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DESCRIPTION

FIELD OF THE INVENTION

BACKGROUND

[0001] Cerebral malaria is a major cause of global morbidity and mortality, typically characterized by loss of blood brain barrier integrity and neurological impairment, followed by death in 15-30% cases despite treatment. Cerebral malaria in humans is caused by *Plasmodium falciparum*. The typical symptoms of cerebral malaria include fever, headache and myalgia followed by drowsiness, confusion, impaired balance or coordination, motor impairment, coma and death. Treatment options are currently limited to quinine or artemisinin derivatives, which control parasitemia but are not as effective in reducing mortality. Accordingly, an unmet need exists in the art for effective therapeutic and preventive approaches without adverse side-effects that prevent or treat cerebral malaria.

BRIEF SUMMARY OF THE INVENTION

[0002] According to the present invention, pharmaceutical compositions strictly according to the appended claims are provided for use in treating, preventing or ameliorating at least one symptom, indication or complication of cerebral malaria (including, *e.g.*, experimental cerebral malaria, falciparum malaria, etc.) in a subject.

[0003] In certain embodiments, the at least one symptom, indication or complication is selected from the group consisting of fever, headache and myalgia followed by drowsiness, confusion, vascular leakage, loss of blood-brain barrier integrity, elevated blood level of an endothelial marker, sequestration of parasitized erythrocytes in the brain, impaired balance or coordination, motor impairment, splenomegaly, loss of reflexes and self-preservation, lack of hygiene-related behavior, acute lung injury, convulsion, coma and death. In certain embodiments, the endothelial marker is selected from the group consisting of angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), angiopoietin receptor Tie2, von Willebrand Factor (vWF), intercellular adhesion molecule-1 (ICAM-1), IP-10, E-selectin and vascular cell adhesion molecule-1 (VCAM-1).

[0004] According to another aspect, methods are provided for improving or increasing survival of a subject following *Plasmodium* infection. The methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an angiopoietin or modified angiopoietin protein or a fragment thereof to the subject in need thereof. In a related aspect, the disclosure provides methods for preventing vascular leakage or protecting blood brain barrier integrity, the method comprising administering a pharmaceutical composition

comprising a therapeutically effective amount of an modified angiopoietin protein or a fragment thereof to a subject in need thereof.

[0005] In another aspect, the disclosure provides for methods for preventing severe cerebral malaria in a subject infected with *Plasmodium* spp., the method comprising selecting a subject with more than 0.1% parasitemia; and administering a pharmaceutical composition comprising a therapeutically effective amount of an modified angiopoietin protein or a fragment thereof to the subject in need thereof.

[0006] In certain embodiments, the administration of the modified angiopoietin to a subject in need thereof prevents at least one indication of neurological impairment selected from the group consisting of impaired balance or coordination, motor impairment, loss of reflexes and self-preservation, long term neurocognitive injury and impairment including memory deficits and affective disorders, lack of hygiene-related behavior, convulsion, and fitting or seizures.

[0007] In certain embodiments, the modified angiopoietin is administered in combination with a second therapeutic agent or therapy. In certain embodiments, the modified angiopoietin is administered as adjunctive therapy along with a second therapeutic agent such as *e.g.*, artesunate.

[0008] Exemplary angiopoietin molecules that can be used in the context of the methods include, *e.g.*, angiopoietin-1, recombinant angiopoietin (*e.g.*, angiopoietin-1 expressed in adenoviral vector), and a modified angiopoietin (*e.g.*, a fusion protein comprising an angiopoietin or a fragment thereof). According to certain embodiments, the modified angiopoietin is a fusion protein consisting of the fibrinogen-like domain of angiopoietin fused to the Fc fragment of human IgG1 and then forced into a tetramer (Davis et al 2003, Nat. Struct. Biol. 10: 38-44). In certain embodiments, the modified angiopoietin comprises a fusion protein comprising a first fibrinogen-like domain of angiopoietin fused at its C-terminal end to the N-terminal end of an Fc fragment and the Fc fragment fused at its C-terminal end to the N-terminal end of a second fibrinogen-like domain of angiopoietin.

[0009] One such type of modified angiopoietin that can be used in the context of the methods of the present invention is AngF1-Fc-F1 (SEQ ID NO: 2).

[0010] In another aspect, the present disclosure includes methods for treating, preventing or ameliorating at least one symptom, indication or complication of cerebral malaria (including, e.g., experimental cerebral malaria, falciparum malaria, etc.) in a subject. The methods according to this aspect of the invention comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-Tie2 antibody or an antigen-binding fragment thereof to a subject in need thereof. In certain embodiments, the anti-Tie2 antibody is an activating or agonist antibody.

BRIEF DESCRIPTION OF THE FIGURES

[0011]

Figure 1 shows the survival curves of C57Bl/6 (Experimental Cerebral Malaria-Susceptible; ECM-S) and BALB/c (Experimental Cerebral Malaria-Resistant; ECM-R) mice infected with *Plasmodium berghei* ANKA (PbA).

Figure 2 shows the percent fold change in Rapid Murine Coma and Behavioral Score (RMCBS) in ECM-S and ECM-R mice infected with PbA.

Figure 3 shows comparative levels of Evans Blue dye extracted from the brains of ECM-R and ECM-S mice infected with PbA.

Figure 4 shows percent parasitemia in ECM-S and ECM-R mice infected with PbA.

Figure 5 shows Ang-1 serum levels over the course of PbA infection. ***$p<0.0001$ (time), *$p<0.05$ (strain) and **$p<0.001$ (2-way ANOVA with Bonferroni post-test for the indicated comparison).

Figure 6(a) RMCBS (%) and serum Ang-1 levels (ng/ml) *$p<0.05$ compared to naive, One-way ANOVA. **Figure 6(b)** depicts scatter plots showing linear regression analysis between serum Ang1 (ng/ml) levels and time to death (hours) (Spearman correlation, r value, $p<0.0001$).

Figure 7 shows relative Ang2 protein levels in ECM-R and ECM-S mice post PbA infection.

Figure 8 shows vWF protein levels in ECM-R and ECM-S mice on Day 6 post infection with PbA.

Figure 9 shows the relative Tie2 mRNA levels in ECM-R and ECM-S mice post PbA infection.

Figure 10 shows the survival curves of C57Bl/6 mice infected with PbA and treated with anti-Ang2 antibody (mAb 'B'), isotype control (mAb 'A') or saline.

Figure 11 shows percent fold change in RMCBS in C57Bl/6 mice infected with PbA and treated with anti-Ang2 antibody (mAb 'B'), isotype control (mAb 'A') or saline.

Figure 12 shows percent parasitemia on Day 7 post PbA infection in C57Bl/6 mice treated with anti-Ang2 antibody (mAb 'B'), isotype control (mAb 'A') or saline.

Figure 13 shows the plasma protein levels of **(a)** vWF and **(b)** E-selectin, ICAM and VCAM in naive C57Bl/6 mice (hatched bars) or mice infected with PbA and treated with anti-Ang2 antibody (mAb 'B') (grey bars) or isotype control (mAb 'A') (black bars).

Figure 14 shows survival curves of C57Bl/6 mice infected with PbA and treated with AngF1-Fc-F1, Fc control, anti-Ang2 antibody, a dual Anti-Ang1/Ang2 antibody ("comparator") or saline.

Figure 15 shows percent parasitemia in C57Bl/6 mice infected with PbA and treated with AngF1-Fc-F1, Fc control, anti-Ang2 antibody, a dual Anti-Ang1/Ang2 antibody ("comparator")

or saline.

Figure 16(a) shows levels of Evans Blue dye extracted from the brains of C57Bl/6 mice infected with PbA and treated with Fc control (A), anti-Ang2 antibody (B), AngF1-Fc-F1 (C), or anti-Ang1/Ang2 comparator antibody (D). **Figure 16(b)** shows the percent parasitemia in C57Bl/6 mice infected with PbA and treated with Fc control (A), anti-Ang2 antibody (B), AngF1-Fc-F1 (C), or anti-Ang1/Ang2 "comparator" antibody (D).

Figure 17(a) shows the percent change in RMCBS on Day 6 post PbA infection in C57Bl/6 mice treated with AngF1-Fc-F1 or isotype control. **Figure 17(b)** shows the percent change over 7 days in RMCBS in C57Bl/6 mice infected with PbA and treated with AngF1-Fc-F1 or isotype control.

Figure 18(a) shows percent parasitemia in C57Bl/6 mice infected with PbA and treated with AngF1-Fc-F1 or isotype control. **Figure 18(b)** shows the percent fold change in weight of C57Bl/6 mice infected with PbA and treated with AngF1-Fc-F1 or isotype control.

Figure 19 shows plasma protein levels of cytokines **T**NFα and IFNγ **(a),** vWF (b), E-selectin, sICAM and VCAM-1 **(c)** of naive C57Bl/6 mice and mice infected with PbA and treated with AngF1-Fc-F1 or Fc control.

Figure 20 shows survival curves of C57Bl/6 mice infected with PbA and treated with artesunate + AngF1-Fc-F1, artesunate + saline, or saline.

Figure 21(a) shows percent parasitemia in C57Bl/6 mice infected with PbA and treated with artesunate + AngF1-Fc-F1 or artesunate + saline. **Figure 21(b)** shows the percent fold change in weight of C57Bl/6 mice infected with PbA and treated with artesunate + AngF1-Fc-F1 or artesunate + saline.

DETAILED DESCRIPTION

[0012] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. 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, since the scope of the present invention will be limited only by the appended claims.

[0013] 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. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0014] Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are now described.

Methods for Treating, Preventing or Ameliorating Cerebral Malaria

[0015] Cerebral malaria pathogenesis is associated with endothelial activation and loss of blood brain barrier integrity. The angiopoietin- Tie2 signaling pathway is a key regulator of endothelial function. Alterations in the angiogenic balance, specifically increased angiopoietin-2 (Ang2) relative to Ang1, has been associated with poor clinical outcome in cerebral malaria (Yeo et al, 2008 PNAS; Lovegrove et al PLoS ONE 2009; Erdman et al PLoS ONE 2011; Conroy et al PLoS ONE 2011). However, it is unclear whether the Ang-Tie2 pathway is causally involved in cerebral malaria pathogenesis. The inventors have hypothesized that dysregulation in angiopoietins contributes to cerebral malaria pathogenesis, and therefore, interventions to maintain Tie2 activation may promote endothelial stability, prevent deleterious effects to the blood brain barrier and improve outcome following *Plasmodium* infection. Accordingly, it is shown herein, that modified angiopoietins, when administered to an infected subject, protect the blood brain barrier integrity and prevent neurological impairment and death. As disclosed elsewhere herein, the inventors have used a well-known murine model of *Plasmodium berghei* ANKA (PbA) - induced experimental cerebral malaria (ECM) to study alterations in angiopoietins associated with disease severity and fatality and to show that dysregulation of the Ang/Tie2 axis is associated with disease severity and fatality. As shown herein, Ang1 levels inversely correlated with morbidity and mortality in the mouse model of cerebral malaria. Based on the studies shown herein, it is established that Ang1 is necessary to maintain blood brain barrier integrity in response to a lethal malaria challenge and can improve survival above that achieved by conventional treatment (*e.g.*, artesunate) alone. Further, administration of a modified angiopoietin enhanced blood brain barrier integrity and promoted maintenance of a quiescent endothelium via down-regulation of pro-adhesive molecules implicated in parasite sequestration and cerebral malaria pathogenesis.

[0016] Accordingly, the present disclosure includes methods for treating, preventing, or ameliorating at least one symptom, indication or complication of cerebral malaria (including, for example, experimental cerebral malaria, falciparum malaria, etc.) in a subject. The methods according to this aspect comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a modified angiopoietin protein or a fragment thereof to the subject in need thereof.

[0017] "Cerebral malaria" (CM), as used herein, means an infectious disease caused by *Plasmodium* species and characterized by loss of blood brain barrier integrity and neurological impairment. The symptoms of CM include, but are not limited to, fever, headache and myalgia followed by drowsiness, confusion, vascular leakage, loss of blood-brain barrier integrity, elevated blood level of an endothelial marker, sequestration of parasitized erythrocytes in the

brain, impaired balance or coordination, motor impairment, splenomegaly, loss of reflexes and self-preservation, lack of hygiene-related behavior, acute lung injury, convulsion, fitting, coma and death. The clinicopathology of CM is characterized by sequestration of infected red blood cells in the venules and capillaries of the brain followed by endothelial activation. The term "cerebral malaria" includes but is not limited to severe cerebral malaria caused in humans by *Plasmodium falciparum*, and experimental cerebral malaria (ECM), caused in mice by *Plasmodium berghei* ANKA.

[0018] As used herein, the terms "treat", "treating", or the like, mean to alleviate a symptom or a complication, eliminate the causation of a symptom or a complication either on a temporary or permanent basis, or to prevent or slow the appearance of a symptom or complication of cerebral malaria in the subject. In the context of the present invention, the terms "treat", "treating", or the like, refer to reducing or decreasing mortality in a subject infected with *Plasmodium* species. The terms also refer to preventing the loss of blood brain barrier integrity and neurological impairment in a subject with cerebral malaria. In certain embodiments, the present methods are useful for treating or ameliorating at least one symptom, indication or complication of cerebral malaria including, but not limited to, fever, headache and myalgia followed by drowsiness, confusion, vascular leakage, loss of blood-brain barrier integrity, elevated blood level of an endothelial marker, sequestration of parasitized erythrocytes in the brain, impaired balance or coordination, motor impairment, splenomegaly, loss of reflexes and self-preservation, lack of hygiene-related behavior, acute lung injury, convulsion, fitting, coma and death.

[0019] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[0020] As used herein, the term "subject" refers to an animal, preferably a mammal, that exhibits one or more symptoms, indications or complications of cerebral malaria, and/or who has been diagnosed with cerebral malaria (CM) and/or in need of amelioration, prevention and/or treatment of cerebral malaria. The term "a subject in need thereof" may also include, *e.g.*, subjects who, prior to treatment, exhibit (or have exhibited) one or more symptoms or indications of cerebral malaria such as, *e.g.*, fever, headache and myalgia followed by drowsiness, confusion, vascular leakage, loss of blood-brain barrier integrity, elevated blood level of an endothelial marker, sequestration of parasitized erythrocytes in the brain, impaired balance or coordination, motor impairment, splenomegaly, loss of reflexes and self-preservation, lack of hygiene-related behavior, acute lung injury, convulsion, fitting, coma and death.

[0021] In the context of the present invention, "a subject in need thereof" may include a subset of population, which may show an elevated level of an endothelial marker. Such a subject population may show an elevated level of an endothelial marker such as, *e.g.*, Ang1, Ang2, Tie2, vWF, ICAM-1, E-selectin and VCAM-1.

[0022] The methods of the present invention may be used to treat cerebral malaria in adults, including the elderly. In some embodiments, the methods of the present invention are used to treat adults more than 50 years, more than 55 years, more than 60 years, more than 65 years, or more than 70 years old.

[0023] In some embodiments, the methods herein may be used to treat cerebral malaria in children who are ≤ 3 years old. For example, the present methods may be used to treat infants who are less than 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months or less than 12 months old. In other embodiments, the methods of the present invention may be used to treat children who are more than 3 years old, more than 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, or more than 15 years old.

[0024] The present disclosure also includes methods for increasing survival in a subject with cerebral malaria. The methods according to this aspect comprise administering to the subject one or more doses of a pharmaceutical composition comprising a modified angiopoietin to increase survival in the subject.

[0025] The present disclosure also includes methods to prevent severe cerebral malaria in a subject infected with *Plasmodium* species, the methods comprising administering a therapeutically effective amount of a pharmaceutical composition comprising a modified angiopoietin to the subject in need thereof. In certain embodiments, the modified angiopoietin is administered as an exogenous protein.

[0026] The term "preventing" as used herein refers to preventing development of disease. The term, as used herein, also includes preventing vascular leakage, protecting blood brain barrier integrity and the onset of neurological symptoms such as seizures and paralysis upon infection with the pathogen. In some embodiments, the term refers to preventing endothelial dysfunction, which is a key pathological feature of cerebral malaria (including experimental cerebral malaria).

[0027] The present disclosure includes methods for treating, preventing or reducing the severity of cerebral malaria comprising administering a therapeutically effective amount of a pharmaceutical composition comprising a modified angiopoietin to a subject in need thereof, wherein the pharmaceutical composition is administered to the subject in multiple doses, *e.g.*, as part of a specific therapeutic dosing regimen. For example, the therapeutic dosing regimen may comprise administering multiple doses of the pharmaceutical composition to the subject at a frequency of about once a day, once every two days, once every three days, once every four days, once every five days, once every six days, once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, or less frequently. In some embodiments, the therapeutic dosing regimen comprises administering multiple doses of the pharmaceutical composition to the subject at a frequency of about once a day, about 2 times a day, about 3

times a day or more than 4 times a day.

[0028] In certain embodiments, the modified angiopoietin is administered subcutaneously, intravenously, intracranially, intraventricularly, or delivered systemically in an adenoviral vector to a subject in need thereof.

[0029] The methods according to certain embodiments, comprise administering to a subject a therapeutically effective amount of a pharmaceutical composition comprising a modified angiopoietin in combination with a second therapeutic agent. The second therapeutic agent may be an agent selected from the group consisting of an artemisinin, quinine, or a variant or derivative thereof (*e.g.*, artesunate), a vascular endothelial growth factor (VEGF) antagonist [*e.g.*, a "VEGF-Trap" such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab)], an activating anti-Tie2 antibody, an Ang2 antagonist, an antihistamine, and a non-steroidal anti-inflammatory drug (NSAID). As used herein, the phrase 'in combination with" means that the pharmaceutical composition comprising a modified angiopoietin is administered to the subject at the same time as, just before, or just after administration of the second therapeutic agent. In certain embodiments, the second therapeutic agent is administered as a co-formulation with the modified angiopoietin.

[0030] The present disclosure also concerns methods for treating, preventing or ameliorating at least one symptom, indication or complication of cerebral malaria in a subject, wherein the methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-Tie2 antibody or an antigen-binding fragment thereof to the subject in need thereof. In certain embodiments, the anti-Tie2 antibody is an activating or agonist antibody, *e.g.*, the antibody upon binding to Tie2 increases the activity of Tie2 or otherwise stimulates Tie2 signaling. In certain embodiments, the anti-Tie2 antibody is an antibody as set forth in US20130209492. The anti-Tie2 antibody may be administered subcutaneously, intravenously, or intracranially at a dosage of from about 0.1 mg/kg to about 100 mg/kg of the subject's body weight. In certain embodiments, the activating anti-Tie2 antibody is administered in combination with a second activating anti-Tie2 antibody to the subject in need thereof.

[0031] In another aspect, the present disclosure includes methods for treating, preventing or ameliorating at least one symptom, indication or complication of a disease or disorder associated with dysfunction of the Ang-Tie2 pathway in a subject. The methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a modified angiopoietin protein or a fragment thereof to the subject in need thereof. In certain embodiments, the angiopoietin or variants thereof may be used to treat, prevent or ameliorate at least one symptom or indication of a disease or disorder including cerebral malaria, sepsis, anthrax, dengue, hemorrhagic fever (including viral hemorrhagic fever, *e.g.*, lassa fever, Yellow fever, and Ebola fever), toxic shock syndrome, HUS, hemorrhagic shock (model for massive blood loss due to traumatic injury, *e.g.*, IED), ischemic reperfusion, hemolytic uremic syndrome, myocardial infarction and stroke.

Modified Angiopoietins

[0032] The methods of the present disclosure comprise administering to a subject in need thereof a therapeutic composition comprising an angiopoietin or a variant thereof. As used herein, an "angiopoietin" includes angiopoietin-1 (Ang1) or angiopoietin-2 (Ang2).

[0033] Non-limiting examples of categories of modified angiopoietins include recombinant angiopoietins (*e.g.*, angiopoietin expressed in an adenoviral vector; Thurston et al 2000, Nat. Med.), mutant and chimeric forms of angiopoietins, and fusion proteins comprising angiopoietin or a fragment thereof that specifically bind Tie1 and/or Tie2 receptors.

[0034] According to certain exemplary embodiments of the present disclosure, the modified angiopoietin is a fusion protein comprising one or more domains of the angiopoietin molecule fused to a multimerizing domain. In general terms, the multimerizing domain(s) of the present invention function to connect the various components of the angiopoietin molecule (*e.g.*, the fibrinogen-like domains) with one another. As used herein, a "multimerizing domain" is any macromolecule that has the ability to associate (covalently or non-covalently) with a second macromolecule of the same or similar structure or constitution. For example, a multimerizing domain may be a polypeptide comprising an immunoglobulin C_H3 domain. A non-limiting example of a multimerizing domain is an Fc portion of an immunoglobulin, *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group. In certain embodiments, the multimerizing domain is an Fc fragment or an amino acid sequence of 1 to about 200 amino acids in length containing at least one cysteine residues. In other embodiments, the multimerizing domain is a cysteine residue or a short cysteine-containing peptide. Other multimerizing domains include peptides or polypeptides comprising or consisting of a leucine zipper, a helix-loop motif, or a coiled-coil motif.

[0035] In certain embodiments, the modified angiopoietin is a fusion protein comprising one or more fibrinogen-like domains of the angiopoietin-1 molecule fused to the Fc fragment of an immunoglobulin comprising any of the amino acid sequences, as set forth in US Patent No. 7,008,781. In certain exemplary embodiments, the fusion protein that can be used in the context of the methods of the present invention comprises a first fibrinogen-like domain of angiopoietin fused at its C-terminal end to the N-terminal end of an IgG Fc fragment and the C-terminal of the Fc fragment fused to the N-terminal end of a second fibrinogen-like domain of angiopoietin (Davis et al, Nat. Struct. Biol. 2003), wherein the angiopoietin is Ang1. According to certain exemplary embodiments, the methods of the present invention comprise the use of the modified angiopoietin referred to and known in the art as AngF1-Fc-F1. In certain embodiments, the modified angiopoietin is a dimer comprising two AngF1-Fc-F1s that associate through intramolecular association of the Fc fragments (also referred to as BowAng1, as disclosed in Davis et al, Nat. Struct. Biol. 2003). According to certain embodiments, the fusion protein comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the fusion protein comprises the amino acid sequence of SEQ ID NO: 4.

[0036] Other modified angiopoietins that can be used in the context of the methods of the present invention include any of the modified angiopoietin molecules as set forth in US Patent Nos. 6265564, 6441137, and 6825008.

[0037] In certain embodiments, the angiopoietin or variants thereof may be used to treat, prevent or ameliorate at least one symptom or indication of a disease or disorder including sepsis, dengue, hemorrhagic fever (including viral hemorrhagic fever, *e.g.*, lassa fever, Yellow fever, and Ebola fever), toxic shock syndrome, HUS, hemorrhagic shock (model for massive blood loss due to traumatic injury, *e.g.*, IED), ischemic repurfusion, hemolytic uremic syndrome, myocardial infarction and stroke.

Pharmaceutical Compositions

[0038] The present disclosure includes methods which comprise administering a modified angiopoietin to a subject wherein the modified angiopoietin is contained within a pharmaceutical composition. The pharmaceutical compositions of the invention may be formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0039] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al., 1987, J. Biol. Chem. 262: 4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0040] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the

cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0041] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0042] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0043] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

Dosage

[0044] The amount of the modified angiopoietin (*e.g.*, AngF1-Fc-F1) administered to a subject according to the methods of the present invention is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means an amount of modified angiopoietin that results in one or more of: (a) a reduction in the severity or duration of a symptom, indication or complication of severe cerebral malaria; (b) increased survival; (c) protection of the blood brain barrier integrity; and (d) prevention of neurological impairment in

the subject.

[0045] In the case of a modified angiopoietin, a therapeutically effective amount can be from about 0.05 mg to about 600 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, or about 600 mg, of the modified angiopoietin.

[0046] The amount of the modified angiopoietin contained within the individual doses may be expressed in terms of milligrams of protein per kilogram of the subject's body weight (*i.e.*, mg/kg). For example, the modified angiopoietin may be administered to a subject at a dose of about 0.0001 to about 100 mg/kg of patient body weight. In certain embodiments, the modified angiopoietin is administered to a subject in need thereof at a dose of about 5 - 25 mg/kg of the subject's body weight.

EXAMPLES

[0047] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Alterations in Endothelial Regulators in the Murine *Plasmodium berghei* ANKA (Pba)-Induced Model of Experimental Cerebral Malaria (ECM)

[0048] In this Example, the alterations in endothelial regulators in *Plasmodium berghei* ANKA (PbA)-induced experimental cerebral malaria in a mouse model were studied. This model is based on the observation that C57Bl/6 mice are susceptible to the murine parasite *P. berghei* ANKA which produces a severe, ultimately fatal disease with neurological symptoms paralleling

the symptoms and disease development of cerebral malaria in humans infected with *Plasmodium falciparum*. In contrast to C57Bl/6 mice, BALB/c mice are resistant to PbA, in that they do not develop encephalopathy, although they become infected and achieve similar or sometimes higher levels of parasite density. This study showed that dysregulation of the Ang/Tie2 axis was associated with disease severity and fatality. Further, Ang1 levels inversely correlated with morbidity and mortality in the mouse model of cerebral malaria.

Materials and Methods

PbA-induced ECM model: Infection

[0049] Cryopreserved *Plasmodium berghei* ANKA (PbA; MR4, Manassas, VA) was passaged through naive C57Bl/6 mice. For the experiment, infection was initiated in C57Bl/6 and BALB/c mice by intraperitoneal (ip) injection of 1 $x10^6$ freshly-isolated parasitized erythrocytes (PE) obtained from donor passage mice. Parasitemia was monitored by thin-blood smear stained with modified Geimsa stain (Protocol Hema3 Stain Set, Sigma, Oakville, ON) by counting at least 1500 erythrocytes. The infected mice were monitored for survival, neurological impairment, vascular leakage, parasite burden, and levels of angiogenic factors and markers of endothelial activation.

Survival and Assessment of Health Status

[0050] Post-infection survival of mice was plotted as Kaplan Meier curves using a log rank test for comparison. Quantitative assessment of ECM-associated neurological impairment (impaired coordination and motor performance) was performed daily using the 10 parameter Rapid Murine Coma and Behavioral Score (RMCBS), as previously described by Carroll RW, et al. 2010 in PLoS ONE 5: e13124. Signs of ECM include impaired balance/coordination, motor impairment (ataxia, hemiplegia/paraplegia), loss of reflexes and self-preservation, lack of hygiene-related behavior (grooming) and/or fitting. For each parameter, a score was assigned from 0 to 2, with 0 indicating the lowest function and a score of 2 the highest. Total scores for each mouse were calculated and provided as a percentage of the total possible score. Mice with a score of 35% or less were deemed to have severe ECM.

Evans Blue Permeability Assay

[0051] Vascular permeability in the brain was assessed using Evans blue (EB) dye. Mice were intravenously injected via tail vein with 0.1 mL of 1% Evans blue dye solution (Evans Blue powder; Sigma-Aldrich in PBS, filter sterilized) when clinical signs of ECM were observed. Mice

were euthanized with isoflurane (99.9% inhalational anesthetic) after 1 hour, perfused with 50 mL of PBS (1X). Brains were dissected aseptically, weighed, photographed and placed in 1 mL N, N-dimethylformamide for 48 hours at room temperature to extract dye from tissue. Absorbance was measured at 620 nm. The concentration was calculated using a standard curve of EB dye and expressed as ng dye per gram of brain tissue.

Detection of Cytokine Markers of Endothelial Activation

[0052] Peripheral whole blood was collected by saphenous venipuncture on day 0 (d 0; prior to PbA infection) and d 6 p.i. into heparinized tubes (Starstedt). Plasma was isolated from whole blood samples by centrifugation at 1000 x *g* for 15 min. Plasma samples were aliquoted and stored at -80°C until analyzed. Levels of mouse intercellular adhesion molecule-1 (sICAM-1/CD54), E-Selectin (sE-Sel/CD62E) and Vascular Cell Adhesion Molecule-1 (sVCAM-1/CD106) in their soluble forms were determined in plasma using commercially available murine ELISA kits (R&D Systems, Minneapolis, USA), according to the manufacturers protocol. Levels of von Willebrand Factor antigen (vWF:Ag) was measured in serum by ELISA as follows: 96-well MaxiSorp plates (Nunc) were coated overnight at 4°C with a polyclonal antibody anti-human vWF (1:600, Dako, Glostrup, Denmark) in 0.01M PBS. Mouse serum was plated in 1% BSA-PBS and bound vWF:Ag was detected with horseradish peroxidase (HRP)-conjugated polyclonal antibody anti-human vWF (1:8000, Dako). Plates were developed with a 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution and the absorbance was read at 450nm, after the colorimetric reaction was stopped with H_2SO_4. vWF:Ag concentration was interpolated from a standard curve created with human vWF of a known concentration from fresh frozen plasma (1:500, American Diagnostica, Stamford, CT) that was included on each plate. IFN-gamma and TNF-alpha were determined in plasma using commercially available murine ELISA kits (eBioscience), according to the manufacturer's protocol.

Statistical Analysis

[0053] Statistical analysis was performed using GraphPad Prism version 4.00 (San Diego, CA). Post-infection survival of mice was plotted as Kaplan Meier curves and assessed using log-rank test. Survival studies were conducted in triplicate and data pooled unless otherwise specified. Shapiro-Wilk test was used to determine normally distributed data and comparisons between groups were assessed using the non-parametric Mann-Whitney test or Kruskal-Wallis test followed by Dunn's post-hoc. Two-way ANOVA was used to compare between groups over multiple time-points. For plasma biomarker testing, the Friedman test with Dunn's multiple comparison was used to compare levels between samples collected from patients at different time points (e.g., admission and convalescence). All data are presented as median and IQR (non-parametric), unless otherwise stated. Normally distributed data is presented as mean and SEM. A $p<0.05$ was considered statistically significant. Data from multiple experiments were normalized to the geometric mean of the infected but untreated group of each experiment for

comparisons.

Results

[0054] As shown in Figure 1, the resistant BALB/c mice (also referred to as "ECM-R") showed significantly prolonged survival (p=0.0007, log rank test) following infection with PbA as compared to the susceptible C57Bl/6 mice (also referred to as "ECM-S"). The resistant BALB/c mice showed 100% survival for 7 days post infection with survival dropping to more than 10% by Day 10 and up to Day 14 (when the mice were sacrificed). In contrast, the susceptible C57Bl/6 mice showed only about 50% survival as early as Day 6 with 0% by Day 8 post infection.

[0055] The ECM-S mice showed neurological impairment as evidenced by scores less than 35% around Day 6 as compared to the resistant ECM-R mice which showed an absence of neurological impairment (Figure 2). Evans Blue (EB) extravasation was used to assess vascular permeability and blood-brain-barrier dysfunction. Following perfusion to remove circulating dye, extravasated EB level in brain parenchyma of PbA-infected ECM-S mice was 2-fold higher than level in ECM-R mice with comparable parasite burdens [mean (SD) EB dye/g tissue: 10.7 (7.9) for ECM-R mice vs. 22.9 (7.2) for ECM-S mice; p=0.005], consistent with increased vascular leakage and loss of blood-brain-barrier integrity (Figure 3). This was independent of observed parasitemia as evidenced by a greater parasite burden in the resistant mice as compared to the susceptible mice (Figure 4).

[0056] The ECM-R mice differed from the ECM-S mice in the levels of angiogenic factors Ang1, Ang2 and vWF upon PbA infection. Longitudinal evaluation over the course of infection showed that Ang1 levels decreased with time ($p<0.0001$, 2-way ANOVA; Figure 5). The kinetics of Ang1 decline following infection was significantly different in ECM-R vs. ECM-S mice (p=0.02, 2-way ANOVA). During the acute phase of disease, ECM-R mice maintained significantly higher Ang1 levels as compared to ECM-S mice ($p<0.001$). For both strains, the loss of circulating Ang1 was associated with the onset of neurological impairment and ECM as determined by a significant decline in the RMCBS. Ang1 serum levels (ng/ml) correlated significantly with RMCBS (%) scores ($p<0.05$; Figure 6a) and with time to death (hours) ($p<0.0001$; Figure 6b). Overall, when assessed on day 6 post-infection, mice with lowest levels of circulating Ang1 were significantly more likely to proceed to a fatal outcome, supporting the hypothesis that Ang1 may be a critical determinant of survival.

[0057] The ECM-R mice showed significantly lower Ang2 and vWF levels (Figures 7 - 9).

Example 2: Blocking Ang2 Does Not Confer Improved Protection against the Development of Experimental CM

[0058] In this Example, the effect of Ang2 blockade on PbA-induced ECM in a mouse model was assessed. Twenty C57Bl/6 mice were infected by *Plasmodium berghei* ANKA (PbA) via an intraperitoneal injection of 1 $x10^6$ freshly-isolated parasitized erythrocytes (PE) obtained from donor passage mice on Day 0. The infected mice were treated with 15 mg/kg of an anti-Ang2 antibody, H1H685, as described in US Patent Application Publication No. US20110027286, on Days -1, 1, 4, and 7 post-infection via subcutaneous injection. The mice were monitored for survival, neurological impairment, parasite burden and plasma protein levels, as described in detail in Example 1.

[0059] Figure 10 shows a Kaplan Meier curve plotting the survival of mice infected with PbA and treated with anti-Ang2 antibody (referred to as "mAb 'B'" in Figure 10), or an isotype control (mAb 'A') or saline. Blocking Ang2 with the anti-Ang2 antibody did not lead to increased survival of infected mice as compared to the isotype control. Treatment with anti-Ang2 antibody also did not prevent neurological impairment in the infected mice (Figure 11), though there was no significant difference in the parasite burden between infected mice treated with anti-Ang2 antibody, the isotype control or saline (Figure 12). The plasma protein levels of vWF, ICAM, VCAM and E-selectin did not differ significantly in the mice treated with the anti-Ang2 antibody, or isotype control (Figure 13).

Example 3: Therapeutic Administration of Ang1 Significantly Improves Survival of Mice Infected with PbA

[0060] In this Example, the effect of Ang1 administration on PbA-induced ECM in a mouse model was studied. Eighty C57Bl/6 mice were infected by *Plasmodium berghei* ANKA (PbA) via an intraperitoneal injection of 1 $x10^6$ freshly-isolated parasitized erythrocytes (PE) obtained from donor passage mice on Day 0. The infected mice were divided into four groups and were treated with either 15 mg/kg of an anti-Ang2 antibody (see Example 2), a "comparator" dual blocking antibody of Ang1/Ang2 or an isotype control, or with 25 mg/kg of AngF1-Fc-F1 (SEQ ID NO: 2) via subcutaneous injection on Day 4 and Day 6 post infection. The "comparator" dual Ang1/Ang2 blocking antibody was the peptibody 2xCon4C (AMG386) as set forth in US Patent No. 7205275 (Amgen). The mice were monitored for survival, neurological impairment, parasite burden, and plasma protein levels, as described in detail in Example 1.

[0061] As shown in Figure 14, therapeutic administration of AngF1-Fc-F1 to mice infected with PbA significantly improved survival ($p<0.05$, log rank test) compared to mice treated with the isotype control, anti-Ang2 antibody or the dual blocking antibody. The mice treated with AngF1-Fc-F1 showed more than 35% survival up to at least Day 15 p.i. as compared to the mice treated with the other three treatments which showed 0% survival by Day 8 post infection, though the percent parasitemia across all treatments was similar (Figure 15). The treatment with AngF1-Fc-F1 also protected blood brain barrier integrity as shown by Evans Blue dye uptake (Figure 16). The mice treated with AngF1-Fc-F1 showed significantly low amount of dye uptake (panel C of Figure 16a), consistent with maintenance of an intact blood brain

barrier and reduced vascular permeability ($p<0.05$, Kruskal-Wallis), even though the percent parasitemia was similar (Figure 16b). This confirmed that exogenous Ang1 is sufficient to maintain blood-brain-barrier integrity following a lethal malaria challenge.

[0062] The therapeutic administration of AngF1-Fc-F1 also prevented ECM-associated morbidity and neurological impairment (Figure 17), independent of changes to peripheral parasitemia (Figure 18). By day 6 post-infection, the majority of control mice had progressed to ECM, whereas disease progression was mitigated in treated mice. Infected AngF1-Fc-F1-treated mice displayed similar weight loss to control-treated mice.

[0063] To determine whether vascular protection with AngF1-Fc-F1 occurred secondary to a reduction in inflammatory cytokines or whether protection was due to a direct enhancement of vascular stability in the face of a systemic inflammatory response, plasma samples from treated and untreated PbA-infected mice were assayed for key pro-inflammatory cytokines. PbA-infected mice showed a significant increase in TNF and IFNγ on day 6 post-infection (3-fold increase in TNF and a 60-fold increase in IFNγ compared to baseline, $p<0.01$ for both, one-way ANOVA) despite treatment. Treatment with AngF1-Fc-F1 did not affect levels of TNF or IFNγ, with both control and treated mice showing a similar up-regulation in response to infection (Figure 19a).

[0064] Pro-inflammatory cytokine stimulation and/or direct endothelial interactions with parasitized erythrocytes may contribute to a number of pathological events implicated in CM, including up-regulation of endothelial cell receptors that mediate parasite cytoadhesion. Disruption of parasite sequestration to host receptors via down-regulation of cell adhesion molecules (CAMs), such as ICAM-1 and/or vascular cellular adhesion molecule-1 (VCAM-1), may lessen microvascular obstruction and endothelial dysfunction. Therefore, the effect of Ang1 treatment on reduction of circulating levels of soluble forms of CAMS, considered as an indicator of endothelial activation and a pro-adhesive vascular phenotype, was investigated. Analysis of plasma samples collected on day 6 post-infection showed a 4-fold increase in circulating levels of sICAM-1 with PbA infection, compared to baseline ($p<0.001$, One-way ANOVA with Bonferroni test for multiple comparisons; Figure 19c). These levels were significantly reduced with Ang1 treatment compared to PbA-infected controls ($p<0.05$; Figure 19c). Similarly, sVCAM-1 levels significantly increased at day 6 post-infection with PbA (5-fold increase, $p<0.001$; Figure 19c) and Ang1 treatment significantly reduced circulating levels of sVCAM-1 compared to PbA-infected controls ($p<0.05$, Figure 19c). These data indicate that Ang-1-Tie-2 interactions help to maintain vascular quiescence in the face of systemic inflammatory response, in part through preservation of an anti-adhesive vascular phenotype via down regulation of adhesion molecules.

[0065] This study showed that therapeutic administration of Ang1 (AngF1-Fc-F1), but not inhibition of Ang2 improved blood brain barrier integrity and survival. It supported investigation of pro-Ang1, but not inhibition of Ang2 as adjunctive therapy for cerebral malaria. Further, it is of interest that Ang1-based treatment was efficacious and preserved blood brain barrier integrity, despite a robust systemic pro-inflammatory response to infection. The data suggest

that anti-inflammatory strategies may not be required to preserve vascular integrity and improve outcome in life-threatening infections associated with systemic inflammation.

[0066] In one further experiment, AngF1-Fc-F1 will be administered in combination with an anti-Tie2 antibody (as set forth in US20130209492) to infected susceptible C57Bl/6 mice to study the involvement of Tie2 in blocking the protective effect of AngF1-Fc-F1. The anti-Tie2 antibody may an activating (or agonist) antibody, *e.g.*, it increases the binding of an angiopoietin and/or increases the activity of Tie2. It is expected that AngF1-Fc-F1 administered in combination with anti-Tie2 antibody will lead to lower survival of infected mice as compared to AngF1-Fc-F1 alone.

[0067] In another further experiment, AngF1-Fc-F1 will be used to treat resistant BALB/c mice infected with PbA to study the effect on survival, blood brain barrier integrity, neurological impairment, and plasma protein markers. It is expected that administration of AngF1-Fc-F1 will lead to improved protection of blood brain barrier integrity and survival of infected BALB/c mice.

[0068] In a third experiment, AngF1-Fc-F1 will be administered in combination with an anti-Tie2 antibody to infected resistant BALB/c mice to study if the resistant mice are rendered susceptible to ECM. It is expected that AngF1-Fc-F1 administered in combination with anti-Tie2 antibody will lead to lower survival of infected BALB/c mice as compared to AngF1-Fc-F1 alone.

Example 4: Therapeutic Administration of AngF1-Fc-F1 as Adjunctive Therapy in Combination with Artesunate

[0069] In this Example, the effect of administration of Ang-F1-Fc-F1 in combination with artesunate on PbA-induced ECM in a mouse model was studied. Twenty C57Bl/6 mice were infected by *Plasmodium berghei* ANKA (PbA) via an intraperitoneal injection of 1 $x10^6$ freshly-isolated parasitized erythrocytes (PE) obtained from donor passage mice on Day 0. The infected mice were treated with 15 mg/kg of AngF1-Fc-F1 (SEQ ID NO: 2) on Days 4 and 6 post infection and with 10 mg/kg of artesunate on Day 5 post-infection via subcutaneous injection. The mice were monitored for survival, neurological impairment, parasite burden, and plasma protein levels, as described in detail in Example 1.

[0070] Adjunctive AngF1-Fc-F1 treatment significantly improved survival of infected mice as compared to artesunate alone (Figure 20). Infected mice treated with AngF1-Fc-F1 in combination with artesunate showed 100% survival at least up to Day 15 post infection. In contrast, infected mice treated with artesunate alone showed less than 60% survival by Day 8 post infection. Despite significantly diminished parasite burden to pre-ECM levels (i.e., <2% parasitemia), 41.7% of artesunate treated mice died of ECM. This effect was independent of percent parasitemia (Figure 21).

[0071] In a further experiment, the effect of adjunctive AngF1-Fc-F1 in combination with artesunate will be studied on neurological impairment in infected mice. It is expected that administration of AngF1-Fc-F1 in combination with artesunate will prevent neurological impairment in infected mice as compared to artesunate alone.

[0072] In another further experiment, AngF1-Fc-F1 will be administered in combination with an anti-Ang2 antibody (as described in Example 2) to study survival of infected mice. It is expected that AngF1-Fc-F1 administered in combination with anti-Ang2 antibody will lead to improved survival of infected mice as compared to AngF1-Fc-F1 alone.

Example 5: Protection from malaria-associated acute lung injury

[0073] In this Example, the effect of AngF1-Fc-F1 administration on vascular permeability in lung and malaria-associated acute lung injury in infected mice will be studied. Twenty C57Bl/6 mice will be infected with *Plasmodium berghei* ANKA (PbA) via an intraperitoneal injection of 1 $x10^6$ freshly-isolated parasitized erythrocytes (PE) obtained from donor passage mice on Day 0. The infected mice will be treated with 15 mg/kg of AngF1-Fc-F1 (SEQ ID NO: 2) on Days 4, and 6 post-infection via subcutaneous injection. The mice will be monitored for survival and vascular permeability in lung by Evans Blue staining.

[0074] It is expected that mice treated with AngF1-Fc-F1 will show absence of Evans Blue dye uptake in lungs pointing to absence of vascular permeability and protection from malaria-associated acute lung injury.

[0075] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

SEQUENCE LISTING

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Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                405                 410                 415
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Phe Arg Asp Cys Ala Asp
        435                 440                 445
Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile
    450                 455                 460
Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn
465                 470                 475                 480
Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp
                485                 490                 495
Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser
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                500                 505                 510
Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln
        515                 520                 525
Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg
    530                 535                 540
Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn
545                 550                 555                 560
Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser
                565                 570                 575
Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn
            580                 585                 590
Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp
        595                 600                 605
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala
    610                 615                 620
Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys
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caccttaaag gacttacagg gacagccggc aaaataagca gcatcagcca accaggaaat 420
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cagaggcaga acacaaataa gttcaacggc attaaatggt actactggaa aggctcaggc 600
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cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac 840
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aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 960
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gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg 1260
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acaattattc agcgacgtga ggatggcagc gttgattttc agaggacttg gaaagaatat 1560
aaagtgggat ttggtaaccc ttcaggagaa tattggctgg gaaatgagtt tgtttcgcaa 1620
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            20                  25                  30
Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu
        35                  40                  45
Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly
    50                  55                  60
Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser
65                  70                  75                  80
Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp
                85                  90                  95
Trp Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser
            100                 105                 110
Ser Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr
        115                 120                 125
Ala Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr
    130                 135                 140
Lys Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu
145                 150                 155                 160
Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly
                165                 170                 175
Met Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys
            180                 185                 190
Trp Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met
        195                 200                 205
Met Ile Arg Pro Ala Asp Phe Gly Gly Pro Gly Glu Pro Lys Ser Cys

    210                 215                 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225                 230                 235                 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    290                 295                 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            340                 345                 350
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        355                 360                 365
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385                 390                 395                 400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                405                 410                 415
```

```
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            420                 425                 430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445
Pro Gly Lys Gly Gly Gly Gly Ser Gly Ala Pro Arg Asp Cys Ala Glu
    450                 455                 460
Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe
465                 470                 475                 480
Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly
                485                 490                 495
Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp
            500                 505                 510
Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser
        515                 520                 525
Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln
    530                 535                 540
Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu
545                 550                 555                 560
Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn
                565                 570                 575
Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser
            580                 585                 590
Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn
        595                 600                 605
Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp
    610                 615                 620
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625                 630                 635                 640
Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys
                645                 650                 655
Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala
            660                 665                 670
Asp Phe
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REFERENCES CITED IN THE DESCRIPTION

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- **CARROLL RW et al.**PLoS ONE, 2010, vol. 5, e13124- [0050]

PATENTKRAV

1. Farmaceutisk sammensætning, der omfatter en terapeutisk effektiv mængde af et modificeret angiopoietinprotein til anvendelse i en fremgangsmåde til behandling, forebyggelse eller bedring af mindst ét symptom på, én indikation eller komplikation af cerebral malaria, hvilken fremgangsmåde omfatter administration af sammensætningen til et individ med behov derfor, hvor det modificerede angiopoietin omfatter et fusionsprotein, der omfatter mindst ét fibrinogen-lignende domæne af angiopoietin-1 kondenseret til et immunoglobulin Fc-fragment.

2. Sammensætning til anvendelse i fremgangsmåde ifølge krav 1, hvor det/den mindst ene symptom, indikation eller komplikation er valgt fra gruppen bestående af feber, hovedpine og myalgi efterfulgt af døsighed, konfusion, vaskulær lækage, tab af blodbarriereintegritet, forhøjet blodniveau af en endotel markør, eventuelt valgt fra gruppen bestående af Ang1, Ang2, Tie2, vWF, ICAM-1, E-selectin og VCAM-1, sekvestrering af parasitiserede erythrocytter i hjernen, nedsat balance eller koordination, motorisk funktionsnedsættelse, splenomegali, tab af reflekser og selvopholdelse, manglende hygiejnerelateret adfærd, akut lungelæsion, konvulsion, anfald, koma og død.

3. Sammensætning til anvendelse i fremgangsmåden ifølge krav 1 eller 2, hvor:

(a) det modificerede angiopoietin administreres ved en dosis på 5 - 50 mg/kg af individets kropsvægt, eventuelt hvor det modificerede angiopoietin administreres som en dosis på 15 mg/kg af individets kropsvægt; og/eller

(b) det modificerede angiopoietin administreres subkutant; og/eller

(c) det modificerede angiopoietin administreres i kombination med et andet terapeutisk middel; og/eller

(d) det modificerede angiopoietin administreres i kombination med et andet terapeutisk middel og det andet terapeutisk middel er valgt fra gruppen bestående af et artemisinin, quinin og en variant eller et derivat deraf, eventuelt hvor det andet terapeutiske middel er artesunat.

4. Sammensætning til anvendelse i fremgangsmåden ifølge et hvilket som helst af krav 1-3, hvor administration af det modificerede angiopoietin øger overlevelse.

5. Sammensætning til anvendelse i fremgangsmåden ifølge et hvilket som helst af krav 1-3, hvor administration af det modificerede angiopoietin beskytter blodbarriereintegritet.

6. Farmaceutisk sammensætning, der omfatter en terapeutisk effektiv mængde af et modificeret angiopoietinprotein til anvendelse i en fremgangsmåde til forebyggelse af svær cerebral malaria hos et individ inficeret med *Plasmodium* spp., hvilken fremgangsmåde omfatter: (a) udvælgelse af et individ med mere end 0,1 % parasitæmi; og (b) administration af den farmaceutiske sammensætning til individet med behov derfor, hvor det modificerede angiopoietin omfatter et fusionsprotein omfattende mindst ét fibrinogen-lignende domæne af angiopoietin-1 kondenseret til et immunoglobulin Fc-fragment.

7. Sammensætning til anvendelse i fremgangsmåden ifølge krav 6, hvor administrationen af det modificerede angiopoietin forhindrer mindst én indikation valgt fra gruppen bestående af neurologisk funktionsnedsættelse og vaskulær lækage, eventuelt hvor den mindst ene indikation af neurologisk funktionsnedsættelse er valgt fra gruppen bestående af nedsat balance eller koordination, motorisk funktionsnedsættelse, tab af reflekser og selvopholdelse, langsigtet neurokognitiv læsion og funktionsnedsættelse, hukommelsesdeficiens, emotionelle forstyrrelser, manglende hygiejnerelateret adfærd, konvulsion og anfald.

8. Sammensætning til anvendelse i fremgangsmåden ifølge krav 6 eller 7, hvor administrationen af det modificerede angiopoietin forhindrer tab af blodbarriereintegritet.

9. Sammensætning til anvendelse i fremgangsmåden ifølge et hvilket som helst af krav 6-8, hvor:

(a) det modificerede angiopoietin administreres ved en dosis på 15 mg/kg af individets kropsvægt; og/eller

(b) det modificerede angiopoietin administreres subkutant; og/eller

(c) det modificerede angiopoietin administreres i kombination med et andet terapeutisk middel, eventuelt hvor det andet terapeutisk middel er artesunat.

10. Sammensætning til anvendelse i fremgangsmåden ifølge et hvilket som helst af krav 1 - 9, hvor fusionsproteinet omfatter et første fibrinogen-lignende domæne af angiopoietin-1 kondenseret ved dets C-terminale ende til den N-terminale ende af et Fc-fragment og Fc-fragmentet kondenseret ved dets C-terminale ende til den N-terminale ende af et andet fibrinogen-lignende domæne af angiopoietin-1.

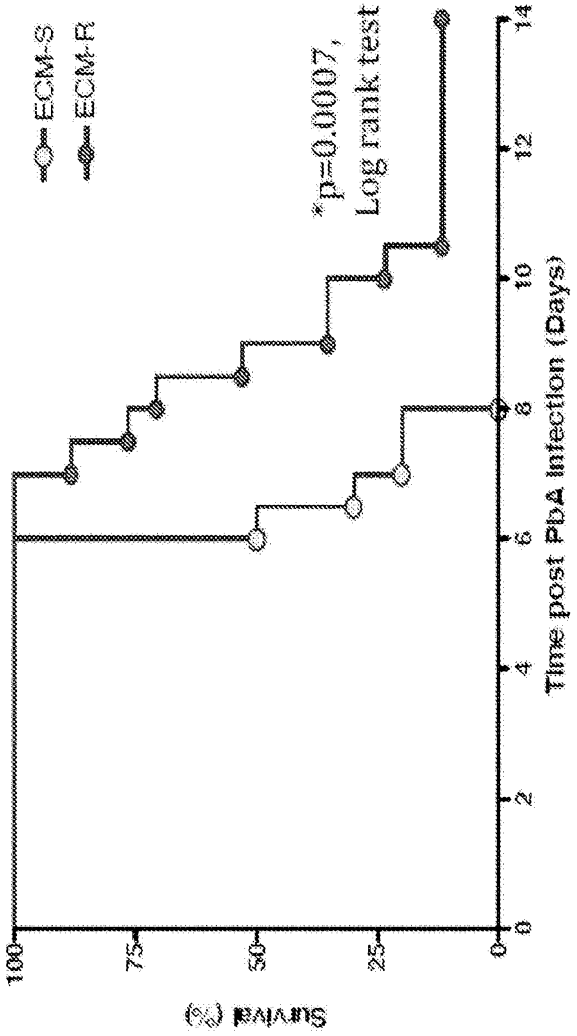
11. Sammensætning til anvendelse i fremgangsmåden ifølge et hvilket som helst af krav 1 - 10, hvor det modificerede angiopoietin-1 er AngF1-Fc-F1.

12. Sammensætning til anvendelse i fremgangsmåden ifølge et hvilket som helst af krav 1-11, hvor Fc-fragmentet omfatter et IgG-Fc-domæne.

13. Sammensætning til anvendelse i fremgangsmåden ifølge krav 12, hvor IgG-Fc-domænet er et humant IgG1-Fc-domæne.

14. Sammensætning til anvendelse i fremgangsmåde ifølge et hvilket som helst af krav 1 - 13, hvor det modificerede angiopoietin-1 består af aminosyresekvensen ifølge SEQ ID NO: 2.

DRAWINGS



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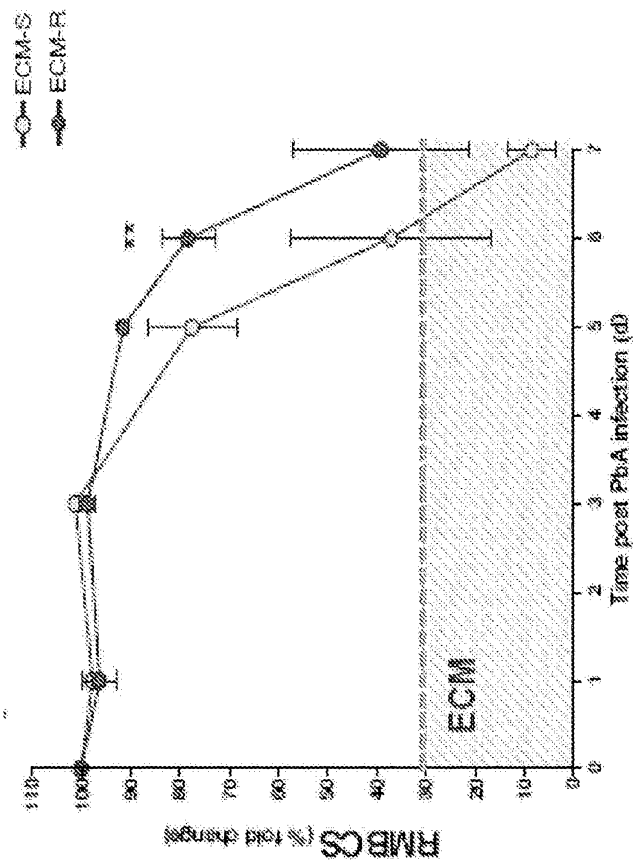
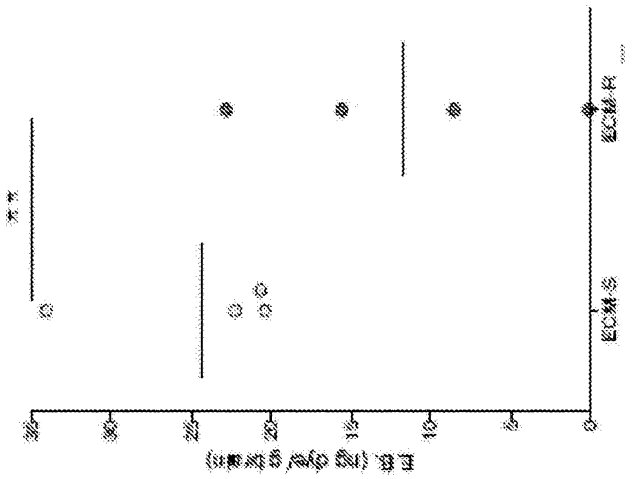

ECM-S
ECM-R
**
ECM
RNBCS (% total strength)
Time post PbA infection (d)
110
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7

Figure 2



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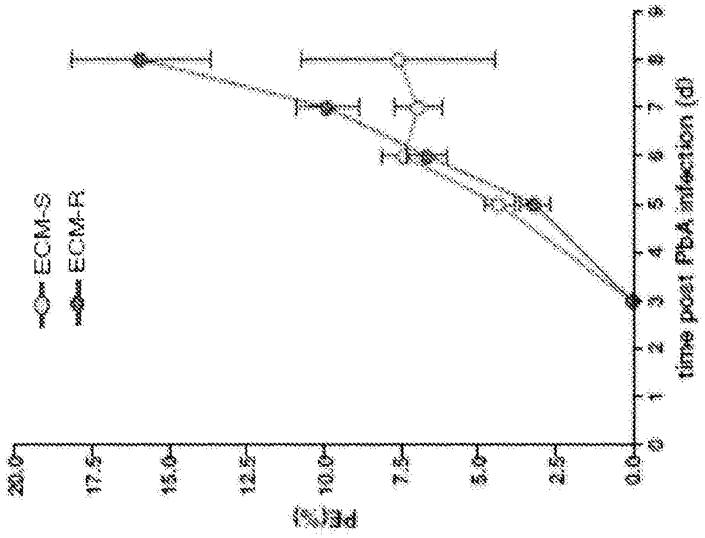

ECM-S
ECM-R
PETAL
time post PbA infection (d)
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
0
1
2
3
4
5
6
7
8
9

Figure 4

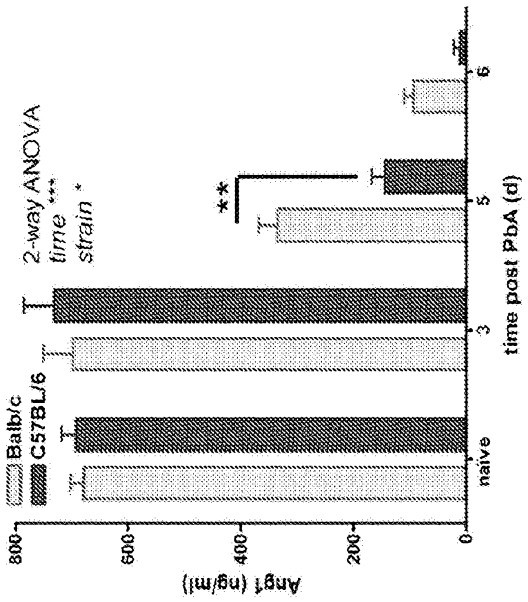

Balb/c
C57BL/6
2-way ANOVA
time ***
strain *
**
Angt (ng/ml)
800
600
400
200
0
naive
3
5
6
time post PbA (d)

Figure 5

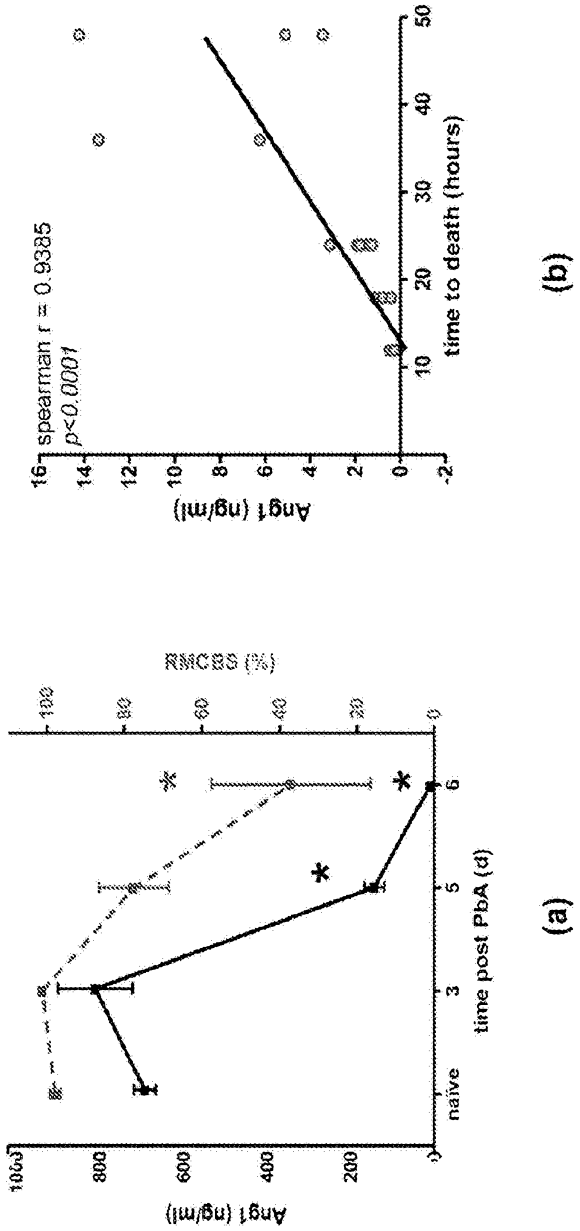
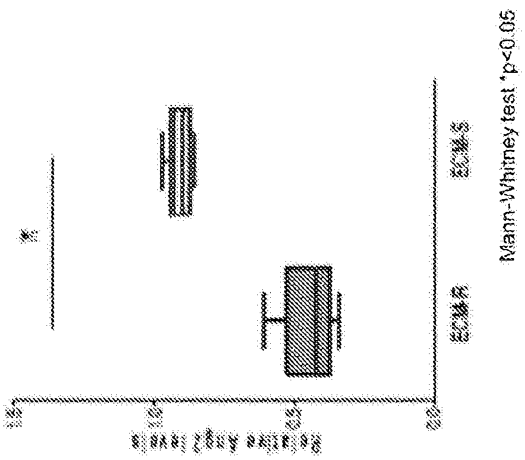

Angt (ng/ml)
spearman r = 0.9385
p<0.0001
time to death (hours)
(b)
RMCBS (%)
Angt (ng/ml)
naive
time post PbA (d)
(a)

Figure 6



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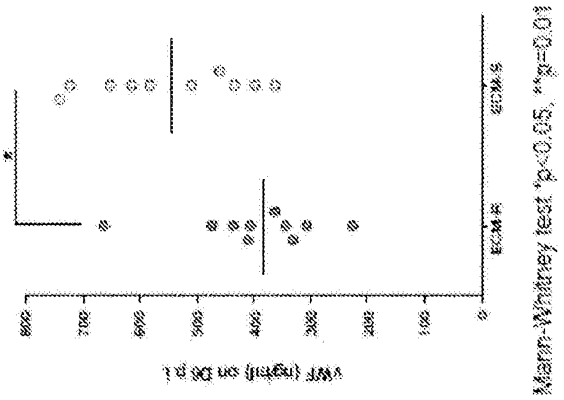

vWF (ng/ml) on D0 p.i.
800
700
600
500
400
300
200
100
0
excl.
excl.
*
Mann-Whitney test *p<0.05, **p=0.01

Figure 8

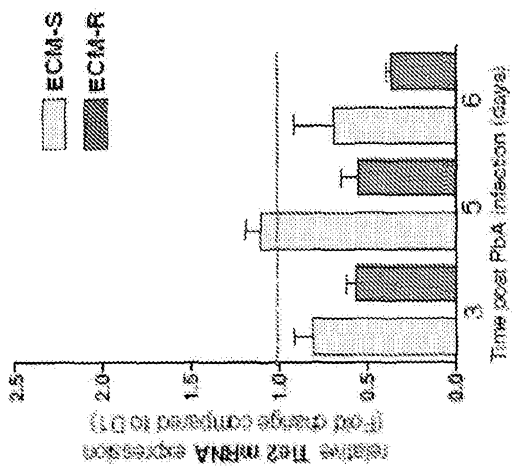

EC14-S
EC14-R
Tie2 mRNA expression
(Fold change compared to D1)
2.5
2.0
1.5
1.0
0.5
0.0
3
6
Time post PbA infection (days)

Figure 9

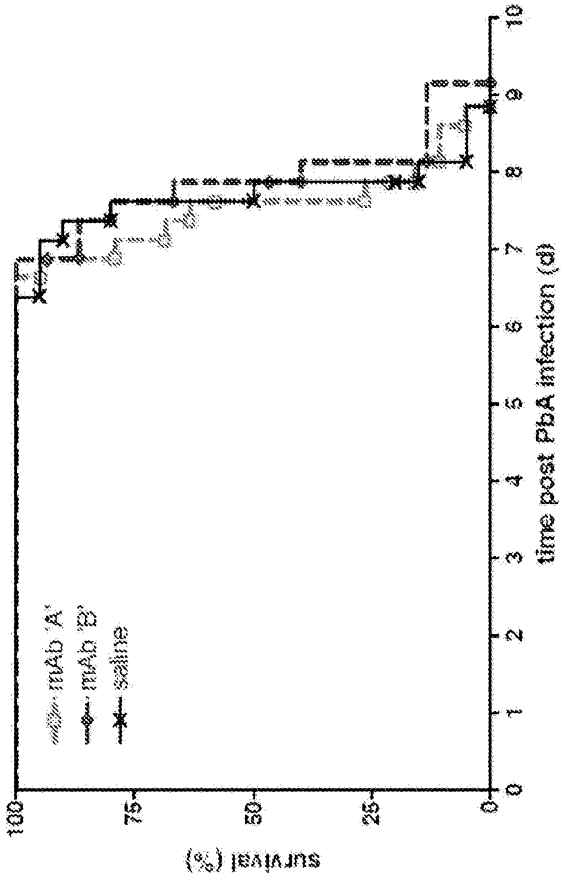

survival (%)
100
75
50
25
0
0
1
2
3
4
5
6
7
8
9
10
time post PbA infection (d)
mAb 'A'
mAb 'B'
saline

Figure 10

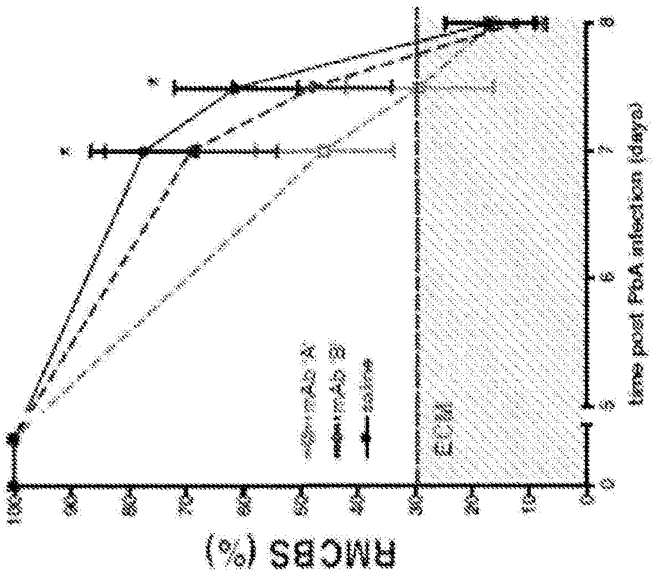


Figure 11

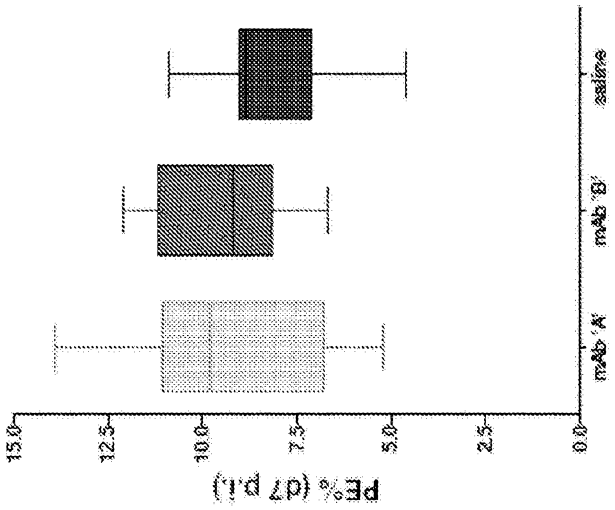


Figure 12

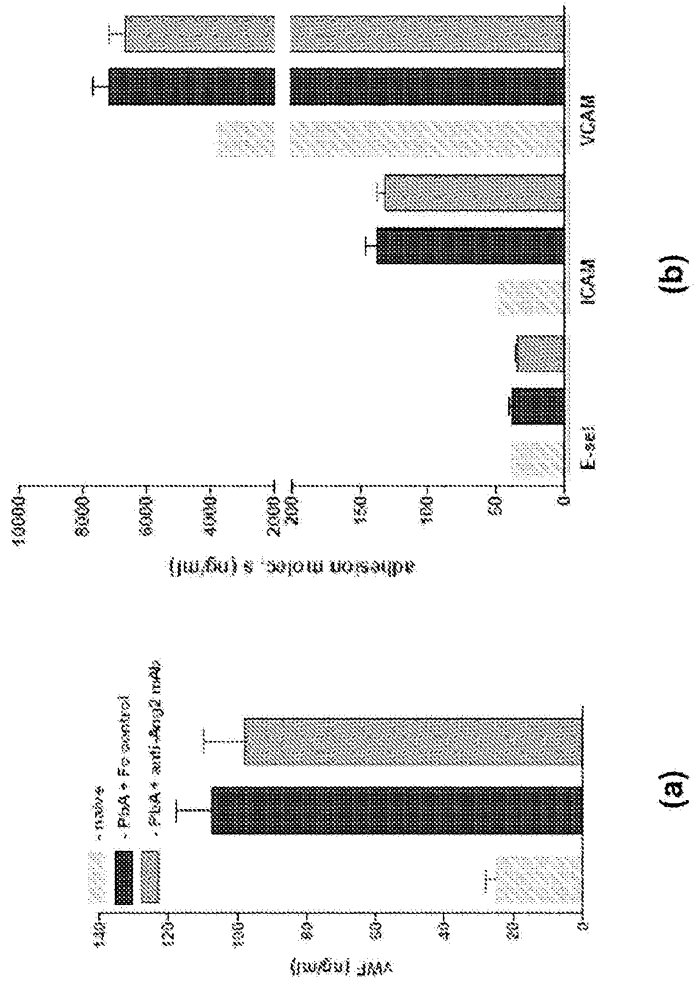

adhesion molec. s (ng/ml)
E-sel
ICAM
VCAM
(b)
vWF (ng/ml)
- mock
- PlgA + Fc control
- PlgA + anti-Angl2 mAb
(a)

Figure 13

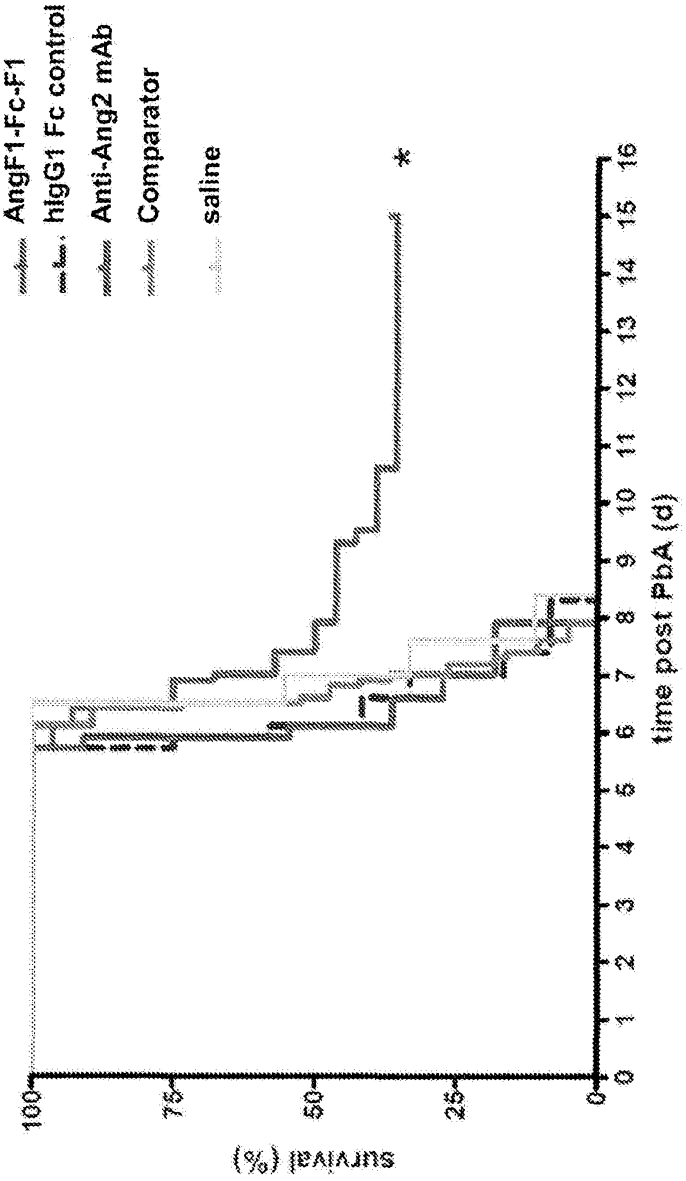

AngF1-Fc-F1
hIgG1 Fc control
Anti-Ang2 mAb
Comparator
saline
*
survival (%)
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
time post PbA (d)

Figure 14

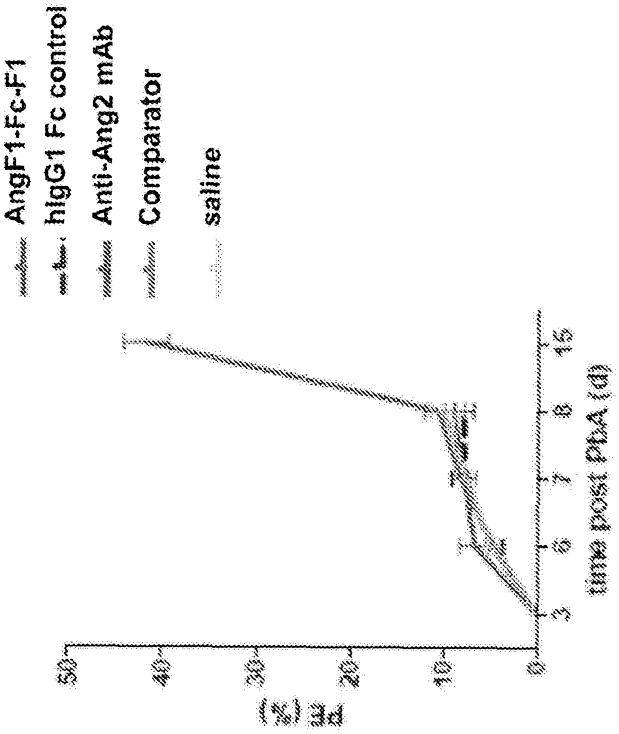

AngF1-Fc-F1
hIgG1 Fc control
Anti-Ang2 mAb
Comparator
saline
PE (%)
50
40
30
20
10
0
3
6
7
8
15
time post PtxA (d)

Figure 15

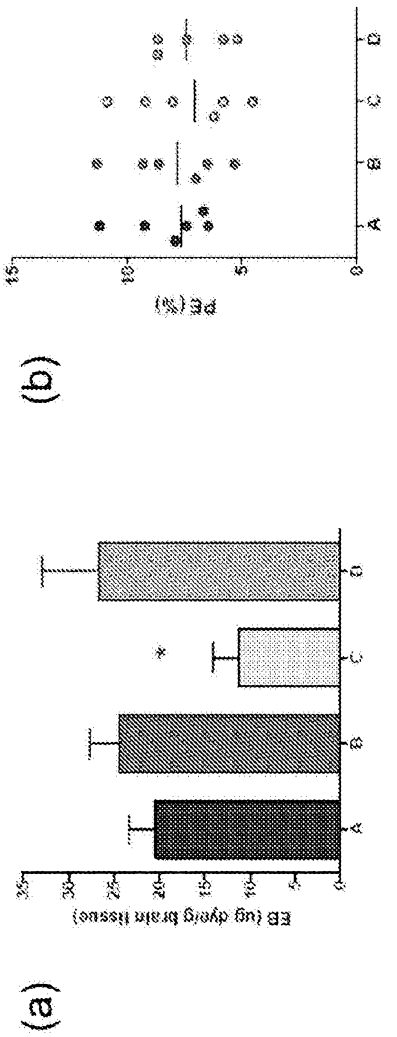

(a)
EB (ug dye/g brain tissue)
0
5
10
15
20
25
30
35
A
B
C
D
*
(b)
PE (%)
0
5
10
15
A
B
C
D

Figure 16

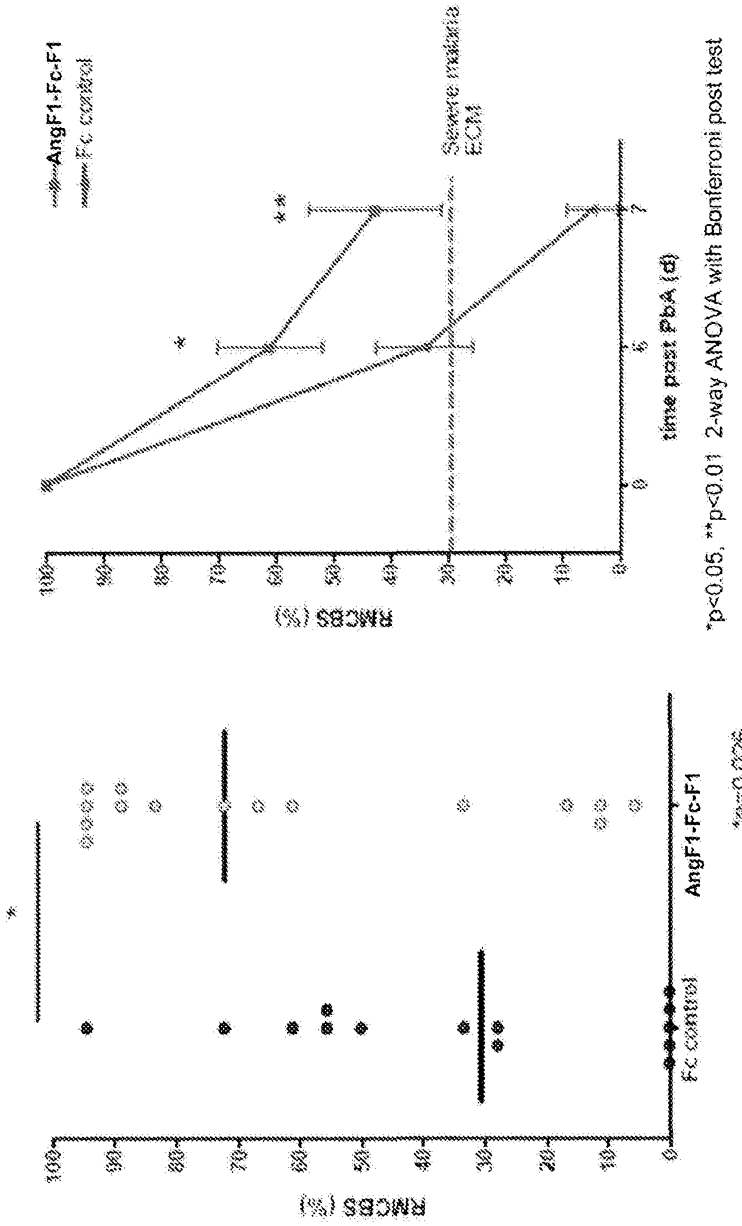

AngF1-Fc-F1
Fc control
Severe malaria ECM
RMCBS (%)
time post PbA (d)
*p<0.05, **p<0.01 2-way ANOVA with Bonferroni post test
(b)
*p=0.026
(a)

Figure 17

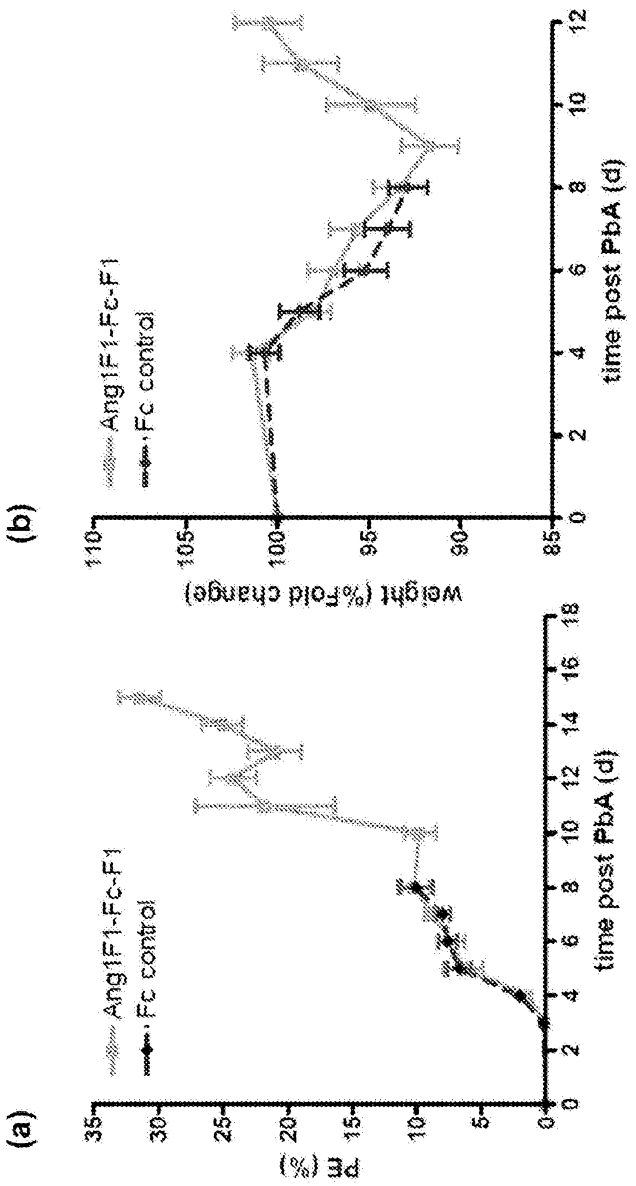

(a)
Ang1F1-Fc-F1
Fc control
PE (%)
time post PbA (d)
(b)
Ang1F1-Fc-F1
Fc control
weight (%Fold change)
time post PbA (d)

Figure 18

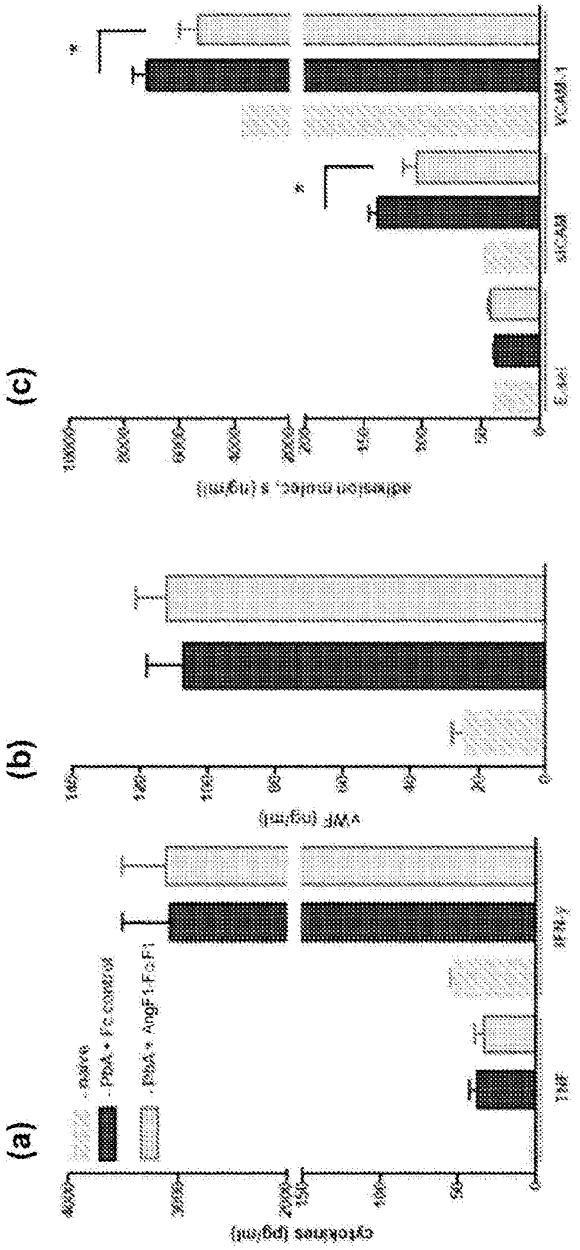
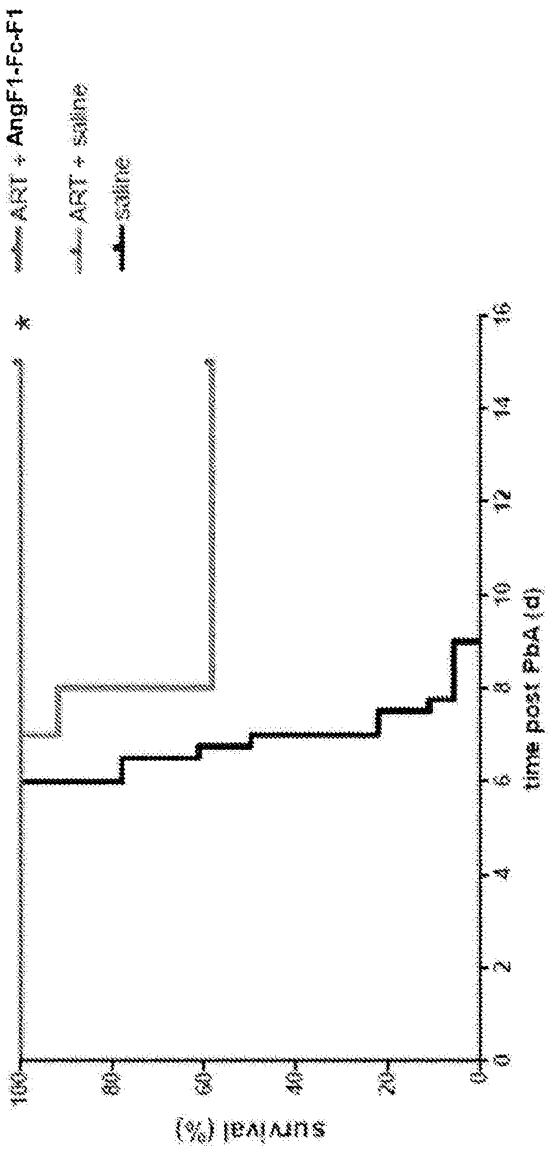

(a)
- saline
- PbA + Fc control
- PbA + AngF1-Fc-F1
cytokines (pg/ml)
TNF
IFN-γ
(b)
vWF (ng/ml)
(c)
adhesion molecules (ng/ml)
E-sel
sICAM
VCAM-1
*
*

Figure 19



* p < 0.05

Figure 20

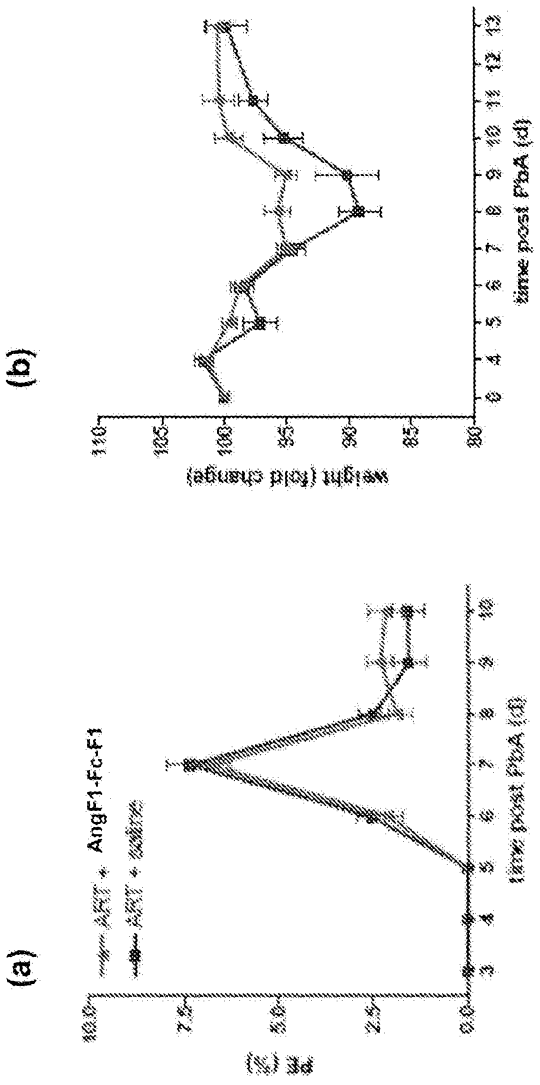

(a)
AKT + AngF1-Fc-F1
AKT + saline
PE (%)
time post PbA (d)
(b)
weight (fold change)
time post PbA (d)

Figure 21