

Binding domain of *Plasmodium* Reticulocyte binding proteins.Field of the invention

The present invention provides isolated polynucleotides, polypeptides, antibodies and/or vaccines for the prevention and/or treatment of malaria caused by *Plasmodium* merozoites, in particular *Plasmodium falciparum* and/or *Plasmodium vivax*.

Background of the invention

Malaria is caused by parasites of the genus *Plasmodium* and causes an estimated 300-500 million clinical cases and 1-3 million deaths annually (Snow et al, 2005). In addition to morbidity and mortality the economic burden due to malaria is immense, with loss of up to 1-2% of GDP per year estimated for some countries where malaria is endemic.

An essential step in the life cycle of malaria parasites is the invasion of host erythrocytes by merozoites. The invasion process is characterized by a multitude of specific, but relatively poorly understood, interactions between protein ligands expressed by the merozoite and receptors on the erythrocyte surface (Cowman AF and Crabb BS, 2006). Several molecules implicated in the invasion process have been identified in the apical organelles (rhoptry, micronemes, and dense granules) of the merozoite. At least two gene families the Reticulocyte Binding Protein homologues (RH) and the family of erythrocyte binding proteins/ligands (EBL) have been shown to mediate specific interactions with host cell receptors thereby defining host cell specificity and are thought to play an important role in parasite virulence and possibly immune evasion (Gaur et al., 2004; Iyer et al., 2007).

In the human parasite *Plasmodium falciparum* two gene families termed Erythrocyte Binding Like Proteins (or EBL) and the Reticulocyte Binding Protein Homologues (RBPH) have been shown to play a crucial role in the selection of suitable host cells. Both EBL and RBPH are thought to directly interact with specific receptors on the red blood cell surface. In the case of EBL the region within the protein that directly mediates binding has been identified. This region called Duffy Binding Like Domain (DBL) is characterized by a number of conserved cysteine residues and is conserved in all members of this gene family. In contrast no binding region of any RBPH member has so far been identified. Considering the large size of these proteins (up to 300kDa) it is crucial to dissect the protein into smaller functional domains.

Numerous studies have indicated that malarial merozoites can invade erythrocytes through several invasion pathways. This ability is dependent on the repertoire of parasite ligands expressed at the surface of the parasite and variations of receptors at the erythrocyte surface. The various alternative invasion pathways are classified according to the nature of the erythrocyte receptors involved in invasion, which in turn are operationally defined by the enzymatic treatments upon erythrocytes which disrupt binding. *P. falciparum* EBA-175, one of the EBL members, is the best characterized receptor and recognizes sialic acid components on Glycophorin A (Sim BKL et al, 1994). Other EBLs have been shown to interact with a Glycophorin B and C as well as the Duffy blood group antigen.

In *P. falciparum* five RH members PfRH1, PfRH2a & 2b, PfRH3, and PfRH4 have been identified (Cowman AF and Crabb BS, 2006). Recognition of erythrocytes by PfRH1 is sialic acid dependent and trypsin resistant (Rayner JC et al, 2001), whereas that of PfRH2b is sialic acid independent and trypsin resistant (Duraisingh et al., 2003), and that of PfRH4 is sialic acid independent and trypsin resistant (Stubbs et al, 2005). While all these studies indicate that

RH recognizes a specific receptor on the erythrocyte surface, only in the case of PfRH1 has direct binding to red blood cells been demonstrated.

How binding of EBL or RH to specific erythrocyte receptors ultimately leads to merozoite invasion is an important question that requires the parasite ligand to be dissected into functional domains.

Summary of the invention

The present invention addresses the problems above and provides at least one *Plasmodium* reticulocyte Binding Protein homologue (RH) fragments that is involved in the binding to and/or invasion of the erythrocytes by the parasite. The present invention therefore provides effective preventive and/or therapeutic measures against Plasmodium invasion.

Accordingly, the present invention provides at least one isolated polypeptide, wherein the polypeptide is a Plasmodium Reticulocyte Binding Protein homologue (RH) fragment, comprising or substantially comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof. In particular the isolated polypeptide comprises at least one amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. More in particular the at least one polypeptide comprises at least the amino acid of SEQ ID NO: 8, a homologue and/or a portion thereof. The isolated polypeptide sequence may be less than 700 amino acids, in particular less than 550 amino acids. The isolated polypeptide sequence may be selected from PfRH1, PvRBP1 and/or a homologue thereof.

According to another aspect, the invention provides an isolated polypeptide comprising or substantially comprising at least one amino acid sequence

selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof, wherein the polypeptide comprises less than 700. In particular, the polypeptide comprises less than 550 amino acids. More in particular, the polypeptide according to the invention consists of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof.

The invention also provides an isolated polynucleotide encoding a polypeptide comprising, substantially comprising or consisting of, at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof. In particular, the polynucleotide may comprise, substantially comprise or consist of at least one nucleic acid sequence selected from SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof. More in particular the at least one nucleic acid sequence may be SEQ ID NO: 7, a homologue and/or a portion thereof. There is also provided a vector comprising the isolated polynucleotide. Further, there is provided at least one host cell comprising vector or the polynucleotide according to the invention.

According to another aspect, the invention provides a method of producing the polypeptide according comprising the steps of:

- (a) culturing the cell comprising the vector, under conditions suitable for expression of the polypeptide; and
- (b) recovering the polypeptide so expressed.

The recovered polypeptide may comprise at least one of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. In particular, the polypeptide may comprise at least the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

According to yet another aspect of the invention, there is provided an isolated antibody, wherein the antibody specifically binds to a polypeptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. In particular, the antibody may specifically bind to a polypeptide comprising, substantially comprising or consisting of the amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 8 a homologue and/or a portion thereof. The antibody may be monoclonal, polyclonal, chimeric, humanised, single chain, Fab, Fab', F(ab)' fragments and/or F(v) portions of the whole antibody.

According to a further aspect, the invention provides a pharmaceutical composition for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes, comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising, substantially comprising or consisting of at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof,

optionally in the presence of at least one pharmaceutically acceptable excipient, diluent, carrier, adjuvant and/or a combination thereof.

There is also provided a method of treating and/or preventing malaria comprising administering to a subject in need a composition comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule capable of hybridizing and/or complementary to any part of the polynucleotide comprising, substantially comprising or consisting of at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, a homologue and/or a portion thereof.

The method may comprise reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes, wherein the subject may be a mammal. In particular the subject may be a human.

According to yet another aspect of the invention there is provided a use of

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

5 (c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising, substantially comprising or consisting of at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof,

10 in preparation of a medicament for use in therapy. In particular, the medicament may be for treating and/or preventing malaria. More in particular, the medicament may be for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes.

There is also provided at least one polypeptide, at least one polynucleotide
15 and/or at least one antibody according to the invention for use in therapy. In particular, the polypeptide, polynucleotide and/or antibody may be for treating and/or preventing malaria, and more in particular, for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes.

According to a further aspect, the invention provides a method of diagnosis
20 and/or prognosis of malaria in a subject, comprising:

(a) providing at least one sample from a subject;

(b) detecting the presence of at least one polynucleotide comprising, substantially comprising or consisting of at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and
25 SEQ ID NO: 7, a homologue and/or a portion thereof; and/or

(c) detecting the presence of at least one polypeptide comprising, substantially comprising or consisting of the amino acid sequence of SEQ

ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, a homologue and/or a portion thereof;

wherein the presence of the polynucleotide and/or the polypeptide is indicative of presence of Plasmodium in the subject. The step (c) may be in the presence
5 of at least one antibody capable of binding to at least one polypeptide comprising, substantially comprising or consisting of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. The at least one polypeptide may
10 comprise, substantially comprise or consists of the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The subject may be a mammal, in particular a human.

According to yet another aspect the invention provides a diagnostic and/or prognostic kit for the diagnosis and/or prognosis of malaria in a subject comprising

15 (a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

20 (b) at least one nucleic acid molecule capable of hybridizing and/or complementary to any part of the polynucleotide comprising, substantially comprising or consisting of at least one of the sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, a homologue and/or a portion thereof,

25 wherein the subject may be a mammal, in particular the subject may be a human.

According to another aspect of the present invention there is provided a method of inducing a protective immune response to Plasmodium merozoites in a

subject comprising administering to the subject an immunologically effective amount of at least one polypeptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. Optionally, the polypeptide may be in combination with at least one pharmaceutically acceptable excipient, carrier, adjuvant and/or additive. There is also provided a pharmaceutical composition comprising the at least one polypeptide in combination with at least one pharmaceutically acceptable excipient, carrier, diluent, adjuvant and/or additive. The at least one polypeptide may comprise, substantially comprise or consist of the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

According to yet another aspect of the invention, there is provided a recombinant DNA vaccine comprising an expression vector for expression of a polynucleotide, the polynucleotide comprising, substantially comprising or consisting of at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof, optionally in the presence of at least one pharmaceutically acceptable excipient, carrier, adjuvant, diluent and/or additive. The at least one polynucleotide may comprise, substantially comprise or consists of the nucleic acid sequence of SEQ ID NO: 7, a homologue and/or a portion thereof. The polynucleotide may encode for an immunogenic peptide, comprising, substantially comprising or consisting of at least one of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof, wherein the at least one amino acid sequence comprises, substantially comprises or consists of the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The recombinant vaccine may be for use in a mammal, in particular a human.

According to a further aspect the invention provides, a method of vaccinating a subject against malaria comprising administering to the subject in need an effective amount of a recombinant DNA vaccine capable of expressing an immunogenic peptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof after administration of the vaccine to a subject. The at least one amino acid sequence may comprise, substantially comprise or consist of the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The subject may be a mammal, in particular a human.

Brief description of the figures

Figure 1 represents chimeric constructs for the expression of different regions of genomic sequence from *PfRH1* with intro spliced out in 3D7 on COS7 cells. *P. falciparum* 3D7 clone with intron spliced out (Genbank accession no: AF533700). Sequence includes signal sequence (SS, black) and Exon2 (grey) encoding a large extracellular domain. Figure represents the extracellular domain divided into eight regions (From I to VIII). Each region (black line with bp no.) is approximately 2Kb with 1Kb overlap between 2 regions except VIII, which is 1.3Kb.

Figure 2 represents the erythrocyte-binding assay on COS7 cells transfected with chimeric constructs, each expressing one of the eight regions of *PfRH1* before and after enzymatic treatment. (A) Typical erythrocyte binding to COS cell expressing binding region or (B) non-binding region (magnification of X 200). (i) Bright field of typical field and corresponding (ii) Cell-associated fluorescence as GFP (green) Bar, 50um. (C) Percentage (%) of erythrocyte binding activity of the various *PfRH1* regions after normalizing transfection efficiency. (D) Comparison of Percentage (%) erythrocyte-binding activity of

different regions of PfRH1 after enzymatic treatment. Data represent percentage of binding activity (%) after normalizing transfection efficiency to 5% in three independent experiments, and the error bar denotes the SE.

- 5 Figure 3 represents erythrocyte-binding assay on COS7 cells transfected with construct expressing full-length region II or deletion constructs of PfRH1 before and after enzymatic treatment. (A) Overall architecture of Region II deletion constructs of PfRH1. *RII-1* was deleted 500bps at 3' end of region II, *RII-2* was deleted 500bps at 5' end of region II and *RII-3* was deleted 500bps at both ends of region II (light gray). (B). Comparison of erythrocyte-binding activity with full-length RII or deletion constructs before and after enzymatic treatment. Data represent percentage of binding activity (%) after normalizing transfection efficiency to 5% in three independent experiments, and the error bar denotes the SE.

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- Figure 4 represents erythrocyte-binding with recombinant protein rRII-3 or rRVIII. Protein bound to erythrocytes was detected by Western blotting with anti-His mouse antibody. Erythrocyte-binding assay with purified rRII-3(A) or rRVIII (B) Protein only (Lane 1), Protein bound to untreated erythrocytes (lane 2), Neuraminidase treated (lane 3), chymotrypsin treated (lane 4) and trypsin treated (lane 5) erythrocytes.

- Figure 5 represents bioinformatic analysis of the minimal binding region RII-3 of PfRH1. (A) Alignment and secondary structural prediction of putative erythrocyte binding region of *P. falciparum* RHs family members, PfRH1-RII-3 SEQ ID NO:8), PfRH2A (SEQ ID NO:38) and PfRH4 (SEQ ID NO:40). Red or dark grey with "*" underneath indicates identical residues, blue or black with ":" underneath indicates conserved residues and green or light grey with "." indicates semi-conserved residues. Secondary structure is shown above the sequence alignment with α -helix in purple or dark grey and β -sheet shown as

arrow in purple or dark grey. Dashed lines indicate regions not observed in the predicted secondary structure. (B) Approximate locations of predicated erythrocyte binding regions of different *plasmodium* species, *P. falciparum* – PfRH1-RII-3; *P. yoelii* – Py235; *P. vivax* – PvRBP1 and PvRBP2. The homologous regions of PfRH1-RII-3 erythrocyte binding region are indicated as light grey boxes. (C) Results of the coiled coils prediction methods for the RII-3 minimal binding protein. The calculation was done using three different window sizes of 14, 21 and 28 amino-acids respectively.

Figure 6 represents the predicted binding regions of the RH orthologues in *P. falciparum* and *P. vivax*. Binding regions were identified using PfRH1-RII-3 (SEQ ID NO: 8) vs predicted binding regions of other RH members (using ClustalW), namely the *P. falciparum* homologues PfRH2A (SEQ ID NO: 38), PfRH2B (SEQ ID NO: 39), PfRH4 (SEQ ID NO: 40), PfRH3 (SEQ ID NO: 41) and the *P. vivax* homologues, PvRBP-1 (SEQ ID NO: 42), PvRBP-2 (SEQ ID NO: 43).

Figure 7 (A) Western analysis of PfRH1 expression on the merozoite extracts supernatant probed with α RII-3 (Lane 1), α tRVIII (Lane 2), Pre-immune serum (Lane 3) and normal RBC lysate supernatant?? (Lane 4). The expected protein of about 240 kDa was detected by both antisera (arrow). Size markers are shown in kilodaltons. (B) IFA analysis of smear of free merozoites reacted with α RII-3, (C) merozoites reacted with α tRVIII and (D) merozoites reacted with preimmune serum. The parasites nuclei stained with DAPI (panel 1) are shown in first column; for the image in the second column, secondary antibody used was red AlexaFlour 594 goat anti-mouse IgG (H+L) (panel 2); the merge column shows the overlap between the first and second columns (panel 3). (E) represents invasion inhibition assay represented as percentage (%) inhibition of the W2mef strain in the presence of antibodies, RII-3 or tRVIII, or preimmune serum at various dilutions.

Brief description of the sequences

SEQ ID NO: 1 refers to the polynucleotide sequence of region II (RII) fragment, encoding the binding domain polypeptide, of *P. falciparum* PfRH1:

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5  AAAGATGTAATAAATAATAAGATAGATATATATAAAACAATAAGTTCTTTTATATCTACTCAGAAACAAT
   TATATTATTTTGAATATATATATATAATGAATAAAAATACATTAAACCTACTTTTCATATAATATACAAAA
   AACAGATATAAATTTCTAGTAGTAAATACACATATACAAAATCTCATTTTTTTAAAAAGATAATCATATATTG
   TTATCTAAATATTATACTGCCAAATTTATTGATATCCTAAATAAAACATATTATTATAATTTATATAAAA
   ATAAAATTTCTTTTATTCAATAAATATATTATAAAGCTTAGAAACGATTTAAAAAGAATATGCATTTAAATC
10  TATACAATTTTATTCAAGATAAAATCAAAAACATAAAGATGAATTATCCATAGAAAAATATATTACAAGAA
   GTTAATAATATATATATAAAATATGATACTTCGATAAATGAAATATCTAAATATAACAATTTAATTATTA
   ATACTGATTTACAAATAGTACAACAAAACTTTTAGAAATCAAACAAAAAATGATATTACACACAA
   AGTACAACCTTATAAATCATATATATAAAAATATACATGATGAAATATTAAACAAAAAATAATGAAATA
   ACAAAGATTATTATAAATAATATAAAAGATCATAAAAAGATTTACAAGATCTCTTACTATTTATACAAC
15  AAATCAAACAATATAATATATTAACAGATCATAAAATTACACAATGTAATAATTATTATAAGGAAATCAT
   AAAAATGAAAGAAGATATAAATCATATTCATATATATATACAACCAATTCTAAATAATTTACACACATTA
   AAACAAGTACAAAATAATAAATCAAATATGAAGAGCACATCAAACAAATATTACAAAAAATTTATGATA
   AAAAGGAATCTTTAAAAAAAATTTCTCTTAAAGATGAAGCACAATTAGACATTACCCCTCCTCGATGA
   CTTAATACAAAAGCAAACAAAAAACAAACACAAACACAAACACAAAAACAAACACTAATACAA
20  AATAATGAGACGATTCAACTTATTTCTGGACAAGAAGATAAACATGAATCCAATCCATTTAATCATATAC
   AAACCTATATTCAACAAAAAGATACACAAAATAAAAACATCCAAATCTTCTTAAATCCTTGATAATGG
   AAATATTAACACATTCATAGACACAATTTCTAAATATATATTAACAAAAAGATATAGAATTAACACAA
   CACGTTTATACAGACGAAAAATTAATGATTATCTTGAAGAAATAAAAAATGAACAAAAACAAATAGATA
   AGACCATCGACGATATAAAAATACAAGAAACATTAACAAATAACTCATATTGTTAACAATATAAAAAAC
25  CATCAAAAAGGATTTGCTCAAAGAATTTATTCAACATTTAATAAAATATATGAACGAAAGATATCAGAAT
   ATGCAACAGGGTTATAATAATTTAACAATTTATTAATCAATATGAAGAAGAAAATAATAATATGAAAC
   AATATATTACTACCATACGAAATATCCAAAAATATATTATGATAATATATATGCTAAGGAAAAGGAAAT
   TCGCTCGGGACAATATTATAAGGATTTTATCACATCAAGGAAAATATTTATAATATAAGGGAAAATATA
   TCCAAAAATGTAGATATGATAAAAAATGAAGAAAAGAAGAAATACAGAATTGTGTAGATAAATATAATT
30  CTATAAAACAATATGTAATAATGCTTAAAAATGGAGACACACAAGATGAAATAATAATAATAATGA
   TATATACGACAAGTTAATTGTCCCCCTTGATTCAATAAAACAAAATATCGATAAATACAACACAGAACAT
   AATTTTATAACATTTACAAATAAAATAAATACACATAATAAGAAGAACCAAGAAATGATGGAAGAATTCA
   TATATGCATATAAAAGGTTAAAAATTTTAAAAATATTAAAT

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35 SEQ ID NO: 2 refer to the polypeptide sequence of region II (RII) fragment of *P. falciparum* PfRH1.

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KDVINNKIDYKTISSFISTQKQLYFFEYIYIMNKNTLNLLSYNIQKTDINSSSKYTYTKSHFLKDNHIL
LSKYTTAKFIDILNKTYYYNLYKNKILLFNKYI IKLRNDLKEYAFKSIQFIQDKIKKHKDELSIENILQE
VNNIYIKYDTSINEISKYNNLIINTDLQIVQQKLEIKQKNDITHKVQLINHIYKNIHDEILNKKNEI
40  TKIIINNIDHKKDLQDLLLFIQQIKQYNILTDHKITQCNNYKEI IKMKEDINHIHIYIQPILNNLHTL
   KQVQNNKIKYEEHIKQILQKIYDKKESLKKIILLKDEAQLDITLLDDLIQKQTKKQTOTOTOTQKQTLIQ
   NNETIQLISGOEDKHESNPFNHIQTYIQQKDTQNKNIQNLLKSLYNGNINTFIDTISKYILKQKDIELTQ
   HVYTDEKINDYLEEIKNEQNKIDKTIDDIKIQETLKQITHIVNNIKTIKKDLLKEFIQHLIKYMNERYQN
   MQQGYNNLTNYINQYEEENNMKQYITTIRNIQKIYYDNIYAKEKEIRSGQYKDFITSRKNINIRENI
45  SKNVDMIKNEEKKKIQNCVDKYSIKQYVKMLKNGDTQDENNNNNNDIYDKLIVPLDSIKQNDIKYNTHE
   NFITFTNKINTHNKKNQEMMEEFIYAYKRLKILKILN

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SEQ ID NO: 3 refers to the polynucleotide sequence of a smaller PfRH1-RII (RII-1) fragment having deletion at the 3' end.

5 AAAGATGTAATAAATAATAAGATAGATATATATAAAACAATAAGTTCTTTTATATCTACTCAGAAACAAT
TATATTATTTTGAATATATATATATAATGAATAAAAATACATTAAACCTACTTTCATATAATATACAAAA
AACAGATATAAATTCTAGTAGTAAATACACATATACAAAATCTCATTTTTAAAAGATAATCATATATTG
10 TTATCTAAATATTATACTGCCAAATTTATTGATATCCTAAATAAAACATATTATTATAATTATATAAAAA
ATAAAATTCCTTTTATTCAATAAATATATTATAAAGCTTAGAAACGATTTAAAAGAATATGCATTTAAATC
TATACAATTTATTCAAGATAAAATCAAAAAACATAAAGATGAATTATCCATAGAAAAATATATTACAAGAA
GTTAATAATATATATATAAAATATGATACTTCGATAAATGAAATATCTAAATATAACAATTTAATTATTA
15 ATACTGATTTACAAATAGTACAACAAAACTTTTAGAAATCAAACAAAAAATGATATTACACACAA
AGTACAACCTTATAAATCATATATATAAAAAATATACATGATGAAATATTAACAAAAAATAATGAAATA
ACAAAGATTATTATAAATAATATAAAAGATCATAAAAAAGATTTACAAGATCTCTTACTATTTATACAAC
AAATCAAACAATATAATATATTAACAGATCATAAAATTACACAATGTAATAATTATTATAAGGAAATCAT
AAAAATGAAAGAAGATATAAATCATATTATATATATACAAACCAATTCTAAATAATTTACACACATTA
20 AAACAAGTACAAAATAATAAATCAATATGAAGAGCACATCAAACAAATATTACAAAAATTTATGATA
AAAAGGAATCTTTAAAAAAATTTATTCTCTTAAAGATGAAGCACAAATTAGACATTACCCTCCTCGATGA
CTTAATACAAAAGCAAAACAAAAAACAAACACAAACACAAACACAAACACAAACACAAACACAAACACTAATACAA
AATAATGAGACGATTCAACTTATTTCTGGACAAGAAGATAAACATGAATCCAATCCATTTAATCATATAC
25 AAACCTATATTCAACAAAAAGATACACAAAATAAAAAACATCCAAATCTTCTTAAATCCTTGTATAATGG
AAATATTAACACATTTCATAGACACAATTTCTAAATATATATTAACAAAAAGATATAGAATTAACACAA
CACGTTTATACAGACGAAAAATTAATGATTATCTTGAAGAAATAAAAAATGAACAAAAACAAATAGATA
AGACCATCGACGATATAAAAAATACAAGAAACATTAACAAATAACTCATATTGTTAACAATATAAAAAAC
CATCAAAAAGGATTTGCTCAAAGAATTTATTCAACATTTAATAAAATATATGAACGAAAGATATCAGAAT
ATGCAACAGGGTTATAATAATTTAACAAT

SEQ ID NO: 4 refer to the polypeptide sequence of a smaller PfRH1-RII (RII-1) fragment having deletion at the 3' end.

30 KDVINNKIDYKTISSFISTQKQLYFFEYIYIMNKNTLNLSSYNIQKTDINSSSKYTYTKSHFLKDNHIL
LSKYTAKFIDILNKTYYNLYKNKILLFNKYI IKLRNDLKEYAFKSIQFIQDKIKKKHDELSIENILQE
VNNIYIKYDTSINEISKYNNLIINTDLQIVQKLLLEIKQKKNDITHKVQLINHIYKNIHDEILNKKNEI
TKIIINNIKDHKKDLQDLLLFIQQIKQYNILTDHKITQCNYYKEI IKMKEDINHIHIYIQPILNNLHTL
KQVQNNKIYEEHIKQILQKIYDKKESLKKIILLKDEAQLDITLLDDLIQKQTKKQTQTQTQKQTLIQ
35 NNETIQLISGQEDKHESNPFNHIQTYIQKQDTQNKNIQNLKSLYNGNINTFIDTISKYILKQKDIELTQ
HVYTDEKINDYLEEIKNEQNKIDKTIDDIKIQETLKQITHIVNNIKTIKKDLLKEFIQHLLIKYMNERYQN
MQQGYNNLTN

SEQ ID NO: 5 refers to the polynucleotide sequence of a smaller PfRH1-RII (RII-2) fragment having deletion at the 5' end.

40 TTACAAATAGTACAACAAAACTTTTAGAAATCAAACAAAAAATGATATTACACACAAAGTACAAC
TTATAAATCATATATATAAAAAATATACATGATGAAATATTAACAAAAAATAATGAAATAACAAAGAT
TATTATAAATAATATAAAAGATCATAAAAAAGATTTACAAGATCTCTTACTATTTATACAACAAATCAAA
CAATATAATATATTAACAGATCATAAAATTACACAATGTAATAATTATTATAAGGAAATCATAAAAAATGA
AAGAAGATATAAATCATATTTCATATATATATACAACCAATTCTAAATAATTTACACACATTAACAAAGT
45 ACAAATAATAAATCAAATATGAAGAGCACATCAAACAAATATTACAAAAATTTATGATAAAAAGGAA
TCTTTAAAAAAATTTATTCTCTTAAAGATGAAGCACAAATTAGACATTACCCTCCTCGATGACTTAATAC
AAAAGCAAAACAAAAAACAAACACAAACACAAACACAAACACAAACACAAACACTAATACAAAATAATGA
GACGATTCAACTTATTTCTGGACAAGAAGATAAACATGAATCCAATCCATTTAATCATATACAAACCTAT

ATTCAACAAAAAGATACACAAAAATAAAAAACATCCAAAAATCTTCTTAAATCCTTGTATAATGGAAATATTA
 ACACATTCATAGACACAATTTCTAAATATATATATTAACAAAAAGATATAGAATTAACACAACACGTTTA
 TACAGACGAAAAAATTAATGATTATCTTGAAGAAATAAAAAATGAACAAAAACAAATAGATAAGACCATC
 GACGATATAAAAAATACAAGAAACATTAAAAACAAATAACTCATATTGTTAACAATATAAAAAACCATCAAAA
 5 AGGATTTGCTCAAAGAATTTATTCAACATTTAATAAAAAATATATGAACGAAAGATATCAGAATATGCAACA
 GGGTTATAATAATTTAACAAATTATATTAATCAATATGAAGAAGAAAAATAATAATATGAAACAATATATT
 ACTACCATACGAAATATCCAAAAATATATTATGATAATATATATGCTAAGGAAAAGGAAATTCGCTCGG
 GACAATATTATAAGGATTTTATCACATCAAGGAAAAATATTTATAATATAAGGGAAAAATATATCCAAAA
 TGTAGATATGATAAAAAATGAAGAAAAGAAGAAAAATACAGAATTGTGTAGATAAATAAATTCTATAAAA
 10 CAATATGTAAAAATGCTTAAAAATGGAGACACACAAGATGAAAAATAATAATAAATAATGATATATACG
 ACAAGTTAATTGTCCCCCTTGATTCAATAAAACAAAAATATCGATAAATACAACACAGAACATAATTTTAT
 AACATTTACAAATAAAATAAATACACATAATAAGAAGAACCAAGAAATGATGGAAGAATTCATATATGCA
 TATAAAAGGTTAAAAATTTTAAAAATATTAAAT

- 15 SEQ ID NO: 6 refer to the polypeptide sequence of a smaller PfRH1-RII (RII-2) fragment having deletion at the 5' end.

LQIVQQKLLLEIKQKKNIDITHKVQLINHIYKNIHDEILNKKNNETKIIINNIDHKDLQDLLLFIQQIK
 QYNILTDHKITQCNNYYKEIIMKEDINHIHIYIQPILNNLHHTLKQVQNNKIKYEEHIKQILQKIYDKKE
 SLKKIILLKDEAQLDITLLDDLIQKQTKQTQTQTQKQTLIQNNETIQLISGQEDKHESNPFNHIQTY
 20 IQQKDTQNKNIQNLKSLYNGNINTFIDTISKYILKQKDIELTQHVYTDEKINDYLEEIKNEQNKIDKTI
 DDIKIQETLQKITHIVNNIKTIKKDLLKEFIQHLIKYMNERYQNMQQGYNNLTNYINQYEEENNNMKQYI
 TTIRNIQKIYYDNIYAKEKEIRSGQYYKDFITSRKNINYIRENISKNVDMIKNEEKKKIQNCVDKYNISIK
 QYVKMLKNGDTQDENNNNNNDIYDKLIVPLDSIKQNIQDKNYTEHNFTFTNKINTHNKKNQEMMEEFIYA
 YKRLKILKILN

25

- SEQ ID NO: 7 refer to the polynucleotide sequence of a smaller PfRH1-RII (RII-3) fragment having deletion at the 5' and the 3' end.

TTACAAATAGTACAACAAAACTTTTAGAAATCAAACAAAAAATGATATTACACACAAAGTACAAC
 TTATAAATCATATATATAAAAAATATACATGATGAAATATTAAACAAAAAATAATGAAATAACAAAGAT
 30 TATTATAAATAATATAAAAGATCATAAAAAAGATTTACAAGATCTCTTACTATTTATACAACAAATCAAA
 CAATATAATATATTAAACAGATCATAAAATTACACAATGTAATAATTATTATAAGGAAATCATAAAATGA
 AAGAAGATATAAATCATATTCATATATATATACAACCAATTCTAAATAATTTACACACATTTAAACAAGT
 ACAAATAATAAAATCAAATATGAAGAGCACATCAAACAAATATTACAAAAAATTTATGATAAAAAGGAA
 TCTTTAAAAAAATTTATTCTCTTAAAAGATGAAGCACAAATTAGACATTACCCTCCTCGATGACTTAATAC
 35 AAAAGCAAACAAAAAACAAACACAAACACAAACACAAACACAAACAAACACTAATACAAAATAATGA
 GACGATTCAACTTATTTCTGGACAAGAAGATAAACATGAATCCAATCCATTTAATCATATACAAACCTAT
 ATTCAACAAAAAGATACACAAAAATAAAAAACATCCAAAAATCTTCTTAAATCCTTGTATAATGGAAATATTA
 ACACATTCATAGACACAATTTCTAAATATATATTAACAAAAAGATATAGAATTAACACAACACGTTTA
 TACAGACGAAAAAATTAATGATTATCTTGAAGAAATAAAAAATGAACAAAAACAAATAGATAAGACCATC
 40 GACGATATAAAAAATACAAGAAACATTAAAAACAAATAACTCATATTGTTAACAATATAAAAAACCATCAAAA
 AGGATTTGCTCAAAGAATTTATTCAACATTTAATAAAAAATATATGAACGAAAGATATCAGAATATGCAACA
 GGGTTATAATAATTTAACAAAT

- 45 SEQ ID NO: 8 refer to the polypeptide sequence of a smaller PfRH1-RII (RII-3) fragment having deletion at the 5' and the 3' end.

5 LQIVQQKLEIKQKKNDITHKVQLINHIYKNIHDEILNKKNEITKIIINNIKDHKDLQDLLLLFIQQIK
QYNILTDHKITQCNYYKEIIMKEDINHIHIYIOPILNNLHTLKQVQNNKIKYEEHIKQILQKIYDKKE
SLKKIILLKDEAQLDITLLDDLIQKQTKKQTQTQTQKQTLIQNNETIQLISGQEDKHESNPFNHIQTY
IQQKDTQNKNIQNLKSLYNGNINTFIDTISKYILKQKDIELTQHVYTDEKINDYLEEIKNEQNKIDKTI
DDIKIQETLKQITHIVNNIKTIKKDLLKEFIQHLLIKYMNERYNMQQGYNNLTN

Primer sequences:

10 SEQ ID NO: 9 (5' GACCAGCTGGAATTTAGCCATGAACAGGAA 3') refers to the forward primer and SEQ ID NO: 10 refers to the reverse primer (5'AACGGGGCCCTTTTGTGTTG CTTTGTATTAA 3') used to amplify the Region I (RI) of *P. falciparum* PfRH1 (PfRH1-RI).

15 SEQ ID NO: 11 (5' GACCAGCTGAAAGATGTAATAATAATAAG 3') refers to the forward primer and SEQ ID NO: 12 refers to the reverse primer (5' AACGGGGCCCATTTAA TATTTTAAAATTTT 3') used to amplify the Region II (RII) of *P. falciparum* PfRH1 (PfRH1-RII).

20 SEQ ID NO: 13 (5' TCTCGTCAGCTGCTAATAACAAAATAATGAGACG 3') refers to the forward primer and SEQ ID NO: 14 refers to the reverse primer (5' ACGATGGGGCC CTATATCGTCAAAATGTTTTGT 3') used to amplify the Region III (RIII) of *P. falciparum* PfRH1 (PfRH1-RIII).

25 SEQ ID NO: 15 (5' GACCAGCTGATATCCTTAAAAGCTTGTGAA 3') refers to the forward primer and SEQ ID NO: 16 refers to the reverse primer (5' AACGGGGCCCTTTAGA TTTGTTTACATCTAT 3') used to amplify the Region IV (RIV) of *P. falciparum* PfRH1 (PfRH1-RIV).

30 SEQ ID NO: 17 (5' GACCAGCTGTACCATGCTGATGATACACGT 3') refers to the forward primer and SEQ ID NO: 18 refers to the reverse primer (5' AACGGGGCCCTATAAA AACATTATATATTTT 3') used to amplify the Region V (RV) of *P. falciparum* PfRH1 (PfRH1-RV).

SEQ ID NO: 19 (5' GACCAGCTGAATAATGCTCAACTATATTTT 3') refers to the forward primer and SEQ ID NO: 20 refers to the reverse primer (5' AACGGGCCCCATTCAT TTGTTCTAATTTGTT 3') used to amplify the Region VI (RVI) of *P. falciparum* PfRH1 (PfRH1-RVI).

5

SEQ ID NO: 21 (5' GACCAGCTGCAATCATATAATTTAATACAA 3') refers to the forward primer and SEQ ID NO: 22 refers to the reverse primer (5' AACGGGCCCCGATGTT GGTTATAT TTCTTG 3') used to amplify the Region VII (RVII) of *P. falciparum* PfRH1 (PfRH1-RVII).

10

SEQ ID NO: 23 (5' GACCAGCTGACAATAATTAATCAAAGTATA 3') refers to the forward primer and SEQ ID NO: 24 refers to the reverse primer (5' AACGGGCCC ATTTTTTTTTTTTGTTC AATTC 3') used to amplify the Region VIII (RVIII) of *P. falciparum* PfRH1 (PfRH1-RVIII).

15

SEQ ID NO: 25 (5' TCTCGTCAGCTGAAAGATGTAATAAATAATAAG 3') refers to the forward primer and SEQ ID NO: 26 (5' AACGGG CCCATTTGTTAAATTATTATAACC 3') refers to the reverse primer used to amplify the smaller fragment of Region II (RII-1) of *P. falciparum* PfRH1 (PfRH1-RII-1).

20

SEQ ID NO: 27 (5' GACCAGCTGTTACAAATAGTACAACAAAAA 3') refers to the forward primer and SEQ ID NO: 28 (5' AACGGGCCCCATTTAATATTTTTTAAAATTTT 3') refers to the reverse primer used to amplify the smaller fragment of Region II, (RII-2) of *P. falciparum* PfRH1 (PfRH1-RII-2).

25

SEQ ID NO: 29 (5' GACCAGCTGTTACAAATAGTACAACAAAAA 3') refers to the forward primer and SEQ ID NO: 30 (5' AACGGGCCCC

ATTTGTTAAATTATTATAACC 3') refers to the reverse primer used to amplify the smaller fragment of Region II, (RII-3) of *P. falciparum* PfRH1 (PfRH1-RII-3).

SEQ ID NO: 31 (5' CGT ATACTCGAGATGGGGGGGACTGCCGCC 3') refers to the universal forward primer and SEQ ID NO: 32 (5' CGTATAGGATC CAAGTAAAACAAGGGCTG 3') refers to the universal reverse primer used to amplify the various regions (RI to RVIII) cloned into pRE4 vector for sub cloning of the respective regions into the pEGFP-N1 vector.

SEQ ID NO: 33 (5' GACCATATGTTACAAATAGTACAACAAAAA 3') refers to forward primer and SEQ ID NO: 34 (5' AACCTCGAGATTTGT TAAATTATTATAACC 3') refers to reverse primer used to amplify the smaller fragment of Region II, (RII-3) for cloning into pET24a+ over expression vector.

SEQ ID NO: 35 (5' GACGAATTCATAAATGAAGAAGCTCTACAA 3') refers to forward primer and SEQ ID NO: 36 (5' AACCTCGAGATTTTTTTT TTTGTTCAATTC 3') refers to reverse primer used to amplify the smaller fragment of Region VIII, (rtRVIII) for cloning into pET24a+ over expression vector.

SEQ ID NO:37: DNA sequence of rtRVIII

ATAAATGAAGAAGCTCTACAATTTACAGGCTCTATGGACACAATCTTATAAGTGAAGATGACAAAAATA
ATTTGGTACATATTATAAAAGAACAAAAGAATATATATACACAAAAGGAAATAGATATTTCTAAAATAAT
TAAACATGTTAAAAAGGATTATATTCATTGAATGAACATGATATGAATCATGATACACATATGAATATA
ATAAATGAACATATAAATAATAATATTTTACAACCATACACACAATTAATAAACATGATAAAAGATATTG
ATAATGTTTTTATAAAAATACAAAATAATAAATTCGAACAAATACAAAAATATATAGAAATTATTAAATC
TTTAGAACAATTAAATAAAAAATATAAACACAGATAATTTAAATAAATTAAGATACACAAAACAAATTA
ATAAATATAGAAACAGAAATGAAACATAAAACAAAAACAATTAATAAACAAATGAATGATATAGAAAAGG
ATAATATTACAGATCAATATATGCATGATGTTTCAGCAAAATATATTTGAACCTATAACATTAAAAATGAA
TGAATATAATACATTATTAAATGATAATCATAATAATAATATAAATAATGAACATCAATTTAATCATTTA
AATAGTCTTCATACAAAATATTTAGTCATAATTATAATAAAGAACAACAACAGAAATATATAACCAACA
TCATGCAAAGAATTGATGTATTCATAAATGATTTAGATACTTACCAATATGAATATTATTTTTATGAATG
GAATCAAGAATATAAACAAATAGACAAAATAAAATAAATCAACATATAAACAAATATTAATAAATCTA
ATTCATGTTAAGAAACAATTTGAACACACCTTAGAAAATATAAAAAATAATGAAAATATTTTCGACAACA
TACAATTGAAAAAAAAGATATTGACGATATTATTATAAACATTAATAATACAAAAGAAACATATCTAAA
AGAATTGAACAAAAAAAAT

Homologous sequences (Figures 5 and 6):

SEQ ID NO: 38 refers to the PfRH2a sequence homologous to the PfRH1-RII-3 binding domain.

5 LEETQDKLLELYENFKKEKNIINNYYKIVHFNKLKEIENSLETYNSISTNFNKINETQNIDILK
NEFNNIKTKINDKVKELVHVDSTLTLESIQTFNNLYGDLMSNIQDVYKYEDINNVELKKVKLYI
ENITNLLGRINTFIKELDKYQDENNGIDKYIEINKENNSYIIKLKEKANNLKENFSKLLQNIKR
NETELYNINNIIKDDIMNTGKSVNNIKQKFSSNLPLKEKLFQMEEMLLNINNIMNETKRISNTAA
10 YTNITLQDIENNKKNENNNMNIETIDKLIDHIKIHNKEIQAEILIIDDAKRKVKEITDNINKAF
NEITENYNNENN

SEQ ID NO: 39 refers to the sequence of the predicted binding region of *P. falciparum* PfRH2b.

15 LEETQDKLLELYENFKKEKNIINNYYKIVHFNKLKEIENSLETYNSISTNFNKINETQNIDILK
NEFNNIKTKINDKVKELVHVDSTLTLESIQTFNNLYGDLMSNIQDVYKYEDINNVELKKVKLYI
ENITNLLGRINTFIKELDKYQDENNGIDKYIEINKENNSYIIKLKEKANNLKENFSKLLQNIKR
NETELYNINNIIKDDIMNTGKSVNNIKQKFSSNLPLKEKLFQMEEMLLNINNIMNETKRISNTDA
YTNITLQDIENNKKNENNNMNIETIDKLIDHIKIHNKEIQAEILIIDDAKRKVKEITDNINKAF
20 NEITENYNNENN

SEQ ID NO: 40 refer to the PfRH4 sequence homologous to the PfRH1-RII-3 binding domain.

LNKFMQNETFFKKNIDDKIKEMNNIYDNIYIILKQKFLNKLNEIIQNHKNKQETKLNTTTTIQELL
QLLKDIKEIQTKQIDTKINTFNMYNDIQQIKIKINQNEKEIKKVLPQLYIPKNEQEYIQIYKN
25 ELKDRIKETQTKINLQKILELKEKEHYITNKHTYLNFTHTKIQQILQQQYKNNTQEKNTLAQF
LYNADIKKYIDELIPITQQIQTKMYTTNNIEHIKQILINYYIQECKPIQNISEHTIYTLYQEIKT
NLENIEQKIMQNIQQTTNRLKINIKKIFDQINQKYDDLTKNINQMND

SEQ ID NO: 41 refers to the predicted sequence of the binding region of *P. falciparum* PfRH3.

30 INEIKSKMDNINEKLKHITDFIDKNVNYIYENHSTQDINIMLNNTISEYNKLEFINSDFDNIS
KKLKKELQDLVTLKESLMKMNHVNLKMDPLKSLNQVLEKYEELKKNINEYSKEENKLYDFKKQM
ESRLNAFITNLNNDETLDVGKNIYDQFVEYKEQLLIKRIIINNEIVIINDEVKKIKDELKSY
NILSYKLENDTSHDVVNSVENTPSSDVATAVSNSSSILSTYNSTELNKLNRFFSEKDDDELNVES
35 KVKQDENIFIEKNKIFDDIIEKDIELYNKKTNAIKNLNNAINGSMNNLSLIDSVKMKNKGDIINRL
SQRSYLIQTDNFDIYEKIFLKDNLNKGLEEIENRLSNTYMNELKIEAEKQNEKYKKLKENINT
YDDTFLEKLIGDNYEWEVLKIELNGLNVNINILQANIDTLIIKPYIDHIDHIIISLIESLKHNE
NKIKKVIPNLERLKDFIQTKFNTNDIKLDHNNLIT

SEQ ID NO: 42 refers to the predicted sequence of the binding region of *P. vivax* PvRBP-1.

5 INDLQDLIDQMKEYKDEIVNNSEFISNRYKNIYENLKETYETELNDIGKLENDTSKVN FYLMQI
RKINTEKTKIDESLOTVEKFYKEILDSKEKIYELKIEFEKSVTEINRLQDGESARDLHEEQIKE
ILDKMAKKVHYLKELLSLKGKSSVYFTEMNELLNTASYDNMEGFSAKKEKADNDINALYNSVYR
EDINALIEEVEKFVTENKESTLEMLKDEEMEEKLQDAKETFAKLN FVSDDKLT DVYTKMSAEVT
NAEGIKKEIAQKQFENVHKKMKEFSDAFSTKFEALQNSMQQYNQEGD

SEQ ID NO: 43 refers to the predicted sequence of the binding region of *P. vivax* PvRBP-2.

10 LQKVESDIYRVELKTLFYVAAKH YADFKFSLEHLKMFENLSKSKEKMLYSTFEKLEGDLLNKIN
TLMGSEQSTSDLT SIIADSEKIIKSAESLINS SSEEI AKYALDSNEKINEIKKNYDQNILKVRE
FINKSNGLI TSVKGTSQLSESDKQOIETKIEEIKKKKKDILERGKEFINIMNEIKKKKSNSSN
15 SSTNSKEFTDKLKELETEFEGLNKT VKGYLQEIEDIKVKENEDRSLKNQIEQHLKYTSDNRDNV
KTLISKND EIQKYIEKIEKLINDAPSGKDKFTTEKTNLQNKVKKI IDEFHKEDLQLLLNSLSKF
YEEHQKLYNEASTIEKIKDLHQKTKEEYKLEKMKFSNFGQILDKLNTEL DNLTLEKNIVEEQ
TNYINKVMSDSL TNLTAEVDNLR S

Detailed description of the invention

- 20 Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content of such bibliographic references is herein incorporated by reference.
- 25 The binding of the merozoites to erythrocytes is mediated by specific binding proteins on the surface of the merozoite and is necessary for the erythrocyte invasion. At least two gene families the Reticulocyte Binding Protein homologues (RH) and the family of erythrocyte binding proteins/ligands (EBL) have been shown to mediate specific interactions with host cell receptors
- 30 thereby defining host cell specificity and are thought to play an important role in parasite virulence and possibly immune evasion. How binding of EBL or RH to specific erythrocyte receptors ultimately leads to merozoite invasion is an important question. In particular, invasion of erythrocytes by malaria parasite *Plasmodium falciparum* depends on recognition of specific erythrocyte surface
- 35 receptors by parasite ligands. The > 300 kDa *P. falciparum* reticulocyte binding

protein homologue 1 (PfRH1) recognizes a so far uncharacterized neuraminidase sensitive, trypsin resistant receptor on the surface of the erythrocyte. PfRH1 is a member of the reticulocyte binding protein homologues (RH) gene family which is found in all malaria species and plays an important
5 role in host cell selection and virulence.

The inventors have further identified the corresponding regions in the *Plasmodium falciparum* and/or *P. vivax* orthologues (Figure 6). They have also shown that the region elicits protective antibodies in mice (Figure 7).
10 Accordingly, the inventors demonstrated that immunization of mice with the correctly folded binding region of PfRH1 elicits strong invasion inhibitory antibodies while other regions of the same protein do not produce such inhibitory antibodies.

Although the invention will be described with specific reference to regions derived from *Plasmodium falciparum* and/or *P. vivax* orthologues, the invention is not limited to regions from only these two parasites. Any other homologous region derived from any other *Plasmodium* known in the art, for example, *Plasmodium ovale*, *Plasmodium malaria*, *Plasmodium yoelii*, *Plasmodium knowlesi*, *Plasmodium reichenowi*, *Plasmodium cynomolgi* or the like may be
20 encompassed by the present invention.

The present invention provides isolated polypeptide fragments of Reticulocyte Binding Protein homologues, PfRH1, involved in the binding and/or invasion of
25 the erythrocytes by Plasmodium.

Accordingly, there is provided an isolated polypeptide, wherein the polypeptide is a Plasmodium Reticulocyte Binding Protein homologue fragment, comprising, substantially comprising or consisting of at least one amino acid sequence
30 selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8,

a homologue or a portion thereof. The isolated polypeptide may comprise, substantially comprise or consist of at least one amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. In particular the at least one amino acid sequence may
5 comprise, substantially comprise or consist of the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The isolated polypeptide sequence may be less than 700 amino acids, in particular less than 550 amino acids. More in particular, the isolated polypeptide may comprise less than 400 or less than 350 amino acids. The isolated polypeptide may be selected from PfRH1, PvRBP1
10 and/or a homologue thereof.

According to another aspect, the invention provides an isolated polypeptide comprising, substantially comprising or consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ
15 ID NO: 8, a homologue or a portion thereof, wherein the polypeptide comprises less than 700. In particular, the polypeptide comprises less than 550 amino acids. More in particular, the isolated polypeptide may comprise less than 400 or less than 350 amino acids. More in particular, the polypeptide according to the invention consists of at least one amino acid sequence selected from SEQ
20 ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof.

The invention also provides an isolated polynucleotide encoding a polypeptide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion
25 thereof. In particular, the polynucleotide may comprise at least one nucleic acid sequence selected from SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof. More in particular the at least one nucleic acid sequence may be SEQ ID NO: 7, a homologue and/or a portion thereof.

With the term “comprising” it is understood that the polypeptide and/or polynucleotide according to the invention comprises at least one indicated sequences (for example a specific sequence indicated with a SEQ ID Number or an homologous sequence or fragment thereof) plus an additional (fixed,
5 variable or chosen) sequence at the 5’ end and/or at the 3’ end of the claimed sequence.

With the term “substantially comprising” it is understood that the polypeptide and/or polynucleotide according to the invention “substantially” comprises the
10 indicated sequence as “essential” element. Additional sequences may be included at the 5’ end and/or at the 3’ end. Accordingly, a polypeptide “substantially comprising” sequence X will be novel in view of a known polypeptide accidentally comprising the sequence X.

15 With the term “consisting of” it is understood that the polypeptide and/or polynucleotide according to the invention corresponds to at least one of the indicated sequence (for example a specific sequence indicated with a SEQ ID Number or an homologous sequence or fragment thereof).

In the present description, the polypeptide, polynucleotide, composition or other
20 products according to the invention will be generally indicated to “comprise” a sequence. However, for the purpose of the present application the use of the term “comprise” will also include the optional limitation “substantially comprise” or “consist of”.

25 The term “nucleic acid” is well known in the art and is used to generally refer to a molecule (one or more strands) of DNA, RNA or a derivative or analog thereof comprising nucleobases. A nucleobase includes, for example, a purine or pyrimidine base found in DNA (e.g., an adenine “A”, a guanine “G”, a thymine “T” or a cytosine “C”) or RNA (e.g., an A, a G, an Uracil “U” or a C). The term
30 nucleic acid encompasses the terms “oligonucleotide” and “polynucleotide”

each as subgenus of the term "nucleic acid". The term "complementary" in the context of nucleic acids refers to a strand of nucleic acid non-covalently attached to another strand, wherein the complementarity of the two strands is defined by the complementarity of the bases. For example, the base A on one strand pairs with the base T or U on the other, and the base G on one strand pairs with the base C on the other. An oligonucleotide or analog is of "substantial complementarity" when there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide or analog to non-target sequences under conditions in which specific binding is desired.

The term "isolated" as used herein refers to a biological component (such as a nucleic acid, peptide or protein) that has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins which have been isolated thus include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids. A "nucleotide" Includes, but is not limited to, a monomer that includes a base linked to a sugar, such as a pyrimidine, purine or synthetic analogs thereof, or a base linked to an amino acid, as in a peptide nucleic acid (PNA). "Polynucleotide" refers to a nucleic acid sequence (such as a linear sequence) of any length. Therefore, a polynucleotide includes oligonucleotides, and also gene sequences found in chromosomes. Accordingly a nucleotide is one monomer in a polynucleotide. A nucleotide or nucleic acid sequence refers to the sequence of bases in a polynucleotide.

A "polypeptide" is a polymer in which the monomers are amino acid residues which are joined together through amide bonds. When the amino acids are

alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. The terms "polypeptide" or "protein" as used herein are intended to encompass any amino acid sequence and include modified sequences such as glycoproteins. The term "polypeptide" is specifically intended to cover naturally occurring proteins, as well as those which are recombinantly or synthetically produced. The term "polypeptide fragment" refers to a portion of a polypeptide which exhibits at least one useful epitope. An "epitope" is a region of a polypeptide capable of binding an immunoglobulin generated in response to contact with an antigen.

"Homologues" of a nucleotide or amino acid sequence will possess a relatively high degree of sequence identity or homology when aligned using standard methods. Methods of alignment of sequences for comparison are well known in the art. Homologues of a nucleotide or amino acid sequence of the PfRH binding region, in particular the homologues of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8 are typically characterized by possession of at least 75 %, for example at least 85 %, 90 %, 95 %, 98 %, or 99 %, sequence identity counted over the full length alignment with the originating NS sequence using the NCBI Blast 2.0, set to default parameters. Methods for determining sequence identity over such short windows are available at the NCBI website on the Internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologues could be obtained that fall outside of the ranges provided. An RH "fragment" as used herein, is meant to cover any nucleotide or amino acid sequence derived from the RH nucleotide or amino acid sequence known in the art. The derived RH "fragment" is shorter by at least one nucleotide or one amino acid compared to the RH sequence known in the art. In particular the RH protein "fragment" may be 700 amino acids in length. More in particular the RH protein "fragment" may be 550 amino acids in length.

Any of the isolated polynucleotide described above may be comprised in a vector. The vector may be comprised in a cell. Accordingly there is provided a method of producing the polypeptide according to any one of embodiment of the invention, the method comprising the steps of:

- 5 (a) culturing the cell according to the invention, under conditions suitable for expression of the polypeptide; and
- (b) recovering the polypeptide so expressed.

The recovered polypeptide may comprise at least one of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ
10 ID NO: 8, a homologue and/or a portion thereof. The at least one amino acid sequence may comprise the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

A "vector" refers to a nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid
15 sequences that permit it to replicate in the host cell, such as an origin of replication. A vector may also include one or more therapeutic genes and/or selectable marker genes and other genetic elements known in the art. A vector can transduce, transform or infect a cell, thereby causing the cell to express nucleic acids and/or proteins (e.g. the polypeptides of the present invention)
20 other than those native to the cell. A vector optionally includes materials to aid in achieving entry of the nucleic acid into the cell, such as a viral particle, liposome, protein coating or the like.

Unless otherwise explained, all technical and scientific terms used herein have
25 the same meaning as commonly understood by one of ordinary skill in the art. It is further to be understood that all base pair sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be

used in the practice or testing of this disclosure, suitable methods and materials are described. The term "comprises" means "includes."

According to another aspect the invention provides an isolated antibody, wherein the antibody specifically binds to polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. In particular, the antibody specifically binds to a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 8 a homologue and/or a portion thereof. The antibody may be a monoclonal, polyclonal, chimeric, humanised, single chain, Fab, Fab', F(ab)' fragments and/or F(v) portions of the whole antibody.

The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a Plasmodium PfRHI-RII. For example, antibody fragments capable of binding to PfRHI-RII and/or portions thereof, include, but not limited to Fab (e. g. , by papain digestion), Fab' (e. g. , by pepsin digestion and partial reduction) and F (ab')₂ (e. g. , by pepsin digestion), facb (e. g. , by plasmin digestion), pFc' (e. g. , by pepsin or plasmin digestion), Fd (e. g. , by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e. g. , by molecular biology techniques) fragments, are encompassed by the invention.

Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a F (ab')₂ heavy chain portion can be designed to include DNA sequences encoding the CH1

domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

"Polyclonal antibodies" are antibodies that are derived from different B-cell lines.

- 5 They are a mixture of immunoglobulin molecules secreted against a specific antigen, each recognising a different epitope. Many methodologies exist for polyclonal antibody production in laboratory animals. Institutional guidelines governing animal use and procedures relating to these methodologies are generally oriented around humane considerations and appropriate conduct for
- 10 adjuvant (agents which modify the effect of other agents while having few if any direct effects when given by themselves) use. This includes adjuvant selection, routes and sites of administration, injection volumes per site and number of sites per animal. Institutional policies generally include allowable volumes of blood per collection and safety precautions including appropriate restraint and
- 15 sedation or anesthesia of animals for injury prevention to animals or personnel.

The primary goal of antibody production in laboratory animals is to obtain high titer, high affinity antibodies from the serum of animals following immunization with the antigens. Adjuvants are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site, antigen depot

20 which allows for a slow release of antigen into draining lymph nodes. Production of polyclonal antibodies is well known in the art.

By contrast, "monoclonal antibodies" are derived from a single cell line and the antibodies are produced by the hybridoma technology well known to those skilled in the art.

- 25 According to a further aspect, the invention provides a pharmaceutical composition for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes, comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; or

(b) at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c). at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof

optionally in the presence of at least one pharmaceutically acceptable excipient, diluent, carrier, additive, adjuvant and/or a combination thereof. The pharmaceutical composition is a pharmaceutical composition formulated for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes. The pharmaceutical composition may be in the presence of at least one pharmaceutically acceptable carrier, diluent, excipient additive and/or adjuvant. Examples of suitable excipients are water, saline, dextrose, glycerol, ethanol and the like as well as combinations thereof. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or alternatively the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carrier, excipient and/or diluent. Excipients normally employed for such formulations, includes mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. In particular, the pharmaceutical composition is useful for treating at least one condition selected from a group comprising of, cancer, infectious diseases, inflammatory diseases and autoimmune diseases. Accordingly, any pharmaceutical composition comprising a drug, compound, or substance capable of reducing and/or

inhibiting the binding to and/or invasion of Plasmodium into erythrocytes is within the scope of the present invention.

5 The "pharmaceutical compositions" referred to herein, are preferably prepared and administered in dose units. For treatment of a subject, such as but not limited to a human subject, and depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of
10 the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administrations of subdivided doses at specific intervals. The pharmaceutical compositions can be administered systemically. The compositions are in general administered topically, intravenously, intramuscularly, orally,
15 parenterally, or as implants, but even rectal use is possible in principle.

Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro) capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solutions
20 in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug
25 delivery systems. In certain embodiments patients with malaria may be treated with the polypeptides and/or polynucleotides of the invention or other specific blocking agents (e.g. monoclonal antibodies) thus preventing the binding of the plasmodium merozoites to the erythrocyte surface and/or their invasion into the erythrocytes.

The polynucleotides mentioned herein are for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes. Accordingly, the method comprises administering to or transfecting *in vivo* or *in vitro* the cells with a nucleic acid construct comprising a nucleic acid molecule and/or hybridising to and/or complementary to any part of the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof.

- 10 The present invention therefore extends to the preparation of anti-sense nucleotides, ribozymes and silencing interference RNA (siRNA) technology that may be used to interfere with the expression of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof. This approach involves anti-sense nucleic acid molecules and ribozymes to block expression, either by masking it with an anti-sense nucleic acid or cleaving it with a ribozyme.

Anti-sense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. In the cell, they hybridise to that mRNA, forming an untranslatable double-stranded molecule. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Anti-sense methods have been used to inhibit the expression of many genes *in vitro*. The reduction and/or inhibition of binding to an/or invasion of plasmodium into erythrocytes can also be carried out by the silencing interference RNA (siRNA) technology. RNA interference technology is well known and consists of a process in which a double stranded RNA (dsRNA) induces the postranscriptional degradation of homologous transcripts. RNAi can be initiated by exposing cells to dsRNA either via transfection or endogenous expression. According to the exemplified embodiment, DNA targeting sequences, are selected and prepared according to standard technology, for example, the DNA

targeting sequence are generated using Ambion siRNA target finder (http://www.ambion.com/techlib/misc/siRNA_finder.html). The DNA targeting sequences may be inserted into a construct and/or vector and used to transfect the cell or cell lines in vitro or in vivo. The RNA polymerase of the cell transcribes the siRNAs complementary to the SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof. These siRNAs form a complex known as the RNA-induced silencing complex or RISC which functions in homologous target RNA destruction. In mammalian systems, the sequence-specific RNAi effect has been observed by the introduction of siRNAs either via transfection or endogenous expression of 19-23 base transcripts capable of forming duplexes, or via expression of short hairpin RNAs. The siRNA expression constructs and/or vectors may be constructed according to any method known in the art, for example by chemical synthesis, in vitro transcription, by digestion of long dsRNA by an RNase III family enzyme (e.g. Dicer, RNase III), by expression in cells from an siRNA expression plasmid or viral vector, and expression in cells from a PCR-derived siRNA expression cassette. The construct is directly transfected into mammalian cells resulting in functional expression of siRNAs.

Accordingly, there is provided a method of treating and/or preventing malaria comprising administering to a subject in need a composition comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, a homologue and/or a portion thereof.

- 5 The method may comprise reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes. The subject in need of the treatment may be a mammal, in particular a human. The method further comprises administering a composition which binds or interacts with the polynucleotide to reduce and/or inhibit the expression of binding domain polypeptide, namely the
- 10 PfRH polypeptide. The composition may further comprise the polypeptides of the invention capable of binding to and blocking the erythrocyte receptor, thus preventing the binding of the polypeptides on the merozoite surface to the erythrocytes. The composition may alternatively comprise antibodies capable of binding to the polypeptides on the merozoite surface thus neutralizing the
- 15 binding of the merozoites to the erythrocyte surface blocking invasion. Increasing the concentration of the antibody or the polypeptide will act as antagonists, preventing the binding of the parasite to the erythrocyte.

A nucleic acid molecule is "hybridisable" to another nucleic acid molecule, when a single-stranded form of the nucleic acid molecule can anneal to the other

20 nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (Sambrook and Russell, 2001). The conditions of temperature and ionic strength determine the "stringency" of the hybridisation. Hybridisation requires the two nucleic acids to contain complementary sequences. Depending on the stringency of the hybridisation, mismatches

25 between bases are possible. The appropriate stringency for hybridising nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability

(corresponding to higher T_m) of nucleic acid hybridisation decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (Sambrook and Russell, 2001). For hybridisation with shorter nucleic acids, i.e. oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (Sambrook and Russell, 2001).

At least one antibody of the invention binds at least one specified epitope specific to at least one peptide sequence, subunit, fragment, portion or any combination thereof as described herein. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of the protein sequences corresponding to the peptide sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8 as described herein.

According to a further aspect there is provided a use of

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof.

in preparation of a medicament for use in therapy. The polypeptides comprising the sequence of SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO:

8, a homologue and/or a portion thereof, the polynucleotides comprising the sequence of SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof or the antibody capable of binding to polypeptides comprising the sequence of SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof may be used in therapy. The use may be for treating and/or preventing malaria, wherein the use may be for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes.

The phrase "reducing and/or inhibiting the binding to and/or invasion" used herein refers to the ability of the polypeptides or antibodies and/or nucleic acid molecules described in the previous section, to measurably reduce and/or inhibit ability of the Plasmodium parasite in making contact with the erythrocyte. In the present invention it contemplates blocking of the erythrocyte receptor by means of the polypeptides of the invention or blocking of the Reticulocyte Binding Protein homologue (ligand), for example the PfRH1, on the surface of the merozoite by means of the antibody. The invention further contemplates reduction and/or inhibition of the expression of the Reticulocyte Binding Protein homologue on the surface of Plasmodium merozoites by means of nucleic acids of the invention that are complementary and/or hybridisable to the nucleic acid and/or transcript sequences that transcribe and/or translate for the expression of the erythrocyte binding protein of the parasite. It is understood that the phrase is relative, and does not require absolute suppression. Thus, in certain aspects, reducing and/or inhibiting expression of the erythrocyte binding protein of the parasite requires that, following application of the nucleic acid molecules mentioned in the previous section, Reticulocyte Binding Protein homologue is expressed at least 5 % less than prior to application these compounds and/or molecules, such as at least 10 % less, at least 15 % less, at least 20 % less, at least 25 % less, or even more reduced. Thus, in some particular aspects, application of the nucleic acid molecules inhibits and/or reduces expression of

the protein by about 30 %, about 40 %, about 50 %, about 60 %, or more. In specific examples, where the nucleic acid molecules are particularly effective, expression is inhibited and/or reduced by 70 %, 85 %, 85 %, 90 %, 95 %, or even more.

5

According to yet another aspect the invention provides a method of diagnosis and/or prognosis of malaria in a subject, comprising:

(a) providing at least one sample from a subject;

10 (b) detecting the presence of polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof; and/or

(c) detecting the presence of polypeptide comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, a homologue and/or a portion thereof;

15 wherein the presence of the polynucleotide and/or the polypeptide is indicative of presence of Plasmodium in the subject. The step c) may be in the presence of at least one antibody capable of binding to at least one polypeptide comprising the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof. The
20 at least one polypeptide may comprise the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The subject may be a mammal, in particular human. The invention also provides a diagnostic and/or prognostic kit for the diagnosis and/or prognosis of malaria in a subject comprising

25 (a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at least one of the sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, a homologue and/or a portion thereof;

5 wherein the subject may be a mammal, in particular human.

A "subject" may be a patient suffering from malaria. A person skilled in the art will know how to select subjects based on their amenability to a particular treatment, or their susceptibility to a particular disease. The control for example, may not be suffering malaria. The control may not have detectable levels of the
10 Reticulocyte Binding Protein homologue (polypeptide) and/or polynucleotide encoding for the protein (polypeptide) that may be indicative of presence of Plasmodium in the subject. The "control value" may also be an average value in obtained from a selected population.

15 "Diagnose" or "diagnosis" used herein, refers to determining the nature or the identity of malaria. A diagnosis may be accompanied by a determination as to the severity of the disease. "Prognostic" or "prognosis" used herein refers to predicting the outcome or prognosis of a disease, such as to give a chance of survival based on observations and results of clinical tests.

20

As used herein "presence of plasmodium" refers to a measurable parameter of the presence of the Reticulocyte Binding Protein homologue (polypeptide) and/or the polynucleotide encoding the protein (polypeptide). For example, presence of nucleic acids may be detected by the use of Southern blots,
25 northern blots, *in situ* hybridization and/or quantitative real time PCR. The polypeptides of the invention can be detected using several well recognized binding assays, for example the COS cell binding assay described in the further sections. Further, labelled monoclonal antibodies to the polypeptides of the invention can be used to detect merozoites in the biological sample obtained

from the subject. Alternatively, labelled polypeptides can be used to detect the presence of antibodies in the biological sample. Cell free assays can be used to measure the binding of the binding domain fragment of the current invention. For example, the erythrocyte proteins may be immobilized on a solid support and binding of labelled polypeptides may be measured. Various "means", for example, fluorometric, flow cytometric means may be used. The assays and means mentioned herein are examples, and by no way limiting.

According to yet another aspect, the present invention provides a method of inducing a protective immune response to Plasmodium merozoites in a subject comprising administering to the subject an immunologically effective amount of a pharmaceutical composition comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof, in combination with pharmaceutically acceptable excipient, carrier and/or additive, optionally in the presence of an adjuvant. The at least one sequence may comprise the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The method of inducing a protective immune response to Plasmodium merozoites may be in a subject wherein the subject may be a mammal, in particular human.

There is further provided a recombinant DNA vaccine comprising an expression vector for expression of a polynucleotide, comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof, optionally in the presence of at least one pharmaceutically acceptable excipient, carrier and/or additive, optionally in the presence of an adjuvant. The at least one nucleic sequence may comprise the nucleic acid sequence of SEQ ID NO: 7, a homologue and/or a portion thereof. The polynucleotide in the recombinant may encode for an immunogenic peptide, comprising at least one of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8,

a homologue and/or a portion thereof. The at least one amino acid sequence may comprise the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The recombinant vaccine may be for administration in a subject, wherein the subject may be a mammal, in particular human.

5

According to a further aspect the invention provides a method of vaccinating a patient against malaria comprising administering to the patient in need an effective amount of a recombinant DNA vaccine capable of expressing an immunogenic peptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof after administration of the vaccine to a patient. The at least one amino acid sequence may comprise the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof. The subject in need of the vaccine may be a mammal, in particular human.

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The term "vaccine" is used herein to describe a preparation intended for active immunological prophylaxis (the protective effect which is preferably long-term, i. e., at least about 6 months and preferably, at least about one year or longer). In the present invention, vaccines comprise an expression vector, which expresses an antigenic protein after administration to a subject, such as a mammal. Vaccines may also comprise chimeric peptides or an immunogenic peptide portion thereof in combination with a signal peptide sequence and/or an anchor peptide sequence and optionally, additional antigenic peptides or immunogenic fragments thereof from *Plasmodium falciparum*. In alternative embodiments according to the present invention, the polypeptide or an immunogenic fragment thereof, is administered to a patient alone, but may be administered in combination with at least one additional immunogenic malaria peptide in combination with a pharmaceutically acceptable carrier, excipient or additive. The method of administering the vaccine (s) according to the present invention may vary and include intravenous, buccal, oral, transdermal and nasal,

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among others, but intramuscular or subcutaneous administration is the most common method of administration.

Methods of inducing an immunogenic response in a patient or vaccinating a patient against a malaria infection are also contemplated by the present invention. In this method, a patient is administered the polypeptides of the invention as an immunogenic fragment, thereof, alone or may be administered in combination with another merozoite surface or immunogenic fragment thereof in combination with a pharmaceutically acceptable carrier, excipient or additive at least once, or in certain instances, at selected intervals to provide a booster to the initial immunization or to maintain immunity in the treated patient for extended periods of time.

The immunogenic response generated preferably will be "substantially protective", i. e., will protect the patient from some of the more severe symptoms and physiological states of the malaria disease, including the death of the patient from malaria. The immune response may include the generation of antibodies; activation of cytotoxic T lymphocytes (CTL) against cells presenting peptides derived from the polypeptide sequences of the present invention, or other mechanisms well known in the art.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Russel, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (2001).

Generation of constructs for Expression of Different Regions of P_fRH1 on Surface of COS7 Cells

The plasmid pRE4 (kindly given by Prof. John Adams, University of Notre Dame, IN) contains the gene for HSV gD. All constructs were designed to express eight different regions of P_fRH1 fused to the secretory signal sequence and transmembrane domain of HSV gD (Chitnis et al, 1994). The pRE4 vector was digested with restriction enzymes *PvuII* and *Apal* to excise the central region of HSV gD. The restriction fragments were separated by gel electrophoresis and the vector was recovered using QIAquick Gel Extraction Kit (Qiagen). *P. falciparum* 3D7 clone genomic DNA (gDNA) was used as a template for generating different regions of P_fRH1 RI to RVIII. The 2 kb overlapping fragments of *PfRH1* comprising the eight regions is shown in Figure 1. The genomic DNA was extracted from infected blood using Easy DNA kit (Invitrogen). PCR primers were designed for generating ~2Kb fragments with 1Kb overlap according to 3D7 clone *RH1* gDNA sequence with intro spliced out (Genbank accession no: AF533700). PCR products encoding different regions of P_fRH1 were digested with *PvuII* and *Apal* and in frame cloned into pRE4 to make the following constructs (I) to (VIII):

(I): SEQ ID NO: 9 and 10 were used to generate the region I of P_fRH1 spanning the region from 55 to 2001bp.

(II): SEQ ID NO: 11 and 12 were used to generate the region RII of P_fRH1 comprising the sequence of SEQ ID NO: 1 spanning the region from 1000 to 3000 bp. The SEQ ID NO: 2 comprises the polypeptide sequence encoded by SEQ ID NO: 1.

(III): SEQ ID NO: 13 and 14 were used to generate the region RIII of P_fRH1 spanning the region from 2041 to 4002bp.

(IV): SEQ ID NO: 15 and 16 were used to generate the region RIV of P_fRH1 spanning the region from 3001 to 5001bp.

(V): SEQ ID NO: 17 and 18 were used to generate the region RV of P_fRH1 spanning the region from 4003 to 5000bp.

(VI): SEQ ID NO: 19 and 20 were used to generate the region RVI of PfRH1 spanning the region from 5002 to 7002bp.

(VII): SEQ ID NO: 21 and 22 were used to generate the region RVII of PfRH1 spanning the region from 6001 to 8001bp.

5 (VIII): SEQ ID NO: 23 and 24 were used to generate the region RVIII of PfRH1 spanning the region from 7003 to 8301bp.

The PCR products encoding the eight different regions of PfRH1 were digested with *PvuII* and *ApaI* and cloned in frame into pRE4. All the eight constructs of
10 *PfRH1* were then subcloned into pEGFP-N1 vector with flanking signal sequence and transmembrane domain from HSV gD so as to target the protein to the surface of transfected COS7 cells as a GFP fusion protein. Plasmid pEGFP-PvDBP II (kindly given by Prof. John Adams, University of Notre Dame, IN) was digested with *XhoI* and *BamHI* restriction enzymes to remove the
15 DBP II. The pEGFP-N1 backbone was purified by gel electrophoresis and recovered using QIAquick Gel Extraction Kit (Qiagen). Universal primers were designed for generating eight GFP constructs of PfRH1 (primer [*XhoI*], 5' CGT ATACTCGAGATGGGGGGGACTGCCGCC 3'; (SEQ ID NO: 31); primer [*BamHI*], 5' CGTATAGGATC CAAGTAAACAAGGGCTG 3' (SEQ ID NO: 32).
20 The amplified PCR fragments were cleaved with *XhoI* and *BamHI* and cloned into pEGFP-N1 vector digested by *XhoI* and *BamHI* restriction enzymes. All the GFP constructs were purified using QIAfilter plasmid Maxi Kit (Qiagen).

25 **Erythrocyte binding assay and the identification of erythrocyte binding region of PfRH1.**

COS7 cells (American Type Culture Collection) were cultured and transfected with pEGFP constructs as described previously (Mayer et al, 2004). 2 kb overlapping fragments of *PfRH1* as shown in Figure 1, cloned into the mammalian cell expression vector, pEGFP-N1, generated GFP fusion proteins.
30 The transfected cells were used for erythrocyte-binding assay 40-60 hours post-

transfection by scoring the number of rosettes under an inverted fluorescent microscope (Nikon) as described previously (Chitnis et al, 1994). Transfected COS7 cells with at least half their surface area covered by erythrocytes were scored as positive for binding. The number of rosettes was counted in 30 fields at 200X magnification using an inverted fluorescent microscope. In each experiment, two wells of COS7 cells were transfected for each construct, and the data shown are from at least 3 separate experiments. The transfection efficiency (%) was calculated as total no. of fluorescent COS7 cells X 100/total no. of COS7 cells, while binding activity (%) was calculated as total no. of fluorescent COS7 cells with rosettes X 100/total no. of COS7 cells. The binding activity (%) was then normalized to 5% transfection efficiency. Figure 2A-ii and 2B-ii shows the binding of the erythrocytes as viewed under inverted fluorescent microscope. Region II (spanning the region from 1000 to 3000bp) of PfRH1 possessed the strongest binding ability to erythrocytes with >70% binding activity (Figure 2C). Regions III, IV and VI showed minimal binding (<10%) while no binding was observed in region I, V, VII and VIII of PfRH1 (Figure 2C). Negative controls of either untransfected COS7 cells or COS7 cells expressing the *P. vivax* Duffy Binding Protein region II (PvDBPII) (Kind gift of Professor John Adams; Michon et al., 2000) with chymotrypsin-treated erythrocytes gave no rosettes. These data strongly suggested that region II of PfRH1 could be the erythrocyte binding region.

To further examine the specificity of erythrocyte binding to region II, we tested the ability of COS7 cells expressing all PfRH1 constructs to bind neuraminidase-, chymotrypsin- and trypsin-treated erythrocytes respectively. PfRH1 protein binds to a neuraminidase-sensitive, chymotrysin and trypsin-resistant receptor on the erythrocyte surface (Rayner et al, 2001). Human erythrocytes were collected in 10% citrate phosphate dextrose and stored at 4°C for up to 4 weeks. The Duffy phenotypes of the erythrocytes were determined by a standard blood banking method (Chitnis et al, 1994). Duffy-

positive human erythrocytes were washed 3 times in RPMI-1640 (Invitrogen) for use in erythrocyte-binding assay described above. Washed human erythrocytes were treated with neuraminidase, chymotrypsin and trypsin respectively as described previously (Rayner et al, 2001). In the current study binding of region II is dramatically affected when erythrocytes are pretreated with neuraminidase (Fig. 2D) with binding being reduced approximately 10 fold. Little or no impact on binding of erythrocytes to region II is seen when the erythrocytes are pretreated with chymotrypsin or trypsin (Fig. 2D). Enzyme treatment of erythrocytes had little impact on the minimal binding seen to any of the other regions (Fig. 2D). For all the experiments described the effectiveness of enzyme treatment was assessed by measuring the effect on binding of pretreated erythrocytes to COS7 cells expressing two known erythrocyte binding domains PvDBPII or EBA-175RII. Binding of erythrocytes to PvDBPII is known to be resistant to neuraminidase and trypsin treatment but sensitive to chymotrypsin while EBA-175RII is neuraminidase and trypsin sensitive but chymotrypsin resistant (Baum et al, 2003).

Taken together, these data clearly shows that RII of PfRH1 has the expected erythrocyte binding specificity previously demonstrated on the full length protein (Rayner et al, 2001).

Generation of constructs for expression of different regions of PfRH1- RII on surface of COS-7 cells.

Similar methods as described above were used to express different regions of PfRH1-RII, namely RII-1, RII-2 and RII-3 (Figure 3A) in pRE4 vector for expression on the surface of COS7 cells. Deletion constructs designed for pRE4 are described below as RII-1, RII-2, and RII-3. RII-1 contains region II DNA sequence 1 (SEQ ID NO: 3) from 1000bp to 2499bp (~ 1.5Kb), but lacks ~500bps at 3' end. RII-2 consists of region II DNA sequence (SEQ ID NO: 5) from 1498bp to 3000bp (~ 1.5Kb), but lacks ~500bps at 5' end. RII-3 contains

region II DNA sequence (SEQ ID NO: 7) from 1498bp to 2498bp (1.0Kb), but lacks ~ 500bps at both ends. PCR primers SEQ ID NO: 25 and 26 were used to amplify the RII-1 region comprising the sequence of SEQ ID NO: 3. The SEQ ID NO: 3 encodes the polypeptide comprising SEQ ID NO: 3. PCR primers SEQ ID NO: 27 and 28 were used to amplify the RII-2 region comprising the sequence of SEQ ID NO: 5. The SEQ ID NO: 5 encodes the polypeptide comprising SEQ ID NO: 5. PCR primers SEQ ID NO: 29 and 30 were used to amplify the RII-3 region comprising the sequence of SEQ ID NO: 7. The SEQ ID NO: 7 encodes the polypeptide comprising SEQ ID NO: 7. The same primers were used to generate deletion constructs of region II as GFP fusion in pEGFP vector.

Identification of a minimal erythrocyte binding region of PfRH1

To further delineate region II binding, the three deletion constructs RII-1, RII-2 and RII-3, cloned into pEGFP-N1 vector were transfected into COS7 cells. Using the same approach as used for the larger construct strong binding was observed with the full-length region II, as well as the deletion constructs RII-1, RII-2 and RII-3 to erythrocytes (Fig. 3B). Neuraminidase treatment significantly decreased all binding activity while treatment with chymotrypsin or trypsin had little effect (Fig. 3B). RII-2 and RII-1 had a slightly reduced binding activity compared to full length RII, whereas RII-3 showed even better binding than RII. These data strongly suggest that RII-3 contains the minimal binding region of PfRH1.

Expression of rRII-3 and rtRVIII Recombinant Proteins

The DNA sequence of RII-3 (SEQ ID NO: 7) was amplified by PCR using primers SEQ ID NO: 33 and 34. The DNA sequence of tRVIII (SEQ ID NO: 37) was amplified by PCR using primers SEQ ID NO: 35 and 36 to generate recombinant constructs in pET24a(+). The PCR products were digested with *EcoRI* and *XhoI* and cloned into *E.coli* expression vector pET24a(+) (Novagen)

to generate a C-terminal His tag. *E.coli* strain, BL21-CodonPlus-RIL (kindly supplied by Prof. Julien Lescar, Nanyang Technological University, Singapore), was transformed with pET24a(+) constructs and used for expression of recombinant rRII-3 and rtRVIII. Luria Bertani (LB) containing kanamycin (Invitrogen) (50ug/ml) and chloramphenicol (Invitrogen) (50 ug/ml) was inoculated with *E. coli* BL21-codonPlus-RIL transformed with pET24a(+) constructs and cultured overnight at 37 °C. Fresh LB containing kanamycin (50ug/ml) and chloramphenicol (50 ug/ml) was inoculated with the overnight culture at a dilution of 1:50 and cultured at 37 °C to an $A_{600\text{ nm}}$ of 0.6 –0.8.

Expression of rRII-3 and rtRVII proteins was induced by adding isopropyl-1-thio- β -galactopyranoside (IPTG) (USB) to the culture at a final concentration of 0.2mM. Induced cultures were allowed to grow overnight at 16°C. After induction, *E. coli* cells were harvested by centrifugation, resuspended in chilled lysis buffer (50 mM Tris, pH 8.0, 200 mM NaCl, 0.1% tween-20 with protease inhibitor cocktail, EDTA-free (Roche Applied Science) for rRII-3; 50 mM Tris, pH 8.5, 200 mM NaCl, with protease inhibitor cocktail, EDTA-free for rtRVIII), and lysed by sonication. The supernatant was collected by centrifugation of lysed cells at 4 °C. Recombinant protein was purified under native conditions using nickel-nitrilotriacetic acid-agarose (Ni-NTA) as described by the manufacturer (Qiagen). The recombinant proteins were further purified by ion-exchange chromatography using a MonoS™ HR 5/5 column (Amersham Biosciences) for rRII-3 and MonoQ™ 5/50 GL column (Amersham Biosciences) for rtRVIII, following the manufacturer's protocol.

Erythrocyte-binding Assay Using rRII-3 and rtRVIII recombinant proteins

The recombinant protein named rRII-3 expressed in a soluble form with the expected molecular weight of approximately 40 kDa protein (Fig. 4A, lane 1) was also tested for its ability to bind erythrocytes. At the same time a construct expressing a similarly sized soluble protein of region VIII named rtRVIII was prepared as negative control (Fig. 4B, lane 1). Only, rRII-3 directly bound to

erythrocytes (Fig 4A) with no binding being detected for tRVIII in any of the conditions tested (Fig 4B). Importantly, chymotrypsin- and trypsin-treated erythrocytes, but not in neuraminidase-treated erythrocytes are bound by rRII-3 (Fig. 4A). These results independently confirm that RII-3 is the erythrocyte binding region of PfRH1.

Amino-Acid Sequence Analysis and identification of a new erythrocyte binding domain in *Plasmodium*

Amino-acid sequence alignments were carried out using program BLAST (Altschul et al, 1997) and ClustalW (Thompson et al, 1994). The coiled-coils prediction was carried out using program COILS available at <http://www.russell.embl.de/cgi-bin/coils-svr.pl>. Secondary structure predictions were carried out according to the method of reference (Rost et al, 2003).

Inspection of the sequence of the 334 amino-acid long minimal binding fragment RII-3 (Figure 5A) reveals a very uneven distribution of residues with Ala, Gly, Met, Cys, Pro, Trp absent or with respective occurrences < 1% and a large excess of Ile (16.2%), Lys (14.7%), Gln (12.3%) and Leu (10.2%) residues. The presence of a heptad repeat motif with an Ile side chain at position "a" could be detected between residues 261 to 289. As a confirmation, the program COILS (Baum J, 2003) was used and indeed unambiguously detected a coiled coil region in the C-terminal domain of RII-3, centred at residue 275 (Figure 5C). In the absence of a 3D structure, the exact length of this α -helical coiled coil is difficult to assess but is likely to span between 28 to 49 residues which translates into a helix of a length comprised between 43 to 75 Å. A weak sequence identity of 27% for 105 aligned amino-acids with the second Heptad repeat region B "HRB" of the parainfluenza virus F protein may be detected between amino-acids 201 to 304 of RII-3. Interestingly the corresponding HRB region spanning residues 378-479 of the parainfluenza F protein, participates in the formation of trimeric coiled coil of α -helices (Yin et al,

2006). Overall the RII-3 protein is predicted to be predominantly α -helical with a possible second coiled coil region centered at residue 125 (Figure 5C). Between these two helical segments lies a region of approximately 70 residues spanning amino-acids 180 till 250 which is predicted to be richer in loop structures (Figure 5C).

Having identified the binding region for PfRH1 it was important to determine whether this domain has conserved features seen in other members of the RH. Alignment and secondary structure prediction of the corresponding regions of other members of *P. falciparum* were performed using ClustalW sequence alignment program at <http://www.ebi.ac.uk/clustalw> (Thompson et al, 1994). PfRH2a/b and PfRH4 indicate both amino acid and secondary structure conservation with approx. 65% similarity (Figure 5A). As noted, PfRH3 is not shown in the alignment as it introduced too many gaps in the resulting alignment; on the other hand pair-alignment of PfRH1-RII-3 and PfRH3 indicates 56% similarity between these 2 regions. This conservation while clearly less pronounced is somewhat analogous to the DBL domains. The conserved region of PfRH1-RII-3 also can be found in other members of RH of different *Plasmodium* species (Figure 5B) with more than 50% similarity. While overall sequence identity for all the regions is low they all are predicted to contain a coiled coil region in the C-terminal domain consistent with an overall structural conservation of the domain. Predicted binding regions of RH orthologues in *P. falciparum* and *P. vivax* are shown in Figure 6. The binding regions were identified using the PfRH1-RII-3 vs predicted binding regions of other RH members (ClustalW).

Generation of antibodies against recombinant proteins rRII-3 and rRVIII

Purified recombinant proteins, rRII-3 and rRVIII were used to immunize mice to raise the antisera α rRII-3 and α rRVIII. In order to determine whether the antisera are able to recognize PfRH1 protein, we performed Western blotting of

P. falciparum W2mef merozoite extracts probed with α R11-3 and α rtRVIII respectively. Mouse pre immune serum and normal RBC lysate were also used to test the antisera specificity. Previous work had shown that an approximately 240 kDa protein is recognized by PfRH1 specific sera in W2mef (Baum et al., 2003) and a similarly sized protein was detected with both antisera α R11-3 and α rtRVIII raised (Figure 7A). No protein was detected by mouse pre-immune serum or in normal RBC lysate using α R11-3 or α rtRVIII. In Immunofluorescence Assays (IFAs) using fixed parasites both α R11-3 and α rtRVIII antisera gave a punctuate pattern in schizonts consistent with the expression at the apical end of merozoites (Figure 7 B and C). No staining was observed in mouse preimmune serum (Figure 7 D). These results show that the two antisera are specific for PfRH1 protein.

Invasion inhibition assay of *P. falciparum* W2mef in the presence of α R11-3 and α rtRVIII.

We then investigated whether the different dilutions of the sera raised exhibited different effects on invasion of the parasites (Figure 7E). Invasion inhibition assays performed on synchronized cultures showed the ability of the α R11-3 and α rtRVIII antisera to inhibit invasion. The pre-immune serum had no significant effect on invasion in all the parasites studied. On the other hand antiserum α R11-3, raised against the minimal binding region of PfRH1, successfully blocked invasion in W2mef up to 70% at a 1:10 dilution. Furthermore, the α rtRVIII showed minimal or no inhibition for at all dilutions of the antibody. The invasion inhibition assay demonstrates that antiserum raised against the binding domain of PfRH1 (R11-3) contains more efficient invasion inhibitory antibodies than those raised against another region of the protein.

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Claims

1. An isolated polypeptide, wherein the polypeptide is a Plasmodium RH fragment, comprising at least one amino acid sequence selected from
5 SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a
homologue or a portion thereof.
2. The isolated polypeptide according to claim 1, wherein the polypeptide
comprises at least one amino acid sequence selected from SEQ ID NO:
10 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion
thereof.
3. The isolated polypeptide according to any one of the preceding claims,
wherein at least one amino acid sequence comprises SEQ ID NO: 8, a
15 homologue and/or a portion thereof.
4. The isolated polypeptide according to any one of the preceding claims,
wherein the polypeptide sequence is less than 700 amino acids.
- 20 5. The isolated polypeptide according to any one of the preceding claims,
wherein the polypeptide sequence is less than 550 amino acids.
6. The isolated polypeptide according to any of the preceding claims,
wherein the polypeptide sequence is selected from PfRH1, PvRBP1
25 and/or a homologue thereof.
7. An isolated polynucleotide encoding a polypeptide of claim 1 to 6.
8. The isolated polynucleotide according to claim 7, wherein the
30 polynucleotide comprises at least one nucleic acid sequence selected

from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof.

5 9. The isolated polynucleotide according to claim 7 or 8, wherein the polynucleotide comprises at least one nucleic acid sequence selected from SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof.

10 10. The isolated polynucleotide according to any one of claims 7 to 9, wherein the at least one nucleic acid sequence is SEQ ID NO: 7, a homologue and/or a portion thereof.

15 11. A vector comprising the polynucleotide according to any one of the claims 7 to 10.

12. A cell comprising the vector according to claim 11.

13. A method of producing the polypeptide according to any one of claims 1 to 6, the method comprising the steps of :

- 20 (a) culturing the cell according to claim 12, under conditions suitable for expression of the polypeptide; and
(b) recovering the polypeptide so expressed.

25 14. The method according to claim 13, wherein the recovered polypeptide comprises at least one of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof.

15. The method according to claim 13 or 14, wherein at least one amino acid sequence comprises the sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

16. An isolated antibody, wherein the antibody specifically binds to a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4 or, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof.

17. The isolated antibody according to claim 16, wherein the antibody specifically binds to a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 8, a homologue and/or a portion thereof.

18. The antibody according to claim 16 or 17, wherein the antibody is a monoclonal, polyclonal, chimeric, humanised, single chain, Fab, Fab', F(ab)' fragments and/or F(v) portions of the whole antibody.

19. A pharmaceutical composition for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes, comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at

least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof;

optionally in the presence of at least one pharmaceutically acceptable excipient, diluent, carrier and/or a combination thereof.

20. A method of treating and/or preventing malaria comprising administering to a subject in need a composition comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, a homologue and/or a portion thereof.

21. The method according to claim 20, wherein the method comprises reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes.

22. The method according to claim 20, wherein the subject is a mammal.

23. The method according to claim 20, wherein the subject is a human.

24. Use of

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; and/or

(b) at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof; and/or

(c) at least one nucleic acid molecule hybridizing and/or complementary to any part of the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof;

in preparation of a medicament for use in therapy.

25.The use according to claim 24, wherein the medicament is for treating and/or preventing malaria.

26.The use according to claim 24 or 25, wherein the medicament is for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes.

27.The polypeptide according to claims 1 to 6, the polynucleotide according to claims 7 to 10 or the antibody according to claims 16 and 17, for use in therapy.

28. The polypeptide, polynucleotide and/or antibody according to claim 27, for treating and/or preventing malaria.

29. The polypeptide, polynucleotide and/or antibody according to claim 27 or 28, for reducing and/or inhibiting the binding to and/or invasion of Plasmodium into erythrocytes.

30. A method of diagnosis and/or prognosis of malaria in a subject, comprising:

(a) providing at least one sample from a subject;

(b) detecting the presence of at least one polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof and/or;

(c) detecting the presence of at least one polypeptide comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, a homologue and/or a portion thereof;

wherein, the presence of the polynucleotide and/or the polypeptide is indicative of presence of Plasmodium in the subject.

31. The method according to claim 30, wherein detecting the presence of at least one polypeptide comprises the presence of at least one antibody capable of binding to the polypeptide.

32. The method according to claim 30 or 31, wherein the at least one polypeptide comprises the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

33. The method according to any one of claims 30 to 32, wherein the subject is a mammal.

34. The method according to any one of claims 30 to 33, wherein the subject is a human.

35. A kit for the diagnosis and/or prognosis of malaria in a subject comprising

(a) at least one antibody and/or portion thereof, capable of binding to at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue or a portion thereof ; or

(b) at least one nucleic acid molecule capable of hybridizing and/or complementary to any part of at least one polynucleotide comprising at least one of the sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, a homologue and/or a portion thereof.

36. The diagnostic and/or prognostic kit according to claim 35, wherein the subject is a mammal.

37. The diagnostic and/or prognostic kit according to claim 35 or 36, wherein the subject is a human.

38. A method of inducing a protective immune response to at least one Plasmodium merozoite in a subject comprising administering to the subject an immunologically effective amount of at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof, optionally in combination with pharmaceutically acceptable excipient, carrier, additive, and/or adjuvant.

39. The method according to claim 38, wherein the at least one polypeptide comprises the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

40. The method according to claims 38 or 39, wherein the subject is a mammal.

41. The method according to any one of claims 38 to 40, wherein the subject is a human.

5

42. A recombinant DNA vaccine comprising an expression vector for expression of a polynucleotide, the polynucleotide comprising at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7, a homologue and/or a portion thereof, optionally in the presence of at least one pharmaceutically acceptable excipient, carrier, additive, and/or adjuvant.

10

43. The recombinant DNA vaccine according to claim 42, wherein the polynucleotide comprises at least the nucleic acid sequence of SEQ ID NO: 7, a homologue and/or a portion thereof.

15

44. The recombinant vaccine according to claims 42 or 43, wherein the polynucleotide encodes for an immunogenic peptide, comprising at least one of the amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof.

20

45. The recombinant vaccine according to claim 44, wherein the peptide comprises the amino acid sequence of at least SEQ ID NO: 8, a homologue and/or a portion thereof.

25

46. The recombinant vaccine according to any one claims 42 to 45, wherein the subject is a mammal.

47. The vaccine according to any one of claims 42 to 46, wherein the subject is a human.

48. A method of vaccinating a subject against malaria comprising administering to the subject in need an effective amount of a recombinant DNA vaccine capable of expressing an immunogenic peptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8, a homologue and/or a portion thereof after administration of the vaccine to the subject.

49. The method according to claim 48, wherein the peptide comprises at least the amino acid sequence of SEQ ID NO: 8, a homologue and/or a portion thereof.

50. The method according to claims 48 or 49, wherein the subject is a mammal.

51. The method according to any one of claims 48 to 50, wherein the subject is a human.