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(54) **Title:** ANTI-MALARIAL COMPOSITIONS

(57) **Abstract:** This disclosure provides antibodies that are useful for preventing and/or treating malaria. The epitope to which the antibodies bind is in close proximity to the conserved proteolytic cleavage site of *P. falciparum* circumsporozoite protein (CSP), and the antibodies provided in this disclosure can prevent cleavage and inhibit *P. falciparum* sporozoites from invading the liver.

ANTI-MALARIAL COMPOSITIONS

[01] This application incorporates by reference the contents of an 11.3 kb text file created on May 14, 2014 and named "14097799substitutesequencelisting2.txt," which is the sequence listing for this application.

[02] Each reference cited in this disclosure is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[03] The invention relates to compositions and methods for preventing and treating malaria.

BRIEF DESCRIPTION OF THE DRAWINGS

[04] **FIG. 1.** Graph of results of biolayer interference assay (BLI) to detect binding of monoclonal antibodies (mAbs) raised against recombinant circumsporozoite protein (rCSP). See Example 2.

[05] **FIGS. 2A-B.** Graphs demonstrating binding of anti-CSP mAbs to regions of *P. falciparum* CSP by ELISA. **FIG. 2A** shows binding of mAb 5D5 strongly to a long CSP N-terminal region peptide. **FIG. 2B** shows binding of mAb 2G9 to a long CSP repeat region peptide. See Example 3.

[06] **FIG. 3.** Photomicrograph of Western Blot showing binding of anti-CSP mAbs 2G9 and 5D5 to *P.falciparum* sporozoites and rCSP under reducing conditions. Lanes 1 and 3, 25,000 *P.falciparum* sporozoites; lanes 2 and 4, 0.25 μg rCSP. See Example 4.

[07] **FIGS. 4A-C.** Mapping of mAb 5D5 to a specific region of the N-terminal CSP domain. **FIG. 4A,** schematic representation of *P.falciparum* CSP. **FIG. 4B,** peptides located in the N terminal region of CSP. Peptide 8, SEQ ID NO:17; peptide 9, SEQ ID NO:18; peptide 10, SEQ ID NO: 19. **FIG. 4C,** graph of results of peptide mapping by ELISA. See Example 5.

[08] **FIG. 5.** Graph showing percent inhibition by 1 μg/ml of mAb 5D5 and control mAb 2A10 of parasites in the liver of mice challenged with transgenic *P. berghei* sporozoites containing a portion of the *P.falciparum* CSP (repeat region and a small portion of the N-terminal domain, including the cleavage site). See Example 6.

[09] **FIGS. 6A-B.** Amino acid sequences of the heavy and light chain variable regions of mAb 5D5. CDR sequences are underlined. **FIG. 6A,** heavy chain variable region (SEQ ID NO: 14). **FIG. 6B,** kappa (light) chain variable region (SEQ ID NO: 16). See Example 7.

DETAILED DESCRIPTION

[10] Malaria is an infectious, febrile disease which is caused by protozoa of the genus *Plasmodium.* Malaria is transmitted by the bites of infected *Anopheles* mosquitoes and can be caused by any *Plasmodium* species that infects humans, including, but not limited to, *Plasmodium vivax* and *Plasmodium falciparum.*

[11] Circumsporozoite protein (CSP) is the major protein on the surface of the *Plasmodium* sporozoite. CSP contains an N-terminal domain, a conserved pentapeptide protease cleavage site at the core of region I, a repeat region, a short conserved sequence termed region III, and a C-terminal region with sequence homology to the thrombospondin type-1 repeat superfamily (Doud *et al, Proc. Natl. Acad. Sci. USA 109,* 7817-22, 2012. CSP is proteolytically processed cysteine protease during invasion of hepatocytes (Coppi *et al., J. Exp. Med. 201, 2*1 -33, 2005). Processing occurs on the sporozoite surface when sporozoites contact hepatocytes, resulting in the removal of the amino-terminal third of the protein. The cleavage site is a five amino acid sequence (KLKQP; SEQ ID NO:20), which is highly conserved among *P. falciparum* isotypes.

[12] This disclosure provides antibodies that bind to an epitope in close proximity to the protease cleavage site of CSP. Binding of the antibodies to this epitope can prevent cleavage and inhibit *Plasmodium* sporozoites from invading the liver. Because the cleavage site among all species of *Plasmodium* that infect humans is conserved, the disclosed antibodies can be used to treat or reduce infection in human by all such *Plasmodium* species.

5D5 Antibodies

[13] Unless otherwise indicated, the term "antibody" means an intact antibody *{e.g.,* an intact monoclonal antibody) or antigen-binding fragment of an antibody *{e.g.,* an antigen-binding fragment of a monoclonal antibody), including intact antibodies or antigen-binding fragments that have been modified or engineered. Modified or engineered antibodies include, but are not limited to, chimeric antibodies, humanized antibodies, and multispecific antibodies *{e.g.,* bispecific antibodies). Examples of antigen-binding

fragments include Fab, Fab', $F(ab')_2$, Fv, single chain antibodies *(e.g.,* scFv), and diabodies. Particularly useful subclasses of antibodies include IgGi, IgG_3, and IgA.

[14] A "5D5 antibody" is an antibody containing the CDR regions of monoclonal antibody (mAb) 5D5, which is described in the specific examples, below. The antibody mAb 5D5 was deposited on November 19, 2013 with The Malaria Research and Reference Reagent Resource Center (MR4), which is managed by managed by ATCC, under Accession No. MRA-1242. Availability of the deposited antibody is not to be construed as a license to use the antibody in contravention of the rights granted under the authority of any government in accordance with its patent laws. mAb 5D5 is a murine IgGi/κ subclass antibody obtained using the full length recombinant CSP of *Plasmodium falciparum* (3D7 strain) as an immunogen.

[15] The immunoglobulin heavy chain of mAb 5D5 antibody comprises **CDR** $_{H_I}$ (GYTFTGYGLS; SEQ ID NO:l), CDR_{H2} (IYPRSGNTYYNEKFKGKAT; SEQ ID NO:2), and CDR_{H3} (SWGNSSFVY; SEQ ID NO:3). The immunoglobulin light chain variable region of mAb 5D5 comprises CDR_Li (KASQSVTNDVT; SEQ ID NO:4), CDR_{L2} (ASNRYTG; SEQ ID NO:5), and CDR_{L3} (QQDYSSPFT; SEQ ID NO:6). Together, these CDR regions define a binding site for the mAb 5D5 epitope EKLRKPKHKKLK (SEQ ID NO:7).

[16] The locations of the mAb 5D5 epitope (bold and underlined) and the protease cleavage site (bold and italicized) within *P.falciparum* CSP (SEQ ID NO: 8) are shown below:

MMRKLAI L SVS S FLFVEALFQEYQCYGS S SNTRVLNELNYDNAGTNLYNE
LEMNYYGKQENWY S LKKNSRS LGENDDGNNE DNE KL RK P KHK***KL***i***KQ***P A DG
NPD PNANPNVDPNAN PNVDPNANPNVDPNANPNANPNANPNANPNANPNANPNA
NPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNVDP
NANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNA
NPNANPNANPNANPNANPNANPNKNNQGNGQGHNMPNDPNRNVDENANAN
SAVKNNNNEE P S DKH I KEYLNKI QNS L S TE W S P C SVTCGNG I QVRI KPGS
ANKPKDELDYAND I EKKI CKMEKC S SVFNVVNS S I GL IMVL S FLFLN

[17] In some variations, a 5D5 antibody is an scFv. Particularly useful 5D5 scFv antibodies are those which comprise an Fc region of the IgGi, IgG_3, or IgA subclass.

[18] In some variations, the CDR sequences of a 5D5 antibody are interposed between human or humanized immunoglobulin framework regions to reduce or eliminate antigenicity when administered to humans. Methods for humanizing antibodies are known in the art. For example, mouse immunoglobulin constant regions can be replaced with human

immunoglobulin constant regions, as described in Morrison *et al, Proc. Natl. Acad. Sci. USA 81,* 6851-55, 1984; Neuberger *et al, Nature 312,* 604-08, 1984; U.S. Patent 6,893,625; U.S. Patent 5,500,362; and U.S. Patent 4,816,567.

[19] Alternatively, CDRs can be grafted into human framework regions. The human framework regions can be consensus human framework regions, created by aligning framework regions from several human heavy chain or light chain amino acid sequences to identify a consensus amino acid sequence. Descriptions of CDR grafting are provided, for example, in U.S. Patent 7,022,500; U.S. Patent 6,982,321; U.S. Patent 6,180,370; U.S. Patent 6,054,297; U.S. Patent 5,693,762; U.S. Patent 5,859,205; U.S. Patent 5,693,761; U.S. Patent 5,565,332; U.S. Patent 5,585,089; U.S. Patent 5,530,101; Jones *et al., Nature 321,* 522-25, 1986; Riechmann *et al., Nature 332,* 323-27, 1988; Verhoeyen *et al., Science 239,* 1534-36, 1988; and Winter, *FEBS Lett. 430,* 92-94, 1998.

[20] Other approaches include SUPERHUMANIZATION™, in which human CDR sequences which are structurally similar to a mouse antibody can be chosen from human germline genes *(e.g.,* U.S. Patent 6,881,557; Tan *et al, J. Immunol. 169,* 1119-25, 2002); "reshaping," "hyperchimerization," or "veneering/resurfacing" (see, e.g., Vaswami *et al, Annals of Allergy, Asthma, & Immunol. 81,* 105 (1998); Roguska *et al, Prot. Engineer. 9,* 895-904 (1996); U.S. Patent 5,639,641; U.S. Patent 6,072,035); ACTIVMAB™ technology (Vaccinex, Inc., Rochester, N.Y.), which involves using a vaccinia virus-based vector to express antibodies in mammalian cells, producing high levels of combinatorial diversity of IgG heavy and light chains *{e.g.,* U.S. Patent 6,706,477; U.S. Patent 6,800,442; and U.S. Patent 6,872,518); and HUMAN ENGINEERING™ technology (XOMA (US) LLC *(e.g.,* WO 93/1 1794; U.S. Patent 5,766,886; U.S. Patent 5,770,196; U.S. Patent 5,821,123; and U.S. Patent 5,869,619).

Production of 5D5 Antibodies

[21] Methods for recombinant production of antibodies are well known in the art. For example, DNA molecules encoding light chain variable regions and heavy chain variable regions can be chemically synthesized using the amino acid sequence information provided in this disclosure. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, *e.g.,* constant region coding sequences and expression control sequences, to produce conventional gene expression constructs encoding the desired antibody. Production of gene expression constructs is within routine skill in the art. Nucleic acid molecules may comprise coding sequences for one or more of the CDRs

of a 5D5 antibody. This disclosure provides the amino acid sequences of the CDRs, and any nucleotide sequence that encodes the desired amino acid sequence may be used to express the desired amino acid sequence. Non-limiting examples of nucleotide sequences include SEQ ID NO: 13 (encoding the V_H region of mAb 5D5) and SEQ ID NO: 15 (encoding the V_L region of mAb 5D5).

[22] Nucleic acid molecules can encode one or more of the 5D5 CDRs. In some variations, a nucleic acid molecule encoding one or more of the 5D5 CDR sequences is a cDNA molecule having no introns.

[23] Expression constructs expressing one or more of the 5D5 CDRs can be introduced into host cells through conventional transfection or transformation techniques. Examples of host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells *{e.g.,* Hep G2), and myeloma cells that do not otherwise produce immunoglobulins. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light or heavy chain variable regions.

[24] Alternatively, amino acid sequences of a 5D5 antibody can be synthesized using a peptide synthesizer, as is known in the art.

Pharmaceutical Compositions and Methods of Use

[25] Pharmaceutical compositions comprising one or more of the 5D5 antibodies disclosed herein typically contain a sufficient concentration of 5D5 antibodies to reduce or prevent cleavage of CSP so that the invasion of the liver by *Plasmodium* sporozoites is slowed, reduced, or prevented. Pharmaceutical compositions comprise a pharmaceutically acceptable vehicle. Descriptions of suitable pharmaceutically acceptable vehicles, and factors involved in their selection, are found in a variety of readily available sources.

[26] Administration typically is parenteral. Pharmaceutical compositions suitable for parenteral administration comprise various aqueous media such as aqueous dextrose and saline solutions and glycol solutions, and may comprise suitable stabilizing and/or buffering agents. In some variations, 5D5 antibodies are provided in a single or a multi-use vial as a lyophilized sterile powder, under vacuum. Packages or kits containing such vials may also include one or more vials of Bacteriostatic Water for Injection (BWFI), USP, which may contain a preservative *{e.g.* benzyl alcohol).

[27] A pharmaceutical composition comprising one or more of the 5D5 antibodies disclosed herein can be administered prophylactically to reduce or prevent cleavage of CSP and thereby slow, reduce, or prevent *Plasmodium* sporozoites from invading the liver. Such compositions can be administered to malaria-naïve individuals *{e.g.,* military personnel, state-department personnel, travelers) entering endemic regions to reduce or prevent infection. In some variations, a pharmaceutical composition comprising a 5D5 antibody can be administered in conjunction with a vaccine composition comprising a malarial antigen such as those disclosed in Mata *et al., BioMed Research Int'l. Vol. 2013,* Article ID 282913, 2013. Unless otherwise indicated, administration "in conjunction with" a traditional malaria vaccine includes sequential administration (either before or after administration of a traditional malaria vaccine) as well as administration of a 5D5 antibody in a composition comprising an agent that raises an immune response against the *Plasmodium.*

[28] A pharmaceutical composition comprising 5D5 antibodies can be administered to treat malaria in an individual already infected with a *Plasmodium* species. In some variations, a pharmaceutical composition comprising 5D5 antibodies is administered in conjunction with one or more other anti-malarial drugs, such as atovaquone and proguanil, chloroquine, doxycycline, mefloquine, and primaquine. Unless otherwise indicated, administration "in conjunction with" an anti-malarial drug includes sequential administration (either before or after administration of an anti-malarial drug) as well as administration of a 5D5 antibody in a composition comprising an anti-malarial drug.

[29] The following examples are provided to illustrate certain particular features and/or embodiments. The examples should not be construed to limit the disclosure to these particular features or embodiments.

Example 1. Antibody Production and Purification

Antibodies were prepared by Precision Antibody, Inc. (Columbia, MD). Three Balb/c mice were immunized with recombinant full-length *P.falciparum* CSP (rCSP; SEQ ID NO:8) produced by Pfenex, Inc. (San Diego, CA) Serum titers were determined from tail bleed samples via ELISA, and splenocytes were harvested and fused once titers exceeded 1:50,000.

Example 2. Reactivity of anti-CSP mAbs

[30] A panel of 14 mAbs generated as described in Example 1 were tested for binding to rCSP by biolayer interference assay (BLI), which measures changes in an interference pattern generated from visible light reflected from an optical layer and a biolayer containing a mAb for characterizing its binding profile of rCSP, and by Western blot. The results of the BLI assays are shown in **FIG. 1.** Most of the antibodies tested demonstrated a strong association to rCSP. Several of the antibodies demonstrated rapid dissociation from rCSP, with 5D5 being the most rapid.

Example 3. Binding of mAbs to CSP domains

[31] The same panel of 14 mAbs was used in an indirect ELISA to determine binding to long peptides corresponding to the N-terminal (SEQ ID NO:9), repeat (SEQ ID NO: 10), and C-terminal (SEQ ID NO: 11) domains of CSP. Only mAb 5D5 bound the N-terminal region of CSP **(FIG. 2A),** while other antibodies bound either to the repeat or C-terminal region of CSP **(FIG. 2B).**

Example 4. Binding of mAb 5D5 to Native CSP

[32] An immunofluorescence assay (IFA) was used to determine if mAb 5D5 bound to native CSP. Transgenic *P. berghei* sporozoites containing a portion of the *P.falciparum* CSP (repeat region and a small portion of the N-terminal domain, including the cleavage site) were the generous gift of Dr. E. Nardin. Sporozoites were obtained by feeding *Anopheles stephensi* mosquitoes on infected Swiss-Webster mice (Taconic Laboratories) and harvesting sporozoites from dissected salivary glands 18-21 days after the last blood meal. Transgenic *P. berghei* sporozoites were air-dried onto multispot glass slides and incubated for 1 hour at room temperature with mAbs diluted in PBS + 1% BSA. Slides were washed 3X in PBS, incubated for 1 hour at room temperature with a FITC-labeled goat anti-mouse IgG antibody (Kirkegaard and Perry, Gaithersburg, MD), washed again, and then viewed under a fluorescence microscope.

[33] *P.falciparum* 3D7 sporozoites air-dried onto multisport glass slides and were incubated for 30 min at 37°C in a humidified chamber with mAbs diluted in PBS. Slides were washed 3X in PBS and incubated for 30 min at 37°C in the dark with a FITC-labeled goat anti-mouse IgG antibody (Kirkegaard and Perry, Gaithersburg, MD) diluted 1:40 in PBS

containing 0.02% Evans blue. Slides were washed again, mounted with VECTASHIELD® mounting media, and viewed under an epifluorescence microscope.

[34] A weak fluorescence signal was detected with mAb 5D5 compared with the signal generated by control mAb 2G9, which recognizes the CSP repeat region.

[35] An IFA was also performed using a chimeric rodent malaria parasite *P. berghei* that expresses a larger portion of the N-terminal region (amino acids 22-92) of the *P. falciparum* CSP. Again, mAb 5D5 generated a weak fluorescence signal as compared to mAb 2G9. The mAb 5D5 recognized *P.falciparum* 3D7 parasites *in situ* but required a higher concentration compared to mAb 2G9 for a signal to be detected.

[36] Binding to native CSP also was examined via western blot with *P.falciparum* 3D7 sporozoite lysate and rCSP under reducing conditions **(FIG. 3).** Differential binding was seen with the mAb 5D5, whereas the control mAb 2G9 bound to rCSP in sporozoite lysate and to rCSP.

Example 5. Peptide Mapping of Anti-CSP mAbs

[37] To define further which area of the N-terminal region mAb 5D5 recognizes, an indirect ELISA was performed with overlapping 15-mer peptides spanning the entire CSP. This mapping was conducted by AscentGene, Rockville, MD, using peptides diluted in DMSO to a concentration of 2μg/ml. Plates were blocked with BSA and incubated with 1.3 μg/well of each mAb. An HRP-conjugated anti-mouse secondary antibody (1:10,000) was used, followed by detection with TMB substrate.

[38] The results are shown in **FIG. 4C.** Strong reactivity of the 5D5 mAb was found only to peptide 9 (SEQ ID NO:18), which consisted of amino acids 81-95 of CSP. No reactivity was seen with mAb 5D5 to peptide 8 (amino acids 71-85 of CSP; SEQ ID NO:17) or peptide 10 (amino acids 91-106 of CSP; SEQ ID NO: 19). The putative 5D5 epitope was identified as EDNEKLRKPKHKKLK (SEQ ID NO:7).

[39] To confirm the epitope, competitive ELISA assays were performed using a series of 9-mer peptides (peptide A, SEQ ID NO:21; peptide B, SEQ ID NO:22; peptide C, SEQ ID NO:23; peptide D, SEQ ID NO:24; peptide E, SEQ ID NO:25) encompassing the sequence corresponding to peptides 8-10 to identify which peptides competed with rCSP for binding to mAb 5D5. The results are shown in Table 1. The results confirm that peptide 9 competes with rCSP for binding to mAb 5D5.

Table 1.

Peptide concentration	A	B	C	D	E	8	9	10	rCSP
0.01μg/ml	3.570	3.510	3.488	3.453	3.392	3.353	0.246	3.336	0.340
0.001μg/ml	1.052	1.026	0.976	0.989	0.925	0.952	0.190	0.920	0.191
NC	0.245	0.204	0.230	0.226	0.211	0.219	0.229	0.211	0.198
PC 0.01μg/ml	3.595	3.405	3.557	3.506	3.471	3.417	3.442	3.393	3.490
PC 0.001μg/ml	1.352	1.315	1.311	1.304	1.228	1.212	1.208	1.168	1.174

Example 6. Passive Transfer of Anti-CSP mAb Decreases Parasite Liver Load

[40] Challenge studies were performed with C57B1/6 mice and live chimeric *P. berghei* sporozoites expressing either the repeat region (and a small portion of the N-terminal domain) or the majority of the N terminal domain of *P.falciparum,* both of which contain the cleavage site and the 5D5 epitope. Each mouse was injected intravenously with 300 μg of mAb 5D5 or one of the positive control mAbs 3D1 1 or 2A10 in PBS just before challenge with 10,000 sporozoites suspended in 100 μï of PBS containing 1% normal mouse serum. Mice were euthanized 40-42 hours post challenge, and their livers were excised. Total RNA was extracted from the livers and used to estimate liver parasite load by real time PCR as described in Bruna-Romero *et al., Int J. Parasitol. 31,* 1499, 2001.

[41] The results are shown in **FIG. 5.** Liver load of parasites in mice to which mAb 5D5 was administered was significantly decreased compared to the naive control group.

Example 7. Analysis of mAb 5D5 Heavy and Light Chains

[42] **Total RNA Extraction.** Total RNA was extracted from hybridoma cells using Trizol Reagent (Invitrogen Catalog Number 15596) and prepared according to the manufacturer's protocol. Isopropanol-precipitated RNA was resuspended in sterile RNAse/DNAse free H_20 and the absorbance at A260 determined. Electrophoresis on a 1% TAE-agarose gel was used to determine quality.

[43] **First-round RT-PCR.** 5 μg of RNA was used for the first strand cDNA preparation using SUPERSCRIPT® III (Invitrogen catalog # 18080-051). Oligo-dT was used as primer. cDNA was purified using Qiagen QIAQUICK® columns and tailed using terminal deoxyribonucleotide transferase (Invitrogen catalog # 10533-065). The polyadenylated first strand cDNA was purified using Qiagen QIAQUICK® columns as before. PCR was performed with reverse primers specific for the heavy and light chains and with a common oligodT primer as a forward primer. Reverse primers were located in the constant regions of heavy and light chains. No restriction sites were engineered into the primers.

[44] PCR Conditions:

i. Forward primer only: 50°C, 5 cycles
ii. Add reverse primer(s)
iii. Denaturation step 94°C, 120 seconds
iv. 35 Cycles of: 94°C, 30 seconds
55°C, 30 seconds
72°C, 120 seconds

[45] **Analysis of PCR Results.** PCR reaction samples were analyzed on a preparative TAE-1% agarose gel to visualize the amplified DNA fragments. The correct antibody variable region DNA fragments should have a size between 600-750 base pairs. The amplified PCR products are excised from the gel and purified using Qiagen QIAQUICK® columns.

[46] The purified PCR products were ligated into EcoRV-digested pBluescript using a QUICK-LIGATION® kit (New England BioLabs, Inc. Catalog #M2200S) and transformed into *E. coli* strain DH5-a on X-Gal/IPTG LB plates. The next day, white colonies were placed into 3 ml of LB+amp broth, grown overnight, and purified using Qiagen minicolumns, followed by restriction digestions and gel electrophoresis. A minimum of 6 correctly sized clones of each light and heavy chain were sequenced.

[47] **Sequencing.** The cloned DNAs were sequenced by Sequetech (Mountain View, CA) using an ABI 3730XL Capillary Sequencer using primers which flank both ends of the cDNA insert. The resulting DNA files were assembled and edited using VECTOR NTI®. The resulting assemblies translated to provide a deduced amino acid sequence.

[48] The heavy chain insert encodes an IgGl isotype murine antibody. Full-length clones included a 51 bp 5' untranslated leader preceding a canonical 19 amino acid signal sequence. The light chain insert encodes an kappa isotype murine antibody. Full-length clones included a 27 bp 5' untranslated leader preceding a canonical 20 amino acid signal sequence.

[49] The amino acid sequences of the heavy and light chains are shown in **FIG. 6A** and **FIG. 6B.** The CDRs of mAb 5D5 were identified using the Kabat database (bioinf.org.uk/abs/#cdrdef; see "Protein Sequence and Structure Analysis of Antibody

Variable Domains" in Antibody Engineering Lab Manual, Dubel & Kontermann, eds., Springer-Verlag, Heidelberg, 2001).

CLAIMS

1. An isolated antibody which:

(a) comprises:

(1) an immunoglobulin heavy chain variable region, comprising a first heavy chain complementarity determining region (CDR_H) comprising the amino acid sequence SEQ ID NO:l; a second CDR_H comprising the amino acid sequence SEQ ID NO:2; and a third CDR_H comprising the amino acid sequence SEQ ID NO:3; and

(2) an immunoglobulin light chain variable region comprising a first light chain complementarity determining region (CDR_L) comprising the amino acid sequence SEQ ID NO:4; a second CDR_L comprising the amino acid sequence SEQ ID NO:5; and CDR_L comprising the amino acid sequence SEQ ID NO:6;

or

(b) comprises the complementarity determining regions of the immunoglobulin heavy and light chains of the antibody deposited under Accession No. MRA-1242.

2. The isolated antibody of claim 1, which is an scFv antibody.

3. The isolated antibody of claim 1, which is humanized.

4. A cDNA molecule, comprising a coding sequence for a CDR_H of the antibody of any of claims 1, 2, 3, and 13.

5. The cDNA molecule of claim 4, which comprises:

a coding sequence for a first CDR_H;

a coding sequence for a second CDR_H;

a coding sequence for a third CDR_H;

a coding sequence for a first CDR_H and a coding sequence for a second CDR_H;

a coding sequence for a first CDR_H and a coding sequence for a third CDR_H;

a coding sequence for a second CDR_H and a coding sequence for a third CDR_H; or

a coding sequence for a first CDR_H, a coding sequence for a second CDR_H, and a coding sequence for a third CDR_H.

6. A cDNA molecule comprising a coding sequence for a CDR_L of the antibody of any of claims 1, 2, 3, and 13.

7. The cDNA molecule of claim 6, which comprises:

a coding sequence for a first CDR_L;

a coding sequence for a second CDR_L;

a coding sequence for a third CDR_L;

a coding sequence for a first CDR_L and a coding sequence for a second CDR_L;

a coding sequence for a first CDR_L and a coding sequence for a third CDR_L;

a coding sequence for a second CDR_L and a coding sequence for a third CDR_L; or

a coding sequence for a first CDR_L, a coding sequence for a second CDR_L, and a coding sequence for the third CDR_L.

8. A pharmaceutical composition, comprising the antibody of any of claims 1, 2, 3, and 13 and a pharmaceutically acceptable carrier.

9. A method of protecting an individual against a *Plasmodium* infection, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising the antibody of any of claims 1, 2, 3, and 13.

10. The method of claim 9, further comprising administering to the individual a malarial antigen.

11. A method of treating malaria, comprising administering to an individual in need thereof an effective amount of a pharmaceutical composition comprising the antibody of any of claims 1, 2, 3, and 13.

12. The method of claim 11, further comprising administering to the individual an anti-malarial drug.

13. The antibody deposited under Accession No. MRA-1242.

14. Use of the antibody of any of claims 1, 2, 3, and 13 in the manufacture of a medicament for protecting an individual against a *Plasmodium* infection.

15. Use of the antibody of any of claims 1, 2, 3, and 13 in the manufacture of a medicament for treating malaria.

16. Use of the antibody of any of claims 1, 2, 3, and 13 for protecting an individual against a *Plasmodium* infection.

17. Use of the antibody of any of claims 1, 2, 3, and 13 for treating malaria.

FIG. 1

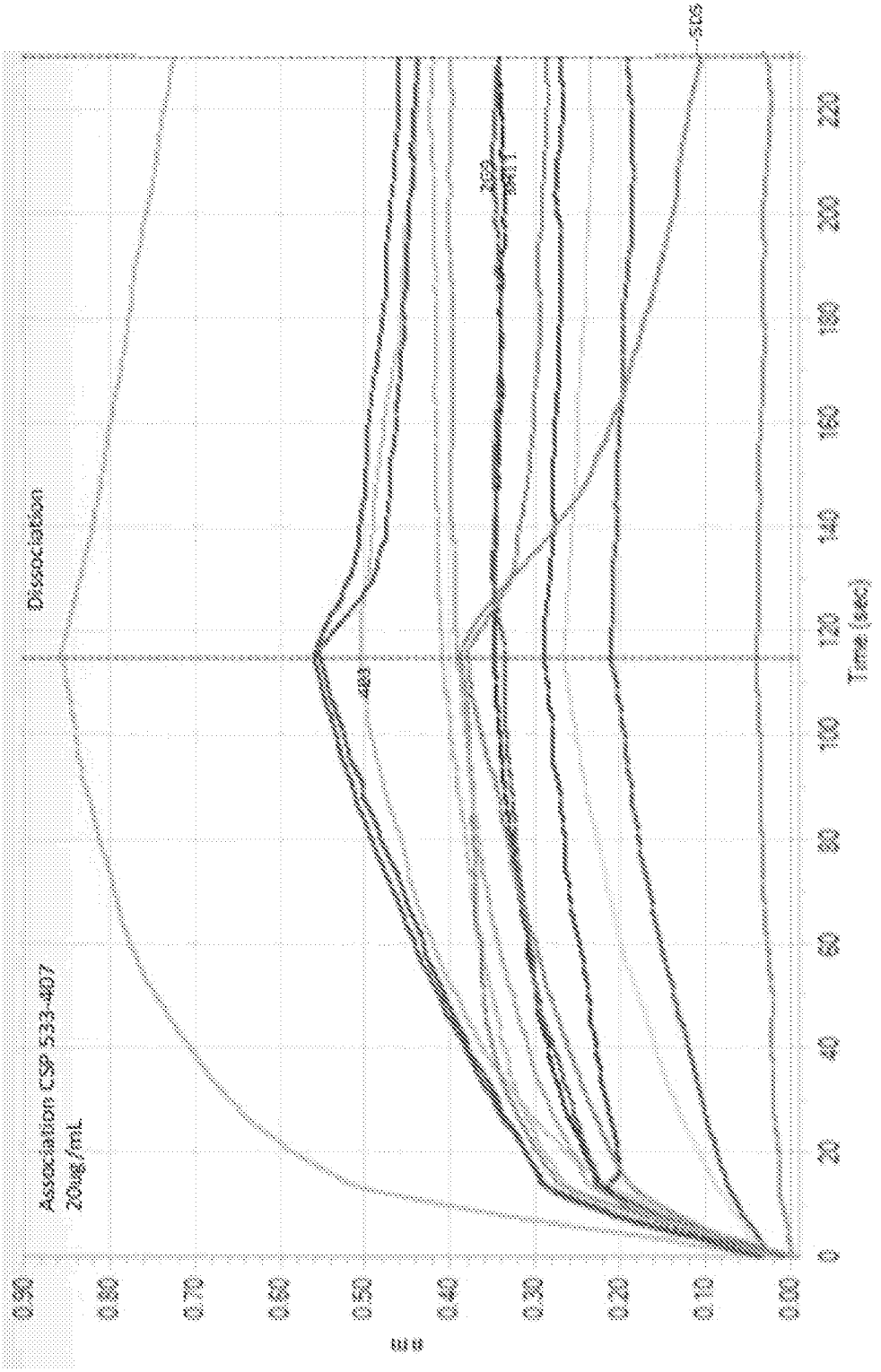
Association CSP 533-4007
20ug/mL
Dissociation
400
200
100
50
25
12.5
6.25
3.13
1.56
0.78
0.39
0.20
0.10
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
nm
0
20
40
60
80
100
120
140
160
180
200
220
Time (sec)

FIG. 2A

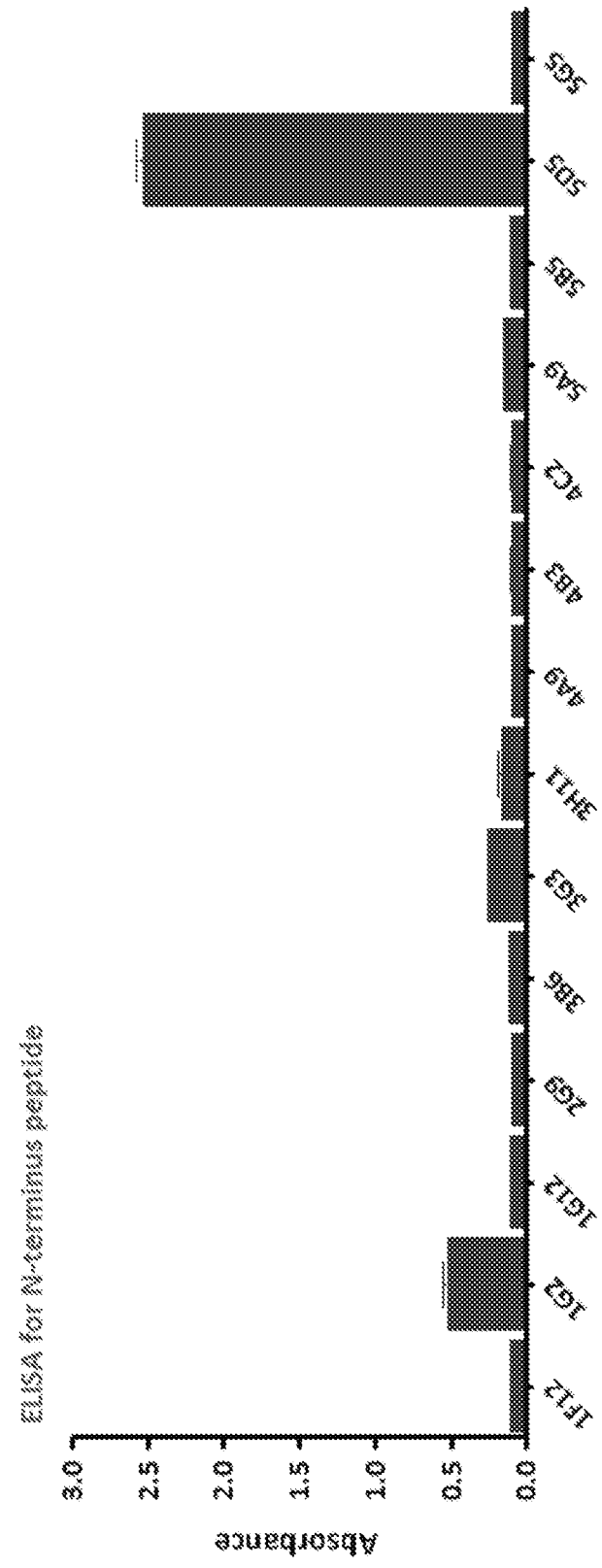
ELISA for N-terminus peptide
Absorbance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1F12
1G2
1G12
7G9
3B6
3G3
3H12
4A9
4B3
4C2
5A9
5B5
5D5
5E5

FIG. 2B

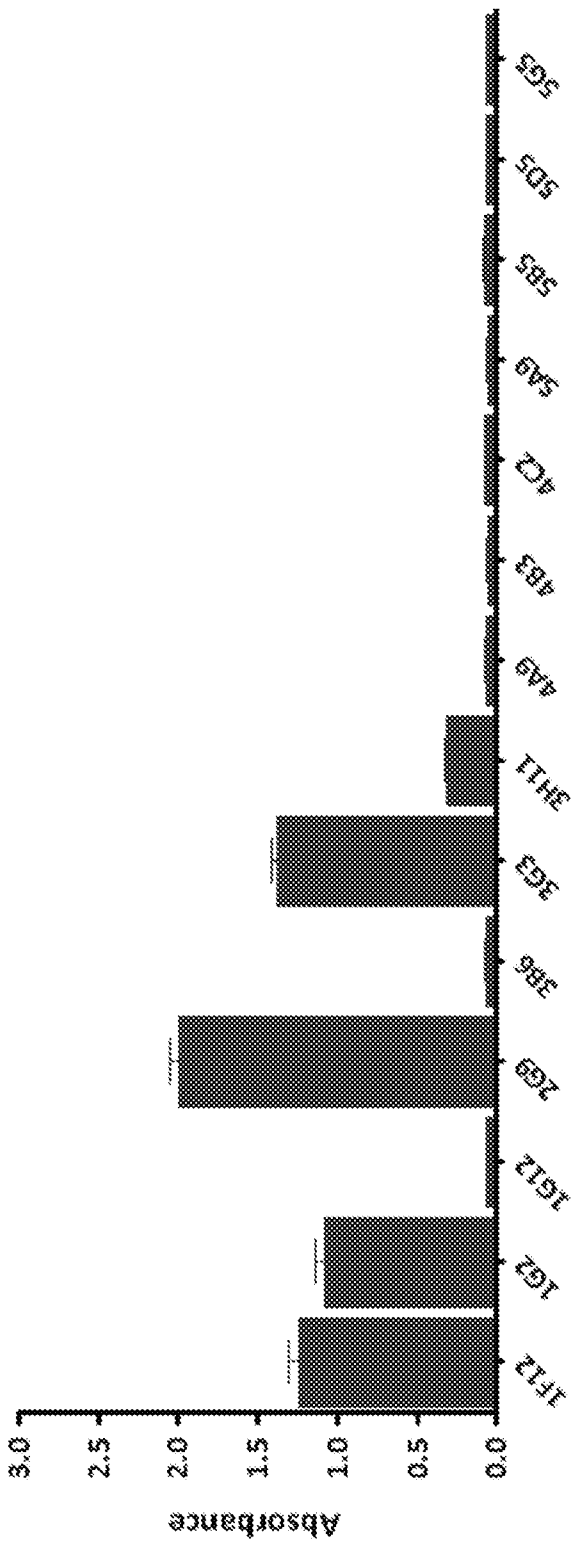
ELISA for Repeat peptide
Absorbance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1F12
1G2
1G12
2G9
3B6
3G3
3H11
4A9
4B3
4C2
5A9
5B5
5D5
5G5

FIG. 3

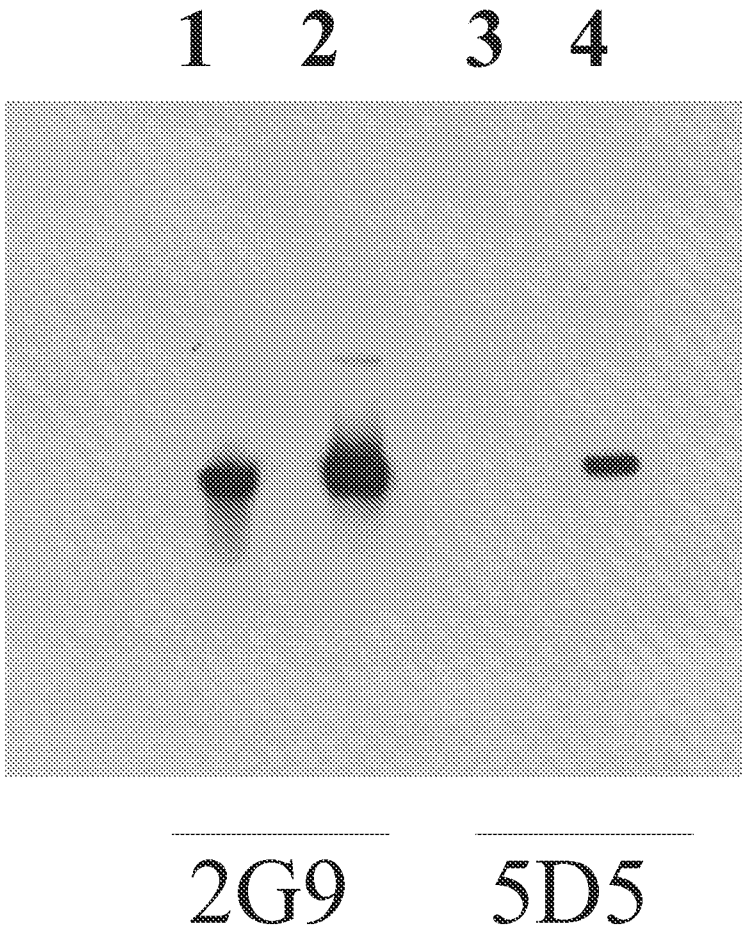

FIG. 4A

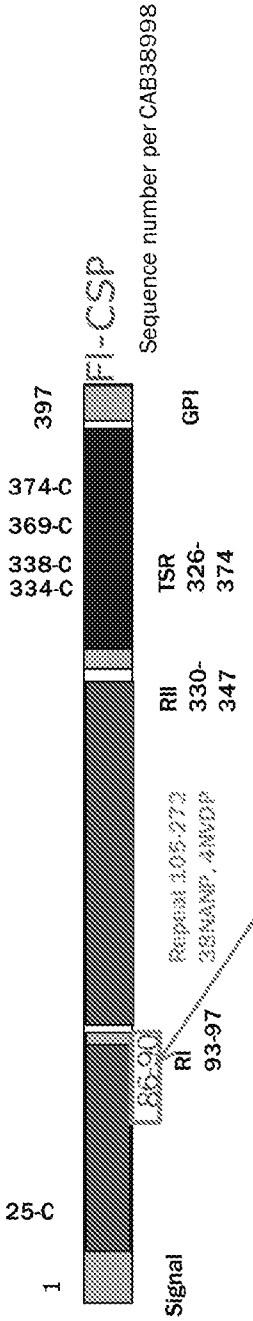

FIG. 4B

Peptide ID #	N-terminus of CSP
8 (71-85 a.a.)	SLYGENDDGNNEDNEK
9 (81-95 a.a.)	EDNEKLRKPKHKKLK
10 (91-105 a.a.)	HKKLKQPADGNPDP

FIG. 4C

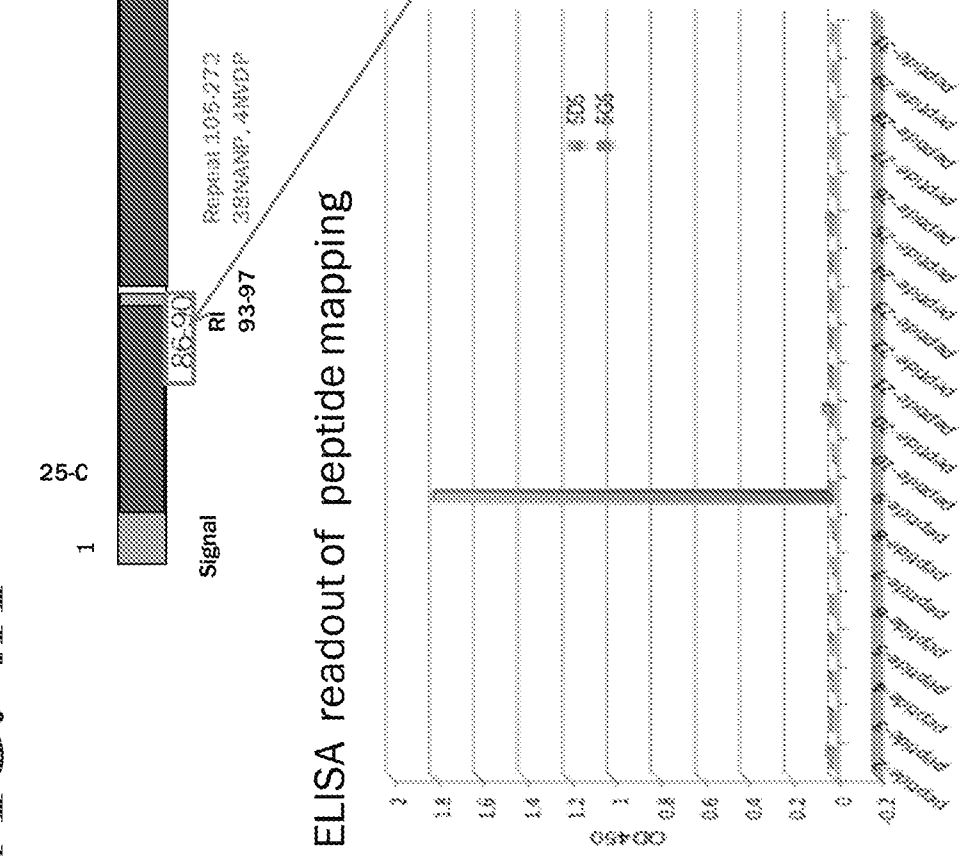

FIG. 5

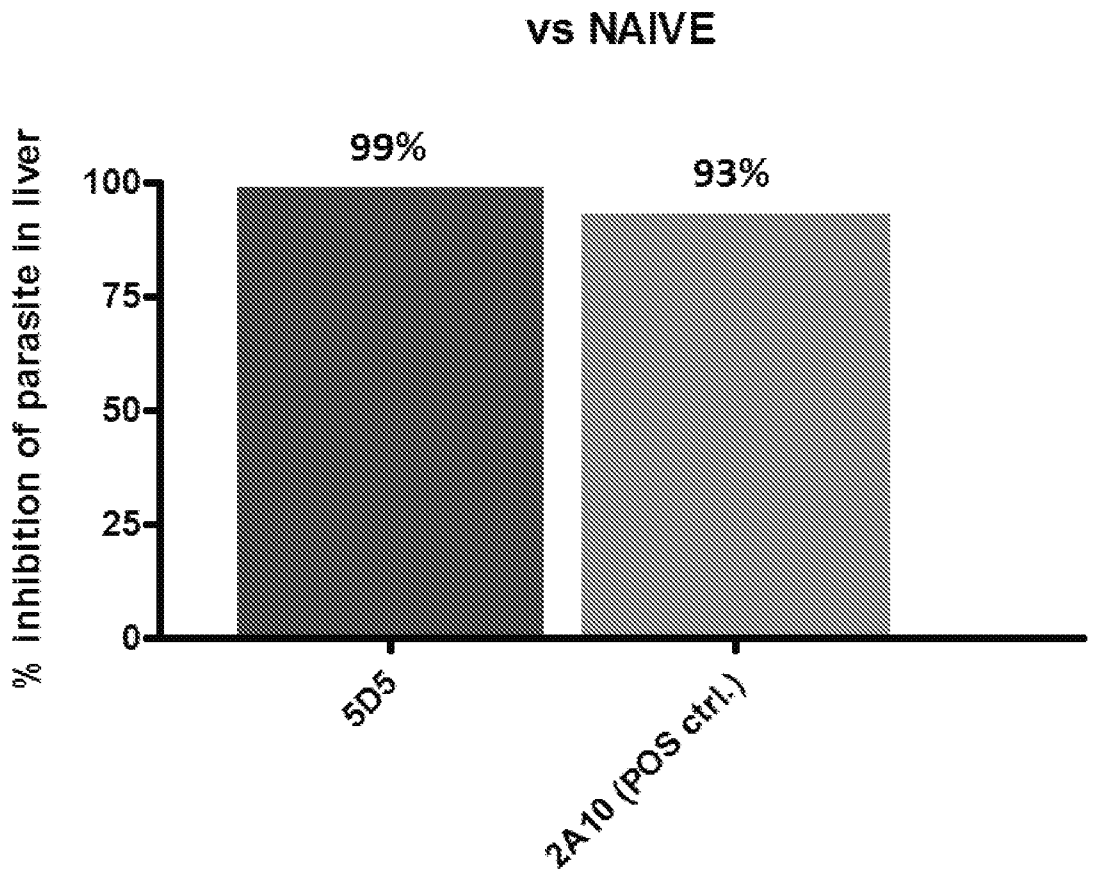
vs NAIVE
% inhibition of parasite in liver
100
75
50
25
0
99%
93%
5D5
2A10 (POS ctrl.)

FIG. 6A

5D5 Heavy Chain

```
        <--Signal Sequence->><---------H1------- -------><--CDR1
  1     MEWIWIFLFI LSGTAGVQSQ VHLQQSGGEV ARPGASVKLS CKASGYTFTG

        --->><----H2-------><-------CDR2----------><------H3--
 51     YGLSWVKQRT GQGLEWIGEI YPRSGNTYYN EKFKGKATLT ADKSSSTAYM

        --------H3-------><--CDR3-->-DJ Joint--><------CH1----
101     ELRSLTSEDS AVYFCARSWG NSSFVYWGQG TLVTVSAAKT TPPSVYPLAP

        -------------CH1-------------->
151     GSAAQTNSMV TLGCLVKGYF PEPVTVTWN
```

FIG. 6B

5D5 Kappa Chain

```
        ----Signal Peptide--->><----Framework 1--------><CDR1-L1
  1     MKSQTQVFVF LLLCVSGAHG SIVMTQTPKF LLVSAGDRVT ITCKASQSVT

        --->><----Framework 2->><-CDR2->><---
 51     NDVTWYQQKP GQSPKLLIYY ASNRYTGVPD RFTGSGYGTD FTFTISTVQA

        -FW3--->><-CRR3--->><--Joint--->><---------Kappa Constant-
101     EDLAVYFCQQ DYSSPFTFGS GTKLEIKRAD AAPTVSIFPP SSEQLSNSCS
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/068731

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/068731

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/20 A61P33/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , BIOSIS, EMBASE, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/063805 Al (US GOV HEALTH & HUMAN SERV [US] ; RATHORE DHARMENDAR [US] ; MCCUTCHAN TH) 14 July 2005 (2005-07-14) page 3, line 5 - line 23; figures 1-7 ; sequence 6 page 4, line 5 - page 6, line 8 page 6, line 27 - page 7, line 19 page 10, line 21 - 1ine 31 page 17, line 7 - line 22 page 21, line 20 - line 30 page 25, line 23 - page 26, line 2 examples 1,2 ----- -/- ·	1-17

X Further documents are listed in the continuation of Box C.

X See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2015	26/03/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Annette

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/068731

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/17204 A1 (US COMMERCE [US]) 15 October 1992 (1992-10-15) page 5, lines 4-12 , 28-32 page 6, line 15 - line 22 page 8, line 13 - line 18 page 9, line 26 - line 38 page 10, line 14 - line 21 claims 7,11,12; figure 3a	1-17
X	A. COPPI : "The Plasmodium circumsporozoite protein is proteolytically processed during cell invasion" , JOURNAL OF EXPERIMENTAL MEDICINE, vol . 201 , no. 1, 3 January 2005 (2005-01-03) , pages 27-33 , XP055175147 , ISSN : 0022-1007 , D0I : 10. 1084/jem. 20040989 the whole document, in particular page 27, right-hand column, last paragraph ; page 31, left-hand column, last paragraph and page 32, left-hand column, second full paragraph	1-17
X	S. B. ALEY: "Synthetic peptides from the circumsporozoite proteins of Plasmodium falciparum and Plasmodium knowlesi recognize the human hepatoma cell line HepG2-A16 in vitro" , JOURNAL OF EXPERIMENTAL MEDICINE, vol . 164, no. 6, 1 December 1986 (1986-12-01) , pages 1915-1922 , XP055175151 , ISSN : 0022-1007 , D0I : 10. 1084/jem. 164. 6. 1915 page 1916, paragraph 4; figure 1 page 1918, paragraph 3 discussion and summary	1-17
X	PATRICK YING ET AL: "The Malaria Circumsporozoite Protein: Interaction of the Conserved Regions I and 11-Plus with Heparin-like Oligosaccharides in Heparan Sulfate" , EXPERIMENTAL PARASITOLOGY, vol . 85, no. 2, 1 February 1997 (1997-02-01) , pages 168-182 , XP055175154, ISSN : 0014-4894, D0I : 10. 1006/expr. 1996.4134 page 170, right-hand column, paragraph 2; figure 1 page 174, left-hand column, paragraph 2 - right-hand column, paragraph 1 page 180, left-hand column, paragraph 2	1-17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/068731

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005063805 A1	14-07-2005	NONE	
W0 9217204 A1	15-10-1992	AU 1762092 A	02-11-1992
		W0 9217204 A1	15-10-1992