnight at 10 pg per plate. Blocking was performed with milk powder in PBS. Detection of the primary *{i.e.* test) antibody was performed using anti-mouse alkaline phosphatase-conjugated antibody produced in goat (Sigma-Aldrich, USA).

The ability of antibodies raised against PfAARP, PfRH5, $PfMSP1_{19}$ and OVA to block asexual-stage development of *P. falciparum* was tested using the GIA assay. IgG from pooled mouse serum was purified using protein G beads and adjusted to 2 mg/mL. Total mouse IgG concentration was diluted to 1.0, 0.5, 0.25 and 0.125 mg/mL. Importantly, antigen-specific IgG probably represents only a small fraction of the total IgG present, e.g. around 1 to 10% of the total (Williams *et al.,* PLoS Pathog. 2012; 8(11):e1002991 ; incorporated herein by reference). It will therefore be appreciated that the EC50 results achieved using the antigen of the invention represent a significant achievement.

GIA assays were performed to the protocol of the MVI international GIA reference centre (Malkin, E.M., *et al.,* Infection and immunity, 2005. 73(6): pp. 3677-85; incorporated herein by reference). Sorbitol and percoll synchronised parasite cultures (3D7 clone and FVO strain) at the trophozoite stage were cultured for ~40 hours (48 hours in the case of FVO) in the presence of test antibody purified using Protein G (Pierce, UK). Detection of parasites was based on the lactate dehydrogenase assay.

When the GIA assay was conducted against the vaccine homologous 3D7 clone, antibodies to PfAARP performed comparably to those against PfRH5 - in this case the apparent EC50 value for total IgG was very similar, although the dilution curves appear to vary, with the data indicating PfAARP may have improved EC70 or EC90 values in comparison to PfRH5 (Figure 4A).

The potency of anti-PfAARP antibodies was also maintained against a vaccine-heterologous strain (FVO) (Figure 4B), suggesting that the ability of vaccine-induced anti-PfAARP antibodies to block parasite growth is not vaccine homologous/3D7-specific, and is likely to be cross-strain neutralising as seen for PfRH5.

Previously, GIA levels comparable to those of anti-PfMSP1$_{42}$ antibodies have been reported for anti-PfAARP antibodies (Wickramarachchi, T., *et al.,* PLoS One, 2008. 3(3): p. e1732; incorporated herein by reference). It can be seen (Figure 4A) that anti-PfMSPI$_{1g}$ antibodies (the antibody-neutralising C-terminal domain of PfMSP1$_{42}$) perform very poorly in the GIA assay by comparison to anti-PfAARP and anti-PfRH5 antibodies, suggesting that the 'Extended' PfAARP protein composition represents a substantial improvement upon the prior art, outperforming the BG98 anti-PfAMA1 standard (as discussed in Example 5, below).

Example 5: GIA assay using a combination of PfAARP with PfRH5

The interaction of anti-PfAARP and anti-PfRH5 antibodies was assessed (using the method of Williams, A.R., *et al.,* PLoS pathogens, 2012. 8(11): p. e1002991; incorporated herein by reference). Mouse anti-PfAARP IgG was mixed with a fixed concentration of rabbit anti-PfRH5 IgG, and the assays of GIA performed against the 3D7 clone and FVO strain of parasite.

When predicting the outcome of an additive interaction between antibodies of two specificities, the following formula was employed (see Bliss, C.I., Annals of applied biology, 1939. 26(3): pp. 585-615; incorporated herein by reference):

$$GIA_{A+B} = [1 - (1-(GIA_A/100)) \times (1-(GIA_B/100))] \times 100$$

where GIA_{A+B} is the predicted percentage GIA of the mixture, and GIA_A and GIA_B are the percentage GIAs of the individual components.

At the higher concentrations of anti-PfAARP IgG, the GIA resulting from the antibody mixture exceeded the predicted level of GIA (assuming an additive interaction between the two components, see Figure 5A), and is thus defined here as synergistic.

At lower concentrations of anti-PfAARP IgG, the mixture performed exactly as would be predicted assuming an additive interaction between the individual components. The similarity of the results against both the vaccine-homologous 3D7 clone and the vaccine-heterologous FVO strain suggests that the potency of the PfAARP/PfRH5 combination is strain-transcending (Figure 5B).

Antibody interactions between PfRH5 and other merozoite antigens are known to be unpredictable - some are sub-additive, some are additive and some can show synergy (Williams, A.R., *et ai,* PLoS pathogens. 2012. 8(11): p. e1002991; incorporated herein by reference). The positive interaction here between anti-PfAARP IgG and anti-PfRH5 IgG is both a new finding and one that could not have been previously expected. A previous report (Pandey, A.K., *et ai,* Infection and immunity, 2013. 81(2): pp. 441-51; incorporated herein by reference) that used the ICGEB' fragment (SEQ ID NO: 4, Figure 1B) found that an optimised combination of anti-PfRH2, anti-PfEBA175 (F2 region), and anti-PfAARP ('ICGEB') antibody specificities required a total IgG concentration of 10 mg/mL in order to provide approximately 80% inhibition in the GIA assay. In the same report, anti-PfAMA1 antibodies were reported to achieve approximately 80% GIA at 5 mg/mL of polyclonal IgG. This prior art suggests that a PfAARP ('ICGEB')-based combination of antibodies can be outperformed by monovalent anti-PfAMA1 antibodies.

By contrast, the anti-PfAARP and anti-PfRH5 combination reported here outperforms the BG98 anti-PfAMA1 standard (Faber, B.W., *et ai,* Infection and immunity. 2013;

incorporated herein by reference) by a considerable margin: whereas BG98 anti-PfAMAI antibodies achieve approximately 90% GIA at 6 mg/mL total IgG, the anti-PfAARP and anti-PfRH5 combination achieves approximately 90% GIA at a total mouse IgG concentration of 0.625 mg/mL.

Thus, whereas the previously reported combination (Pandey, A.K., *et al.,* Infection and immunity , 2013. 81(2): pp. 441-51 ; incorporated herein by reference) achieved 'anti-PfAMAI equivalence' at double the total IgG concentration of the anti-PfAMA1 antibodies, the anti-PfAARP and anti-PfRH5 combination here achieves 'anti-PfAMA1 equivalence' at around a tenth of the total IgG concentration of the anti-PfAMA1 antibodies.

CLAIMS

1. A vaccine composition comprising a *Plasmodium falciparum* apical asparagine rich protein (PfAARP) antigen having at least 90% identity with SEQ ID NO: 2 or a fragment thereof that has a common antigenic cross-reactivity with said PfAARP antigen.

2. The vaccine composition of claim 1, wherein the PfAARP antigen comprises the amino acid sequence of SEQ ID NO: 3.

3. The vaccine composition of claim 1, wherein the PfAARP antigen fragment is a C-terminal, which comprises or consists of at least 80 consecutive amino acid residues from:
   i. amino acid residues N108 to P217 of SEQ ID NO: 1;
   ii. amino acid residues N91 to P200 of SEQ ID NO: 2; or
   iii. amino acid residues N91 to P174 of SEQ ID NO: 3.

4. The vaccine composition of any one of claims 1 to 3, wherein the PfAARP antigen or fragment thereof is in the form of a recombinant protein, a protein particle, a fusion protein, or a combination thereof.

5. The vaccine composition of any one of the preceding claims, wherein the PfAARP fragment comprises at least 90 consecutive amino acid residues of SEQ ID NO: 2

6. The vaccine composition of any one of the preceding claims, wherein the composition further comprises a PfRH5 antigen or a fragment thereof that has a common antigenic cross-reactivity with said PfRH5 antigen.

7. The vaccine composition of claim 6, wherein the PfRH5 antigen has at least 90% identity with SEQ ID NO: 5 or a fragment thereof.

8. The vaccine composition of claim 6 or claim 7 wherein the PfRH5 antigen or fragment thereof is in the form of a recombinant protein, a protein particle, a fusion protein, or a combination thereof.

9. The vaccine composition of any one of claims 6 to 8, comprising a fusion of the PfAARP antigen or fragment thereof and the PfRH5 antigen or fragment thereof.

10. A viral vector, RNA vaccine or DNA plasmid which expresses a *Plasmodium falciparum* apical asparagine rich protein (PfAARP) antigen having at least 90% identity with SEQ ID NO: 2 or a fragment thereof that has a common antigenic cross-reactivity with said PfAARP antigen.

11. The viral vector, RNA vaccine or DNA plasmid of claim 10, which expresses a PfAARP antigen comprising the amino acid sequence of SEQ ID NO: 3.

12. The viral vector, RNA vaccine or DNA plasmid of claim 10, wherein the PfAARP antigen fragment is a C-terminal, which comprises or consists of at least 80 consecutive amino acid residues from:

i. amino acid residues N108 to P217 of SEQ ID NO: 1;
ii. amino acid residues N91 to P200 of SEQ ID NO: 2; or
iii. amino acid residues N91 to P174 of SEQ ID NO: 3.

13. The viral vector, RNA vaccine or DNA plasmid of any one of claims 9 to 12, which expresses a PfAARP antigen or fragment thereof further comprising a signal peptide.

14. The viral vector, RNA vaccine or DNA plasmid of claim 13, wherein the signal peptide directs secretion from human cells.

15. The viral vector, RNA vaccine or DNA plasmid of claim 13 or claim 14, wherein the signal peptide is a mammalian signal peptide from tissue plasminogen activator.

16. The viral vector, RNA vaccine or DNA plasmid of any one of claims 10 to 15, wherein the viral vector, RNA vaccine or DNA plasmid further expresses a PfRH5 antigen, or a fragment thereof that has a common antigenic cross-reactivity with said PfRH5 antigen.

17. The viral vector, RNA vaccine or DNA plasmid of claim 16, which expresses a PfAARP antigen or a fragment thereof and a PfRH5 antigen or a fragment thereof as a fusion protein.

18. The viral vector, RNA vaccine or DNA plasmid of any one of claims 10 to 15, in combination with a viral vector, RNA vaccine or DNA plasmid that expresses a PfRH5 antigen, or a fragment thereof that has a common antigenic cross-reactivity with said PfRH5 antigen.

19. The viral vector, RNA vaccine or DNA plasmid of any one of claims 16 to 18, wherein the PfRH5 antigen has at least 90% identity with SEQ ID NO: 5 or a fragment thereof.

20. The viral vector of any of claims 10 to 19, wherein said viral vector is a human or simian adenovirus, or a pox virus.

21. The viral vector of claim 20, wherein said viral vector is an AdHu5, ChAd63 or modified vaccinia Ankara (MVA) vector.

22. The RNA vaccine or DNA plasmid of any of claims 10 to 21, wherein the RNA vaccine or DNA plasmid is capable of expression in an immunised mammalian cell.

23. The DNA plasmid of any of claims 10 to 21, wherein the DNA plasmid is capable of expression in a heterologous protein expression system.

24. An antibody, or binding fragment thereof, raised against a *Plasmodium falciparum* apical asparagine rich protein (PfAARP) antigen having at least 90% identity with SEQ ID NO: 1, or against a fragment thereof that has a common antigenic cross-reactivity with said PfAARP antigen.

25. The antibody, or binding fragment thereof, of claim 24, wherein the antibody is raised against a PfAARP antigen having at least 90% identity with the amino acid sequence of SEQ ID NO: 2, or a fragment thereof; or SEQ ID NO: 3, or a fragment thereof.

26. The antibody, or binding fragment thereof, of claim 24 or claim 25, wherein the antibody is raised against a PfAARP C-terminal fragment that comprises at least 80 consecutive amino acid residues from:

i. amino acid residues N108 to P217 of SEQ ID NO: 1;

ii. amino acid residues N91 to P200 of SEQ ID NO: 2; or

iii. amino acid residues N91 to P174 of SEQ ID NO: 3.

27. The antibody, or binding fragment thereof, of claim 24 or claim 25, wherein the antibody is raised against a fragment that comprises at least 90 consecutive amino acids of SEQ ID NO: 1, or SEQ ID NO: 2, or SEQ ID NO: 3.

28. The antibody, or binding fragment thereof, of any one of claims 24 to 27, in combination with a further antibody, or binding fragment thereof, raised against a PfRH5 antigen or fragment thereof.

29. The antibody or binding fragment thereof of claim 28, wherein the further antibody or binding fragment thereof is raised against a PfRH5 antigen having at least 90% identity with SEQ ID NO: 5 or a fragment thereof.

30. An antibody, or binding fragment thereof, of any one of claims 24 to 29, wherein the antibody is monoclonal or polyclonal.

31. An antibody or binding fragment of any one of claims 24 to 30, wherein the antibody is an Fab, F(ab')2, Fv, scFv, Fd or dAb.

32. An oligonucleotide aptamer that binds to a *Plasmodium falciparum* apical asparagine rich protein (PfAARP) antigen having at least 90% identity with SEQ ID NO: 1 or a fragment thereof.

33. The aptamer of claim 32, wherein the aptamer binds to a PfAARP antigen having at least 90% identity with the amino acid sequence of SEQ ID NO: 2, or a fragment thereof; or SEQ ID NO: 3, or a fragment thereof.

34. A vaccine composition comprising the viral vector, RNA vaccine or DNA plasmid of any one of claims 10 to 21.

35. The vaccine composition of any one of claims 1 to 9 and/or claim 34, and/or the viral vector, RNA vaccine or DNA plasmid of any one of claims 10 to 23, and/or the antibody of any one of claims 24 to 31, and/or the aptamer of any one of claims 32 to 34, for use in treatment or prevention of malaria.

36. Use of the vaccine composition of any one of claims 1 to 9 and/or claim 34, and/or the viral vector, RNA vaccine or DNA plasmid of any one of claims 10 to 23, and/or the antibody of any one of claims 24 to 31, and/or the aptamer of any one of claims 32 to 34, in the preparation of a medicament for the prevention or treatment of malaria.

Figure 1A

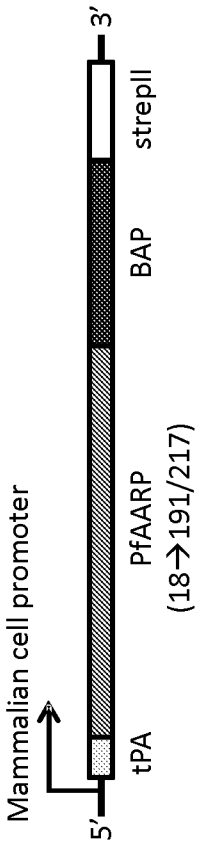

Mammalian cell promoter
5'
3'
tPA
PfAARP
(18→191/217)
BAP
strepII

Figure 1B

```
ICGEB       --ILRNNKSHNNLPISKTNEEEEGKININNLKPIKQHDNIIEDVHIKENKFISIKNKDKN 77
Full        KSILRNNKSHNNLPISKTNEEEEGKININNLKPIKQHDNIIEDVHIKENKFISIKNKDKN 77
Extended    KSILRNNKSHNNLPISKTNEEEEGKININNLKPIKQHDNIIEDVHIKENKFISIKNKDKN 77
              ************************************************************

ICGEB       GSFIDLSMRYNEKESDNDEEEEEDEEDNED---------------------------------- 107
Full        GSFIDLSMRYNEKESDNDEEEEEDEEDNEDNNTNNNNNNNNNNNNNDDDHHNDDDHHDNNND 137
Extended    GSFIDLSMRYNEKESDNDEEEEEDEEDNEDNNTNNNNNNNNNNNNDDDHHNDDDHHDNNND 137
            ********************************

ICGEB       ---------------------------------------------------------------
Full        NHDNDNNDNNNNNNDNNNNNNDNNNNNNDNNNNNSSAAFTALPPPPPPVPPPPPPTLTPSG 197
Extended    NHDNDNNDNNNNNNDNNNNNNDNNNNNNDNNNNNSSAAFTALPPPPPPVPPPPPP------ 197

ICGEB       ---------------------
Full        IVGNVLSTFVSHGLKLIGVP 217
Extended    ---------------------
```

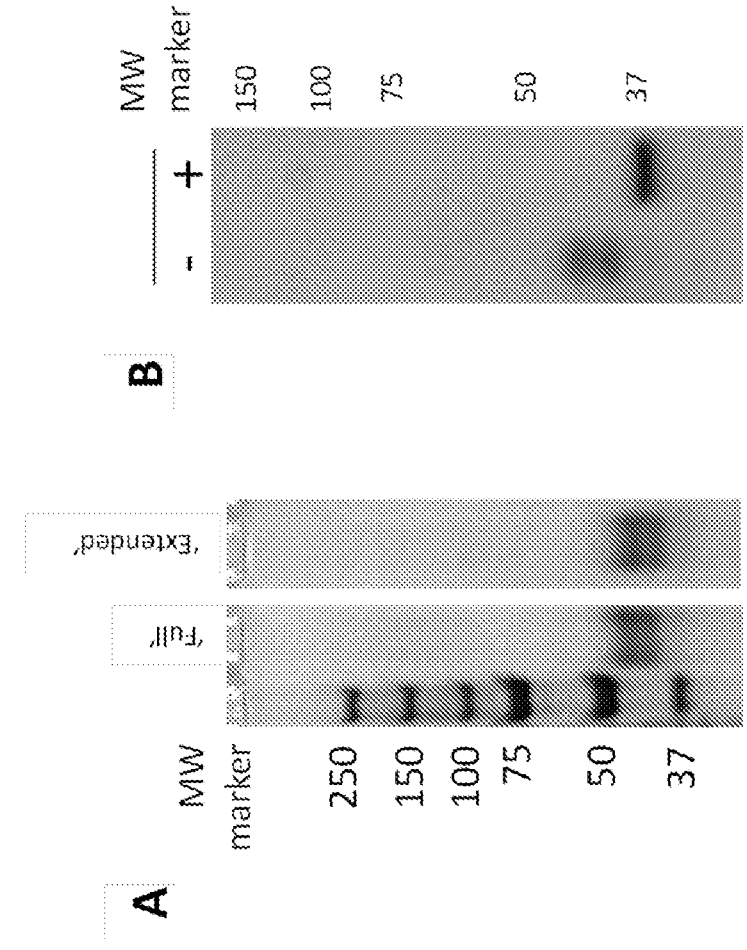

A
MW marker
250
150
100
75
50
37
'Full'
'Extended'
B
MW marker
150
100
75
50
37
-
+

Figure 2

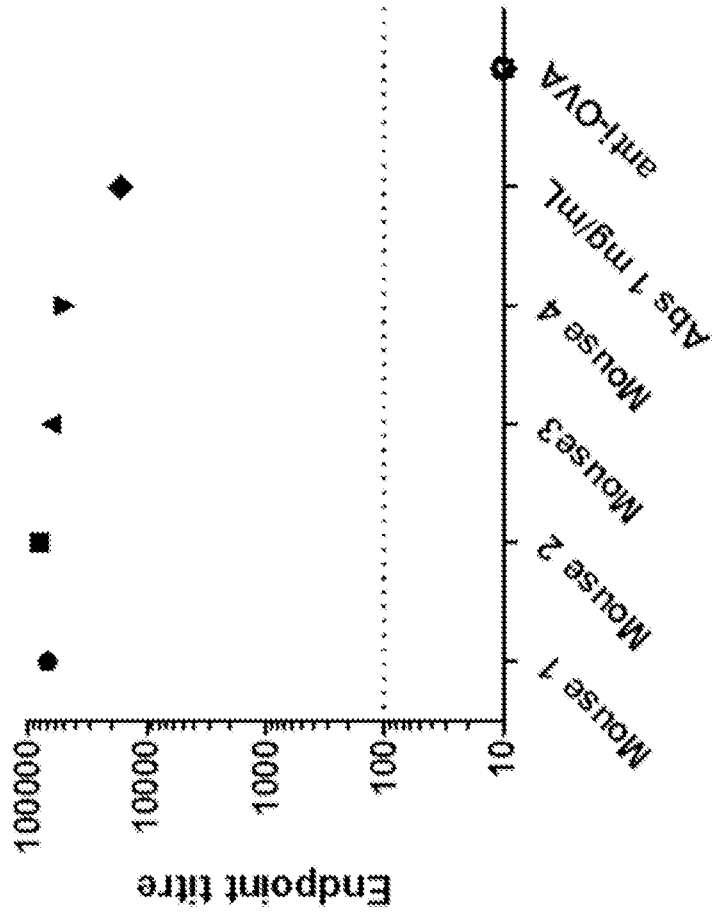

Endpoint titre
100000
10000
1000
100
10
Mouse 1
Mouse 2
Mouse3
Mouse 4
Abs 1 mg/mL
anti-OVA

Figure 3

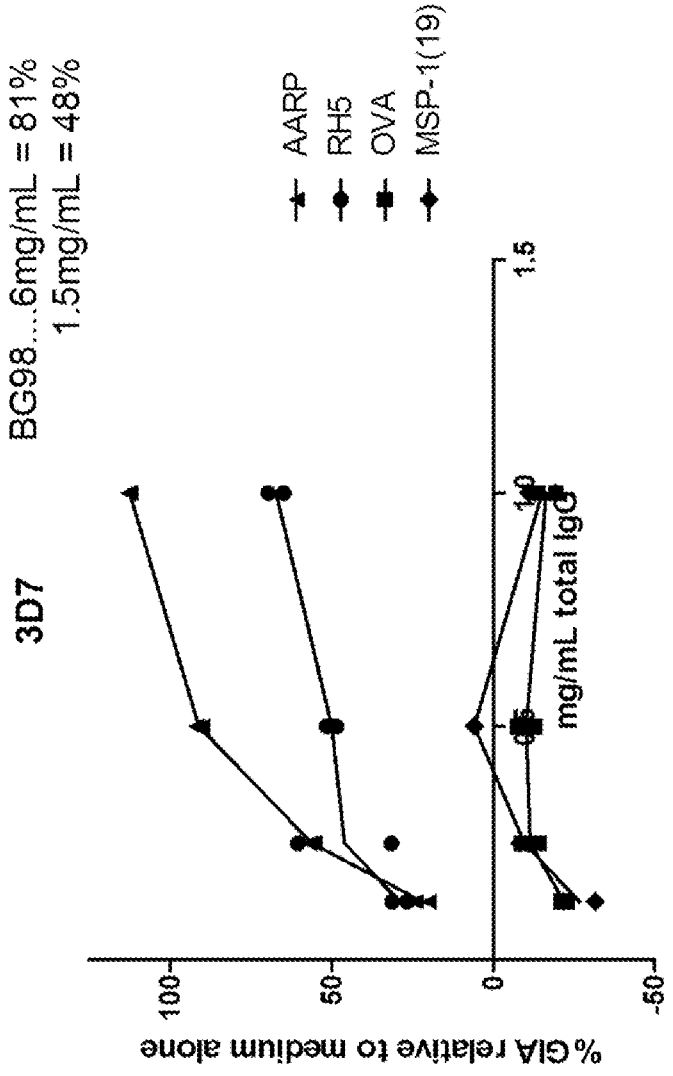


Figure 4A

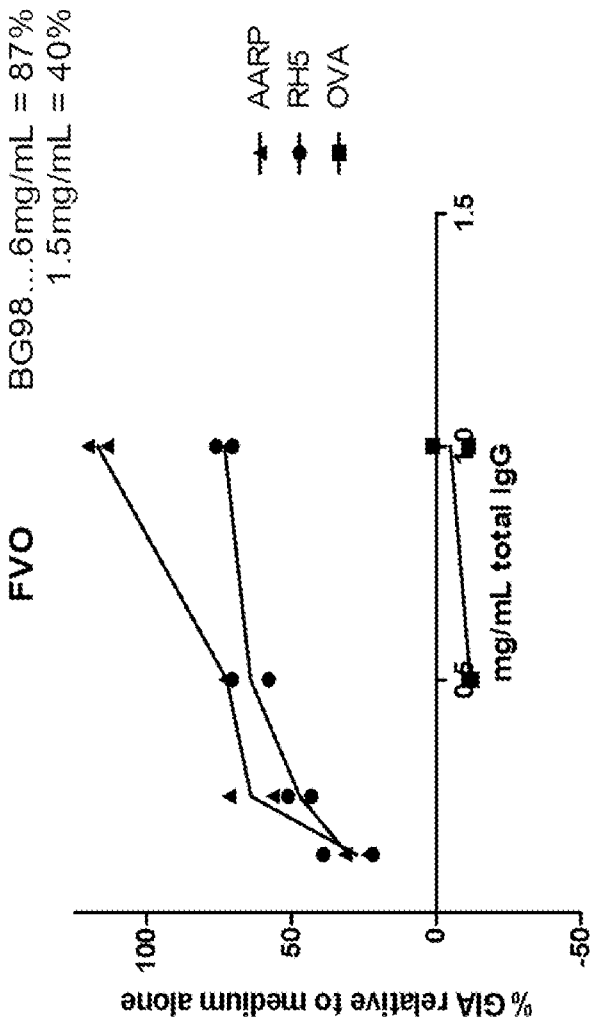


Figure 4B

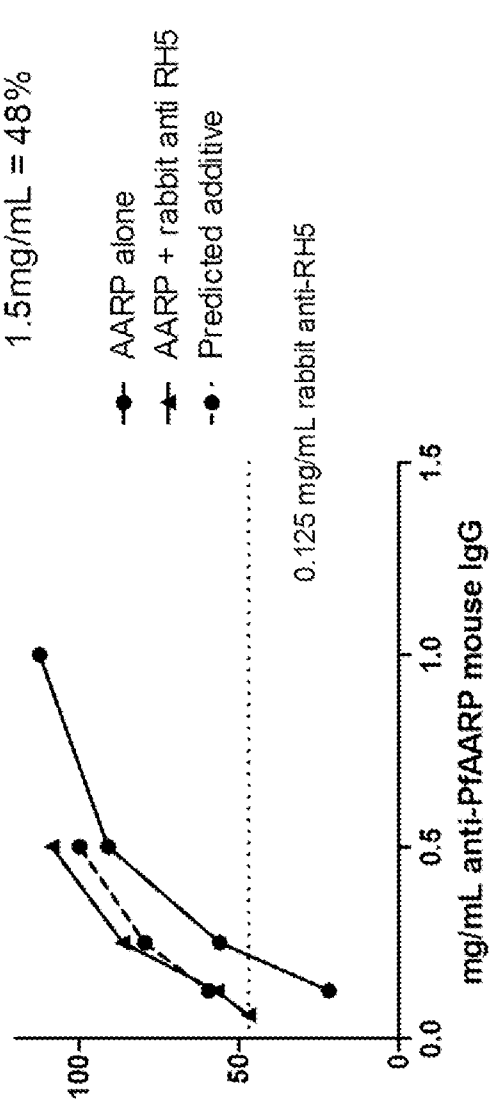


Figure 5A

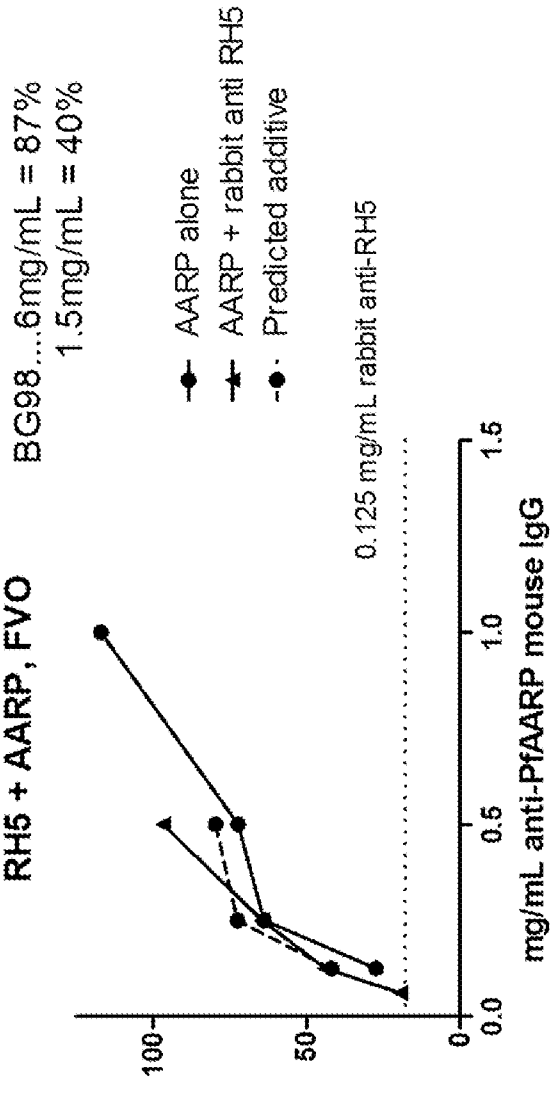


Figure 5B