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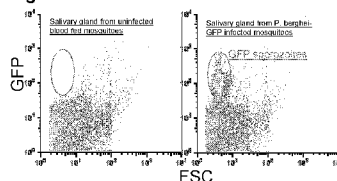
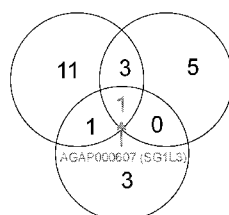
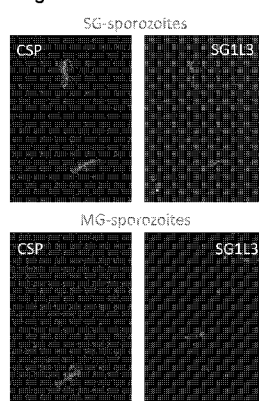
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(54) **Title:** MOSQUITO SALIVA PROTEIN MALARIA VACCINE

Figure 1A**Figure 1B****Figure 1C**

(57) **Abstract:** The invention provides compositions and methods for preventing and treating infection by Plasmodium sporozoites.

TITLE OF THE INVENTION
MOSQUITO SALIVA PROTEIN MALARIA VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application No. 62/110,756, filed February 2, 2015, the contents of which are incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

10 Malaria is a mosquito-borne infectious disease caused by a eukaryotic parasite of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, there are more than 250 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-Saharan Africa. Despite efforts to reduce transmission
15 and to increase treatment, there has been little change in which areas are at risk of this disease since 1992. Indeed, if the prevalence of malaria stays on its present course, the death rate could double in the next twenty years. Precise statistics are unknown because many cases occur in rural areas where people do not have access to hospitals or the means to afford healthcare. As a consequence, the majority of cases are undocumented.

20 Five species of the *Plasmodium* parasite can infect humans. The most serious forms of the disease are caused by *Plasmodium falciparum*, a protozoan parasite. It is transmitted by the female *Anopheles* mosquito. *P. falciparum* has the highest rate of complications and mortality; as of 2006, it has accounted for 91% of all 247 million human malarial infections (98% in Africa) and 90% of the deaths.

25 A wide variety of antimalarial drugs are available to treat malaria. Treatment of *P. falciparum* infections in endemic countries have recently been transformed by the use of combinations of drugs containing an artemisinin derivative. Severe malaria is treated with intravenous or intramuscular quinine, or increasingly, the artemisinin derivative artesunate. Several drugs are also available to prevent malaria in
30 travelers to malaria-endemic countries (prophylaxis). Resistance has developed to several antimalarial drugs, most notably chloroquine.

Vaccines for malaria are under development, with no completely effective vaccine yet available. The first promising studies demonstrating the potential for a malaria vaccine were performed in 1967 by immunizing mice with live, radiation-attenuated sporozoites, providing protection to about 60% of the mice upon subsequent
5 injection with normal, viable sporozoites. Since the 1970s, there has been a considerable effort to develop similar vaccination strategies within humans. However, the most advanced malaria vaccine candidates confer only partial protection against the clinical disease.

There is a need in the art for an effective malaria vaccine to treat or
10 prevent Plasmodium sporozoite infection in patients. The present invention addresses this unmet need.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods to prevent and
15 treat infection by a Plasmodium sporozoite, i.e., malaria.

In one aspect, the invention relates to a vaccine comprising at least one mosquito salivary protein associated with a Plasmodium sporozoite.

In another aspect, the invention relates to a vaccine comprising at least one polypeptide selected from the group consisting of: AGAP000607-PA, or a
20 AGAP000607-PA variant; AGAP001374-PA, or a AGAP001374-PA variant; AGAP009257-PA, or a AGAP009257-PA variant; AGAP009918-PA, or a AGAP009918-PA variant; AGAP010750-PA, or a AGAP010750-PA variant; AGAP001424-PA, or a AGAP001424-PA variant; AGAP001566-PA, or a AGAP001566-PA variant; AGAP007347-PA, or a AGAP007347-PA variant;
25 AGAP008227-PA, or a AGAP008227-PA variant; AGAP008279-PA, or a AGAP008279-PA variant; AGAP008283-PA, or a AGAP008283-PA variant; AGAP011017-PA, or a AGAP011017-PA variant; AGAP011976-PA, or a AGAP011976-PA variant; AGAP012081-PA, or a AGAP012081-PA variant; AGAP013499-PA, or a AGAP013499-PA variant; AGAP002998-PA, or a
30 AGAP002998-PA variant; AGAP003937-PA, or a AGAP003937-PA variant; AGAP004605-PA, or a AGAP004605-PA variant; AGAP011107-PA, or a

AGAP011107-PA variant; AGAP013013-PA, or a AGAP013013-PA variant;
AGAP009974-PA, or a AGAP009974-PA variant; AGAP000528-PA, or a
AGAP000528-PA variant; and AGAP011026-PA, or a AGAP011026-PA variant.

In another aspect, the invention relates to a method of inducing an immune
5 response in a subject against a Plasmodium sporozoite, the method comprising
administering to the subject at least one of the vaccines of the present invention. In one
embodiment, the subject is currently infected with Plasmodium sporozoites and the
vaccine induces an immune response against Plasmodium sporozoites. In one
embodiment, the subject is not currently infected with Plasmodium sporozoites and the
10 vaccine induces an immune response against Plasmodium sporozoites.

In another aspect, the invention relates to a method of treating a subject
infected with Plasmodium sporozoites, the method comprising administering to the
subject at least one of the vaccines of the present invention. In one embodiment, the
method further comprises the administration of an antibiotic.

15 In another aspect, the invention relates to a method of treating a subject
infected with Plasmodium sporozoites, the method comprising administering to the
subject at least one antibody, wherein the at least one antibody specifically binds to at
least one of the polypeptides of the present invention. In one embodiment, the method
further comprises the administration of an antibiotic.

20 In another aspect, the invention relates to an inhibitor composition useful
for treating, reducing, or preventing Plasmodium sporozoite infection, wherein the
inhibitor composition inhibits the interaction between a mosquito salivary protein
associated with a Plasmodium sporozoite and the Plasmodium sporozoite. In one
embodiment, the inhibitor composition specifically binds to the mosquito salivary protein
25 associated with a Plasmodium sporozoite. In one embodiment, the inhibitor composition
specifically binds to the Plasmodium sporozoite. In one embodiment, the inhibitor
composition is at least one selected from the group consisting of a chemical compound, a
protein, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical
compound, a glycan, and an antisense nucleic acid molecule.

30 In another aspect, the invention relates to a method of treating a subject
infected with Plasmodium sporozoites, the method comprising administering to the

subject at least one of the inhibitor compositions of the present invention. In one embodiment, the method further comprises the administration of an antibiotic.

BRIEF DESCRIPTION OF THE DRAWINGS

5 The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the
10 embodiments shown in the drawings.

 Figure 1A through Figure 1C depict the results of experiments performed to identify mosquito saliva proteins that are associated with a Plasmodium sporozoite. Figure 1A depicts the results of fluorescence-activated cell sorting (FACS) to isolate and purify GFP-expressing Plasmodium berghei sporozoites from Anopheles gambiae
15 mosquito salivary glands. Figure 1B depicts a Venn diagram showing the number of A. gambiae proteins identified in three separate experiments that could potentially be associated with a Plasmodium sporozoite, as well as the number of proteins that were common to each experiment. Figure 1C depicts the results of staining sporozoites purified from mosquito salivary glands (SG) and midguts (MG) with rabbit antibody
20 against SG1L3.

 Figure 2A through Figure 2D depict the results of SG1L3 expression analyses in A. gambiae salivary glands. Figure 2A and Figure 2B depict SG1L3 mRNA and protein expression, respectfully, in different regions of A. gambiae. Figure 2C and Figure 2D depict SG1L3 mRNA and protein expression, respectfully, in A. gambiae
25 salivary glands with and without the invasion of Plasmodium sporozoites.

 Figure 3A through Figure 3D depict the results of experiments demonstrating the effects of immunizing mice with SG1L3 antibodies. Figure 3A compares Plasmodium liver burden in mice with no treatment, with a known Plasmodium sporozoite transmission inhibitor (3D11 mAb against P. berghei CSP) treatment, with an
30 SG1L3 antibody treatment, and with an SG1L3 antibody and 3D11 mAb combination treatment. Figure 3B depicts the effect of SG1L3 antibodies on parasitemia in mice.

Figure 3C and Figure 3D depict the effects of a SG1L3 mAb (2A10) on parasitemia and Plasmodium infection, respectively, in mice.

Figure 4A through Figure 4B depict the antibody response by mice in response to being bitten by *A. gambiae*. Figure 4A shows that mice generate strong reactions to whole mosquito salivary gland extracts and barely a detectable reaction to SG1L3. Figure 4B shows gel analysis of immunogens from mosquito saliva.

Figure 5 depicts the effect of SG1L3 antibody on Plasmodium sporozoite invasion (measured by quantity of exoerythrocytic forms) and traverse into host cells.

Figure 6 is list of the *A. gambiae* proteins identified in three separate experiments that could potentially be associated with a Plasmodium sporozoite.

Figure 7 depicts the results of experiments demonstrating that subcutaneous treatment of SG1L3 antibodies in mice is effective at reducing the infection rate of Plasmodium sporozoite as compared to an ovalbumin (OVA) control.

DETAILED DESCRIPTION

The present invention provides compositions and methods to prevent and treat infection by a Plasmodium sporozoite, i.e., malaria. In one embodiment, the composition of the invention is a vaccine that induces the cell-mediated and/or humoral immunity directed against at least one mosquito salivary protein, or variants thereof, which is associated with a Plasmodium sporozoite. In various embodiments, the composition comprises at least one of the group consisting of AGAP000607-PA (a salivary gland-1 like 3 protein; hereinafter “SG1L3”), AGAP001374-PA (hereinafter “TRIO”), AGAP009257-PA, AGAP009918-PA, AGAP010750-PA, AGAP001424-PA, AGAP001566-PA, AGAP007347-PA, AGAP008227-PA, AGAP008279-PA, AGAP008283-PA, AGAP011017-PA, AGAP011976-PA, AGAP012081-PA, AGAP013499-PA, AGAP002998-PA, AGAP003937-PA, AGAP004605-PA, AGAP011107-PA, AGAP013013-PA, AGAP009974-PA, AGAP000528-PA, and AGAP011026-PA, or variants thereof. In a particular embodiment, the composition comprises SG1L3, or a variant thereof.

In another embodiment, the composition of the invention comprises at least one nucleic acid encoding a mosquito salivary protein, or variants thereof,

associated with a Plasmodium sporozoite. In another embodiment, the composition of the invention comprises a nucleic acid sequence encoding SG1L3, or a variant thereof. In one embodiment, the composition comprises an antibody that specifically binds to SG1L3, or a variant thereof.

5 The invention provides methods of inducing an immune response for preventing or treating infection by a Plasmodium sporozoite. In one embodiment, the methods comprise administering at least one mosquito salivary protein, or variants thereof, associated with a Plasmodium sporozoite to a subject. In one embodiment, the methods comprise administering at least SG1L3, or a variant thereof, to a subject. In
10 another embodiment, the methods comprise administering a nucleic acid encoding at least one mosquito salivary protein, or variants thereof, associated with a Plasmodium sporozoite to a subject. In another embodiment, the methods comprise administering a nucleic acid encoding at least SG1L3, or a variant thereof, to a subject.

 The invention also includes inhibitor compositions and methods for
15 inhibiting the interaction between the mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite.

 The invention also provides methods of treating infection by a Plasmodium sporozoite in a subject in need thereof. In one embodiment, the method comprises administering to the subject at least one antibody that binds to a mosquito
20 salivary protein, or variants thereof, associated with a Plasmodium sporozoite. In another embodiment, the method comprises administering to the subject at least one antibody that specifically binds to SG1L3, or a variant thereof.

Definitions

25 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The articles “a” and “an” are used herein to refer to one or to more than
5 one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$
10 from the specified value, as such variations are appropriate to perform the disclosed methods.

The term “analog” or “functional analog” refers to a related modified form of a polypeptide, wherein at least one amino acid substitution, deletion, or addition has been made such that said analog retains substantially the same biological activity as the
15 unmodified form, in vivo and/or in vitro.

The term “antibody,” as used herein, refers to an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. The antibodies in the present
20 invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab)₂, as well as single chain antibodies and humanized antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl.
25 Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

The term “antigen” or “Ag” as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or
30 peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a

nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used herein.

Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the

5 present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample.

10 The term “agent” includes any substance, metabolite, molecule, element, compound, or a combination thereof. It includes, but is not limited to, e.g., protein, oligopeptide, small organic molecule, glycan, polysaccharide, polynucleotide, and the like. It can be a natural product, a synthetic compound, a chemical compound, or a combination of two or more substances. Unless otherwise specified, the terms “agent,”
15 “substance,” and “compound” can be used interchangeably. Further, a “test agent” or “candidate agent” is generally a subject agent for use in an assay of the invention.

The term “binding” refers to a direct association between at least two molecules, due to, for example, covalent, electrostatic, hydrophobic, ionic and/or hydrogen-bond interactions.

20 “CDRs” are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions)
25 in the variable portion of an immunoglobulin. Thus, “CDRs” as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989)
30 Conformations of immunoglobulin hypervariable regions; Nature 342, p 877-883.

A “chimeric antibody” refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

5 “Contacting” refers to a process in which two or more molecules or two or more components of the same molecule or different molecules are brought into physical proximity such that they are able undergo an interaction. Molecules or components thereof may be contacted by combining two or more different components containing molecules, for example by mixing two or more solution components, preparing a solution
10 comprising two or more molecules such as target, candidate or competitive binding reference molecules, and/or combining two or more flowing components.

As used herein, by “combination therapy” is meant that a first agent is administered in conjunction with another agent. “In conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As
15 such, “in conjunction with” refers to administration of one treatment modality before, during, or after delivery of the other treatment modality to the individual. Such combinations are considered to be part of a single treatment regimen or regime.

As used herein, the term “concurrent administration” means that the administration of the first therapy and that of a second therapy in a combination therapy
20 overlap temporally with each other.

A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate. In contrast, a “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of
25 health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

The term “donor antibody” refers to an antibody (monoclonal, and/or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as
30 to provide the altered immunoglobulin coding region and resulting expressed altered

antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

The term “acceptor antibody” refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion,
5 but in some embodiments all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. In certain embodiments a human antibody is the acceptor antibody.

An “effective amount” as used herein, means an amount which provides a
10 therapeutic or prophylactic benefit.

The term “expression” as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

“Expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a
15 nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate
20 the recombinant polynucleotide.

As used herein, the term “heavy chain antibody” or “heavy chain antibodies” comprises immunoglobulin molecules derived from camelid species, either by immunization with a peptide and subsequent isolation of sera, or by the cloning and expression of nucleic acid sequences encoding such antibodies. The term “heavy chain
25 antibody” or “heavy chain antibodies” further encompasses immunoglobulin molecules isolated from an animal with heavy chain disease, or prepared by the cloning and expression of VH (variable heavy chain immunoglobulin) genes from an animal.

“Homologous” refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in
30 both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by

adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared multiplied by 100. For example, if 6 of 10 of the positions in two sequences are matched or

5 homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

A “humanized antibody” refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining
10 immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., 1989, Queen et al., Proc. Natl. Acad Sci USA, 86:10029-10032; 1991, Hodgson et al., Bio/Technology, 9:421). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT database,
15 Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable
20 of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanized antibodies (see for example EP-A-0239400 and EP-A-054951).

25 The term “immunoglobulin” or “Ig,” as used herein, is defined as a class of proteins, which function as antibodies. Antibodies expressed by B cells are sometimes referred to as the BCR (B cell receptor) or antigen receptor. The five members included in this class of proteins are IgA, IgG, IgM, IgD, and IgE. IgA is the primary antibody that is present in body secretions, such as saliva, tears, breast milk, gastrointestinal secretions
30 and mucus secretions of the respiratory and genitourinary tracts. IgG is the most common circulating antibody. IgM is the main immunoglobulin produced in the primary immune

response in most subjects. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is the immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is the immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

As used herein, the term “immune response” includes T-cell mediated and/or B-cell mediated immune responses. Exemplary immune responses include T cell responses, e.g., cytokine production and cellular cytotoxicity, and B cell responses, e.g., antibody production. In addition, the term immune response includes immune responses that are indirectly affected by T cell activation, e.g., antibody production (humoral responses) and activation of cytokine responsive cells, e.g., macrophages. Immune cells involved in the immune response include lymphocytes, such as B cells and T cells (CD4+, CD8+, Th1 and Th2 cells); antigen presenting cells (e.g., professional antigen presenting cells such as dendritic cells, macrophages, B lymphocytes, Langerhans cells, and non-professional antigen presenting cells such as keratinocytes, endothelial cells, astrocytes, fibroblasts, oligodendrocytes); natural killer cells; myeloid cells, such as macrophages, eosinophils, mast cells, basophils, and granulocytes.

As used herein, an “inhibitory-effective amount” is an amount that results in a detectable (e.g., measurable) amount of inhibition of an activity. In some instance, the activity is its ability to bind with another component.

“Isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

A “mutation,” as used herein, refers to a change in nucleic acid or polypeptide sequence relative to a reference sequence (which is preferably a naturally-occurring normal or “wild-type” sequence), and includes translocations, deletions, insertions, and substitutions/point mutations. A “mutant” as used herein, refers to either a

nucleic acid or protein comprising a mutation.

“Parenteral” administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intradermal (i.d.) injection, or infusion techniques.

5 The terms “patient,” “subject,” “individual,” and the like are used interchangeably herein, and refer to any animal, or cells thereof, whether in vitro or in situ, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

 By the term “specifically binds,” as used herein with respect to an
10 antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically
15 binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific. In some instances, the terms “specific binding” or “specifically binding,” can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g.,
20 an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope “X,” the presence of a molecule containing epitope X (or free, unlabeled A), in a reaction containing labeled “X” and the antibody, will reduce the amount of labeled X bound to the antibody.

25 By the term “synthetic antibody” as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein,
30 or an amino acid sequence specifying the antibody, wherein the DNA or amino acid

sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

The term “therapeutic” as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, diminution, remission, or eradication of a disease state.

The term “therapeutically effective amount” refers to the amount of the subject compound that will elicit the biological or clinical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term “therapeutically effective amount” includes that amount of a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease being treated. The therapeutically effective amount will vary depending on the compound, the disease and its severity and the age, weight, etc., of the subject to be treated.

To “treat” a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

“Variant” of the polypeptides according to the present invention may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, (ii) one in which there are one or more modified amino acid residues, e.g., residues that are modified by the attachment of substituent groups, (iii) one in which the polypeptide is an alternative splice variant of the polypeptide of the present invention, (iv) fragments of the polypeptides and/or (v) one in which the polypeptide is fused with another polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification (for example, His-tag) or for detection (for example, Sv5 epitope tag). The

fragments include polypeptides generated via proteolytic cleavage (including multi-site proteolysis) of an original sequence. Variants may be post-translationally, or chemically modified. Such variants are deemed to be within the scope of those skilled in the art from the teaching herein.

5 Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual
10 numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

15

Description

 The invention relates to the administration of at least one mosquito salivary protein associated with a Plasmodium sporozoite, to a subject to induce an immune response. Thus, the present invention provides a polypeptide or a combination of
20 polypeptides, or a polynucleotide or a combination of polynucleotides, which are useful in inducing an immune response, for the treatment or prevention of infection by a Plasmodium sporozoite.

 The invention provides an immunological composition comprising a polypeptide or combination of polypeptides derived from at least one mosquito salivary
25 protein associated with a Plasmodium sporozoite, useful in eliciting an immune response. The compositions comprising one or more polypeptide of the invention not only are useful as a prophylactic therapeutic agent for immunoprotection, but are also useful as a therapeutic agent for treatment of an ongoing disease or disorder associated with infection by a Plasmodium sporozoite in a subject.

30 In one embodiment, the composition of the invention comprises a nucleic acid sequence encoding a mosquito salivary protein, or variants thereof, associated with a

Plasmodium sporozoite. In various embodiments, the composition comprises at least one of the group consisting of AGAP000607-PA (a salivary gland-1 like 3 protein; hereinafter “SG1L3”), AGAP001374-PA (hereinafter “TRIO”), AGAP009257-PA, AGAP009918-PA, AGAP010750-PA, AGAP001424-PA, AGAP001566-PA, AGAP007347-PA, AGAP008227-PA, AGAP008279-PA, AGAP008283-PA, AGAP011017-PA, AGAP011976-PA, AGAP012081-PA, AGAP013499-PA, AGAP002998-PA, AGAP003937-PA, AGAP004605-PA, AGAP011107-PA, AGAP013013-PA, AGAP009974-PA, AGAP000528-PA, and AGAP011026-PA, or variants thereof. In a particular embodiment, the composition of the invention comprises a nucleic acid sequence encoding SG1L3, or a variant thereof. The skilled artisan will understand that the least one mosquito salivary protein associated with a Plasmodium sporozoite, useful in eliciting an immune response, can be used each alone or in any combination for eliciting an immune response.

The present invention also provides methods of preventing, inhibiting, and treating infection by a Plasmodium sporozoite in a subject in need thereof. In one embodiment, the methods of the invention induce immunity against Plasmodium sporozoites in the subject, by generating an immune response in the subject directed to at least one polypeptide, such as SG1L3. In one embodiment, the methods of the invention induce production of SG1L3-specific antibodies in the subject.

In one embodiment, the methods of the invention prevent Plasmodium sporozoite related disease or disorder in a subject in need thereof. In one embodiment, the methods of the invention comprise administering to the subject a composition comprising at least a portion of at least one mosquito salivary protein associated with a Plasmodium sporozoite to a subject. In another embodiment, the methods of the invention comprise administering to the subject a composition comprising a nucleic acid sequence encoding at least one mosquito salivary protein associated with a Plasmodium sporozoite to a subject.

The invention also includes inhibitor compositions and methods for inhibiting the interaction between the mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite.

Compositions

The present invention provides compositions, including polypeptides, nucleotides, vectors, and vaccines, that when administered to a subject, elicit an immune response directed against Plasmodium sporozoites, including an immune response
5 directed against at least one mosquito salivary protein associated with a Plasmodium sporozoite. Further, when the compositions are administered to a subject, they elicit an immune response that serves to protect the subject against diseases or disorders associated with Plasmodium sporozoite infection.

In one embodiment, the present invention provides compositions that are
10 useful as immunomodulatory agents, for example, in initiating or stimulating an immune response and in preventing or diminishing Plasmodium sporozoite related disease or disorder. In various embodiments, the immunomodulatory agents comprise at least one mosquito salivary protein associated with a Plasmodium sporozoite. In various
15 embodiments, the immunomodulatory agents comprise at least one of the group consisting of AGAP000607-PA (a salivary gland-1 like 3 protein; hereinafter “SG1L3”), AGAP001374-PA (hereinafter “TRIO”), AGAP009257-PA, AGAP009918-PA, AGAP010750-PA, AGAP001424-PA, AGAP001566-PA, AGAP007347-PA, AGAP008227-PA, AGAP008279-PA, AGAP008283-PA, AGAP011017-PA, AGAP011976-PA, AGAP012081-PA, AGAP013499-PA, AGAP002998-PA,
20 AGAP003937-PA, AGAP004605-PA, AGAP011107-PA, AGAP013013-PA, AGAP009974-PA, AGAP000528-PA, and AGAP011026-PA, or variants thereof. In one embodiment, the immune response is not detrimental to the host and therefore the compositions of the invention are useful as a vaccine, to prevent or to diminish the likelihood of a subject becoming infected. In one embodiment, the immunomodulatory
25 agents are administered in combination with an adjuvant. In another embodiment, the immunomodulatory agents are administered in the absence of an adjuvant.

Mosquito salivary proteins associated with a Plasmodium sporozoite can be used as immunostimulatory agents to induce the production of antibodies to protect, prevent, or reduce the likelihood of developing a Plasmodium sporozoite induced disease
30 or disorder. In one embodiment, the composition of the invention comprises a SG1L3 polypeptide, or a variant thereof. The skilled artisan will understand that the least one

mosquito salivary protein associated with a Plasmodium sporozoite, useful in eliciting an immune response, can each be used alone or in any combination for eliciting an immune response.

5 The present invention also provides polynucleotides that encode the polypeptides described herein. Therefore, in one embodiment, the composition of the invention comprises a nucleic acid sequence encoding SG1L3, or a variant thereof. The skilled artisan will understand that the least one mosquito salivary protein associated with a Plasmodium sporozoite, useful in eliciting an immune response, can each be used alone or in any combination for eliciting an immune response.

10 In various embodiments, the invention provides a polypeptide, or a fragment of a polypeptide, a homolog, a mutant, a variant, a derivative or a salt of a polypeptide as elsewhere described herein, wherein the immunogenic activity of the polypeptide or fragment thereof is retained.

The invention should also be construed to include any form of a polypeptide having substantial homology to the polypeptides disclosed herein. Preferably, a polypeptide which is “substantially homologous” is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous, more preferably about 90% homologous, even more preferably, about 95% homologous, and even more preferably about 99% homologous to amino acid sequence of the polypeptides disclosed herein.

20 In one embodiment, the polypeptide or combination of polypeptides of the present invention are capable of generating a specific immune response. In another embodiment, the polypeptide or combination of polypeptides of the present invention are capable of generating specific antibodies.

25 Polypeptides of the present invention can be prepared using well known techniques. For example, the polypeptides can be prepared synthetically, using either recombinant DNA technology or chemical synthesis. Polypeptides of the present invention may be synthesized individually or as longer polypeptides composed of two or more polypeptides. The polypeptides of the present invention can be isolated, i.e.,
30 substantially free of other naturally occurring host cell proteins and fragments thereof.

The polypeptides of the present invention may contain modifications, such as glycosylation, aglycosylation, side chain oxidation, or phosphorylation; so long as the modifications do not destroy the immunologic activity of the polypeptides. Other modifications include incorporation of D-amino acids or other amino acid mimetics that
5 can be used, for example, to increase the serum half-life of the polypeptides.

The polypeptides of the invention can be modified whereby the amino acid is substituted for a different amino acid in which the properties of the amino acid side-chain are conserved (a process known as conservative amino acid substitution). Examples of properties of amino acid side chains are hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side
10 chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain
15 (H, F, Y, W). Note that the parenthetic letters indicate the one-letter codes of amino acids. As used herein, X stands for any amino acid.

The polypeptides of the invention can be prepared as a combination, which includes two or more of polypeptides of the invention, for use as a vaccine for the reduction, prevention, or treatment of Plasmodium sporozoite infection. The polypeptides
20 may be in a cocktail or may be conjugated to each other using standard techniques. For example, the polypeptides can be expressed as a single polypeptide sequence. The polypeptides in the combination may be the same or different.

The present invention should also be construed to encompass “analogs,” “mutants,” “derivatives,” and “variants” of the polypeptides of the invention (or of the
25 DNA encoding the same) which analogs, mutants, derivatives and variants are polypeptides which are altered in one or more amino acids (or, when referring to the nucleotide sequence encoding the same, are altered in one or more base pairs) such that the resulting polypeptide (or DNA) is not identical to the sequences recited herein, but has the same biological property as the polypeptides disclosed herein.

30 The nucleic acid sequences include both the DNA sequence that is transcribed into RNA and the RNA sequence that is translated into a polypeptide.

According to other embodiments, the polynucleotides of the invention are inferred from the amino acid sequence of the polypeptides of the invention. As is known in the art several alternative polynucleotides are possible due to redundant codons, while retaining the biological activity of the translated polypeptides.

5 Further, the invention encompasses an isolated nucleic acid encoding a polypeptide having substantial homology to the polypeptides disclosed herein. Preferably, the nucleotide sequence of an isolated nucleic acid encoding a polypeptide of the invention is “substantially homologous,” that is, is about 60% homologous, more preferably about 70% homologous, even more preferably about 80% homologous, more
10 preferably about 90% homologous, even more preferably, about 95% homologous, and even more preferably about 99% homologous to a nucleotide sequence of an isolated nucleic acid encoding a polypeptide of the invention.

 It is to be understood explicitly that the scope of the present invention encompasses homologs, analogs, variants, fragments, derivatives and salts, including
15 shorter and longer polypeptides and polynucleotides, as well as polypeptide and polynucleotide analogs with one or more amino acid or nucleic acid substitution, as well as amino acid or nucleic acid derivatives, non-natural amino or nucleic acids and synthetic amino or nucleic acids as are known in the art, with the stipulation that these modifications must preserve the immunologic activity of the original molecule.
20 Specifically any active fragments of the active polypeptides as well as extensions, conjugates and mixtures are included and are disclosed herein according to the principles of the present invention.

 The invention should be construed to include any and all isolated nucleic acids which are homologous to the nucleic acids described and referenced herein,
25 provided these homologous nucleic acids encode polypeptides having the biological activity of the polypeptides disclosed herein.

 The skilled artisan would understand that the nucleic acids of the invention encompass an RNA or a DNA sequence encoding a polypeptide of the invention, and any modified forms thereof, including chemical modifications of the DNA
30 or RNA which render the nucleotide sequence more stable when it is cell free or when it is associated with a cell. Chemical modifications of nucleotides may also be used to

enhance the efficiency with which a nucleotide sequence is taken up by a cell or the efficiency with which it is expressed in a cell. Any and all combinations of modifications of the nucleotide sequences are contemplated in the present invention.

Further, any number of procedures may be used for the generation of
5 mutant, derivative or variant forms of a protein of the invention using recombinant DNA methodology well known in the art such as, for example, that described in Sambrook et al. (2012, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Procedures for the introduction of amino acid changes in a
10 polypeptide or polypeptide by altering the DNA sequence encoding the polypeptide are well known in the art and are also described in these, and other, treatises.

Vectors

The nucleic acids encoding the polypeptide or combinations of
15 polypeptides of the invention of the invention can be incorporated into suitable vectors, including but not limited to, plasmids and retroviral vectors. Such vectors are well known in the art and are therefore not described in detail herein.

In one embodiment, the invention includes a nucleic acid sequence encoding one or more polypeptides of the invention operably linked to a nucleic acid
20 comprising a promoter/regulatory sequence such that the nucleic acid is preferably capable of directing expression of the protein encoded by the nucleic acid. Thus, the invention encompasses expression vectors and methods for the introduction of exogenous DNA into cells with concomitant expression of the exogenous DNA in the cells such as those described, for example, in Sambrook et al. (2012, Molecular Cloning: A Laboratory
25 Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York). The incorporation of a desired polynucleotide into a vector and the choice of vectors is well-known in the art as described in, for example, Sambrook et al. (2012), and in Ausubel et al. (1997).

30 The polynucleotide can be cloned into a number of types of vectors. However, the present invention should not be construed to be limited to any particular

vector. Instead, the present invention should be construed to encompass a wide plethora of vectors which are readily available and/or well-known in the art. For example, the polynucleotide of the invention can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation
5 vectors, and sequencing vectors.

In specific embodiments, the expression vector is selected from the group consisting of a viral vector, a bacterial vector and a mammalian cell vector. Numerous expression vector systems exist that comprise at least a part or all of the compositions
10 discussed above. Prokaryote- and/or eukaryote-vector based systems can be employed for use with the present invention to produce polynucleotides, or their cognate polypeptides. Many such systems are commercially and widely available.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for
15 example, in Sambrook et al. (2012), and in Ausubel et al. (1997), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and
20 one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193.

For expression of the desired nucleotide sequences of the invention, at least one module in each promoter functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a
25 TATA box, such as the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 genes, a discrete element overlying the start site itself helps to fix the place of initiation.

Additional promoter elements, i.e., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of
30 the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter

elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function
5 either co-operatively or independently to activate transcription.

A promoter may be one naturally associated with a gene or polynucleotide sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a polynucleotide sequence,
10 located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding polynucleotide segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a polynucleotide sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a
15 polynucleotide sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," i.e., containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid
20 sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the compositions disclosed herein (U.S. Patent 4,683,202, U.S. Patent 5,928,906). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria,
25 chloroplasts, and the like, can be employed as well.

Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen for expression. Those of skill in the art of molecular biology generally know how to use promoters, enhancers, and cell type combinations for protein
30 expression, for example, see Sambrook et al. (2012). The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to

direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or polypeptides. The promoter may be heterologous or endogenous.

One example of a constitutive promoter sequence is the immediate early
5 cytomagalovirus (CMV) promoter sequence. This promoter sequence is a strong
constitutive promoter sequence capable of driving high levels of expression of any
polynucleotide sequence operatively linked thereto. However, other constitutive promoter
sequences may also be used, including, but not limited to the simian virus 40 (SV40)
early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus
10 (HIV) long terminal repeat (LTR) promoter, Moloney virus promoter, the avian leukemia
virus promoter, Epstein-Barr virus immediate early promoter, Rous sarcoma virus
promoter, as well as human gene promoters such as, but not limited to, the actin
promoter, the myosin promoter, the hemoglobin promoter, and the muscle creatine
promoter. Further, the invention should not be limited to the use of constitutive
15 promoters. Inducible promoters are also contemplated as part of the invention. The use of
an inducible promoter in the invention provides a molecular switch capable of turning on
expression of the polynucleotide sequence which it is operatively linked when such
expression is desired, or turning off the expression when expression is not desired.
Examples of inducible promoters include, but are not limited to a metallothionine
20 promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline
promoter. Further, the invention includes the use of a tissue-specific promoter, where the
promoter is active only in a desired tissue. Tissue-specific promoters are well known in
the art and include, but are not limited to, the HER-2 promoter and the PSA associated
promoter sequences.

25 In order to assess the expression of the nucleotide sequences encoding the
polypeptide or combinations of polypeptides of the invention, the expression vector to be
introduced into a cell can also contain either a selectable marker gene or a reporter gene
or both to facilitate identification and selection of expressing cells from the population of
cells sought to be transfected or infected through viral vectors. In other embodiments, the
30 selectable marker may be carried on a separate piece of DNA and used in a co-
transfection procedure. Both selectable markers and reporter genes may be flanked with

appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are known in the art and include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for
5 evaluating the functionality of regulatory sequences. Reporter genes that encode for easily assayable proteins are well known in the art. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a protein whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the
10 DNA has been introduced into the recipient cells.

Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (see, e.g., Ui-Tei et al., 2000 FEBS Lett. 479:79-82). Suitable expression systems are well known and may be prepared using well known
15 techniques or obtained commercially. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of siRNA polynucleotide and/or polypeptide expression. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as
20 the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

In some embodiments, the expression vector is modified to increase the expression of the desired polypeptide. For example, the vector can undergo codon optimization to improve expression in a given mammal. For example, the vector can be
25 codon-optimized for human expression. In another embodiment, the expression vector comprises an effective secretory leader. An exemplary leader is an IgE leader sequence. In another embodiment, the expression vector comprises a Kozak element to initiate translation. In another embodiment, the nucleic acid is removed of cis-acting sequence motifs/RNA secondary structures that would impede translation. Such modifications, and
30 others, are known in the art for use in DNA vaccines (Kutzler et al, 2008, Nat. Rev. Gen. 9: 776-788; PCT App. No. PCT/US2007/000886; PCT App. No.; PCT/US2004/018962).

Vaccine

For an antigenic composition to be useful as a vaccine, the antigenic composition must induce an immune response to the antigen in a cell, tissue or subject (e.g., a human). In some embodiments, the vaccine induces a protective immune response in the subject. As used herein, an “immunological composition” may comprise, by way of examples, an antigen (e.g., a polypeptide), a nucleic acid encoding an antigen (e.g., an antigen expression vector), or a cell expressing or presenting an antigen. In particular embodiments the antigenic composition comprises or encodes all or part of any polypeptide antigen described herein, or an immunologically functional equivalent thereof. In other embodiments, the antigenic composition is in a mixture that comprises an additional immunostimulatory agent or nucleic acids encoding such an agent. Immunostimulatory agents include but are not limited to an additional antigen, an immunomodulator, an antigen presenting cell or an adjuvant. In other embodiments, one or more of the additional agent(s) is covalently bonded to the antigen or an immunostimulatory agent, in any combination. In certain embodiments, the antigenic composition is conjugated to or comprises an HLA anchor motif amino acids.

In the context of the present invention, the term “vaccine” (also referred to as an immunogenic composition) refers to a substance that induces anti-Plasmodium sporozoite immunity or suppresses Plasmodium sporozoites upon inoculation into an animal. In various embodiments, the vaccine of the invention comprises at least one mosquito salivary protein associated with a Plasmodium sporozoite that can be administered to a subject to induce an immune response. In various embodiments, the vaccine of the invention comprises at least one of the group consisting of AGAP000607-PA (a salivary gland-1 like 3 protein; hereinafter “SG1L3”), AGAP001374-PA (hereinafter “TRIO”), AGAP009257-PA, AGAP009918-PA, AGAP010750-PA, AGAP001424-PA, AGAP001566-PA, AGAP007347-PA, AGAP008227-PA, AGAP008279-PA, AGAP008283-PA, AGAP011017-PA, AGAP011976-PA, AGAP012081-PA, AGAP013499-PA, AGAP002998-PA, AGAP003937-PA, AGAP004605-PA, AGAP011107-PA, AGAP013013-PA, AGAP009974-PA, AGAP000528-PA, and AGAP011026-PA, or variants thereof. In one embodiment, the

vaccine is administered in combination with an adjuvant. In another embodiment, the vaccine is administered in the absence of an adjuvant.

A vaccine of the present invention may vary in its composition of nucleic acid and/or cellular components. In a non-limiting example, a nucleic encoding an antigen might also be formulated with an adjuvant. Of course, it will be understood that various compositions described herein may further comprise additional components. For example, one or more vaccine components may be comprised in a lipid or liposome. In another non-limiting example, a vaccine may comprise one or more adjuvants. A vaccine of the present invention, and its various components, may be prepared and/or administered by any method disclosed herein or as would be known to one of ordinary skill in the art, in light of the present disclosure.

In one embodiment, the polypeptide vaccine of the invention includes, but is not limited to at least one polypeptide, or a fragment thereof, optionally mixed with adjuvant substances. In some embodiments, the polypeptide is introduced together with an antigen presenting cell (APC). The most common cells used for the latter type of vaccine are bone marrow and peripheral blood derived dendritic cells, as these cells express costimulatory molecules that help activation of T cells. WO00/06723 discloses a cellular vaccine composition which includes an APC presenting tumor associated antigen polypeptides. Presenting the polypeptide can be effected by loading the APC with a polynucleotide (e.g., DNA, RNA) encoding the polypeptide or loading the APC with the polypeptide itself.

Thus, the present invention also encompasses a method of inducing Plasmodium sporozoite immunity using one or more of polypeptides described herein. When a certain polypeptide or combination of polypeptides induces a Plasmodium sporozoite immune response after inoculation into an animal, the polypeptide or combination of polypeptides is understood to have an immunity inducing effect. The induction of Plasmodium sporozoite immunity by a polypeptide or combination of polypeptides can be detected by observing the response of the immune system, in vivo or in vitro, by the host against the polypeptide, or combination of polypeptides.

In another embodiment, the methods of the invention comprise administering to the subject a bacterium or virus comprising a nucleic acid sequence

encoding at least one mosquito salivary protein associated with a Plasmodium sporozoite. In another embodiment, the methods of the invention comprise administering to the subject a bacterium or virus expressing at least a portion of at least one mosquito salivary protein associated with a Plasmodium sporozoite. In another embodiment, the methods of the invention comprise administering to the subject a bacterium or virus comprising at least a portion of at least one mosquito salivary protein associated with a Plasmodium sporozoite.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of APCs. T cells that respond to the antigen presented by APC in an antigen-specific manner differentiate into cytotoxic T cells (also referred to as cytotoxic T lymphocytes or CTLs) due to stimulation by the antigen. These antigen stimulated cells then proliferate. This process is referred to herein as “activation” of T cells. Therefore, CTL induction by a certain polypeptide or combination of polypeptides of the invention can be evaluated by presenting the polypeptide to a T cell by APC, and detecting the induction of CTL. Furthermore, APCs have the effect of activating CD4⁺ T cells, CD8⁺ T cells, macrophages, eosinophils and NK cells.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the polypeptide or combination of polypeptides is initially contacted with DC and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the polypeptide or combination of polypeptides has an activity of inducing the cytotoxic T cells. Furthermore, the induced immune response can be also examined by measuring IFN-gamma produced and released by CTL in the presence of antigen-presenting cells that carry immobilized polypeptide or combination of polypeptides by visualizing using anti-IFN-gamma antibodies, such as an ELISPOT assay.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported to be enhanced by culturing PBMC in

the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The polypeptide or combination of polypeptides, confirmed to possess CTL inducing activity by these methods, are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, a polypeptide or combination of polypeptides that induce CTL against Plasmodium sporozoite A and Plasmodium sporozoite B are useful as vaccines against Plasmodium sporozoite associated disease or disorder. Furthermore, CTL that have acquired cytotoxicity due to presentation of the polypeptide or combination of polypeptides by APC can be also used as vaccines against Plasmodium sporozoite infection.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction can be increased by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous in certain embodiments to use a mixture of multiple types of fragments.

The induction of Plasmodium sporozoite immunity by a polypeptide or combination of polypeptides can be further confirmed by observing the induction of antibody production against the Plasmodium sporozoites. For example, when antibodies against a polypeptide or combination of polypeptides are induced in a laboratory animal immunized with the polypeptide or combination of polypeptides, and when Plasmodium sporozoite associated disease or disorder is suppressed by those antibodies, the polypeptide or combination of polypeptides are understood to induce anti-Plasmodium sporozoite immunity.

Plasmodium sporozoite immunity can be induced by administering a vaccine of the invention, and the induction of Plasmodium sporozoite immunity enables treatment and prevention of pathologies associated with Plasmodium sporozoite. Thus, the invention provides a method for treating or preventing infection by a Plasmodium sporozoite. The therapeutic compounds or compositions of the invention may be administered prophylactically or therapeutically to subjects suffering from, at risk of developing, or susceptible to developing, Plasmodium sporozoite infection. Such subjects may be identified using standard clinical methods. In the context of the present invention,

prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or alternatively delayed in its progression. In the context of the field of medicine, the term “prevent” encompasses any activity which reduces the burden of mortality or morbidity from disease. Prevention can
5 occur at primary, secondary and tertiary prevention levels. While primary prevention avoids the development of a disease, secondary and tertiary levels of prevention encompass activities aimed at preventing the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already established disease by restoring function and reducing disease-related complications.

10 The polypeptide or combination of polypeptides of the invention having immunological activity, or a polynucleotide or vector encoding such a polypeptide or combination of polypeptides, may optionally be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the polypeptide or combination of polypeptides when administered together (or successively) with the
15 polypeptide having immunological activity. Non-limiting examples of suitable adjuvants include cholera toxin, salmonella toxin, alum and such, but are not limited thereto. Furthermore, a vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the vaccine
20 may contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration or boosted by multiple administrations.

Administration

25 In one embodiment, the methods of the present invention comprise administering to a subject a composition comprising at least one polypeptide of the invention, and/or at least one polynucleotide encoding at least one polypeptide of the invention. Administration of the composition can comprise, for example, intramuscular, intravenous, peritoneal, subcutaneous, intradermal, as well as topical administration.

30 The actual dose and schedule can vary depending on whether the compositions are administered in combination with other pharmaceutical compositions,

or depending on inter-individual differences in pharmacokinetics, drug disposition, and metabolism. Similarly, amounts can vary in in vitro applications depending on the particular cell line utilized (e.g., based on the number of vector receptors present on the cell surface, or the ability of the particular vector employed for gene transfer to replicate
5 in that cell line). Furthermore, the amount of vector to be added per cell will likely vary with the length and stability of the therapeutic gene inserted in the vector, as well as also the nature of the sequence, and is particularly a parameter which needs to be determined empirically, and can be altered due to factors not inherent to the methods of the present invention (for instance, the cost associated with synthesis). One skilled in the art can
10 easily make any necessary adjustments in accordance with the exigencies of the particular situation.

These methods described herein are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the effective amount of the compositions can be further approximated through
15 analogy to compounds known to exert the desired effect.

Therapeutic Inhibitor Compositions and Methods

In various embodiments, the present invention includes inhibitor compositions for inhibiting with the interaction between the mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite. In various
20 embodiments, the inhibited mosquito salivary protein associated with a Plasmodium sporozoite is at least one of the group consisting of AGAP000607-PA (a salivary gland-1 like 3 protein; hereinafter “SG1L3”), AGAP001374-PA (hereinafter “TRIO”), AGAP009257-PA, AGAP009918-PA, AGAP010750-PA, AGAP001424-PA, AGAP001566-PA, AGAP007347-PA, AGAP008227-PA, AGAP008279-PA,
25 AGAP008283-PA, AGAP011017-PA, AGAP011976-PA, AGAP012081-PA, AGAP013499-PA, AGAP002998-PA, AGAP003937-PA, AGAP004605-PA, AGAP011107-PA, AGAP013013-PA, AGAP009974-PA, AGAP000528-PA, and AGAP011026-PA, or variants thereof. Inhibition of the interaction between the mosquito
30 salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite

can be assessed using a wide variety of methods, including those disclosed herein, as well as methods known in the art or to be developed in the future.

One skilled in the art, based upon the disclosure provided herein, would understand that the inhibitor compositions and methods of the invention are useful in
5 treating preventing and infection by a Plasmodium sporozoite.

The inhibitor compositions and methods of the invention that interfere with the interaction between the mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite include, but should not be construed as being limited to, a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, a
10 ribozyme, a small molecule chemical compound, a glycan, an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), or combinations thereof. One of skill in the art would readily appreciate, based on the disclosure provided herein, that the inhibitor compositions of the invention include those that interfere with the interaction between the protein and the Plasmodium sporozoite. In some embodiments, the inhibitor compositions
15 bind to the protein and interfere with the interaction between the protein and the Plasmodium sporozoite. In other embodiments, the inhibitor compositions bind to the Plasmodium sporozoite and interfere with the interaction between the protein and the Plasmodium sporozoite.

In various embodiments, the treatment of Plasmodium sporozoite infection
20 in a subject is accomplished through passive antibody therapy (i.e., the transfer of antibodies to the Plasmodium sporozoite infected subject). In various embodiments, the inhibitor compositions and methods of the invention that interfere with the interaction between the mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite are used in combination with an antibiotic therapy. When used in
25 combination, the antibiotic therapy can be administered before, during or after the administration of the inhibitor compositions of the invention.

Further, one of skill in the art would, when equipped with this disclosure and the methods exemplified herein, appreciate that an inhibitor composition includes such inhibitors as discovered in the future, as can be identified by well-known criteria in
30 the art of pharmacology. Therefore, the present invention is not limited in any way to any particular inhibitor composition as exemplified or disclosed herein; rather, the invention

encompasses those inhibitor compositions that would be understood by the routineer to be useful as are known in the art and as are discovered in the future.

Further methods of identifying and producing inhibitor compositions are well known to those of ordinary skill in the art, including, but not limited, obtaining an inhibitor from a naturally occurring source (i.e., *Streptomyces* sp., *Pseudomonas* sp., *Stylorella aurantium*). Alternatively, an inhibitor can be synthesized chemically. Further, the routineer would appreciate, based upon the teachings provided herein, that an inhibitor composition can be obtained from a recombinant organism. Compositions and methods for chemically synthesizing inhibitors and for obtaining them from natural sources are well known in the art and are described in the art.

Numerous vectors and other compositions and methods are well known for administering a protein or a nucleic acid construct encoding a protein to cells or tissues. Therefore, the invention includes a method of administering a protein or a nucleic acid encoding a protein that is an inhibitor. (Sambrook et al., 2012, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1997, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York).

One of skill in the art will appreciate that inhibitors of the invention can be administered singly or in any combination. Further, inhibitors can be administered singly or in any combination in a temporal sense, in that they may be administered concurrently, or before, and/or after each other.

In various embodiments, any of the inhibitors of the invention described herein can be administered alone or in combination with other inhibitors of other molecules associated with *Plasmodium* sporozoite infection.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of a disease or disorder that is already established. Particularly, the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant disease or disorder does not have to occur before the present invention may provide benefit. Therefore, the present invention includes a method for preventing

infection in a subject, in that an inhibitor composition, as discussed previously elsewhere herein, can be administered to a subject prior to the onset of the infection.

The invention encompasses administration of an inhibitor to practice the methods of the invention; the skilled artisan would understand, based on the disclosure provided herein, how to formulate and administer the appropriate inhibitor to a subject. Indeed, the successful administration of the inhibitor has been reduced to practice herein. However, the present invention is not limited to any particular method of administration or treatment regimen.

10 Pharmaceutical Compositions

The present invention includes the treatment of a Plasmodium sporozoite infection in a subject by the administration of a therapeutic composition of the invention to a subject in need thereof. In one embodiment, the therapeutic composition of the invention is an inhibitor composition. In one embodiment, the therapeutic composition of the invention for the treatment of Plasmodium sporozoite infection is at least one antibody that specifically binds to at least one mosquito salivary protein associated with a Plasmodium sporozoite. In various embodiments, the therapeutic composition comprises at least one antibody that specifically binds to at least one of the group consisting of AGAP000607-PA (a salivary gland-1 like 3 protein; hereinafter "SG1L3"), AGAP001374-PA (hereinafter "TRIO"), AGAP009257-PA, AGAP009918-PA, AGAP010750-PA, AGAP001424-PA, AGAP001566-PA, AGAP007347-PA, AGAP008227-PA, AGAP008279-PA, AGAP008283-PA, AGAP011017-PA, AGAP011976-PA, AGAP012081-PA, AGAP013499-PA, AGAP002998-PA, AGAP003937-PA, AGAP004605-PA, AGAP011107-PA, AGAP013013-PA, AGAP009974-PA, AGAP000528-PA, and AGAP011026-PA, or variants thereof. In various embodiments, the treatment of Plasmodium sporozoite infection in a subject is accomplished through passive antibody therapy (i.e., the transfer of antibodies to the Plasmodium sporozoite infected subject).

Administration of the therapeutic composition in accordance with the present invention may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is

therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the compositions of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated. The amount administered will vary depending on various factors including, but not limited to, the composition chosen, the particular disease, the weight, the physical condition, and the age of the subject, and whether prevention or treatment is to be achieved. Such factors can be readily determined by the clinician employing animal models or other test systems which are well known to the art.

When the compositions of the invention are prepared for administration, they are preferably combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. The total active ingredients in such formulations include from 0.1 to 99.9% by weight of the formulation. A "pharmaceutically acceptable" is a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof. The active ingredient for administration may be present as a powder or as granules; as a solution, a suspension or an emulsion.

Pharmaceutical formulations containing the compositions of the invention can be prepared by procedures known in the art using well known and readily available ingredients. The compositions of the invention can also be formulated as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes.

Thus, the composition may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The active ingredients may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

In general, water, suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain the active ingredient, suitable stabilizing agents and, if necessary, buffer substances.

- 5 Antioxidizing agents such as sodium bisulfate, sodium sulfite or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium ethylenediaminetetraacetic acid (EDTA). In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl- or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in Remington's
- 10 Pharmaceutical Sciences, a standard reference text in this field.

These methods described herein are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the effective amount of the compositions can be further approximated through analogy to compounds known to exert the desired effect.

15

EXPERIMENTAL EXAMPLES

- The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should
- 20 in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1: Mosquito Saliva Protein SG1L3 as a Novel Malaria Vaccine Target

- 25 The results described herein demonstrate that mosquito saliva proteins associated with a Plasmodium sporozoite are novel vaccine targets for immunization against malaria.

Identification of Anopheles proteins that interact with Plasmodium sporozoites

- 30 To identify mosquito saliva proteins that are associated with a Plasmodium sporozoite, fluorescence-activated cell sorting (FACS) was used to isolate

and purify GFP-expressing *P. berghei* sporozoites from *A. gambiae* salivary glands (Figure 1A). NanoLC/MS-MS analysis was performed to categorize mosquito proteins that were co-purified with *Plasmodium* sporozoites. Three separate experiments identified a total of 24 *A. gambiae* proteins that could potentially be associated with the sporozoites (Figure 1B, Figure 6). One protein (AGAP000607, SG1L3) was identified in all 3 independent studies, and was therefore selected for in-depth characterization.

To validate whether SG1L3 was associated with *Plasmodium* sporozoites, the sporozoites purified from salivary glands (SG) and midguts (MG) were stained. Rabbit antibody against SG1L3 could detect a signal from mosquito SG sporozoites but not those from MG (Figure 1C).

SG1L3 was only expressed in *A. gambiae* salivary glands (Figure 2A, Figure 2B). The invasion of *Plasmodium* sporozoites into the salivary glands does not alter the expression of SG1L3, both at the mRNA and protein levels (Figure 2C, Figure 2D).

SG1L3 antibodies interfere with sporozoites transmission

It was assessed whether passive immunization with SG1L3 antibodies could interfere with *Plasmodium* sporozoites transmission from mosquito to the mammalian host. Rabbit IgG against SG1L3 were transferred to naïve mice, and these mice were challenged with *P. berghei*-infected *A. gambiae* mosquitoes. A lower *Plasmodium* liver burden was observed in SG1L3 antibody receiving animals (Figure 3A), suggesting that fewer sporozoites had reached the liver and invaded the hepatocytes. Furthermore, these animals had lower parasitemia levels at the early stage of the disease (Figure 3B). In addition, when combined with 3D11 mAb against *P. berghei* CSP, a known reagent that inhibits the *Plasmodium* sporozoite transmission, antibodies against SG1L3 further reduced the *Plasmodium* liver burden (Figure 3A). These results demonstrate that antibodies to *Plasmodium* antigen CSP and mosquito saliva protein have a synergetic effect.

Monoclonal antibodies to SG1L3 were then generated. When transferred to the mice, mAb (2A10) appeared able to not only reduce the *Plasmodium* burden (Figure 3C), but also to protect 3 out of 5 mice from infection (Figure 3D).

Vertebrate hosts generate minimal amount of IgG antibodies to SG1L3 by mosquito bites

SG1L3 is a component of mosquito saliva and is injected into mammalian hosts during blood feeding. It was thus investigated whether mosquito bites could elicit antibody responses to SG1L3. The sera from mice that were repeatedly bitten by A. gambiae mosquitoes were tested. ELISA and Western blot results showed that, though mice could generate strong reactions to whole mosquito salivary gland extracts, the antibodies to SG1L3 were barely detectable (Figure 4A). The major immunogens from mosquito saliva were proteins with sizes of 300 KDa, 65 KDa, and 15 KDa (Figure 4B), which may correspond to gSG5, 5'-nucleotidase, and gSG6, as previously reported.

SG1L3 facilitates Plasmodium sporozoite traversal through host cells

It was observed that the antibody to SG1L3 interfered with Plasmodium sporozoite transmission in vivo. It was next assessed whether SG1L3 antibody inhibited sporozoite traversal or invasion of host cells. Figure 5 shows that the SG1L3 antibody does not prevent the sporozoites invasion into hepatoma cells, even at the highest concentration tested (5 mg/mL). However, when cells were incubated with SG1L3 antibody, the number of cells traversed by sporozoites (measured by influx of fluorescent Dextran) was significantly reduced (Figure 5). These results are consistent with the explanation that the SG1L3 binding to sporozoites facilitates the cell traversal by sporozoites, which is required for sporozoites to reach the hepatocytes in vivo.

Example 2: Mosquito Saliva Protein SG1L3 is an Effective Novel Malaria Vaccine Target

The data described herein demonstrate that mosquito saliva proteins associated with a Plasmodium sporozoite are effective as novel vaccine targets for immunization against malaria as compared to an ovalbumin (OVA) control.

Immunization

Recombinant SG1L3 or OVA were emulsified in Complete Freund's Adjuvant (CFA) and administered subcutaneously to mice (10 µg protein per mouse).

Two subsequent immunizations were emulsified in Incomplete Freund's Adjuvant (IFA). Emulsions were administered at 200 µL per mouse, 3 times at two week intervals.

Plasmodium Challenge

5 Infected mosquitoes were selected based on GFP signals expressed by parasites in their thorax, then randomly aliquoted into individual coffee cups with mesh covers. The infectious mosquito bite challenges were performed at the following day with two infectious bites per mouse. The blood parasite burdens were quantified by daily measurement of parasitemia starting from 4 days post infection. Results are shown in
10 Figure 7.

 The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is
15 apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A vaccine comprising at least one mosquito salivary protein associated with a Plasmodium sporozoite.
2. A vaccine comprising at least one polypeptide selected from the group consisting of:
 - a. AGAP000607-PA, or a AGAP000607-PA variant;
 - b. AGAP001374-PA, or a AGAP001374-PA variant;
 - c. AGAP009257-PA, or a AGAP009257-PA variant;
 - d. AGAP009918-PA, or a AGAP009918-PA variant;
 - e. AGAP010750-PA, or a AGAP010750-PA variant;
 - f. AGAP001424-PA, or a AGAP001424-PA variant;
 - g. AGAP001566-PA, or a AGAP001566-PA variant;
 - h. AGAP007347-PA, or a AGAP007347-PA variant;
 - i. AGAP008227-PA, or a AGAP008227-PA variant;
 - j. AGAP008279-PA, or a AGAP008279-PA variant;
 - k. AGAP008283-PA, or a AGAP008283-PA variant;
 - l. AGAP011017-PA, or a AGAP011017-PA variant;
 - m. AGAP011976-PA, or a AGAP011976-PA variant;
 - n. AGAP012081-PA, or a AGAP012081-PA variant;
 - o. AGAP013499-PA, or a AGAP013499-PA variant;
 - p. AGAP002998-PA, or a AGAP002998-PA variant;
 - q. AGAP003937-PA, or a AGAP003937-PA variant;
 - r. AGAP004605-PA, or a AGAP004605-PA variant;
 - s. AGAP011107-PA, or a AGAP011107-PA variant;
 - t. AGAP013013-PA, or a AGAP013013-PA variant;
 - u. AGAP009974-PA, or a AGAP009974-PA variant;
 - v. AGAP000528-PA, or a AGAP000528-PA variant; and

w. AGAP011026-PA, or a AGAP011026-PA variant.

3. A method of inducing an immune response in a subject against a Plasmodium sporozoite, the method comprising administering to the subject the vaccine of at least one of claims 1 and 2.

4. The method of claim 3, wherein the subject is currently infected with Plasmodium sporozoites and the vaccine induces an immune response against Plasmodium sporozoites.

5. The method of claim 3, wherein the subject is not currently infected with Plasmodium sporozoites and the vaccine induces an immune response against Plasmodium sporozoites.

6. A method of treating a subject infected with Plasmodium sporozoites, the method comprising administering to the subject the vaccine of at least one of claims 1 and 2.

7. The method of claim 6, further comprising the administration of an antibiotic.

8. A method of treating a subject infected with Plasmodium sporozoites, the method comprising administering to the subject at least one antibody, wherein the at least one antibody specifically binds to at least one of the polypeptides of claim 2.

9. The method of claim 8, further comprising the administration of an antibiotic.

10. An inhibitor composition useful for treating, reducing, or preventing Plasmodium sporozoite infection, wherein the inhibitor composition inhibits

the interaction between a mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite.

11. The inhibitor composition of claim 10, wherein the inhibitor composition specifically binds to the mosquito salivary protein associated with a Plasmodium sporozoite.

12. The inhibitor composition of claim 10, wherein the inhibitor composition specifically binds to the Plasmodium sporozoite.

13. The inhibitor composition of claim 10, wherein the inhibitor composition is at least one selected from the group consisting of a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, a glycan, and an antisense nucleic acid molecule.

14. A method of treating a subject infected with Plasmodium sporozoites, the method comprising administering to the subject the inhibitor composition of at least one of claims 10-13.

15. The method of claim 14, further comprising the administration of an antibiotic.

Figure 1A

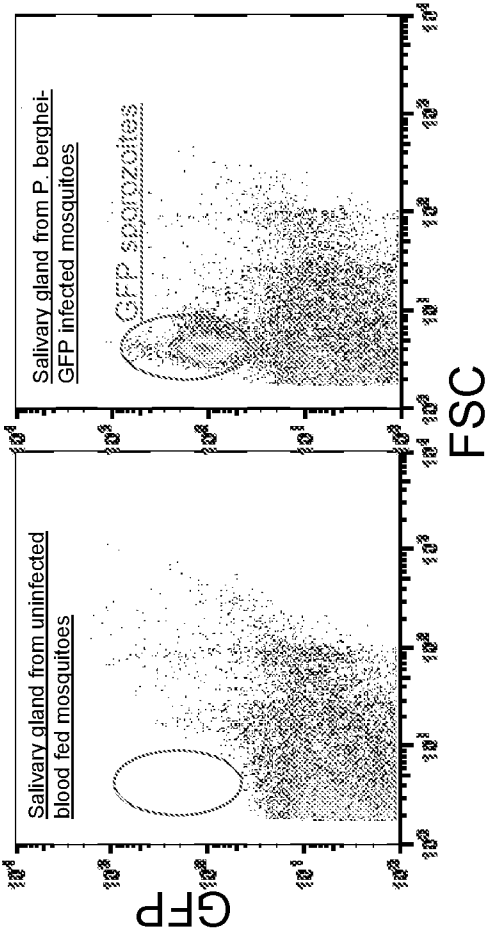


Figure 1C

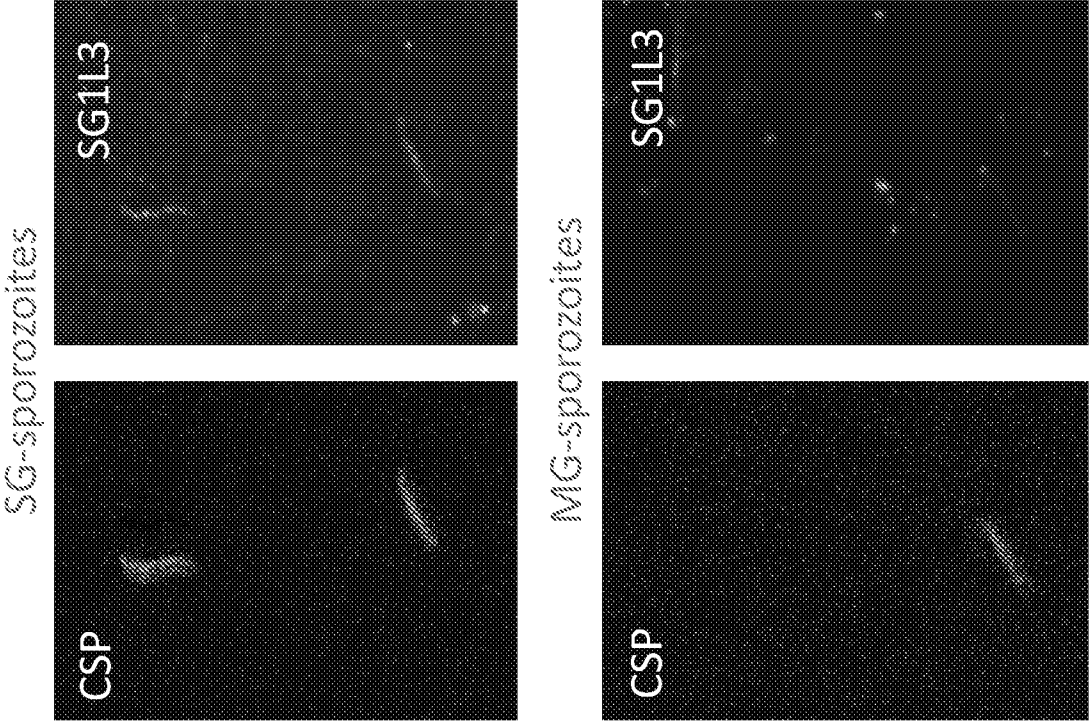


Figure 1B

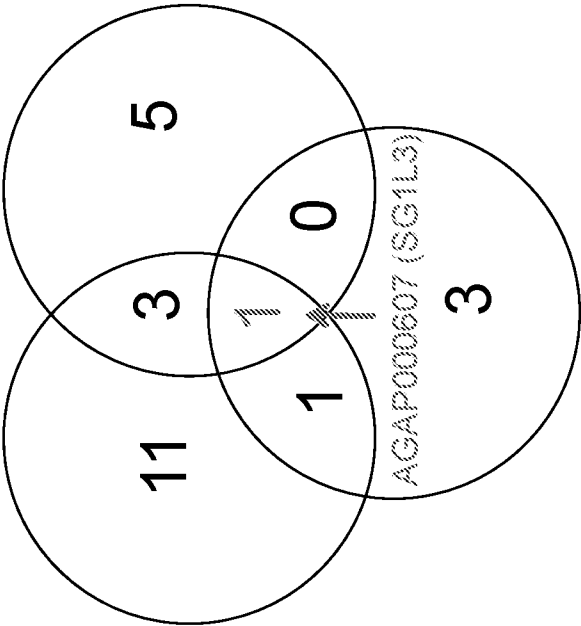


Figure 2B

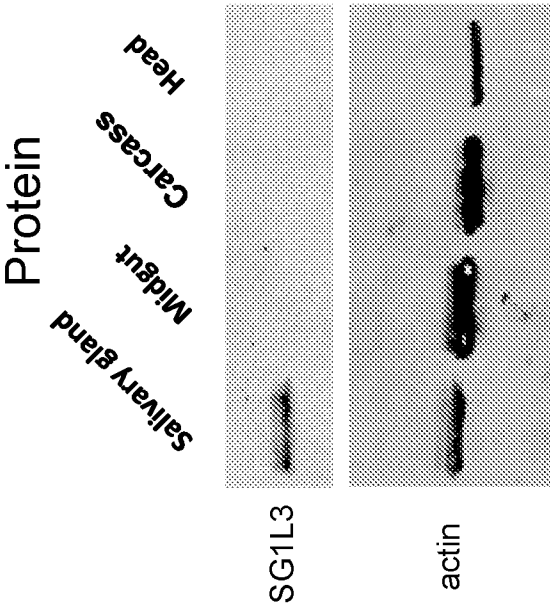


Figure 2A

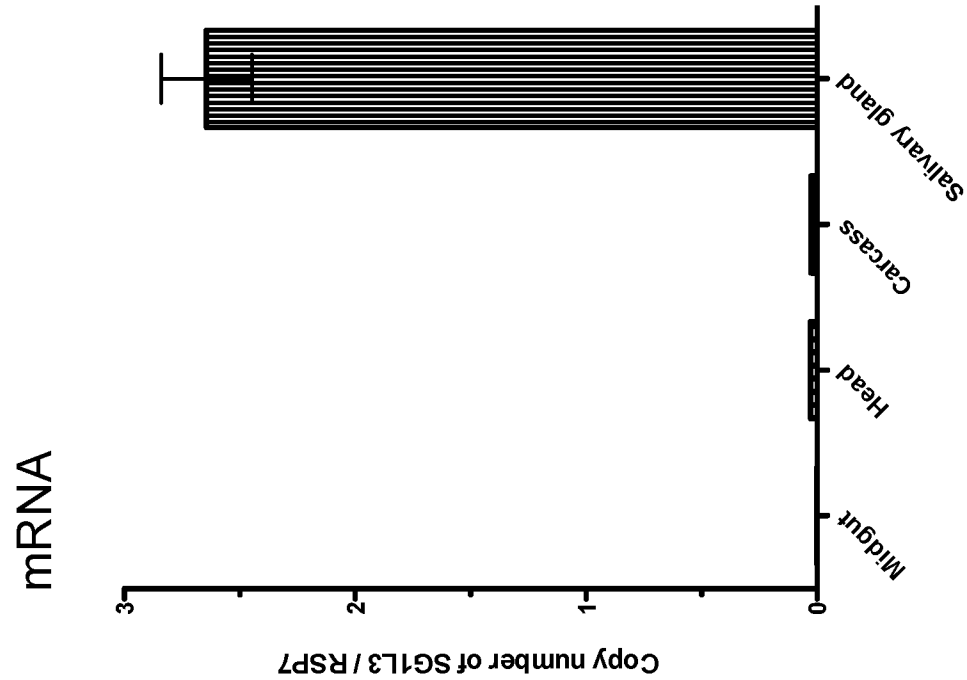


Figure 2C

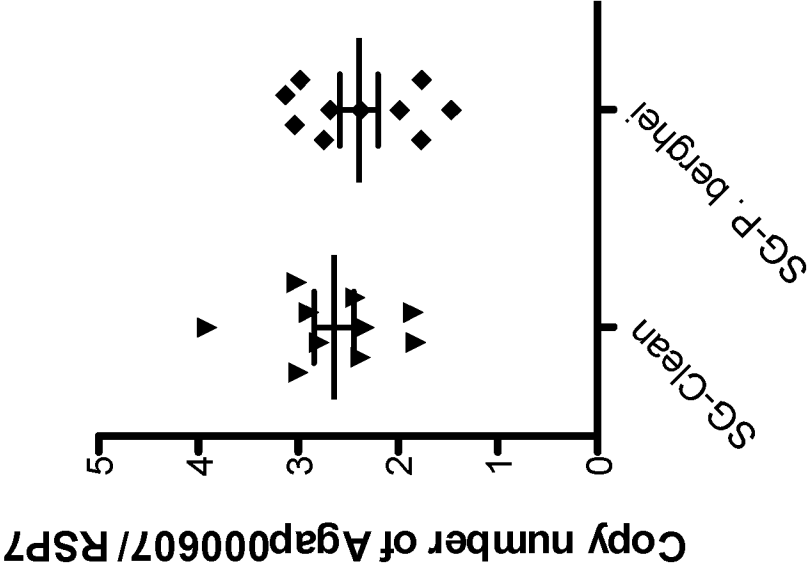


Figure 2D

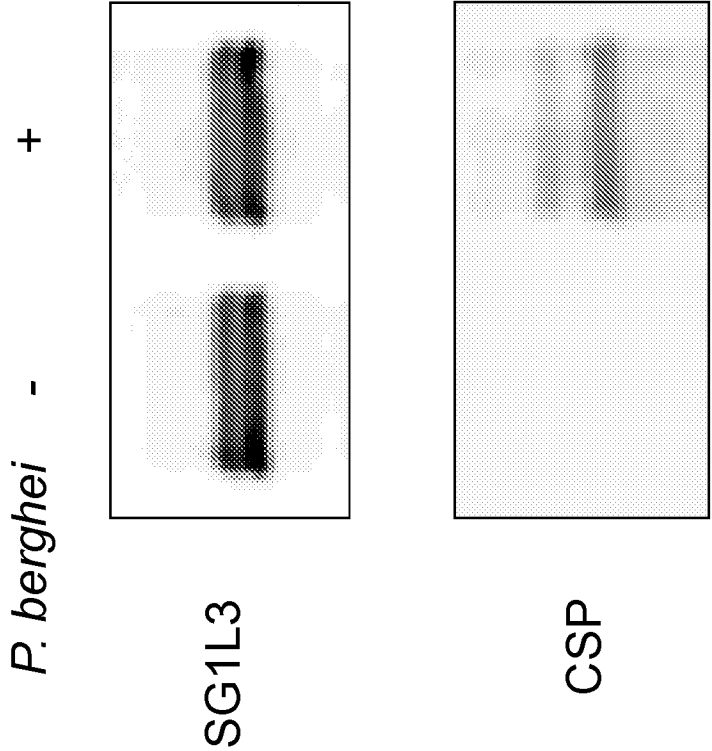


Figure 3A

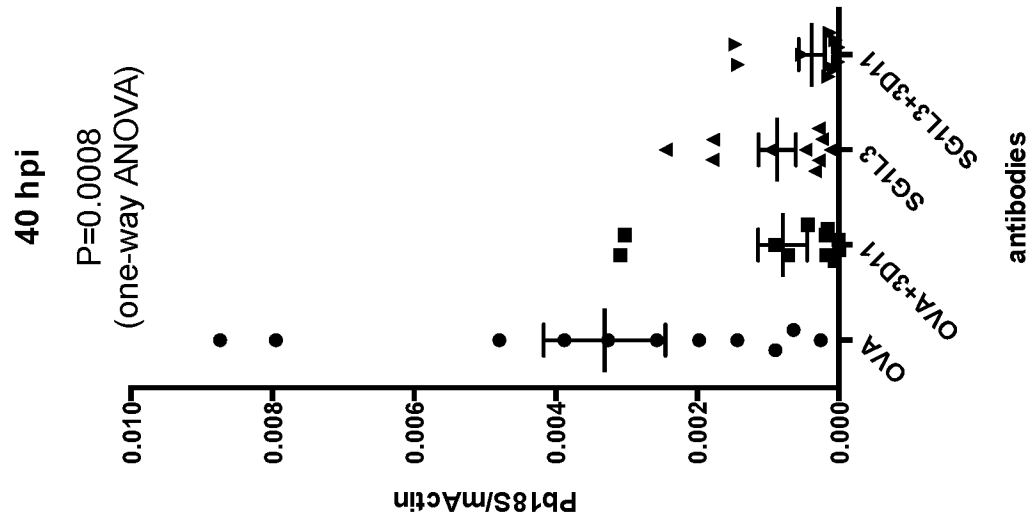


Figure 3B

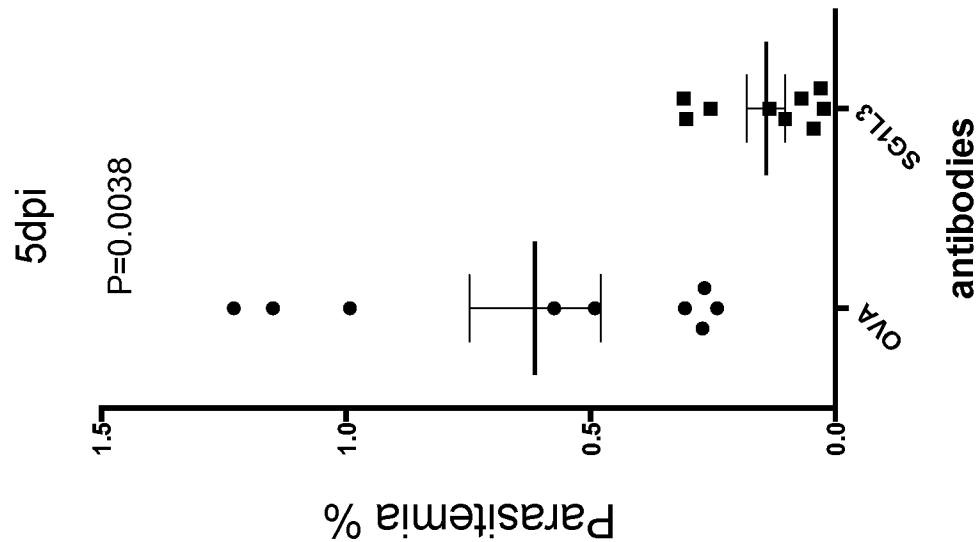


Figure 3D

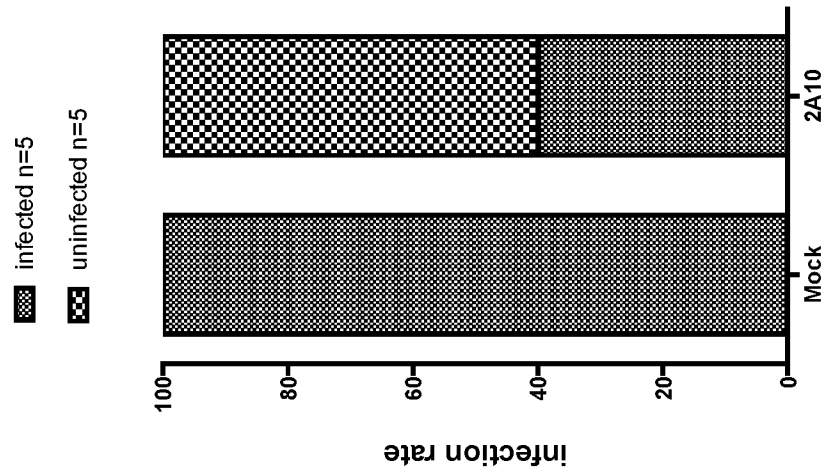
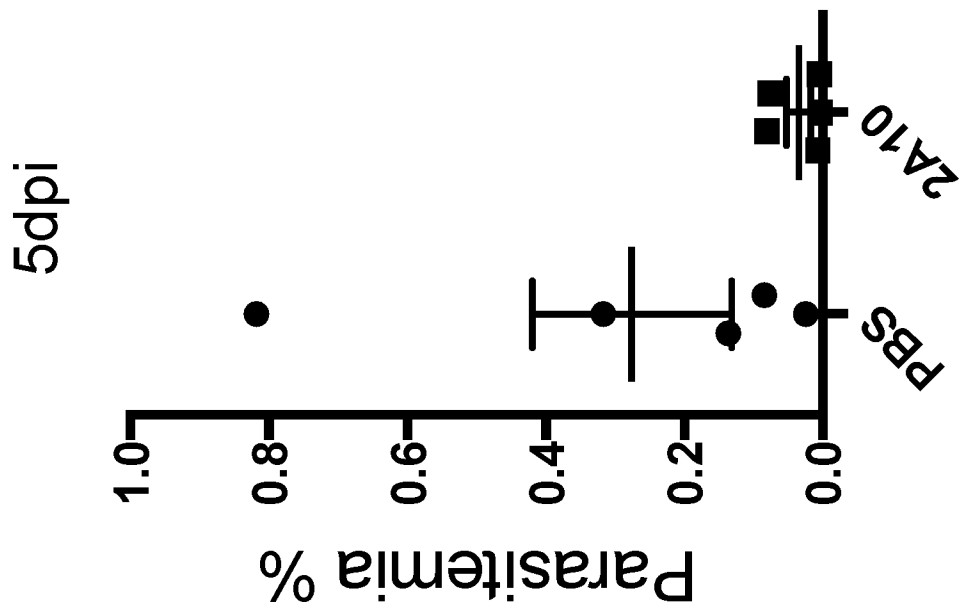


Figure 3C



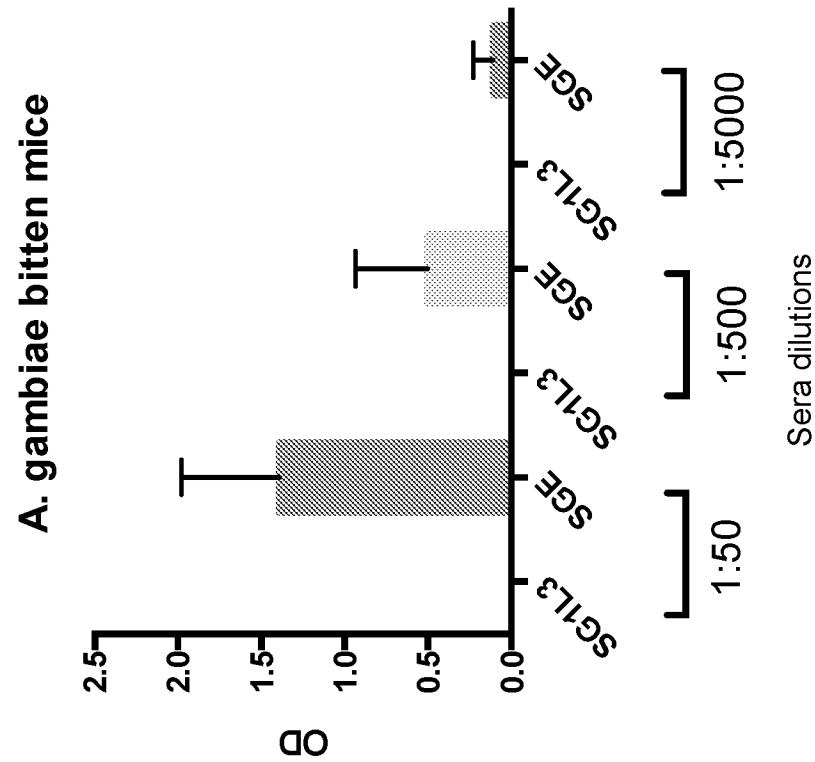


Figure 4A

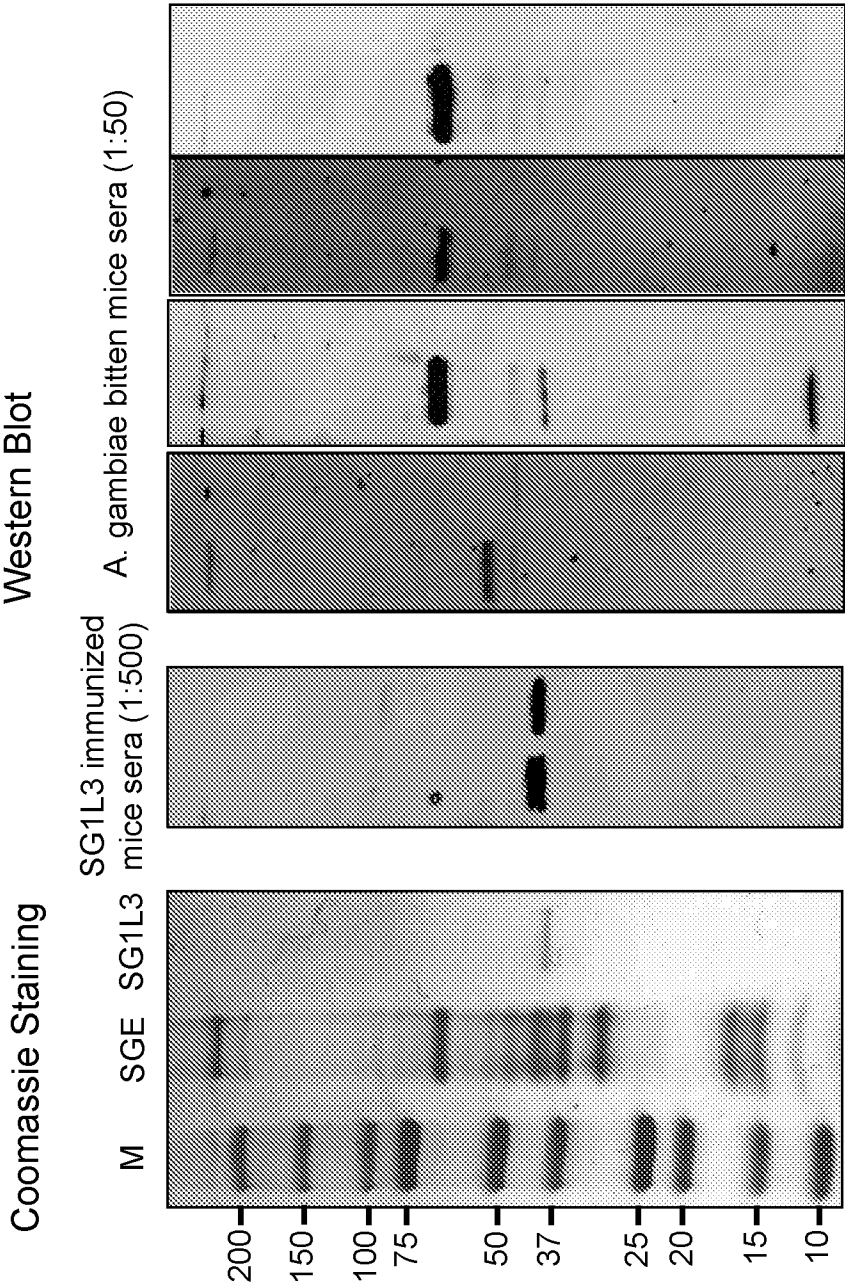


Figure 4B

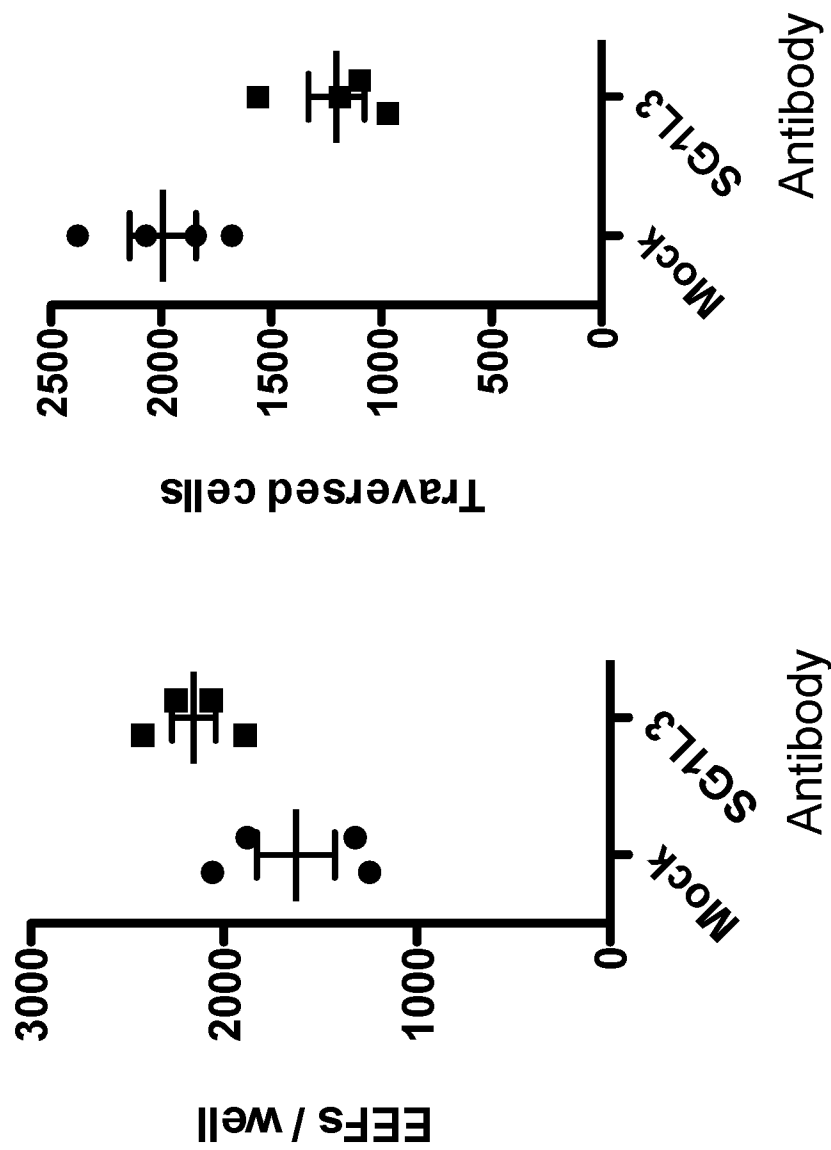


Figure 5

Hit that appeared three times
AGAP000607-PA [*Anopheles gambiae* str. PEST] SG1L3
Hits that appeared twice
AGAP001374-PA TRIO protein
AGAP009257-PA
AGAP009918-PA
AGAP010750-PA
Hits that appeared once
AGAP001424-PA
AGAP001566-PA
AGAP007347-PA
AGAP007374-PA
AGAP008227-PA
AGAP008279-PA
AGAP008283-PA
AGAP011017-PA
AGAP011976-PA
AGAP012081-PA
AGAP013499-PA
AGAP002998-PA
AGAP003937-PA
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AGAP013013-PA
AGAP009974-PA
AGAP000548-PA
AGAP011026-PA

Figure 6

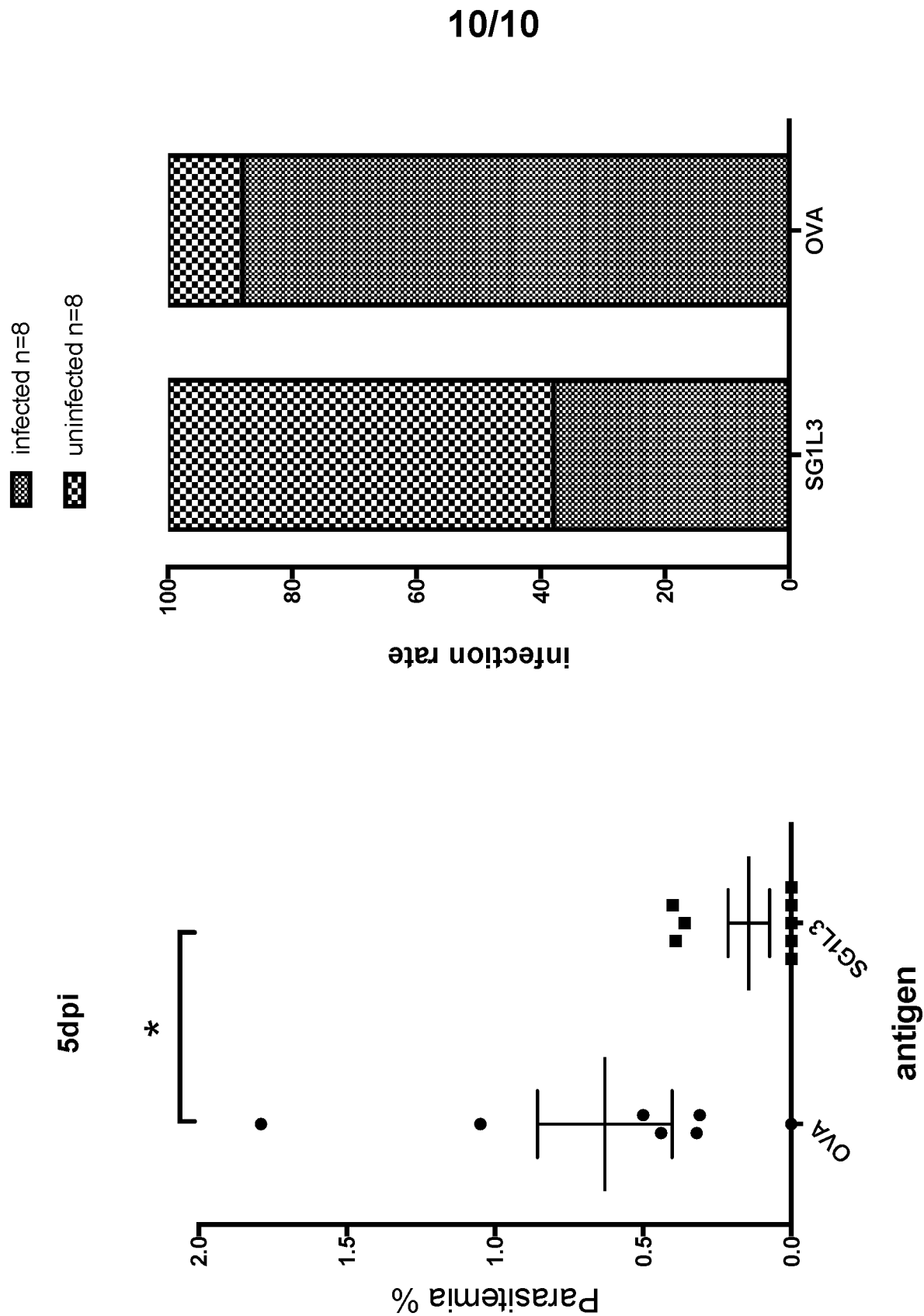


Figure 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/14839

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/01; C07K 14/435 (2016.01)

CPC - C07K 14/435, 14/43577

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)

IPC(8): A61K 38/01, 39/005; C07K 14/435 (2016.01)

CPC: A61K 39/005, 2039/53; C07K 14/435, 14/43577.

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)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO Discovery; PubMed; Google; Google Scholar; Google Patents; The Lens ; ENA; NCBI Blast; KEYWORDS: vaccine, mosquito, salivary, protein, Plasmodium, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	EP 2 567 706 A2 (PEPTCELL LIMITED) March 13, 2013; paragraphs [0001], [0023], [0031], [0033], [0137] Table 1; claim 1	1-2, 3/1-2, 4/3/1-2, 5/3/1-2, 6/1-2 --- 7/6/1-2, 8-9
Y	US 5,270,037 A (BIENZLE) December 14, 1993; column 7, lines 3-11; column 4, lines 3-5; column 4, lines 3-5; column 7, lines 3-11	7/6/1-2, 9
Y	(WANG, J, et al.) Anopheles Gambiae Circumsporozoite Protein-Binding Protein Facilitates Plasmodium Infection Of Mosquito Salivary Glands. Journal of Infectious Diseases. Vol. 208, October 1, 2013, pp 1161-1169; page 1162, column 2, paragraph 4. DOI: 10.1093/infdis/jit284.	8-9

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which 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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

03 May 2016 (03.05.2016)

Date of mailing of the international search report

29 JUN 2016

Name and mailing address of the ISA/

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P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/14839

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

---Please See Supplemental Page---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-2, 3/1-2, 4/3/1-2, 5/3/1-2, 6/1-2, 7/6/1-2, 8-9, and AGAP000607-PA

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

-***-Continued from Box No. III Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-9 and AGAP000607-PA are directed toward vaccine comprising at least one mosquito salivary protein associated with a Plasmodium sporozoite; and methods of treating a subject infected with Plasmodium sporozoites, inducing an immune response in a subject against a Plasmodium sporozoite, and treating a subject infected with Plasmodium sporozoites.

The vaccine and methods will be searched to the extent that they encompass AGAP000607-PA (first exemplary mosquito salivary protein). Applicant is invited to elect additional mosquito salivary protein(s), and/or variant(s) thereof, to be searched. Additional salivary protein(s) and/or variant(s) thereof will be searched upon the payment of additional fees. It is believed that claims 1, 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part) and 9 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass AGAP000607-PA (mosquito salivary protein). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a mosquito salivary protein encompassing AGAP001374-PA (first exemplary elected mosquito salivary protein).

Group II, Claims 10-15 are directed toward an inhibitor composition useful for treating, reducing, or preventing Plasmodium sporozoite infection, wherein the inhibitor composition inhibits the interaction between a mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Groups I+ include AGAP000607-PA, not present in Group II; the special technical features of Group II include an inhibitor, wherein the inhibitor composition inhibits the interaction between a mosquito salivary protein associated with a Plasmodium sporozoite and the Plasmodium sporozoite, not present in Groups I+.

Groups I+ and II share the technical features including: treating, reducing, or preventing Plasmodium sporozoite infection. Groups I+ share the technical features including: a vaccine comprising at least one mosquito salivary protein associated with a Plasmodium sporozoite; a vaccine comprising at least one polypeptide selected from the group consisting of: a. AGAP000607-PA, or a AGAP000607-PA variant; b. AGAP001374-PA, or a AGAP001374-PA variant; c. AGAP009257-PA, or a AGAP009257-PA variant; d. AGAP009918-PA, or a AGAP009918-PA variant; e. AGAP010750-PA, or a AGAP010750-PA variant; f. AGAP001424-PA, or a AGAP001424-PA variant; g. AGAP001566-PA, or a AGAP001566-PA variant; h. AGAP007347-PA, or a AGAP007347-PA variant; i. AGAP008227-PA, or a AGAP008227-PA variant; j. AGAP008279-PA, or a AGAP008279-PA variant; k. AGAP008283-PA, or a AGAP008283-PA variant; l. AGAP011017-PA, or a AGAP011017-PA variant; m. AGAP011976-PA, or a AGAP011976-PA variant; n. AGAP012081-PA, or a AGAP012081-PA variant; o. AGAP013499-PA, or a AGAP013499-PA variant; p. AGAP002998-PA, or a AGAP002998-PA variant; q. AGAP003937-PA, or a AGAP003937-PA variant; r. AGAP004605-PA, or a AGAP004605-PA variant; s. AGAP011107-PA, or a AGAP011107-PA variant; t. AGAP013013-PA, or a AGAP013013-PA variant; u. AGAP009974-PA, or a AGAP009974-PA variant; v. AGAP000528-PA, or a AGAP000528-PA variant; and w. AGAP011026-PA, or a AGAP011026-PA variant; a method of treating a subject infected with Plasmodium sporozoites, the method comprising administering to the subject at least one antibody, wherein the at least one antibody specifically binds to at least one of the polypeptides; a method of inducing an immune response in a subject against a Plasmodium sporozoite, the method comprising administering to the subject the vaccine; and a method of treating a subject infected with Plasmodium sporozoites, the method comprising administering to the subject the vaccine.

However, these shared technical features are previously disclosed by EP 2 567 706 A2 to Peptcell Limited (hereinafter 'Peptcell') in view of the article 'Anopheles gambiae Circumsporozoite Protein-Binding Protein Facilitates Plasmodium Infection of Mosquito Salivary Glands' by Wang et al. (hereinafter 'Wang').

Peptcell discloses a method of treating, reducing, or preventing Plasmodium sporozoite infection (treating, reducing, or preventing Plasmodium sporozoite infection; Table 1; paragraphs [0119], [0128]); a vaccine comprising at least one mosquito salivary protein (a vaccine comprising at least one mosquito salivary protein; paragraphs [0001], [0031]); a vaccine comprising at least one peptide consisting of: an AGAP000607-PA peptide (a vaccine comprising at least one peptide including a peptide of SEQ ID NO: 19 (a vaccine comprising at least one peptide consisting of: an AGAP000607-PA peptide); paragraphs [0001], [0033]; wherein SEQ ID NO: 19 is 100% identical to residues 91-121 of AGAP000607-PA (see GenBank Accession XP_565733 for AGAP000607-PA sequence)); a method of treating a subject infected with Plasmodium sporozoites (a method of treating a subject infected with Plasmodium sporozoites; Table 1; paragraphs [0119], [0128]); a method of inducing an immune response in a subject against a Plasmodium sporozoite (a method of inducing an immune response in a subject against a Plasmodium sporozoite; paragraphs [0031], [0119], [0128], Table 1), the method comprising administering to the subject the vaccine (the method comprising administering to the subject the vaccine; paragraph [0128]); and a method of treating a subject infected with Plasmodium sporozoites (a method of treating a subject infected with Plasmodium sporozoites; Table 1, paragraphs [0119], [0128]), the method comprising administering to the subject the vaccine (the method comprising administering to the subject the vaccine; paragraph [0128]).

-***-Continued Within the Next Supplemental Box-***-

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/14839

---Continued from Box No. III Observations where unity of invention is lacking---

Peptcell does not disclose at least one polypeptide consisting of: a. AGAP000607-PA; at least one mosquito salivary protein associated with a Plasmodium sporozoite; the method comprising administering to the subject at least one antibody, wherein the at least one antibody specifically binds to at least one of the polypeptides;

Wang discloses wherein an Anopheles salivary protein associated with a Plasmodium sporozoite (wherein an Anopheles salivary protein associated with a Plasmodium sporozoite; abstract); the production of at least one antibody, wherein the antibody binds to the salivary protein associated with the Plasmodium sporozoite (the production of at least one antibody, wherein the antibody binds to the salivary protein associated with the Plasmodium sporozoite; page 1162, column 2, paragraph 4), and reduces the load of Plasmodium in the salivary glands of mosquitoes (reduces the load of Plasmodium in the salivary glands of mosquitoes; page 1166, column 2, paragraph 3 - page 1167, column 1, paragraph 1).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Peptcell to have used the full-length immunogenic polypeptides disclosed by Peptcell, including AGAP000607-PA, in order to ensure the generation of antibodies to all available epitopes of the polypeptide in order to enable the selection of one or more high affinity antibodies that produce an effective immune response. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Peptcell to have included the generation of antibodies to target salivary polypeptides, which interact with Plasmodium sporozoites, as disclosed by Wang, in order to provide both a method of vaccination, and a method of treatment comprising providing the antibodies to a subject at immediate risk of incurring a Plasmodium infection, or for a subject unable to produce an immune response, such as an immunocompromised individual, in order to provide a more rapid and effective means of treatment or prevention of infection for said individuals.

Since none of the special technical features of the Groups I+ and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Peptcell and Wang references, unity of invention is lacking.