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(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF GRAFT-VERSUS-HOST DISEASE

(57) Abstract: The present invention relates to compositions and methods for treatment and/or prevention of Graft- Versus-Host Disease. In particular, the invention provides compositions and methods for the treatment and/or prevention of Graft- Versus-Host Disease, based on the use of angiotensin-(1-7) peptides or functional equivalents. In particular, the present invention can be used to facilitate transplantation and reduce or prevent the occurrence of GVHD, mucositis and other diseases, disorders and conditions.

COMPOSITIONS AND METHODS FOR TREATMENT OF GRAFT-VERSUS-HOST DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Patent Application Serial No. 61/635,786, filed April 19, 2012, the disclosure of which is hereby incorporated in its entirety.

BACKGROUND

[0002] Graft-Versus-Host Disease (GVHD) is a major cause of transplantation associated morbidity and mortality. GVHD can occur after allogeneic cell transplants, such as stem cell transplants and/or bone marrow transplants. GVHD can also occur after blood transfusion. In GVHD, the transplanted immune cells can recognize and attack the cells of the host as foreign. Currently, treatment of GVHD typically involves administration of steroids. Steroids are effective for acute GVHD but may cause serious side effects such as infection with bacteria and viruses. Therefore, there is a great need for more effective and safer treatment of GVHD.

SUMMARY OF THE INVENTION

[0003] The present invention provides improved methods for treating or preventing GVHD, in particular, based on the use of an angiotensin (1-7) peptide. As described in the Examples section below, the present invention is, in part, based on the surprising discovery that treatment with an angiotensin (1-7) peptide significantly reduced the occurrence of GVHD in cord blood transplant recipients. Therefore, the present invention represents a more effective and safer treatment for GVHD.

[0004] In one aspect, the present invention provides methods of treating or reducing risk for Graft-Versus-Host Disease comprising administering to a subject who is suffering from or susceptible to Graft-Versus-Host Disease (GVHD) an angiotensin (1-7) peptide. In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose periodically at an administration interval such that at least one symptom or feature of Graft-Versus-Host Disease is

reduced in intensity, severity, duration, or frequency or has delayed in onset. In some embodiments, the at least one symptom or feature of Graft-Versus-Host Disease is selected from liver damage, skin rash, jaundice, intestinal inflammation, sloughing of the mucosal membrane, diarrhea, abdominal pain, nausea, and vomiting. In some embodiments, the GVHD is acute GVHD. In some embodiments, the GVHD is chronic GVHD. In some embodiments, the subject being treated is immune-compromised recipient of transplant.

[0005] In some embodiments, the Graft-Versus-Host Disease is associated with hematopoietic stem cell (HSC) (e.g., bone marrow, peripheral blood (PBSC), and cord blood) transplantation. In some embodiments, the Graft-Versus-Host Disease is associated with cord blood transplantation. In some embodiments, the cord blood transplantation is selected from the group consisting of single cord blood transplantation, double cord blood transplantation, manipulated cord blood transplantation, and combination thereof. In some embodiments, the manipulated cord blood transplantation comprises *ex vivo* expanded cord blood transplantation. In some embodiments, the manipulated cord blood transplantation comprises treatment of the cord blood with prostaglandins prior to transplant. In some embodiments, the manipulated cord blood transplantation comprises depleting T-cells from the cord blood prior to transplant.

[0006] In some embodiments, the Graft-Versus-Host Disease is associated with bone marrow transplantation. In some embodiments, the Graft-Versus-Host Disease is associated with peripheral blood transplantation. Suitable bone marrow or peripheral blood may be obtained from either children or adults.

[0007] In some embodiments, the Graft-Versus-Host Disease is associated with adult stem cell transplantation. In some embodiments, the adult stem cell transplantation is allogeneic adult stem cell transplantation. In some embodiments, the Graft-Versus-Host Disease is associated with embryonic stem cell transplantation. In some embodiments, the Graft-Versus-Host Disease is associated with organ transplantation. In some embodiments, the Graft-Versus-Host Disease is associated with blood transfusion.

[0008] In some embodiments, the angiotensin (1-7) peptide is administered concurrently (e.g., on the same day) with the transplantation. In some embodiments, angiotensin (1-7) peptide is administered periodically subsequent to transplantation. In some embodiments, the angiotensin

(1-7) peptide is administered for at least 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 15 weeks, 18 weeks, 21 weeks, or 24 weeks subsequent to the transplantation. In some embodiments, the angiotensin (1-7) peptide is administered for at least 1 month, 2 months, 3 months, 6 months, 1 year, 2 years, or longer subsequent to the transplantation. In some embodiments, the angiotensin (1-7) peptide is administered daily, twice a week, weekly, once every two weeks, once every three weeks, monthly, or at a variable interval.

[0009] In some embodiments, the angiotensin (1-7) peptide is administered periodically prior to the transplantation. In some embodiments, the angiotensin (1-7) peptide is administered for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks prior to the transplantation. In some embodiments, the angiotensin (1-7) peptide is administered daily, once every two days, twice a week, weekly, or at a variable interval.

[0010] In some embodiments, the angiotensin (1-7) peptide is administered intravenously, intradermally, orally, by inhalation, transdermally (topical), subcutaneously (e.g., abdomen or thigh), and/or transmucosally.

[0011] In some embodiments, the angiotensin (1-7) peptide is administered at the effective dose of 5-1,500 μg/kg/day (e.g., 5-1,200 μg/kg/day, 5-1,000 μg/kg/day, 50-1,200 μg/kg/day, 50-1,000 μg/kg/day, 50-800 μg/kg/day, 100-1200 μg/kg/day, 100-800 μg/kg/day, 100-600 μg/kg/day, 200-1,500 μg/kg/day, 200-1,200 μg/kg/day, 200-1,000 μg/kg/day, 200-800 μg/kg/day, 200-600 μg/kg/day, 300-1,500 μg/kg/day, 300-1,200 μg/kg/day, 300-1,000 μg/kg/day, 300-800 μg/kg/day, or 300-600 μg/kg/day). In some embodiments, the angiotensin (1-7) peptide is administered at the effective dose of 100-1,000 μg/kg/day. In some embodiments, the angiotensin (1-7) peptide is administered at the effective dose of 300-1,000 μg/kg/day. In some embodiments, the angiotensin (1-7) peptide is administered at the effective dose of 1, 2.5, 5, 10, 20, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, or 1,500 μg/kg/day.

[0012] In some embodiments, the administration of the angiotensin (1-7) peptide results in substantially free of Stage 4, 3, 2, or 1 acute GVHD in the subject 100 days (e.g., at least 120 days, 130 days, 140 days, 150 days, 160 days, 170 days, 180 days or more) following

transplantation. In some embodiments, the administration of the angiotensin (1-7) peptide results in substantially free of Grade IV, III, II, or I acute GVHD in the subject 100 days (e.g., at least 120 days, 130 days, 140 days, 150 days, 160 days, 170 days, 180 days or more) following transplantation. In some embodiments, the administration of the angiotensin (1-7) peptide results in substantially free of acute GVHD symptom in the subject 100 days (e.g., at least 120 days, 130 days, 140 days, 150 days, 160 days, 170 days, 180 days or more) following transplantation. In some embodiments, the administration of the angiotensin (1-7) peptide results in substantially free of chronic GVHD symptoms in the subject at least 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 4 years, 5 years or more following transplantation.

[0013] In another aspect, the present invention provides methods of transplantation comprising administering an angiotensin (1-7) peptide in conjunction with introducing an allogeneic organ, tissue or cell into a subject.

[0014] In some embodiments, the allogeneic tissue or cell comprises cord blood. In some embodiments, the allogeneic organ, tissue or cell comprises bone marrow. In some embodiments, the allogeneic organ, tissue or cell comprises adult stem cell. In some embodiments, the allogeneic organ, tissue or cell comprises embryonic stem cell. In some embodiments, the allogeneic organ, tissue or cell comprises an organ (i.e., solid organ).

[0015] In some embodiments, the angiotensin (1-7) peptide is administered prior to introducing the allogeneic organ, tissue or cell. In some embodiments, the angiotensin (1-7) peptide is administered concurrently with introducing the allogeneic organ, tissue or cell. In some embodiments, the angiotensin (1-7) peptide is administered subsequent to introducing the allogeneic organ, tissue or cell.

[0016] In some embodiments, the angiotensin (1-7) peptide is administered in combination with an immunosuppressant. In some embodiments, the immunosuppressant is selected from the group consisting of antithymocyte globulin, anti-TNF agents, azathioprine or other inosine 5'-monophosphate dehydrogenase inhibitors, azodiacarbonide, bisindolyl maleimide VIII, brequinar, chlorambucil, CTLA4-Ig, corticosteroids, cyclophosphamide, cyclosporine A, deoxyspergualin, dexamethasone, FK506, glucocorticoids, IL-2 antagonists,

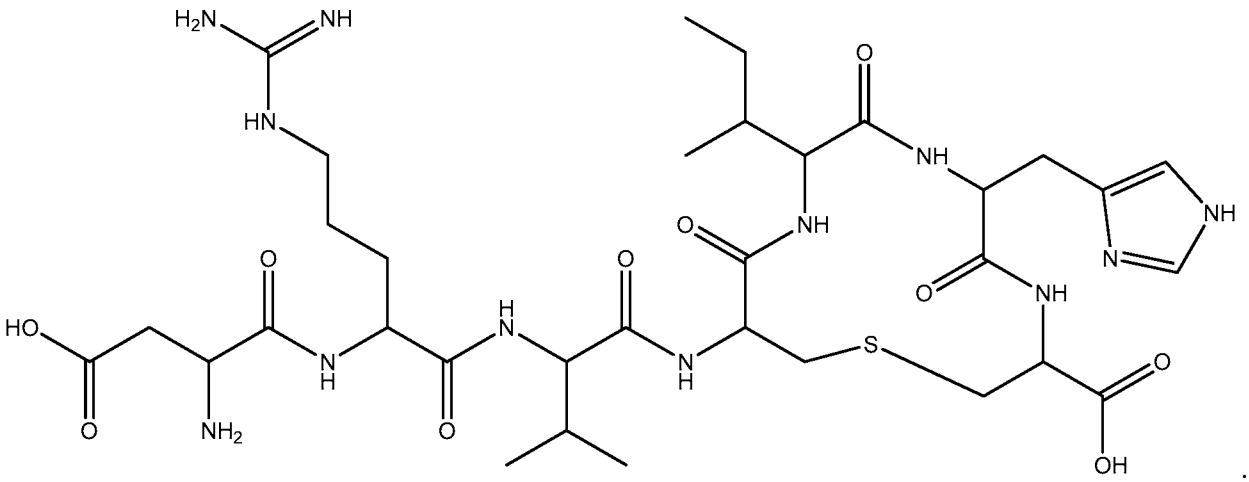
leflunomide, mercaptopurine, 6-mercaptopurine, methotrexate, methylprednisolone, mizoribine, mizoribine monophosphate, muromonab CD3, mycophenolate mofetil, OKT3, prednisone, sirolimus, rapamycin, rho (D) immune globin, vitamin D analogs, and combination thereof.

[0017] In some embodiments, the administration of the angiotensin (1-7) peptide results in reduced intensity, severity, duration, or frequency or delayed onset of at least one symptom or feature of GVHD (e.g., acute or chronic GVHD). In some embodiments, the administration of the angiotensin (1-7) peptide results in reduced intensity, severity, duration, or frequency or delayed onset of at least one symptom or feature of mucositis.

[0018] In various embodiments, an angiotensin (1-7) peptide suitable for the present invention comprises the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:1).

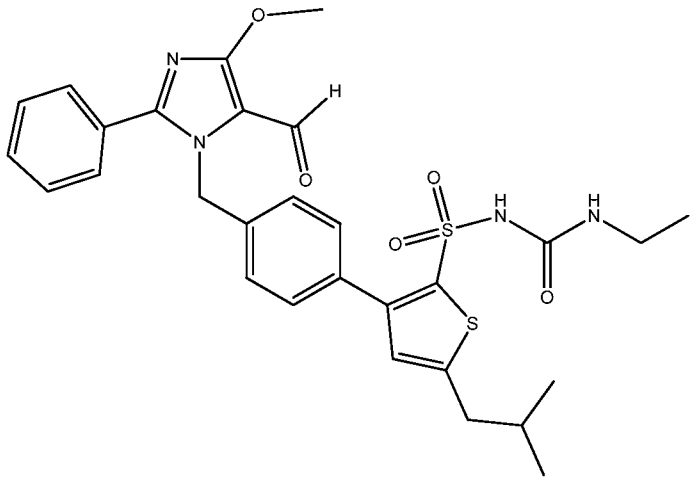
[0019] In some embodiments, the functional equivalent is a linear peptide. In some embodiments, the linear peptide comprises a sequence that includes at least four amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least four amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide comprises a sequence that includes at least five amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least five amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide comprises a sequence that includes at least six amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least six amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide contains 4-25 amino acids. In some embodiments, the linear peptide is a fragment of the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide contains amino acid substitutions, deletions and/or insertions in the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide has an amino acid sequence of Asp^{1}-Arg^{2}-Nle^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:2). In some embodiments, the linear peptide has an amino acid sequence of Asp^{1}-Arg^{2}-Val^{3}-Ser^{4}-Ile^{5}-His^{6}-Cys^{7} (SEQ ID NO:3).

[0020] In some embodiments, the functional equivalent is a cyclic peptide. In some embodiments, the cyclic peptide comprises a linkage between amino acids. In some embodiments, the linkage is located at residues corresponding to positions Tyr^4 and Pro^7 in naturally-occurring Angiotensin (1-7). In some embodiments, the linkage is a thioether bridge. In some embodiments, the cyclic peptide comprises an amino acid sequence otherwise identical to the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:1). In some embodiments, the cyclic peptide comprises a norleucine (Nle) replacing position Val^3 in naturally-occurring Angiotensin (1-7). In some embodiments, the cyclic peptide is a 4,7-cyclized angiotensin (1-7) with the following formula:



[0021] In some embodiments, the angiotensin (1-7) peptide comprises one or more chemical modifications to increase protease resistance, serum stability and/or bioavailability. In some embodiments, the one or more chemical modifications comprise pegylation.

[0022] In some embodiments, the present invention provides methods of treating GVHD including administering to a subject who is suffering from or susceptible to GVHD an angiotensin (1-7) receptor agonist. In some embodiments, the angiotensin (1-7) receptor agonist is a non-peptidic agonist. In some embodiments, the non-peptidic agonist is a compound with the following structure:



or a pharmaceutically acceptable salt thereof.

[0023] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0024] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

DEFINITIONS

[0025] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0026] *Acute:* As used herein, the term "acute" when used in connection with tissue damage and related diseases, disorders, or conditions, has the meaning understood by any one skilled in the medical art. For example, the term typically refers to a disease, disorder, or condition in which there is sudden or severe onset of symptoms. In some embodiments, acute

damage is due to an ischemic or traumatic event. Typically, the term "acute" is used in contrast to the term "chronic."

[0027] *Allogeneic:* As used herein, the term "allogeneic" means from a different organism of the same species. In the context of transplantation, the term is used to mean that the cells, tissues and/or organs referred to as "allogeneic" are from a different individual than a recipient into which said cells, tissues and/or organs are being transplanted. Typically, allogeneic cells, tissues or organs have different genotype than the recipient.

[0028] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0029] *Approximately or about:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0030] *Autologous:* As used herein, the term "autologous" means from the same organism. In the context of transplantation, the term is used to mean that the cells, tissues and/or organs referred to as "autologous" are derived from the recipient itself. Typically, autologous cells, tissues and/or organs do not contain any substantial amount of material which could be regarded as allogeneic or xenogeneic, that is to say derived from a "foreign" cellular source.

[0031] *Biologically active*: As used herein, the phrase "biologically active" refers to a characteristic of any agent that has activity in a biological system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, where a peptide is biologically active, a portion of that peptide that shares at least one biological activity of the peptide is typically referred to as a "biologically active" portion. In certain embodiments, a peptide has no intrinsic biological activity but that inhibits the effects of one or more naturally-occurring angiotensin compounds is considered to be biologically active.

[0032] *Carrier or diluent*: As used herein, the terms "carrier" and "diluent" refers to a pharmaceutically acceptable (e.g., safe and non-toxic for administration to a human) carrier or diluting substance useful for the preparation of a pharmaceutical formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0033] *Chronic:* As used herein, the term "chronic," when used in connection with tissue damage or related diseases, disorders, or conditions has the meaning as understood by any one skilled in the medical art. Typically, the term "chronic" refers to diseases, disorders, or conditions that involve persisting and/or recurring symptoms. Chronic diseases, disorders, or conditions typically develop over a long period of time. The term "chronic" is used in contrast to the term "acute." In some embodiments, a chronic disease, disorder, or condition results from cell degeneration. In some embodiments, a chronic disease, disorder, or condition results from age-related cell degeneration.

[0034] *Dosage form:* As used herein, the terms "dosage form" and "unit dosage form" refer to a physically discrete unit of a therapeutic agent for the patient to be treated. Each unit contains a predetermined quantity of active material calculated to produce the desired therapeutic effect. It will be understood, however, that the total dosage of the composition will be decided by the attending physician within the scope of sound medical judgment.

[0035] *Dosing regimen:* A "dosing regimen" (or "therapeutic regimen"), as that term is used herein, is a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent

has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regime comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, the therapeutic agent is administered continuously over a predetermined period. In some embodiments, the therapeutic agent is administered once a day (QD) or twice a day (BID).

[0036] *Functional equivalent or derivative*: As used herein, the term "functional equivalent" or "functional derivative" denotes, in the context of a functional derivative of an amino acid sequence, a molecule that retains a biological activity (either function or structural) that is substantially similar to that of the original sequence. A functional derivative or equivalent may be a natural derivative or is prepared synthetically. Exemplary functional derivatives include amino acid sequences having substitutions, deletions, or additions of one or more amino acids, provided that the biological activity of the protein is conserved. The substituting amino acid desirably has chemico-physical properties which are similar to that of the substituted amino acid. Desirable similar chemico-physical properties include, similarities in charge, bulkiness, hydrophobicity, hydrophilicity, and the like.

[0037] *Improve, increase, or reduce*: As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with the same form of disease as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0038] *In vitro*: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.

[0039] *In vivo*: As used herein, the term "*in vivo*" refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0040] *Isolated*: As used herein, the term "isolated" refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, substantially 100%, or 100% of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, substantially 100%, or 100% pure. As used herein, a substance is "pure" if it is substantially free of other components. As used herein, the term "isolated cell" refers to a cell not contained in a multi-cellular organism.

[0041] *Prevent*: As used herein, the term "prevent" or "prevention", when used in connection with the occurrence of a disease, disorder, and/or condition, refers to reducing the risk of developing the disease, disorder and/or condition. See the definition of "risk."

[0042] *Polypeptide*: The term "polypeptide" as used herein refers a sequential chain of amino acids linked together via peptide bonds. The term is used to refer to an amino acid chain of any length, but one of ordinary skill in the art will understand that the term is not limited to lengthy chains and can refer to a minimal chain comprising two amino acids linked together via a peptide bond. As is known to those skilled in the art, polypeptides may be processed and/or modified.

[0043] *Protein*: The term "protein" as used herein refers to one or more polypeptides that function as a discrete unit. If a single polypeptide is the discrete functioning unit and does not require permanent or temporary physical association with other polypeptides in order to form the discrete functioning unit, the terms "polypeptide" and "protein" may be used interchangeably. If

the discrete functional unit is comprised of more than one polypeptide that physically associate with one another, the term "protein" refers to the multiple polypeptides that are physically coupled and function together as the discrete unit.

[0044] *Risk:* As will be understood from context, a "risk" of a disease, disorder, and/or condition comprises a likelihood that a particular individual will develop a disease, disorder, and/or condition (*e.g.,* GVHD). In some embodiments, risk is expressed as a percentage. In some embodiments, risk is from 0,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 up to 100%. In some embodiments risk is expressed as a risk relative to a risk associated with a reference sample or group of reference samples. In some embodiments, a reference sample or group of reference samples have a known risk of a disease, disorder, condition and/or event (e.g., GVHD). In some embodiments a reference sample or group of reference samples are from individuals comparable to a particular individual. In some embodiments, relative risk is 0,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

[0045] *Stability:* As used herein, the term "stable" refers to the ability of the therapeutic agent to maintain its therapeutic efficacy (e.g., all or the majority of its intended biological activity and/or physiochemical integrity) over extended periods of time. The stability of a therapeutic agent, and the capability of the pharmaceutical composition to maintain stability of such therapeutic agent, may be assessed over extended periods of time (e.g., for at least 1, 3, 6, 12, 18, 24, 30, 36 months or more). In certain embodiments, pharmaceutical compositions described herein have been formulated such that they are capable of stabilizing, or alternatively slowing or preventing the degradation, of one or more therapeutic agents formulated therewith. In the context of a formulation a stable formulation is one in which the therapeutic agent therein essentially retains its physical and/or chemical integrity and biological activity upon storage and during processes (such as freeze/thaw, mechanical mixing and lyophilization).

[0046] *Subject*: As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre and post natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or

"patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0047] *Substantially*: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0048] *Suffering from*: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of the disease, disorder, and/or condition.

[0049] *Susceptible to*: An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, condition, or event (for example, GVHD) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, condition, and/or event (5) having undergone, planning to undergo, or requiring a transplant. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0050] *Therapeutically effective amount*: As used herein, the term "therapeutically effective amount" of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat,

diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0051] *Transplant:* As used herein, the term "transplant" or "transplantation" refers to the transfer of cells, tissues and/or organ(s) to an individual. In some embodiments, the transferred cells, tissues, and/or organ(s) are allogeneic cells, tissues and/or organ(s) from another individual (e.g., an individual of the same species as the recipient). In some embodiments, the transferred cells, tissues and/or organ(s) are autologous cells, tissues and/or organ(s) taken from the same individual. In some embodiments, the autologous cells, tissues and/or organ(s) from the individual are taken at an earlier time point. In some embodiments, the transferred cells are blood cells (e.g., cord blood cells). In some embodiments, the transferred cells are manipulated or expanded blood cells (e.g., cord blood cells). In some embodiments, the transferred cells are bone marrow cells. In some embodiments, the transferred cells are adult stem cells. In some embodiments the transferred cells are embryonic stem cells. In other embodiments, the transferred cells are induced pluripotent stem cells.

[0052] *Treating*: As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0053] The present invention provides, among other things, improved compositions and methods for treating or reducing risk of GVHD based on the use of angiotensin-(1-7) peptides, angiotensin (1-7) receptor agonists, and/or functional equivalents, analogs or derivatives thereof.

[0054] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Graft-Versus-Host Disease

[0055] An angiotensin (1-7) peptide and/or angiotensin (1-7) receptor agonist as described herein can be used for treating or reducing risk of GVHD. GVHD can occur when an immune response is mounted in response to allogeneic transplantation including, but not limited to, hematopoietic stem cell (HSC) transplantation such as bone marrow, peripheral blood (PBSC), or cord blood transplantation, other types of stem cell transplantation, and/or solid organ transplantation. In some embodiments, GVHD may also occurs with lower frequency following syngeneic and autologous transplant.

[0056] GVHD is well known in the art (see, for example, Ferrara, J. et al., "Graft-versus-host disease" Lancet. 2009;373(9674):1550-61; MacMillan, M. et al., "Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors," Blood, 2009, 113(11): 2410-2415; Matsumura, T. .et al., "Allogeneic cord blood transplantation for adult acute lymphoblastic leukemia: retrospective survey involving 256 patients in Japan," Leukemia. 2012 Jan 17 epub; Kobayashi, K. et al., "Clinical outcomes of unrelated donor umbilical cord blood transplantation for 30 adults with hematological malignancies," Anticancer Res., 2009 (5):1763-70)). An individual's immune system functions through recognition of certain cell surface proteins, some of which are termed major histocompatibility complex proteins, or MHC proteins. Additional minor histocompatibility proteins exist which can also contribute to immunological recognition events. The individual mammal's immune system can recognize its own MHC proteins, or those of its identical twin, as self and thus does not destroy its own cells or those of its identical twin. Members of the same species may share major and/or minor histocompatibility antigens, and thus an individual may not recognize the cells of another member of its species as non-self, depending on the degree of the differences between the MHC proteins of the two individuals. When an individual's immune system recognizes the cells of other members of the same species as non-self, the first individual's immune system can proceed

to destroy the cells of the second individual. In humans, the major histocompatibility proteins are known as "HLA" antigens.

[0057] When tissues such as bone marrow, blood cells, or solid organs are transplanted from one individual to another, the recipient may recognize the donor's cells as non-self and the recipient's immune system may destroy the donor's cells. For this reason, in a tissue transplantation, the recipient can be subjected to immunosuppressive drugs and/or irradiation. However, in GVHD, transplantation patients can also be subject to immunologic recognition in the opposite direction, that is, the donor tissue may contain immunologically competent cells which proceed to destroy the recipient's cells.

[0058] GVHD can develop when any allogeneic cells, for example cord blood, bone marrow, peripheral blood, adult stem cells, embryonic stem cells, blood products, and/or solid organs containing immunocompetent cells are transferred from a donor to a recipient. Thus, when MHC antigenic differences exist between the donor and recipient, the recipient is at risk for the development of GVHD. T-lymphocytes from the donor recognize the differences based HLA antigens and attack the new body, i.e., the recipient's body. Although most patients and donors are matched as closely as possible for HLA markers. In particular, GVHD results when immunocompetent T cells in the donor graft are infused into an immuno-compromised recipient. Many minor markers, however, differ between donors and patients except when the patient and donor are identical twins. Before a transplant, extensive typing of the donor and recipient is performed to make sure that the donor and recipient are very close immunologically. GVHD may also develop when there are antigenic differences between donor and recipient for the minor histocompatibility antigens. Thus, GVHD can also develop between MHC-matched persons. Moreover, surgery patients who receive directed blood transfusion, for example, transfusion of blood from an HLA homozygous child to a heterozygous parent, may also develop GVHD. In some embodiments, GVHD occurs when the blood is transfused into an immuno-compromised patient (e.g. organ transplant patients on high dose immunosuppressives, children with primary immunodeficiencies, or (years ago) into HIV infected patients with AIDS.

[0059] There are two known forms of GVHD - acute and chronic GVHD. Acute GVHD can occur within the first 100 days following a transplant. Without wishing to be held to a

particular theory, it is thought that T-cells present in the donor's tissue and/or cells at the time of transplant can attack the patient's skin, liver, stomach, and/or intestines. The earliest signs of acute GVHD can be a skin rash that appears on the hand, feet and face. Other than blistering skin, patients with severe GVHD can also develop large amounts of watery or bloody diarrhea with cramping from donor T-cells attacking the stomach and intestines. Jaundice (yellowing of the skin and eyes) is a usual indication that GVHD disease involves the liver. The severity of acute GVHD disease can be assessed by the number of organs involved and the degree of symptoms.

[0060] Cases of acute GVHD can be categorized into stage depending on clinical severity (see, for example, Irani, J. et al., "Severe acute gastrointestinal graft-vs-host disease: an emerging surgical dilemma in contemporary cancer care," Arch Surg. 2008;143(11):1041-5). Stage 1 comprises a skin rash over less than 25% of the body. Stage 2 comprises a skin rash over more than 25% of the body accompanied by mild liver or stomach and intestinal disorders. Stage 3 comprises redness of the skin, similar to a severe sunburn, and moderate liver, stomach and intestinal problems. Stage 4 comprises blistering, peeling skin, and severe liver, stomach, and intestinal problems. Additionally or alternatively, acute GVHD can also be characterized into five Clinical Grades 0, I, II, III, and IV. Typically, Grade 0 is substantially symptom free with respect to skin, liver, gut or functional impairment. Grade I is considered mild with skin stage of 1 to 2. Grade II is considered moderate and characterized with skin stage of 1 to 3, liver and gut stage of 1 and functional impairment stage of 1. Grade III is considered severe and characterized with skin stage of 2 to 3, liver and gut stage of 2-3, and functional impairment stage of 2. Grade IV is considered life-threatening and characterized with skin stage of 2 to 4, liver and gut stage of 2 to 4, and functional impairment stage of 3. Exemplary detailed staging and grading are further described in the Examples section.

[0061] Chronic GVHD can occur after the first 100 days following a transplant. Without wishing to be held to a particular theory, it is thought that Chronic GVHD can be caused by T-cells produced by engrafted tissue and/or cells. The same organs and systems can be attacked as in acute GVHD and additionally chronic GVHD can be associated with damage to connective tissue. Patients with chronic GVHD can experience skin problems that may include a dry itching rash, a change in skin color, and tautness or tightening of the skin. Partial hair loss or premature

graying may also occur. Similarly to patients with acute GVHD, patients with chronic GVHD may show jaundice as a sign of liver involvement. Chronic GVHD can also attack glands in the body that secrete mucous, saliva or other lubricants. Patients with chronic GVHD can experience dryness or stinging in their eyes due to impairment of the lacrimal gland. Glands that secrete saliva in the mouth can also be affected by chronic GVHD and, less often, those that lubricate the esophagus, making swallowing and eating difficult. Patients with chronic GVHD can experience a burning sensation in their mouths when using toothpaste or eating acidic foods. Chronic GVHD can attack glands that lubricate the stomach lining and intestines, interfering with the body's ability to properly absorb nutrients. Symptoms can include heartburn, stomach pain and/or weight loss. Occasionally patients with chronic GVHD can experience "contractures," a tightening of tendons that makes extending or contracting their arms and legs difficult. Chronic GVHD can also affect the lungs, causing wheezing, bronchitis, and/or pneumonia.

[0062] The incidence of GVHD can increase with increasing degree of mismatch between donor and recipient HLA antigens, increasing donor age, and increasing patient age. However, the disease may be underdiagnosed and underreported.

Transplantation and GVHD

[0063] As described above, GVHD can occur after a recipient receives a transplant of allogeneic cells, tissues or organs (for example cord blood, bone marrow, adult stem cells, embryonic stem cells, blood products, and/or solid organs). One type of transplant that can result in GVHD is a cord blood transplant.

Cord Blood Transplantation

[0064] Cord blood comprises red blood cells, white blood cells, plasma, platelets, and hematopoietic stem cells. Cord blood is of particular interest in treating bone marrow malignancies because of the high proportion of hematopoietic stem cells.

[0065] Cord blood can be obtained from the placenta or umbilical cord after childbirth. In some embodiments, cord blood can be obtained by sterilely puncturing the vein of the severed umbilical cord and collecting the blood. In some embodiments, the retrieved blood can be

cryopreserved. Methods of obtaining and storing cord blood are well known in the art (see, for example, Sirchia, G. et al., "Placental/umbilical cord blood transplantation," Haematologica. 1999 Aug;84(8):738-47). Cord blood is transplanted intraveneously.

[0066] Cord blood transplantation can involve the transplantation of cord blood cells into patients with naturally and/or artificially reduced numbers of hematopoietic stem cells to increase their number of hematopoietic stem cells and/or into patients whose naturally occurring hematopoietic stem cells are suffering from a disease, disorder or condition to replace their hematopoietic stem cells with healthy stem cells.

[0067] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a cord blood transplantation to treat hematologic malignancies. Hematologic malignancies are a closely related group of cancers that affect blood, bone marrow, and/or lymph nodes. In some embodiments, hematologic malignancies can include leukemias, including but not limited to acute lymphoblastic leukemia (also called acute lymphocytic leukemia); acute myeloid leukemia (also called acute myelogenous leukemia) such as but not limited to myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia leukemias and myelodysplastic syndrome; chronic lymphocytic leukemia (also called chronic lymphocytic leukemia); chronic myelogenous leukemia (also called chronic myeloid leukemia) and/ or hairy cell leukemia. In some embodiments, hematologic malignancies can include lymphomas, including but not limited to Hodgkin's lymphoma and non-Hodgkin's lymphoma. In some embodiments, hematologic malignancies can include myelomas.

[0068] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a cord blood transplantation to treat diseases that affect bone marrow production. Diseases that affect bone marrow production can include, but are not limited to sickle cell anemia, aplastic anemia, thalassemia, congenital neutropenia, and/or severe immunodeficiency syndromes.

[0069] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a cord blood transplantation to treat destruction and/or depletion of bone marrow by chemotherapy.

[0070] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a cord blood transplantation preceded by myeloablative therapy. In myeloablative therapy, substantially all of a patient's hematopoietic stem cells can be eliminated before transplantation, through cell killing or cell inactivation. This can be done through high doses of chemotherapy or radiation therapy. In some embodiments, a combination of cyclophosphamide with busulfan can be used. In some embodiments, total body irradiation can be used. In addition to ridding a patient of any diseased bone marrow, myeloablation can also suppress a subject's immune response, reducing the chance of rejection by the host. Methods of myeloablation are well known in the art as described, for example, in Sirchia et al. ("Placental/umbilical cord blood transplantation," Haematologica. 1999 Aug;84(8):738-47).

[0071] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a cord blood transplantation preceded with myeloablative therapy. In non-myeloablative therapy, chemotherapy and radiation can be given before transplantation at dosages sufficient to transiently injure but not eliminate a patient's hematopoietic stem cells. Methods for non-myeloablative therapy are well known in the art, as described for example, in Spitzer, T. "Nonmyeloablative Allogeneic Stem Cell Transplant Strategies and the Role of Mixed Chimerism," (The Oncologist, June 2000, 5(3): 215-223).

[0072] In transplantation preceded by non-myeloablative therapy, once the cord blood cells are transplanted, a chimeric hematopoietic stem cell population can exist, consisting of the patient's original cells and donor cells. Non-myeloablative transplantations relies on the principal of the "graft- versus-malignancy" (GVM), "graft-versus-tumor," or "graft-versus-leukemia" effect. Without wishing to be held to any particular theory, once the donor stem cells are infused into the recipient, the "new" immune system can recognize that any remaining cancer cells are abnormal and destroys them.

[0073] Transplantations preceded by non-myeloablative therapy can be used in patients who are older or who have other medical conditions that would make them unable to tolerate the toxic chemotherapy effects of regular transplants. Transplantations preceded by non-

myeloablative therapy can also have a role in treating patients who are in remission with a high-risk cancer, such as acute myelogenous leukemia, or who have had a relapse after a previous transplant.

Types of cord blood transplantations

[0074] The success of cord blood transplantations can be highly dependent on the number of cells transplanted. One disadvantage of cord blood transplants is that the number of cells contained in any one cord blood unit is small. On average, banked cord blood units have 120×10^7 nucleated cells, whereas the nucleated cell dose required for successful engraftment is 3.7×10^7 nucleated cells/kg. (Locatelli, F. et al., "Factors Associated With Outcome After Cord Blood Transplantation in Children With Acute Leukemia," Blood, 1999, 93:3662-3671 and Kobayashi, K. et al., "Clinical outcomes of unrelated donor umbilical cord blood transplantation for 30 adults with hematological malignancies," Anticancer Res., 2009 (5):1763-70). Therefore, only a fraction of banked units have sufficient cells for a transplant.

Single cord blood transplantation

[0075] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a single cord blood transplantation. In single cord blood transplantations, a single unit of cord blood from a single individual is transplanted to a patient. As discussed above, the single unit must have a sufficient number of cells for the patient. The use of a single cord blood transplant can be advantageous, given that only a single type of donor cells is introduced.

Manipulated cord blood transplantation

[0076] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a manipulated cord blood transplantation (e.g., manipulated to deplete T-cells or treated with prostaglandins). T-cell depletion can reduce the risk and/or severity of GVHD. Methods for depleting T-cells *ex vivo* can rely on negative selection with anti-T cell antibodies and are well known in the art as described in Ho, V. et al., "The history and future of T-cell depletion as graft-versus-host disease

prophylaxis for allogeneic hematopoietic stem cell transplantation", (Blood, 98(12): 3192-3204, 2001).

[0077] In some embodiments, manipulated cord blood comprises cord blood treated with prostaglandins. Treatment with prostaglandins can expand the population of hematopoietic stem cells in the cord blood. Experimental data in zebrafish and mouse models has demonstrated that pretreatment with prostaglandin can be used to increase hematopoietic stem cell formation *ex vivo* (for example, see North, T. et al., "Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis", Nature 447, 1007-1011, 2007). In some embodiments, cord blood cells are treated with PGE2.

Expanded cord blood transplantation

[0078] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving an expanded cord blood transplantation. In some embodiments, expanded cord blood comprises cord blood in which hematopoietic stem cells in cord blood are expanded *ex vivo* prior to administration by selection for CD34+ cells, as is well known in the art and described, for example, in Shpall, E. et al., "Transplantation of Ex Vivo Expanded Cord Blood," Biology of Blood and Marrow Transplantation 8:368-376, 2002. In some embodiments, expanded cord blood comprises cord blood in which hematopoietic stem cells in cord blood are expanded *ex vivo* prior to administration by proliferation of CD34+ cells, via activation of Notch signaling, as is well known in the art and described, for example, in Delaney, C.et al., "Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution," (Nature Medicine 2010; 16, 232-236). In some embodiments, expanded cord blood comprises cord blood in which hematopoietic stem cells in cord blood are expanded *ex vivo* prior to administration by treatment with nicotinamide, as described, for example, in Peled, T. et al., "Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment" (Exp Hematol. 2012;40(4):342-355).

Double cord blood transplantation

[0079] In some embodiments, an angiotensin (1-7) peptide described herein can be administered to treat or reduce the risk of GVHD in patients receiving a double cord blood transplantation. Methods for double cord blood transplantation are well known in the art, as described, for example, by Sideri, A. et al., in "An overview of the progress on double umbilical cord blood transplantation", (Haematologica. 2011 Aug;96(8):1213-20) and MacMillan, M. et al. in "Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors," (Blood, 2009, 113(11): 2410-2415). In some embodiments, a double cord blood transplantation comprises simultaneous administration of two units of cord blood from separate donors. When such a transplant is performed, there can be an initial state of chimerism, with blood cells from both of the donors being present, followed by predominance of blood from one of the donors. While double cord blood transplantations over single cord blood transplantations can increase cells for transplantation, they can also increase risk of graft versus graft disease.

[0080] One important consideration in cord blood transplantation is HLA typing. The risk of GVHD has been shown to decrease with increased HLA matching. A match of at least 4 of 6 markers is required at HLA-A,-B, and-DRB1. These minimum requirements are based on research studies of transplant outcomes. Patients receiving transplants preceded with non-myeloablative therapy can often develop at least a mild form of chronic GVHD that is thought to be associated with the "graft-versus-tumor" effect.

Other Types of Transplantation

[0081] GVHD can also occur when a patient receives additional types of transplants known in the art. Accordingly, in some embodiments, an angiotensin (1-7) peptide described herein is administered to treat or reduce the risk of GVHD in patients receiving these types of transplants. Non-limiting examples of such transplants include, for example, bone marrow transplantation (see, for example, Blazar, B. et al., "Bone marrow transplantation and approaches to avoid graft-versus-host disease (GVHD)," Philos Trans R Soc Lond B Biol Sci., 2005, 360(1461):1747-67) adult stem cell transplantation (see, for example, Krstevska, S. et al., "Acute graft versus host disease in hematopoietic stem cell allotransplant recipients," Med Arh. 2011;65(5):260-4), embryonic stem cell transplantation (see, for example, Burt, R., et al.,

"Embryonic stem cells as an alternate marrow donor source: engraftment without graft-versus-host disease," J Exp Med. 2004, 199(7):895-904), organ transplantation (see, for example, Kim, J. et at., "Graft-versus-host disease after kidney transplantation", J Korean Surg Soc. 2011, Suppl 1:S36-9), and/or blood transfusion (see, for example, Sun, X. et al., "Transfusion-associated graft-versus-host-disease: case report and review of literature", Transfus Apher Sci. 2010, 43(3):331-4). It is well within the skill of those in the art to determine when and/or to what extent a transplant described above should be administered to a subject.

Mucositis

[0082] In some transplant recipients, mucositis can occur. Generally, mucositis is caused by damage to mucosal tissues throughout the body including, but not limited to, oral, buccal, sublingual, nasal, vaginal, rectal, aural, lung, and gastrointestinal mucosa. Typically, damage to mucosal tissues are caused by inflammation and involves redness and ulcerative sores in the soft tissues of the mucosa. For example, mucositis can occur anywhere along the gastrointestinal (GI) tract. In some embodiments, mucositis occurs in the mouth, also referred to as oral mucositis. In addition to transplantation, mucositis usually occurs as an adverse effect of chemotherapy and radiotherapy treatment for cancer.

[0083] Mucositis can be categorized into clinical grade depending on clinical severity. Various methods an instruments have been developed to stage and measure mucositis severity including, but not limited to, National Cancer Institute's Common Toxicity Criteria (NCI CTC), World Health Organization's (WHO's) Oral Toxicity Scale (OTS). In particular, WHO's oral toxicity scale includes Grade 1, 2,3 and 4. Grade 1 involves soreness with or without erythema. Grade 2 includes erythema and ulcers. Patients can swallow solid food. Grade 3 involves ulcers with extensive erythema. Patient cannot swallow food. Grade 4 involves mucositis to the extent that alimentation is not possible. Generally, Grade 3 and 4 mucositis are considered severe. Exemplary detailed grading is further described in the Example section.

Angiotensin (1-7) peptides

[0084] As used herein, the term "angiotensin (1-7) peptide" refers to both naturally-occurring Angiotensin (1-7) and any functional equivalent, analogue or derivative of naturally-

occurring Angiotensin (1-7). As used herein, "peptide" and "polypeptide" are interchangeable terms and refer to two or more amino acids bound together by a peptide bond. As used herein, the terms "peptide" and "polypeptide" include both linear and cyclic peptide. The terms "angiotensin-(1-7)", "Angiotensin-(1-7)", "Ang-(1-7)", and "TXA-127" are used interchangeably.

Naturally-occurring Angiotensin (1-7)

[0085] Naturally-occurring Angiotensin (1-7) (also referred to as Ang-(1-7)) is a seven amino acid peptide shown below:

Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:1)

It is part of the renin-angiotensin system and is converted from a precursor, also known as Angiotensinogen, which is an α-2-globulin that is produced constitutively and released into the circulation mainly by the liver. Angiotensinogen is a member of the serpin family and also known as renin substrate. Human angiotensinogen is 452 amino acids long, but other species have angiotensinogen of varying sizes. Typically, the first 12 amino acids are the most important for angiotensin activity:

Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}-His^{9}-Leu^{10}-Val^{11}-Ile^{12} (SEQ ID NO:4)

[0086] Different types of angiotensin may be formed by the action of various enzymes. For example, Angiotensin (1-7) is generated by action of Angiotensin-converting enzyme 2 (ACE 2).

[0087] Ang-(1-7) is an endogenous ligand for Mas receptors. Mas receptors are G-protein coupled receptor containing seven transmembrane spanning regions. As used herein, the term "angiotensin-(1-7) receptor' encompasses the G Protein-Coupled Mas Receptors.

[0088] As used herein, the term "naturally-occurring Angiotensin (1-7)" includes any Angiotensin (1-7) peptide purified from natural sources and any recombinantly produced or chemically synthesized peptides that have an amino acid sequence identical to that of the naturally-occurring Angiotensin (1-7).

Functional equivalents, analogs or derivatives of Ang-(1-7)

[0089] In some embodiments, an angiotensin (1-7) peptide suitable for the present invention is a functional equivalent of naturally-occurring Ang-(1-7). As used herein, a functional equivalent of naturally-occurring Ang-(1-7) refers to any peptide that shares amino acid sequence identity to the naturally-occurring Ang-(1-7) and retain substantially the same or similar activity as the naturally-occurring Ang-(1-7). For example, in some embodiments, a functional equivalent of naturally-occurring Ang-(1-7) described herein has pro-angiogenic activity as determined using methods described herein or known in the art, or an activity such as nitric oxide release, vasodilation, improved endothelial function, antidiuresis, or one of the other properties discussed herein, that positively impacts angiogenesis. In some embodiments, a functional equivalent of naturally-occurring Ang-(1-7) described herein can bind to or activate an angiotensin-(1-7) receptor (e.g., the G protein-coupled Mas receptor) as determined using various assays described herein or known in the art. In some embodiments, a functional equivalent of Ang-(1-7) is also referred to as an angiotensin (1-7) analogue or derivative, or functional derivative.

[0090] Typically, a functional equivalent of angiotensin (1-7) shares amino acid sequence similarity to the naturally-occurring Ang-(1-7). In some embodiments, a functional equivalent of Ang-(1-7) according to the invention contains a sequence that includes at least 3 (e.g., at least 4, at least 5, at least 6, at least 7) amino acids from the seven amino acids that appear in the naturally-occurring Ang-(1-7), wherein the at least 3 (e.g., at least 4, at least 5, at least 6, or at least 7) amino acids maintain their relative positions and/or spacing as they appear in the naturally-occurring Ang-(1-7).

[0091] In some embodiments, a functional equivalent of Ang-(1-7) also encompasses any peptide that contains a sequence at least 50% (e.g., at least 60%, 70%, 80%, or 90%) identical to the amino acid sequence of naturally-occurring Ang-(1-7). Percentage of amino acid sequence identity can be determined by alignment of amino acid sequences. Alignment of amino acid sequences can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring

alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Preferably, the WU-BLAST-2 software is used to determine amino acid sequence identity (Altschul *et al.*, Methods in Enzymology 266, 460-480 (1996); http://blast.wustl/edu/blast/README.html). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=11. HSP score (S) and HSP S2 parameters are dynamic values and are established by the program itself, depending upon the composition of the particular sequence, however, the minimum values may be adjusted and are set as indicated above.

[0092] In some embodiments, a functional equivalent, analogue or derivative of Ang-(1-7) is a fragment of the naturally-occurring Ang-(1-7). In some embodiments, a functional equivalent, analogue or derivative of Ang-(1-7) contains amino acid substitutions, deletions and/or insertions in the naturally-occurring Ang-(1-7). Ang-(1-7) functional equivalents, analogues or derivatives can be made by altering the amino acid sequences by substitutions, additions, and/or deletions. For example, one or more amino acid residues within the sequence of the naturally-occurring Ang-(1-7) (SEQ ID NO:1) can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitution for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the positively charged (basic) amino acids include arginine, lysine, and histidine. The nonpolar (hydrophobic) amino acids include leucine, isoleucine, alanine, phenylalanine, valine, proline, tryptophan, and methionine. The uncharged polar amino acids include serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The negatively charged (acid) amino acids include glutamic acid and aspartic acid. The amino acid glycine may be included in either the nonpolar amino acid family or the uncharged (neutral) polar amino acid family. Substitutions made within a family of amino acids are generally understood to be conservative substitutions. For example, the amino acid sequence of a peptide inhibitor can be modified or substituted.

[0093] Examples of Ang-(1-7) functional equivalents, analogues and derivatives are described in the section entitled “Exemplary Angiotensin(1-7) Peptides” below.

[0094] An angiotensin-(1-7) peptide can be of any length. In some embodiments, an angiotensin-(1-7) peptide according to the present invention can contain, for example, from 5-25 amino acid residues, such as 5-20, 5-15 or 5-10 amino acid residues. In some embodiments, an Ang-(1-7) peptide according to the present invention contain 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 residues.

[0095] In some embodiments, an angiotensin-(1-7) peptide contains one or more modifications to increase protease resistance, serum stability and/or bioavailability. In some embodiments, suitable modifications are selected from pegylation, acetylation, glycosylation, biotinylation, substitution with D-amino acid and/or un-natural amino acid, and/or cyclization of the peptide.

[0096] As used herein, the term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In certain embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In certain embodiments, an amino acid is a naturally-occurring amino acid. In certain embodiments, an amino acid is a synthetic or un-natural amino acid (e.g., α,α-disubstituted amino acids, N-alkyl amino acids); in some embodiments, an amino acid is a D-amino acid; in certain embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard amino acids commonly found in naturally occurring peptides including both L- and D- amino acids which are both incorporated in peptides in nature. "Nonstandard" or "unconventional amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, "synthetic or un-natural amino acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting its activity. Examples of unconventional or un-natural amino acids include, but are not limited to, citrulline, ornithine, norleucine, norvaline, 4-(*E*)-butenyl-4(*R*)-methyl-N-methylthreonine (MeBmt), N-methyl-leucine (MeLeu), aminoisobutyric acid, statine, and N-methyl-alanine (MeAla). Amino acids may participate in a disulfide bond. The term "amino acid" is used interchangeably with "amino

acid residue," and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0097] In certain embodiments, angiotensin-(1-7) peptides contain one or more L-amino acids, D-amino acids, and/or un-natural amino acids.

[0098] In addition to peptides containing only naturally occurring amino acids, peptidomimetics or peptide analogs are also encompassed by the present invention. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. The non-peptide compounds are termed "peptide mimetics" or peptidomimetics (Fauchere et al., *Infect. Immun.* 54:283-287 (1986); Evans et al., *J. Med. Chem.* 30:1229-1239 (1987)). Peptide mimetics that are structurally related to therapeutically useful peptides and may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to the paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity) such as naturally-occurring receptor-binding polypeptides, but have one or more peptide linkages optionally replaced by linkages such as $–CH_2NH–$, $–CH_2S–$, $–CH_2–CH_2–$, –CH=CH– (cis and trans), $–CH_2SO–$, $–CH(OH)CH_2–$, $–COCH_2–$ etc., by methods well known in the art (Spatola, Peptide Backbone Modifications, Vega Data, 1(3):267 (1983); Spatola et al. *Life Sci.* 38:1243-1249 (1986); Hudson et al. *Int. J. Pept. Res.* 14:177-185 (1979); and Weinstein. B., 1983, Chemistry and Biochemistry, of Amino Acids, Peptides and Proteins, Weinstein eds, Marcel Dekker, New-York,). Such peptide mimetics may have significant advantages over naturally-occurring polypeptides including more economical production, greater chemical stability, enhanced pharmacological properties (e.g., half-life, absorption, potency, efficiency, etc.), reduced antigenicity and others.

[0099] Angiotensin (1-7) peptides also include other types of peptide derivatives containing additional chemical moieties not normally part of the peptide, provided that the derivative retains the desired functional activity of the peptide. Examples of such derivatives include (1) N-acyl derivatives of the amino terminal or of another free amino group, wherein the acyl group may be an alkanoyl group (e.g., acetyl, hexanoyl, octanoyl) an aroyl group (e.g.,

benzoyl) or a blocking group such as F-moc (fluorenylmethyl–O–CO–); (2) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (3) amide of the carboxy-terminal or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (4) phosphorylated derivatives; (5) derivatives conjugated to an antibody or other biological ligand and other types of derivatives; and (6) derivatives conjugated to a polyethylene glycol (PEG) chain.

[0100] Angiotensin (1-7) peptides may be obtained by any method of peptide synthesis known to those skilled in the art, including synthetic (e.g., exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, classical solution synthesis, native-chemical ligation) and recombinant techniques. For example, the peptides or peptides derivatives can be obtained by solid phase peptide synthesis, which in brief, consist of coupling the carboxyl group of the C-terminal amino acid to a resin (e.g., benzhydrylamine resin, chloromethylated resin, hydroxymethyl resin) and successively adding N-alpha protected amino acids. The protecting groups may be any such groups known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. Such solid phase synthesis has been disclosed, for example, by Merrifield, *J. Am. Chem. Soc.* 85: 2149 (1964); Vale et al., *Science* 213:1394-1397 (1981), in U.S. Patent Numbers 4,305,872 and 4,316,891, Bodonsky et al. *Chem. Ind.* (London), 38:1597 (1966); and Pietta and Marshall, *Chem. Comm.* 650 (1970) by techniques reviewed in Lubell et al. "Peptides" Science of Synthesis 21.11, *Chemistry of Amides*. Thieme, Stuttgart, 713-809 (2005). The coupling of amino acids to appropriate resins is also well known in the art and has been disclosed in U.S. Patent Number 4,244,946. (Reviewed in Houver-Weyl, *Methods of Organic Chemistry.* Vol E22a. Synthesis of Peptides and Peptidomimetics, Murray Goodman, Editor-in-Chief, Thieme. Stuttgart. New York 2002).

[0101] Unless defined otherwise, the scientific and technological terms and nomenclature used herein have the same meaning as commonly understood by a person of ordinary skill to which this invention pertains. Generally, the procedures of cell cultures, infection, molecular biology methods and the like are common methods used in the art. Such standard techniques can be found in reference manuals such as, for example, Ausubel *et al., Current Protocols in*

Molecular Biology, Wiley Interscience, New York, 2001; and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001.

[0102] During any process of the preparation of an Angiotensin (1-7) peptide, it may be desirable to protect sensitive reactive groups on any of the molecule concerned. This may be achieved by means of conventional protecting groups such as those described in Protective Groups In Organic Synthesis by T.W. Greene & P.G.M. Wuts, 1991, John Wiley and Sons, New-York; and Peptides: chemistry and Biology by Sewald and Jakubke, 2002, Wiley-VCH, Wheinheim p.142. For example, alpha amino protecting groups include acyl type protecting groups (e.g., trifluoroacetyl, formyl, acetyl), aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (BOC), cyclohexyloxycarbonyl), aromatic urethane type protecting groups (e.g., fluorenyl-9-methoxy-carbonyl (Fmoc), benzyloxycarbonyl (Cbz), Cbz derivatives) and alkyl type protecting groups (e.g., triphenyl methyl, benzyl). The amino acids side chain protecting groups include benzyl (for Thr and Ser), Cbz (Tyr, Thr, Ser, Arg, Lys), methyl ethyl, cyclohexyl (Asp, His), Boc (Arg, His, Cys) etc. The protecting groups may be removed at a convenient subsequent stage using methods known in the art.

[0103] Further, Angiotensin (1-7) peptides may be synthesized according to the FMOC protocol in an organic phase with protective groups. Desirably, the peptides are purified with a yield of 70% with high-pressure liquid chromatography (HPLC) on a C18 chromatography column and eluted with an acetonitrile gradient of 10-60%. The molecular weight of a peptide can be verified by mass spectrometry (reviewed in Fields, G.B. "Solid-Phase Peptide Synthesis" *Methods in Enzymology.* Vol. 289, Academic Press, 1997).

[0104] Alternatively, Angiotensin (1-7) peptides may be prepared in recombinant systems using, for example, polynucleotide sequences encoding the polypeptides. It is understood that a polypeptide may contain more than one of the above-described modifications within the same polypeptide.

[0105] While peptides may be effective in eliciting a biological activity *in vitro*, their effectiveness *in vivo* might be reduced by the presence of proteases. Serum proteases have specific substrate requirements. The substrate must have both L-amino acids and peptide bonds for cleavage. Furthermore, exopeptidases, which represent the most prominent component of the

protease activity in serum, usually act on the first peptide bond of the peptide and require a free N-terminus (Powell et al., *Pharm. Res.* 10:1268-1273 (1993)). In light of this, it is often advantageous to use modified versions of peptides. The modified peptides retain the structural characteristics of the original L-amino acid peptides that confer the desired biological activity of Ang-(1-7) but are advantageously not readily susceptible to cleavage by protease and/or exopeptidases.

[0106] Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. Thus, a peptide derivative or peptidomimetic of the present invention may be all L, all D or mixed D, L peptide, in either forward or reverse order. The presence of an N-terminal or C-terminal D-amino acid increases the *in vivo* stability of a peptide since peptidases cannot utilize a D-amino acid as a substrate (Powell et al., *Pharm. Res.* 10:1268-1273 (1993)). Reverse-D peptides are peptides containing D-amino acids, arranged in a reverse sequence relative to a peptide containing L-amino acids. Thus, the C-terminal residue of an L-amino acid peptide becomes N-terminal for the D-amino acid peptide, and so forth. Reverse D-peptides retain the same secondary conformation and therefore similar activity, as the L-amino acid peptides, but are more resistant to enzymatic degradation *in vitro* and *in vivo*, and thus can have greater therapeutic efficacy than the original peptide (Brady and Dodson, *Nature* 368:692-693 (1994); Jameson et al., *Nature* 368:744-746 (1994)). Similarly, a reverse-L peptide may be generated using standard methods where the C-terminus of the parent peptide becomes takes the place of the N-terminus of the reverse-L peptide. It is contemplated that reverse L-peptides of L-amino acid peptides that do not have significant secondary structure (e.g., short peptides) retain the same spacing and conformation of the side chains of the L-amino acid peptide and therefore often have the similar activity as the original L-amino acid peptide. Moreover, a reverse peptide may contain a combination of L- and D-amino acids. The spacing between amino acids and the conformation of the side chains may be retained resulting in similar activity as the original L-amino acid peptide.

[0107] Another effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a peptide is to add chemical groups at the peptide termini, such that the modified peptide is no longer a substrate for the peptidase. One such chemical

modification is glycosylation of the peptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of peptides in human serum (Powell et al., *Pharm. Res.* 10:1268-1273 (1993)). Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-terminal alkyl group, consisting of a lower alkyl of from one to twenty carbons, such as an acetyl group, and/or the addition of a C-terminal amide or substituted amide group. In particular, the present invention includes modified peptides consisting of peptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

[0108] Substitution of non-naturally-occurring amino acids for natural amino acids in a subsequence of the peptides can also confer resistance to proteolysis. Such a substitution can, for instance, confer resistance to proteolysis by exopeptidases acting on the N-terminus without affecting biological activity. Examples of non-naturally-occurring amino acids include α,α -disubstituted amino acids, N-alkyl amino acids, C-α-methyl amino acids, β-amino acids, and β-methyl amino acids. Amino acids analogs useful in the present invention may include, but are not limited to, β-alanine, norvaline, norleucine, 4-aminobutyric acid, orithine, hydroxyproline, sarcosine, citrulline, cysteic acid, cyclohexylalanine, 2-aminoisobutyric acid, 6-aminohexanoic acid, t-butylglycine, phenylglycine, o-phosphoserine, N-acetyl serine, N-formylmethionine, 3-methylhistidine and other unconventional amino acids. Furthermore, the synthesis of peptides with non-naturally-occurring amino acids is routine in the art.

[0109] In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods well known in the art (Rizo and Gierasch, *Ann. Rev. Biochem.* 61:387-418 (1992)). For example, constrained peptides may be generated by adding cysteine residues capable of forming disulfide bridges and, thereby, resulting in a cyclic peptide. Cyclic peptides can be constructed to have no free N- or C-termini. Accordingly, they are not susceptible to proteolysis by exopeptidases, although they may be susceptible to endopeptidases, which do not cleave at peptide termini. The amino acid sequences of the peptides with N-terminal or C-terminal D-amino acids and of the cyclic peptides are usually identical to the sequences of the peptides to which they correspond, except for the presence of N-terminal or C-terminal D-amino acid residue, or their circular structure, respectively.

Cyclic Peptides

[0110] In some embodiments, a functional equivalent, analogue or derivative of naturally-occurring Ang-(1-7) is a cyclic peptide. As used herein, a cyclic peptide has an intramolecular covalent bond between two non-adjacent residues. The intramolecular bond may be a backbone to backbone, side-chain to backbone or side-chain to side-chain bond (i.e., terminal functional groups of a linear peptide and/or side-chain functional groups of a terminal or interior residue may be linked to achieve cyclization). Typical intramolecular bonds include disulfide, amide and thioether bonds. A variety of means for cyclizing polypeptides are well known in the art, as are many other modifications that can be made to such peptides. For a general discussion, see International Patent Publication Nos. WO 01/53331 and WO 98/02452, the contents of which are incorporated herein by reference. Such cyclic bonds and other modifications can also be applied to the cyclic peptides and derivative compounds of this invention.

[0111] Cyclic peptides as described herein may comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be from natural or non-natural sources, provided that at least one amino group and at least one carboxyl group are present in the molecule; α- and β-amino acids are generally preferred. Cyclic peptides may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (e.g., benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (e.g., acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (e.g., methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Suitable derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide prior to cyclization is acetylated) and/or a C-terminal amide group (i.e., the carboxy terminus of the linear peptide prior to cyclization is amidated). Residues other than common amino acids that may be present with a cyclic peptide include, but are not limited to, penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-

mercaptoaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, α-aminoadipic acid, m-aminomethylbenzoic acid and α,β-diaminopropionic acid.

[0112] Following synthesis of a linear peptide, with or without N-acetylation and/or C-amidation, cyclization may be achieved by any of a variety of techniques well known in the art. Within one embodiment, a bond may be generated between reactive amino acid side chains. For example, a disulfide bridge may be formed from a linear peptide comprising two thiol-containing residues by oxidizing the peptide using any of a variety of methods. Within one such method, air oxidation of thiols can generate disulfide linkages over a period of several days using either basic or neutral aqueous media. The peptide is used in high dilution to minimize aggregation and intermolecular side reactions. Alternatively, strong oxidizing agents such as I_2 and $K_3Fe(CN)_6$ can be used to form disulfide linkages. Those of ordinary skill in the art will recognize that care must be taken not to oxidize the sensitive side chains of Met, Tyr, Trp or His. Within further embodiments, cyclization may be achieved by amide bond formation. For example, a peptide bond may be formed between terminal functional groups (i.e., the amino and carboxy termini of a linear peptide prior to cyclization). Within another such embodiment, the linear peptide comprises a D-amino acid. Alternatively, cyclization may be accomplished by linking one terminus and a residue side chain or using two side chains, with or without an N-terminal acetyl group and/or a C-terminal amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), α-amino adipic acid, m-aminomethylbenzoic acid, α,β-diaminopropionic acid, glutamate or aspartate. Methods for forming amide bonds are generally well known in the art. Within one such method, carbodiimide-mediated lactam formation can be accomplished by reaction of the carboxylic acid with DCC, DIC, ED AC or DCCI, resulting in the formation of an O-acylurea that can be reacted immediately with the free amino group to complete the cyclization. Alternatively, cyclization can be performed using the azide method, in which a reactive azide intermediate is generated from an alkyl ester via a hydrazide. Alternatively, cyclization can be accomplished using activated esters. The presence of electron withdrawing substituents on the alkoxy carbon of esters increases their susceptibility to aminolysis. The high reactivity of esters of p-nitrophenol, N-hydroxy compounds and polyhalogenated phenols has made these "active esters" useful in the synthesis of amide bonds. Within a further embodiment, a thioether linkage may be formed between the side chain of a thiol-containing residue and an appropriately derivatized α-amino acid. By way of example, a lysine side chain can be coupled

to bromoacetic acid through the carbodiimide coupling method (DCC, EDAC) and then reacted with the side chain of any of the thiol containing residues mentioned above to form a thioether linkage. In order to form dithioethers, any two thiol containing side-chains can be reacted with dibromoethane and diisopropylamine in DMF.

Exemplary Angiotensin-(1-7) Peptides

Linear Angiotensin(1-7) Peptides

[0113] In certain aspects, the invention provides linear angiotensin-(1-7) peptides. As discussed above, the structure of naturally-occurring Ang-(1-7) is as follows:

Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:1)

[0114] The peptides and peptide analogs of the invention can be generally represented by the following sequence:

Xaa^1-Xaa^2-Xaa^3-Xaa^4-Xaa^5-Xaa^6-Xaa^7 (SEQ ID NO:5),

or a pharmaceutically acceptable salt thereof.

[0115] Xaa^1 is any amino acid or a dicarboxylic acid. In certain embodiments, Xaa^1 is Asp, Glu, Asn, Acpc (1-aminocyclopentane carboxylic acid), Ala, Me_2Gly (N,N-dimethylglycine), Pro, Bet (betaine, 1-carboxy-N,N,N-trimethylmethanaminium hydroxide), Glu, Gly, Asp, Sar (sarcosine) or Suc (succinic acid). In certain such embodiments, Xaa^1 is a negatively-charged amino acid, such as Asp or Glu, typically Asp.

[0116] Xaa^2 is Arg, Lys, Ala, Cit (citrulline), Orn (ornithine), acetylated Ser, Sar, D-Arg and D-Lys. In certain embodiments, Xaa^2 is a positively-charged amino acid such as Arg or Lys, typically Arg.

[0117] Xaa^3 is Val, Ala, Leu, Nle (norleucine), Ile, Gly, Lys, Pro, HydroxyPro (hydroxyproline), Aib (2-aminoisobutyric acid), Acpc or Tyr. In certain embodiments, Xaa^3 is an aliphatic amino acid such as Val, Leu, Ile or Nle, typically Val or Nle.

[0118] Xaa^4 is Tyr, Tyr(PO_3), Thr, Ser, homoSer (homoserine), azaTyr (aza-α^1-homo-L-tyrosine) or Ala. In certain embodiments, Xaa^4 is a hydroxyl-substituted amino acid such as Tyr, Ser or Thr, typically Tyr.

[0119] Xaa^5 is Ile, Ala, Leu, norLeu, Val or Gly. In certain embodiments, Xaa^5 is an aliphatic amino acid such as Val, Leu, Ile or Nle, typically Ile.

[0120] Xaa^6 is His, Arg or 6-NH_2-Phe (6-aminophenylalanine). In certain embodiments, Xaa^6 is a fully or partially positively-charged amino acid such as Arg or His.

[0121] Xaa^7 is Cys, Pro or Ala.

[0122] In certain embodiments, one or more of Xaa^1-Xaa^7 is identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In certain such embodiments, all but one or two of Xaa^1-Xaa^7 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa^1-Xaa^6 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7).

[0123] In certain embodiments, Xaa^3 is Nle. When Xaa^3 is Nle, one or more of Xaa^1-Xaa^2 and Xaa^{4-7} are optionally identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In certain such embodiments, all but one or two of Xaa^1-Xaa^2 and Xaa^{4-7} are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa^1-Xaa^2 and Xaa^{4-7} are identical to the corresponding amino acid in naturally-occurring Ang-(1-7), resulting in the amino acid sequence: Asp^1-Arg^2-Nle^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:2).

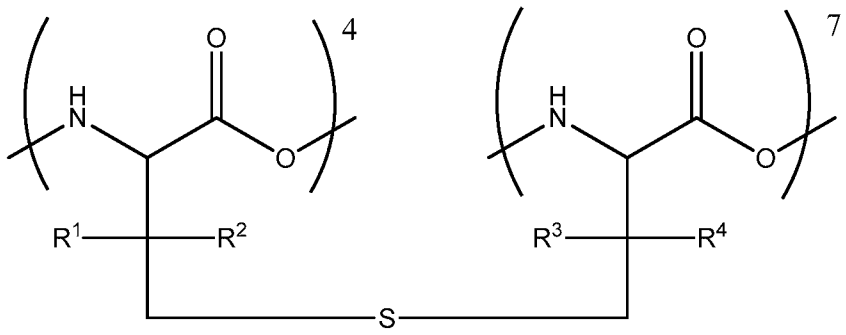
[0124] In certain embodiments, the peptide has the amino acid sequence Asp^1-Arg^2-Val^3-Ser^4-Ile^5-His^6-Cys^7 (SEQ ID NO:3) or Asp^1-Arg^2-Val^3-ser^4-Ile^5-His^6-Cys7 (SEQ ID NO:6).

Exemplary Cyclic Angiotensin (1-7) Peptides

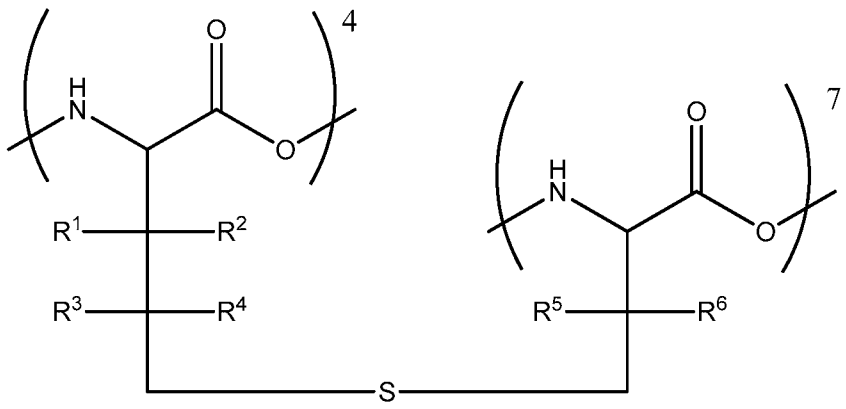
[0125] In certain aspects, the invention provides a cyclic angiotensin-(1-7) (Ang-(1-7)) peptide analog comprising a linkage, such as between the side chains of amino acids corresponding to positions Tyr^4 and Pro^7 in Ang. These peptide analogs typically comprise 7 amino acid residues, but can also include a cleavable sequence. As discussed in greater detail

below, the invention includes fragments and analogs where one or more amino acids are substituted by another amino acid (including fragments), for example, Asp^{1}-Arg^{2}-Val^{3}-Ser^{4}-Ile^{5}-His^{6}-Cys^{7} (SEQ ID NO:22), wherein a linkage is formed between Ser^{4} and Cys^{7}.

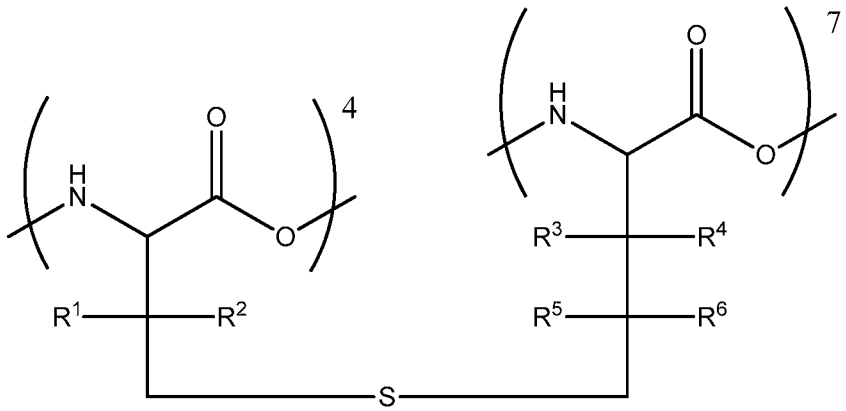
[0126] Although the following section describes aspects of the invention in terms of a thioether bond linking residues at the 4- and 7-positions, it should be understood that other linkages (as described above) could replace the thioether bridge and that other residues could be cyclized. A thioether bridge is also referred to as a monosulfide bridge or, in the case of Ala-S-Ala, as a lanthionine bridge. Thioether bridge-containing peptides can be formed by two amino acids having one of the following formulas:



Formula (I)



Formula (II)



Formula (III)

[0127] In these formulae, R^1, R^2, R^3, R^4, R^5 and R^6 are independently -H, an alkyl (e.g., C_1-C_6 alkyl, C_1-C_4 alkyl) or an aralkyl group, where the alkyl and aralkyl groups are optionally substituted with one or more halogen, -OH or –NRR' groups (where R and R' are independently –H or C_1-C_4 alkyl). In certain embodiments, R^1, R^2, R^3, R^4, R^5 and R^6 are each independently -H or -CH_3, such where all are –H.

[0128] In certain embodiments, the invention provides an Ang analog or derivative comprising a thioether bridge according to formula (I). Typically, R^1, R^2, R^3 and R^4 are independently selected from -H and -CH_3. Peptides comprising a thioether bridge according to formula (I) can be produced, for example, by lantibiotic enzymes or by sulfur extrusion of a disulfide. In one example, the disulfide from which the sulfur is extruded can be formed by D-cysteine in position 4 and L-cysteine in position 7 or by D-cysteine in position 4 and L-penicillamine in position 7 (see, e.g., Galande, Trent and Spatola (2003) *Biopolymers* 71, 534-551).

[0129] In other embodiments, the linkage of the two amino acids can be the bridges depicted in Formula (II) or Formula (III). Peptides comprising a thioether bridge according to Formula (II) can be made, for example, by sulfur extrusion of a disulfide formed by D-homocysteine in position 4 and L-cysteine in position 7. Similarly, peptides comprising a thioether bridge as in Formula (III) can be made, for example, by sulfur extrusion of a disulfide formed by D-cysteine in position 4 and L-homocysteine in position 7.

[0130] As discussed above, the Ang analogs and derivatives of the invention vary in length and amino acid composition. The Ang analogs and derivatives of the invention preferably have biological activity or are an inactive precursor molecule that can be proteolytically activated (such as how angiotensin (I), with 10 amino acids, is converted to active fragments by cleavage of 2 amino acids). The size of an Ang analog or derivative can vary but is typically between from about 5 to 10 amino acids, as long as the "core" pentameric segment comprising the 3-7 Nle-thioether-ring structure is encompassed. The amino acid sequence of an analog or derivative of the invention can vary, typically provided that it is biologically active or can become proteolytically activated. Biological activity of an analog or derivative can be determined using methods known in the art, including radioligand binding studies, *in vitro* cell activation assays and *in vivo* experiments. See, for example, Godeny and Sayeski, (2006) *Am. J. Physiol. Cell. Physiol.* 291:C1297-1307; Sarr *et al.*, *Cardiovasc. Res.* (2006) 71:794-802; and Koziarz *et al.*, (1933) *Gen. Pharmacol.* 24:705- 713.

[0131] Ang analogs and derivatives where only the length of the peptide is varied include the following:

a 4,7-cyclized analog designated [Cyc^{4-7}]Ang-(1-7), which is derived from natural Ang-(1-7) (Asp^1-Arg^2-Val^3-Cyc^4-Ile^5-His^6-Cyc^7, SEQ ID NO:7).

a 4,7-cyclized analog designated [Nle^3, Cyc^{4-7}]Ang-(1-10), which is derived from natural Angiotensin I (Ang-(1-10)) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8-His^9-Leu^{10}, SEQ ID NO:8);

a 4,7-cyclized analog designated [Nle^3, Cyc^{4-7}]Ang-(1-8), which is derived from natural Angiotensin II (Ang-(1-8)) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8, SEQ ID NO:9);

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(2-8), which is derived from natural Angiotensin III (Ang-(2-8)) (Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8, SEQ ID NO:10);

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(3-8), which is derived from natural Angiotensin IV (Ang-(3-8)) (Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8, SEQ ID NO:11);

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(1-7) derived from natural Ang-(1-7) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7, SEQ ID NO:12); and

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(1-9) derived from natural Ang-(1-9) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8-His^9, SEQ ID NO:13).

These analogs can have one of the thioether bridges shown in Formulae (I)-(III) as the Cyc^{4-7} moiety, for example, where Cyc^4 and Cyc^7 are represented by Formula (I), such as where R^1-R^4 are each –H or $–CH_3$, typically -H.

[0132] As compared to the amino acid sequence of the natural angiotensin peptide, the amino acids at positions 4 and 7 of the Cyc^{4-7} analog are modified to allow introduction of the thioether-ring structures shown above. In addition to the length of the Ang analogs, the amino acids at positions other than 3, 4 and 7 can be the same or different from the naturally-occurring peptide, typically provided that the analog retains a biological function. One example is Asp^1-Arg^2-Val^3-Ser^4-Ile^5-His^6-Cys^7 (SEQ ID NO:22). For analogs of inactive precursors, like [Cyc^{4-7}]Ang-(l-10), biological function refers to one or both of an analog's susceptibility to angiotensin-converting enzymes that can cleave it to a biologically active fragment (e.g. Ang-(l-8) or Ang-(l-7)) or the biological activity of the fragment itself. In certain embodiments, an Ang analog or derivative of the invention has no intrinsic function but inhibits the effects of one or more naturally-occurring angiotensin compounds.

[0133] In certain embodiments, an Ang analog of the invention is represented by Formula (IV):

$$Xaa^1\text{-}Xaa^2\text{-}Xaa^3\text{-}Cyc^4\text{-}Xaa^5\text{-}Xaa^6\text{-}Cyc^7 \text{ (IV, SEQ ID NO:14)}$$

[0134] Xaa^1 is any amino acid, but typically a negatively-charged amino acid such as Glu or Asp, more typically Asp.

[0135] Xaa^2 is a positively-charged amino acid such as Arg or Lys, typically Arg.

[0136] Xaa^3 is an aliphatic amino acid, such as Leu, Ile or Val, typically Val.

[0137] Cyc^4 forms a thioether bridge in conjunction with Cyc^7. Cyc^4 can be a D-stereoisomer and/or a L-stereoisomer, typically a D-stereoisomer. Examples of Cyc^4 (taken with Cyc^7) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulae (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0138] Xaa^5 is an aliphatic amino acid, such as Leu, Ile or Val, typically Ile.

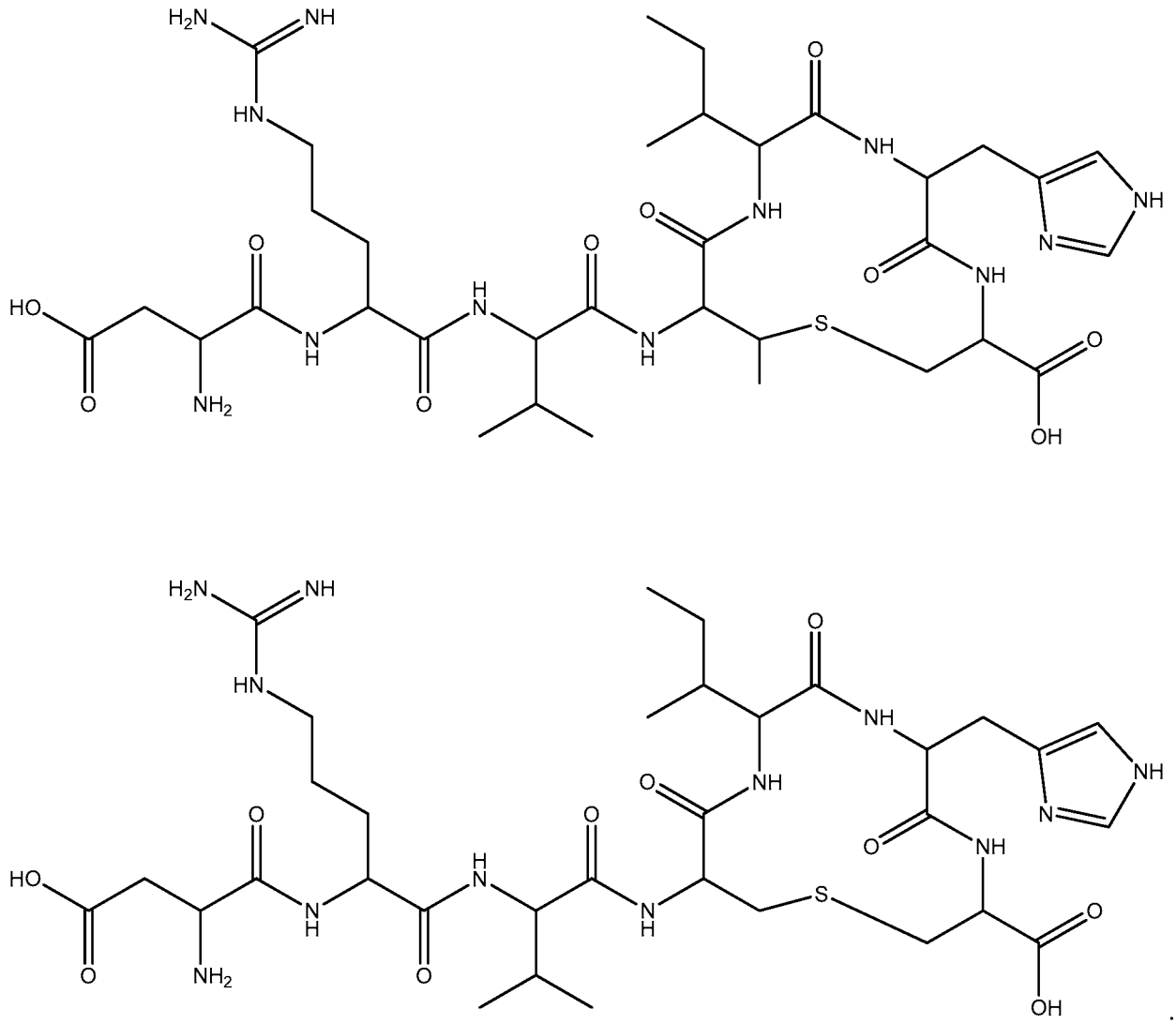
[0139] Xaa^6 is His.

[0140] Cyc^7 forms a thioether bridge in conjunction with Cyc^4, such as in Formula (I), (II) or (III). Cyc^7 can be a D-stereoisomer and/or a L-stereoisomer, typically a L-stereoisomer. Examples of Cyc^7 (taken with Cyc^4) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulas (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0141] In certain embodiments, one or more of Xaa^1-Xaa^6 (excluding Cyc^4 and Cyc^7) is identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In certain such embodiments, all but one or two of Xaa^1-Xaa^6 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa^1-Xaa^6 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7).

[0142] In certain embodiments, Cyc^4 and Cyc^7 are independently selected from Abu (2-aminobutyric acid) and Ala (alanine), where Ala is present in at least one position. Thus, cyclic analogs can have a thioether linkage formed by -Ala^4-S-Ala^7- (Formula (I), where R^1-R^4 are each -H); -Ala^4-S-Abu^7- (Formula (I): R^1-R^3 are -H and R^4 is $-CH_3$) or -Abu^4-S-Ala^7- (Formula (I): R^1, R^3 and R^4 are –H and R^2 is $–CH_3$). Specific examples of cyclic analogs comprise a -Abu^4-S-Ala^7- or -Ala^4-S-Ala^7- linkage.

[0143] In certain embodiments, the invention provides an Ang-(1-7) analog with a thioether-bridge between position 4 and position 7 having the amino acid sequence Asp^1-Arg^2-Val^3-Abu^4-Ile^5-His^6-Ala^7 (SEQ ID NO:15) or the amino acid sequence Asp^1-Arg^2-Val^3-Ala^4-Ile^5-His^6-Ala^7 (SEQ ID NO:16), which are represented by the following structural diagrams:



[0144] In certain embodiments, an Ang analog or derivative of the invention is represented by Formula (V):

Xaa^1-Xaa^2-Nle^3-Cyc^4-Xaa^5-Xaa^6-Cyc^7-Xaa^8-Xaa^9-Xaa^{10} (V, SEQ ID NO:17)

As discussed above, one or more of Xaa^1, Xaa^2, Xaa^8, Xaa^9 and Xaa^{10} are absent in certain embodiments. For example, (1) Xaa^{10} is absent, (2) Xaa^9 and Xaa^{10} are absent, (3) Xaa^8, Xaa^9 and Xaa^{10} are absent, (4) Xaa^1 is absent, (5) Xaa^1 and Xaa^{10} are absent, (6) Xaa^1, Xaa^9 and Xaa^{10} are absent, (7) Xaa^1, Xaa^8, Xaa^9 and Xaa^{10} are absent, (8) Xaa^1 and Xaa^2 are absent, (9) Xaa^1, Xaa^2 and Xaa^{10} are absent, (10) Xaa^1, Xaa^2, Xaa^9 and Xaa^{10} are absent, or (11) Xaa^1, Xaa^2, Xaa^8, Xaa^9 and Xaa^{10} are absent. For each of these embodiments, the remaining amino acids have the values described below.

[0145] Xaa^1, when present, is any amino acid, but typically a negatively charged amino acid such as Glu or Asp, more typically Asp.

[0146] Xaa^2, when present, is a positively charged amino acid such as Arg or Lys, typically Arg.

[0147] Nle^3 is norleucine.

[0148] Cyc^4 forms a thioether bridge in conjunction with Cyc^7. Cyc^4 can be a D-stereoisomer and/or a L-stereoisomer, typically a D-stereoisomer. Examples of Cyc^4 (taken with Cyc^7) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulae (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0149] Xaa^5 is an aliphatic amino acid, such as Leu, Nle, Ile or Val, typically Ile.

[0150] Xaa^6 is His.

[0151] Cyc^7 forms a thioether bridge in conjunction with Cyc^4, such as in Formula (I), (II) or (III). Cyc^7 can be a D-stereoisomer and/or a L-stereoisomer, typically a L-stereoisomer. Examples of Cyc^7 (taken with Cyc^4) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulae (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0152] Xaa^8, when present, is an amino acid other than Pro, typically Phe or Ile. In certain embodiments, Ile results in an inhibitor of Ang(1-8). In certain embodiments, Phe maintains the biological activity of Ang(1-8) or Ang(1-10).

[0153] Xaa^9, when present, is His.

[0154] Xaa^{10}, when present, is an aliphatic residue, for example, Ile, Val or Leu, typically Leu.

[0155] In certain embodiments, one or more of Xaa^1-Xaa^{10} (excluding Nle^3, Cyc^4 and Cyc^7) is identical to the corresponding amino acid in naturally-occurring Ang (including Ang-(1-7), Ang(1-8), Ang(1-9), Ang(1-10), Ang(2-7), Ang(2-8), Ang(2-9), Ang(2-10), Ang(3-8), Ang(3-9) and Ang(3-10). In certain such embodiments, all but one or two of Xaa^1-Xaa^{10} (for those

present) are identical to the corresponding amino acid in naturally-occurring Ang. In other embodiments, all of Xaa^1-Xaa^{10} (for those present) are identical to the corresponding amino acid in naturally-occurring Ang.

[0156] In certain embodiments, Cyc^4 and Cyc^7 are independently selected from Abu (2-aminobutyric acid) and Ala (alanine), where Ala is present at at least one position. Thus, encompassed are cyclic analogs comprising a thioether linkage formed by -Ala^4-S-Ala^7- (Formula (I), where R^1-R^4 are each -H); -Ala^4-S-Abu^7- (Formula (I): R^1-R^3 are -H and R^4 is $-CH_3$) or -Abu^4-S-Ala^7- (Formula (I): R^1, R^3 and R^4 are –H and R^2 is $–CH_3$). Specific cyclic analogs comprise a -Abu^4-S-Ala^7- or -Ala^4-S-Ala^7- linkage.

[0157] In particular, the invention provides an Ang-(l-7) analog or derivative with a thioether-bridge between position 4 and position 7 having the amino acid sequence Asp^1-Arg^2-Nle^3-Abu^4-Ile^5-His^6-Ala^7 (SEQ ID NO:18) or the amino acid sequence Asp^1-Arg^2-Nle^3-Ala^4-Ile^5-His^6-Ala^7 (SEQ ID NO:19).

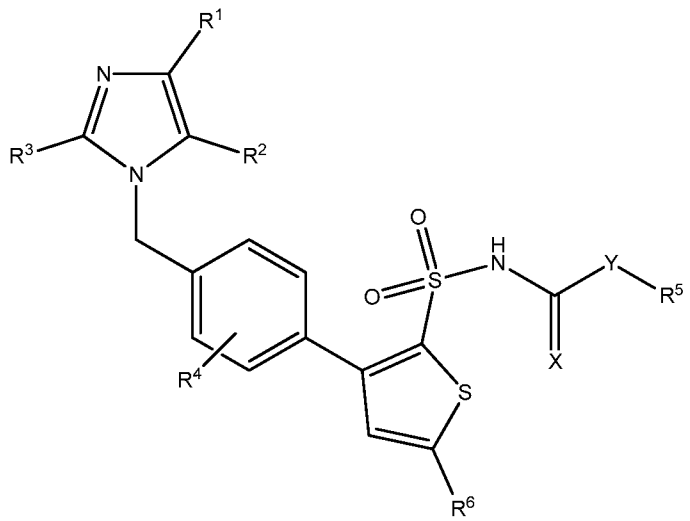
[0158] In another aspect, the invention provides an Ang-(l-8) analog or derivative with a thioether-bridge between position 4 and position 7 having Ang-(l-8) antagonistic activity, in particular an Ang(l-8) analog or derivative having the amino acid sequence Asp^1-Arg^2-Nle^3-Abu^4-Ile^5-His^6-Ala^7-Ile^8 (SEQ ID NO:20) or the amino acid sequence Asp^1-Arg^2-Nle^3-Ala^4-Ile^5-His^6-Ala^7-Ile^8 (SEQ ID NO:21).

Ang (1-7) Receptor Agonists

[0159] In some embodiments, the present invention provides methods of treating GVHD including administering to a subject who is suffering from or susceptible to GVHD an angiotensin (1-7) receptor agonist. As used herein, the term "angiotensin-(1-7) receptor agonist" encompasses any molecule that has a positive impact in a function of an angiotensin-(1-7) receptor, in particular, the G-protein coupled Mas receptor. In some embodiments, an angiotensin-(1-7) receptor agonist directly or indirectly enhances, strengthens, activates and/or increases an angiotensin-(1-7) receptor (i.e., the Mas receptor) activity. In some embodiments, an angiotensin-(1-7) receptor agonist directly interacts with an angiotensin-(1-7) receptor (i.e., the Mas receptor). Such agonists can be peptidic or non-peptidic including, e.g., proteins,

chemical compounds, small molecules, nucleic acids, antibodies, drugs, ligands, or other agents. In some embodiments, the angiotensin (1-7) receptor agonist is a non-peptidic agonist.

[0160] An exemplary class of angiotensin-(1-7) receptor agonists are 1-(p-thienylbenzyl)imidazoles. Examples of these non-peptide angiotensin-(1-7) receptor agonists are represented by Structural Formula (VI):



(VI),

or pharmaceutically acceptable salts thereof, wherein:

R^1 is halogen, hydroxyl, (C_1-C_4)-alkoxy, (C_1-C_8)-alkoxy wherein 1 to 6 carbon atoms are replaced by the heteroatoms O, S, or NH (preferably by O), (C_1-C_4)-alkoxy substituted by a saturated cyclic ether such as tetrahydropyran or tetrahydrofuran, O-(C_1-C_4)-alkenyl, O-(C_1-C_4)-alkylaryl, or aryloxy that is unsubstituted or substituted by a substituent selected from halogen, (C_1-C_3)-alkyl, (C_1-C_3)-alkoxy and trifluoromethyl;

R^2 is CHO, COOH, or (3) CO-O-(C_1-C_4)-alkyl;

R^3 is (C_1-C_4)-alkyl or aryl;

R^4 is hydrogen, halogen (chloro, bromo, fluoro), or (C_1-C_4)-alkyl;

X is oxygen or sulfur;

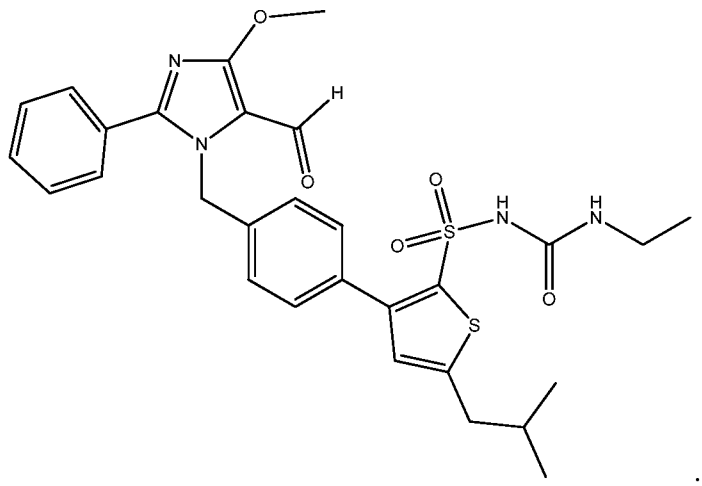
Y is oxygen or -NH-;

R^5 is hydrogen, $(C_1\text{-}C_6)$-alkyl; or $(C_1\text{-}C_4)$-alkylaryl, where R^5 is hydrogen when Y is -NH-; and

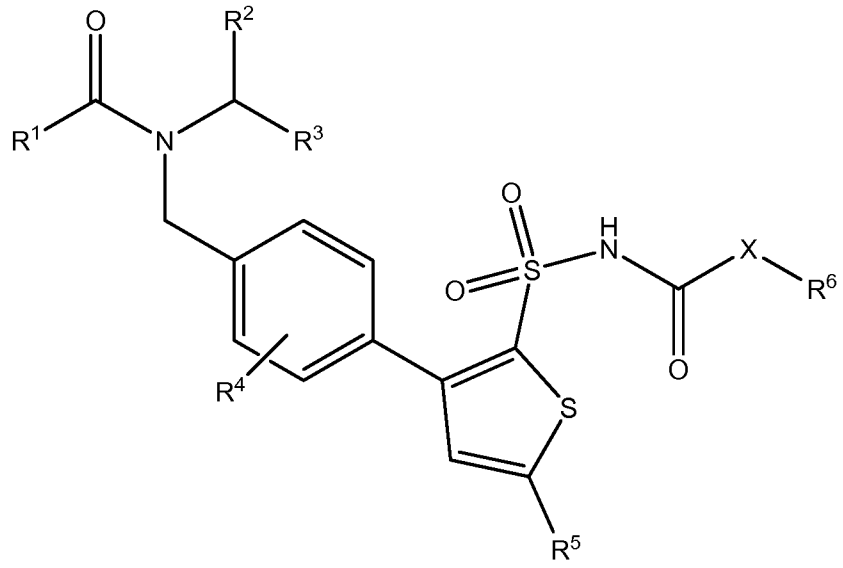
R^6 is $(C_1\text{-}C_5)$-alkyl.

[0161] In certain embodiments, R^1 is not halogen when R^2 is COOH or CO-O-$(C_1\text{-}C_4)$-alkyl.

[0162] In some embodiments, an angiotensin-(1-7) receptor agonist is AVE 0991, 5-formyl-4-methoxy-2-phenyl-1[[4-[2-(ethylaminocarbonylsulfonamido)-5-isobutyl-3-thienyl]-phenyl]-methyl]-imidazole, which is represented by the following structure:



[0163] Another exemplary class of angiotensin-(1-7) receptor agonists are p-thienylbenzylamides. Examples of these non-peptide angiotensin-(1-7) receptor agonists are represented by Structural Formula (VII):



(VII),

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is (C_1-C_5)-alkyl that is unsubstituted or substituted by a radical chosen from NH_2, halogen, O-(C_1-C_3)-alkyl, CO-O-(C_1-C_3)-alkyl and CO_2H, (C_3-C_8)-cycloalkyl, (C_1-C_3)-alkyl-(C_3-C_8)-cycloalkyl, (C_6-C_{10})-aryl that is unsubstituted or substituted by a radical chosen from halogen and O-(C_1-C_3)-alkyl, (C_1-C_3)-alkyl-(C_6-C_{10})-aryl where the aryl radical is unsubstituted or substituted by a radical chosen from halogen and O-(C_1-C_3)-alkyl, (C_1-C_5)-heteroaryl, or (C_1-C_3)-alkyl-(C_1-C_5)-heteroaryl;

R^2 is hydrogen, (C_1-C_6)-alkyl that is unsubstituted or substituted by a radical chosen from halogen and O-(C_1-C_3)-alkyl, (C_3-C_8)-cycloalkyl, (C_1-C_3)-alkyl-(C_3-C_8)-cycloalkyl, (C_6-C_{10})-aryl that is unsubstituted or substituted by a radical chosen from among halogen, O-(C_1-C_3)-alkyl and CO-O-(C_1-C_3)-alkyl, or (C_1-C_3)-alkyl-(C_6-C_{10})-aryl that is unsubstituted or substituted by a radical chosen from halogen and O-(C_1-C_3)-alkyl;

R^3 is hydrogen, COOH, or COO-(C_1-C_4)-alkyl;

R^4 is hydrogen, halogen; or (C_1-C_4)-alkyl;

R^5 is hydrogen or (C_1-C_6)-alkyl;

R^6 is hydrogen, (C_1-C_6)-alkyl, (C_1-C_3)-alkyl-(C_3-C_8)-cycloalkyl, or (C_2-C_6)-alkenyl; and

X is oxygen or NH.

[0164] Additional examples of angiotensin-(1-7) receptor agonists are described in U.S. Patent No. 6,235,766, the contents of which are incorporated by reference herein.

[0165] Various angiotensin-(1-7) receptor agonists described above can be present as pharmaceutically acceptable salts. As used herein, "a pharmaceutically acceptable salt" refers to salts that retain the desired activity of the peptide or equivalent compound, but preferably do not detrimentally affect the activity of the peptide or other component of a system, which uses the peptide. Examples of such salts are acid addition salts formed with inorganic acids, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like. Salts may also be formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, and the like. Salts formed from a cationic material may utilize the conjugate base of these inorganic and organic acids. Salts may also be formed with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel and the like or with an organic cation formed from N,N'- dibenzylethylenediamine or ethylenediamine, or combinations thereof (e.g., a zinc tannate salt). The non-toxic, physiologically acceptable salts are preferred.

[0166] The salts can be formed by conventional means such as by reacting the free acid or free base forms of the product with one or more equivalents of the appropriate acid or base in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying, or by exchanging the cations of an existing salt for another cation on a suitable ion exchange resin.

[0167] An alkyl group is a straight chained or branched non-aromatic hydrocarbon that is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C1-C4 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

[0168] An alkenyl group is a straight chained or branched non-aromatic hydrocarbon that is includes one or more double bonds. Typically, a straight chained or branched alkenyl group has from 2 to about 20 carbon atoms, preferably from 2 to about 10. Examples of straight chained and branched alkenyl groups include ethenyl, n-propenyl, and n-butenyl.

[0169] Aromatic (aryl) groups include carbocyclic aromatic groups such as phenyl, naphthyl, and anthracyl, and heteroaryl groups such as imidazolyl, thienyl, furyl, pyridyl, pyrimidyl, pyranyl, pyrazolyl, pyrrolyl, pyrazinyl, thiazolyl, oxazolyl, and tetrazolyl. Aromatic groups also include fused polycyclic aromatic ring systems in which a carbocyclic aromatic ring or heteroaryl ring is fused to one or more other heteroaryl rings. Examples include benzothienyl, benzofuryl, indolyl, quinolinyl, benzothiazole, benzoxazole, benzimidazole, quinolinyl, isoquinolinyl and isoindolyl.

[0170] An aralkyl group is an alkyl group substituted by an aryl group. Aromatic (aryl) groups include carbocyclic aromatic groups such as phenyl, naphthyl, and anthracyl, and heteroaryl groups such as imidazolyl, thienyl, furyl, pyridyl, pyrimidyl, pyranyl, pyrazolyl, pyrrolyl, pyrazinyl, thiazolyl, oxazolyl, and tetrazolyl. Aromatic groups also include fused polycyclic aromatic ring systems in which a carbocyclic aromatic ring or heteroaryl ring is fused to one or more other heteroaryl rings. Examples include benzothienyl, benzofuryl, indolyl, quinolinyl, benzothiazole, benzoxazole, benzimidazole, quinolinyl, isoquinolinyl and isoindolyl.

Treatment of GVHD with Ang (1-7)

[0171] The present invention provides methods and compositions for treatment of GVHD. By treating GVHD, it is meant that the compositions of the present invention may affect one or more aspects of GVHD, including decreasing resultant symptoms. For example, treatment with an angiotensin (1-7) peptide described herein can reduce the severity of GVHD, such as by reducing a stage of severity of GVHD. In one embodiment, treatment of GVHD with an angiotensin (1-7) peptide described herein reduces GVHD by one, two, three, or four stages (e.g., stage 4 to stage 3, 2, 1, or 0) as compared to a control. In some embodiments, treatment

with an angiotensin (1-7) peptide results in substantially free of Stage 4, 3, 2, or 1 acute GVHD in the subject 100 days (e.g., at least 120 days, 130 days, 140 days, 150 days, 160 days, 170 days, 180 days or more) following transplantation. In some embodiments, treatment with an angiotensin (1-7) peptide results in results in substantially free of Grade IV, III, II, or I acute GVHD in the subject 100 days (e.g., at least 120 days, 130 days, 140 days, 150 days, 160 days, 170 days, 180 days or more) following transplantation. In some embodiments, treatment with an angiotensin (1-7) peptide results in substantially free of acute GVHD symptom in the subject 100 days (e.g., at least 120 days, 130 days, 140 days, 150 days, 160 days, 170 days, 180 days or more) following transplantation. In some embodiments, treatment with an angiotensin (1-7) peptide results in substantially free of chronic GVHD symptoms in the subject at least 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 4 years, 5 years or more following transplantation. Determining the stage of GVHD is within the skill of those in the art, such as a medical practitioner. The present invention may be used as a monotherapy or as part of a combination therapy with one or more other prophylactic or therapeutic materials routinely used with transplantation or used to treat or reduce risk of GVHD.

[0172] In some embodiments, the present invention comprises a method of treating or reducing the risk of GVHD by administering to a subject who is suffering from or susceptible to GVHD an angiotensin (1-7) peptide described herein.

[0173] In some embodiments, a subject is any multicellular organism. In some embodiments, a subject is a mouse, rat, dog, non-human primate or other animal commonly used for laboratory experiments. In some embodiments, a subject is an individual. In some embodiments, an individual is a human. In some embodiments, a subject has or is susceptible to a disease, disorder, or condition. In some embodiments, a subject has or is susceptible to GVHD. In some embodiments, a subject who is susceptible to GVHD comprises a subject who has had a transplantation. In some embodiments, a subject who is susceptible to GVHD comprises a subject who is going to have a transplantation. In some embodiments, a subject who is susceptible to GVHD comprises a subject having a disease, disorder, or condition that may require a transplantation. In some embodiments, a subject who is susceptible to GVHD comprises a subject at risk for a disease, disorder, or condition that may require a transplantation.

In some embodiments, a transplantation is a cord blood transplantation. In some embodiments, a transplantation is a double cord blood transplantation. In some embodiments, a transplantation is a bone marrow transplantation. In some embodiments, a transplantation is an adult stem cell transplantation. In some embodiments, a transplantation is an embryonic stem cell transplantation. In some embodiments, a transplantation is an organ transplantation. In some embodiments, a transplantation is a blood transfusion.

[0174] In some embodiments, an angiotensin (1-7) peptide described herein is administered to a subject having or at risk of GVHD at an effective dose periodically at an administration interval such that at least one symptom or feature of GVHD is reduced in intensity, severity, duration, or frequency or has delayed in onset.

Pharmaceutical Compositions

[0175] In accordance with the methods of the invention, an angiotensin (1-7) peptide described herein of the invention can be administered to a subject alone (e.g., as a purified peptide), or as a component of a composition or medicament (*e.g.*, in the manufacture of a medicament for the treatment of the disease), as described herein. The compositions can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration. Methods of formulating compositions are known in the art (see, e.g., Remington's Pharmaceuticals Sciences, 17th Edition, Mack Publishing Co., (Alfonso R. Gennaro, editor) (1989)).

[0176] Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (*e.g.*, NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, sugars such as mannitol, sucrose, or others, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, *etc.*, as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents (*e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like) which do not deleteriously react with the active compounds or

interference with their activity. In a preferred embodiment, a water-soluble carrier suitable for intravenous administration is used.

[0177] The composition or medicament, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can also be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, *etc.*

[0178] The composition or medicament can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, in a preferred embodiment, a composition for intravenous administration typically is a solution in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0179] In some embodiments, provided compositions, including those provided as pharmaceutical formulations, comprise a liquid carrier such as but not limited to water, saline, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous physiologically balanced solutions, oils, esters and glycols.

[0180] An angiotensin (1-7) peptide and/or angiotensin (1-7) receptor agonist as described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to, those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl

groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

Routes of Administration

[0181] An angiotensin (1-7) peptide described herein (or a composition or medicament containing an angiotensin (1-7) peptide described herein) is administered by any appropriate route. In some embodiments, an angiotensin (1-7) peptide described herein is administered subcutaneously. As used herein, the term "subcutaneous tissue", is defined as a layer of loose, irregular connective tissue immediately beneath the skin. For example, the subcutaneous administration may be performed by injecting a composition into areas including, but not limited to, thigh region, abdominal region, gluteal region, or scapular region. In some embodiments, an angiotensin (1-7) peptide described herein is administered intravenously. In some embodiments, an angiotensin (1-7) peptide described herein is administered orally. In other embodiments, an angiotensin (1-7) peptide described herein is administered by direct administration to a target tissue, such as heart or muscle (*e.g.*, intramuscular), tumor (intratumorallly), nervous system (*e.g.*, direct injection into the brain; intraventricularly; intrathecally). Alternatively, an angiotensin (1-7) peptide described herein (or a composition or medicament containing an angiotensin (1-7) peptide described herein) can be administered by inhalation, parenterally, intradermally, transdermally, or transmucosally (*e.g.*, orally or nasally). More than one route can be used concurrently, if desired.

Dosing

[0182] In some embodiments, a composition is administered in a therapeutically effective amount and/or according to a dosing regimen that is correlated with a particular desired outcome (e.g., with treating or reducing risk for GVHD).

[0183] Particular doses or amounts to be administered in accordance with the present invention may vary, for example, depending on the nature and/or extent of the desired outcome, on particulars of route and/or timing of administration, and/or on one or more characteristics (e.g., weight, age, personal history, genetic characteristic, lifestyle parameter, severity of cardiac defect and/or level of risk of cardiac defect, etc., or combinations thereof). Such doses or

amounts can be determined by those of ordinary skill. In some embodiments, an appropriate dose or amount is determined in accordance with standard clinical techniques. For example, in some embodiments, an appropriate dose or amount is a dose or amount sufficient to reduce severity of GVHD by one, two, three, or four stages. Alternatively or additionally, in some embodiments, an appropriate dose or amount is determined through use of one or more *in vitro* or *in vivo* assays to help identify desirable or optimal dosage ranges or amounts to be administered.

[0184] In various embodiments, angiotensin (1-7) peptides or angiotensin (1-7) receptor agonists, including derivatives, analogs, and/or salts are administered at a therapeutically effective amount. As used herein, the term "therapeutically effective amount" is largely determined based on the total amount of the therapeutic agent contained in the pharmaceutical compositions of the present invention. Generally, a therapeutically effective amount is sufficient to achieve a meaningful benefit to the subject (e.g., treating, modulating, curing, preventing and/or ameliorating the underlying disease or condition). In some particular embodiments, appropriate doses or amounts to be administered may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. In some embodiments, a therapeutically effective dosage amount of angiotensin (1-7) peptides or angiotensin (1-7) receptor agonists, including derivatives, analogs, and/or salts can be, for example, between about 0.001-10 mg/kg, about 0.005-1.5 mg/kg, about 0.1-1 mg/kg, or 300-1,000 μg/kg. In some embodiments, the therapeutically effective dosage amount of angiotensin (1-7) peptides or angiotensin (1-7) receptor agonists, including derivatives, analogs, and/or salts can be, for example, about 1 μg/kg, 2.5 μg/kg, 5 μg/kg, 10 μg/kg, 20 μg/kg, 30 μg/kg, 40 μg/kg, 50 μg/kg, 60 μg/kg, 70 μg/kg, 80 μg/kg, 90 μg/kg, 100 μg/kg, 150 μg/kg, 200 μg/kg, 250 μg/kg, 300 μg/kg, 400 μg/kg, 500 μg/kg, 600 μg/kg, 700 μg/kg, 800 μg/kg, 900 μg/kg, 1000 μg/kg, or 1500 μg/kg. The effective dose for a particular individual can be varied (*e.g.*, increased or decreased) over time, depending on the needs of the individual.

[0185] Therapeutically effective dosage amounts of angiotensin (1-7) peptides or angiotensin (1-7) receptor agonists, including derivatives, analogs, and/or salts may be present in varying amounts in various embodiments. In some embodiments, a therapeutically effective dosage amount can be, for example, about 1-10,000 μg/kg, about 5-1,500 μg/kg, about 100-

1,000 μg/kg, or 50-500 μg/kg. In some embodiments, the therapeutically effective dosage amount can be, for example, about 1 μg/kg, 2.5 μg/kg, 5 μg/kg, 10 μg/kg, 20 μg/kg, 30 μg/kg, 40 μg/kg, 50 μg/kg, 60 μg/kg, 70 μg/kg, 80 μg/kg, 90 μg/kg, 100 μg/kg, 150 μg/kg, 200 μg/kg, 250 μg/kg, 300 μg/kg, 400 μg/kg, 500 μg/kg, 600 μg/kg, 700 μg/kg, 800 μg/kg, 900 μg/kg, 1,000 μg/kg, or 1,500 μg/kg. The effective dose for a particular individual can be varied (*e.g.*, increased or decreased) over time, depending on the needs of the individual. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0186] In other embodiments, a therapeutically effective dosage amount may be, for example, about 0.001 mg/kg weight to 500 mg/kg weight, e.g., from about 0.001 mg/kg weight to 400 mg/kg weight, from about 0.001 mg/kg weight to 300 mg/kg weight, from about 0.001 mg/kg weight to 200 mg/kg weight, from about 0.001 mg/kg weight to 100 mg/kg weight, from about 0.001 mg/kg weight to 90 mg/kg weight, from about 0.001 mg/kg weight to 80 mg/kg weight, from about 0.001 mg/kg weight to 70 mg/kg weight, from about 0.001 mg/kg weight to 60 mg/kg weight, from about 0.001 mg/kg weight to 50 mg/kg weight, from about 0.001 mg/kg weight to 40 mg/kg weight, from about 0.001 mg/kg weight to 30 mg/kg weight, from about 0.001 mg/kg weight to 25 mg/kg weight, from about 0.001 mg/kg weight to 20 mg/kg weight, from about 0.001 mg/kg weight to 15 mg/kg weight, from about 0.001 mg/kg weight to 10 mg/kg weight. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0187] In still other embodiments, a therapeutically effective dosage amount may be, for example, about 0.001 mg/kg weight to about 1 mg/kg weight, e.g. from about 0.001 mg/kg weight to about 0.9 mg/kg weight, from about 0.001 mg/kg weight to about 0.8 mg/kg weight, from about 0.001 mg/kg weight to about 0.8 mg/kg weight, from about 0.001 mg/kg weight to about 0.7 mg/kg weight, from about 0.001 mg/kg weight to about 0.6 mg/kg weight, from about 0.001 mg/kg weight to about 0.5 mg/kg weight, from about 0.01 mg/kg weight to about 1 mg/kg weight, from about 0.01 mg/kg weight to about 0.9 mg/kg weight, from about 0.01 mg/kg weight to about 0.8 mg/kg weight, from about 0.01 mg/kg weight to about 0.7 mg/kg weight, from about 0.01 mg/kg weight to about 0.6 mg/kg weight, from about 0.01 mg/kg weight to about 0.5 mg/kg

weight, from about 0.02 mg/kg weight to about 1 mg/kg weight, from about 0.02 mg/kg weight to about 0.9 mg/kg weight, from about 0.02 mg/kg weight to about 0.8 mg/kg weight, from about 0.02 mg/kg weight to about 0.7 mg/kg weight, from about 0.02 mg/kg weight to about 0.6 mg/kg weight, from about 0.02 mg/kg weight to about 0.5 mg/kg weight, from about 0.03 mg/kg weight to about 1 mg/kg weight, from about 0.03 mg/kg weight to about 0.9 mg/kg weight, from about 0.03 mg/kg weight to about 0.8 mg/kg weight, from about 0.03 mg/kg weight to about 0.7 mg/kg weight, from about 0.03 mg/kg weight to about 0.6 mg/kg weight, from about 0.03 mg/kg weight to about 0.5 mg/kg weight, from about 0.04 mg/kg weight to about 1 mg/kg weight, from about 0.04 mg/kg weight to about 0.9 mg/kg weight, from about 0.04 mg/kg weight to about 0.8 mg/kg weight, from about 0.04 mg/kg weight to about 0.7 mg/kg weight, from about 0.04 mg/kg weight to about 0.6 mg/kg weight, from about 0.04 mg/kg weight to about 0.5 mg/kg weight, from about 0.05 mg/kg weight to about 1 mg/kg weight, from about 0.05 mg/kg weight to about 0.9 mg/kg weight, from about 0.05 mg/kg weight to about 0.8 mg/kg weight, from about 0.05 mg/kg weight to about 0.7 mg/kg weight, from about 0.05 mg/kg weight to about 0.6 mg/kg weight, from about 0.05 mg/kg weight to about 0.5 mg/kg weight. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0188] In still other embodiments, a therapeutically effective dosage amount may be, for example, about 0.0001 mg/kg weight to 0.1 mg/kg weight, e.g. from about 0.0001 mg/kg weight to 0.09 mg/kg weight, from about 0.0001 mg/kg weight to 0.08 mg/kg weight, from about 0.0001 mg/kg weight to 0.07 mg/kg weight, from about 0.0001 mg/kg weight to 0.06 mg/kg weight, from about 0.0001 mg/kg weight to 0.05 mg/kg weight, from about 0.0001 mg/kg weight to about 0.04 mg/kg weight, from about 0.0001 mg/kg weight to 0.03 mg/kg weight, from about 0.0001 mg/kg weight to 0.02 mg/kg weight, from about 0.0001 mg/kg weight to 0.019 mg/kg weight, from about 0.0001 mg/kg weight to 0.018 mg/kg weight, from about 0.0001 mg/kg weight to 0.017 mg/kg weight, from about 0.0001 mg/kg weight to 0.016 mg/kg weight, from about 0.0001 mg/kg weight to 0.015 mg/kg weight, from about 0.0001 mg/kg weight to 0.014 mg/kg weight, from about 0.0001 mg/kg weight to 0.013 mg/kg weight, from about 0.0001 mg/kg weight to 0.012 mg/kg weight, from about 0.0001 mg/kg weight to 0.011 mg/kg weight, from about 0.0001 mg/kg weight to 0.01 mg/kg weight, from about 0.0001 mg/kg weight to 0.009 mg/kg weight, from about 0.0001 mg/kg weight to 0.008 mg/kg weight, from about 0.0001

mg/kg weight to 0.007 mg/kg weight, from about 0.0001 mg/kg weight to 0.006 mg/kg weight, from about 0.0001 mg/kg weight to 0.005 mg/kg weight, from about 0.0001 mg/kg weight to 0.004 mg/kg weight, from about 0.0001 mg/kg weight to 0.003 mg/kg weight, from about 0.0001 mg/kg weight to 0.002 mg/kg weight. In some embodiments, the therapeutically effective dose may be 0.0001 mg/kg weight, 0.0002 mg/kg weight, 0.0003 mg/kg weight, 0.0004 mg/kg weight, 0.0005 mg/kg weight, 0.0006 mg/kg weight, 0.0007 mg/kg weight, 0.0008 mg/kg weight, 0.0009 mg/kg weight, 0.001 mg/kg weight, 0.002 mg/kg weight, 0.003 mg/kg weight, 0.004 mg/kg weight, 0.005 mg/kg weight, 0.006 mg/kg weight, 0.007 mg/kg weight, 0.008 mg/kg weight, 0.009 mg/kg weight, 0.01 mg/kg weight, 0.02 mg/kg weight, 0.03 mg/kg weight, 0.04 mg/kg weight, 0.05 mg/kg weight, 0.06 mg/kg weight, 0.07 mg/kg weight, 0.08 mg/kg weight, 0.09 mg/kg weight, or 0.1 mg/kg weight. The effective dose for a particular individual can be varied (*e.g.*, increased or decreased) over time, depending on the needs of the individual. In some embodiments, the therapeutically effective amount described herein is provided in one dose. In some embodiments, the therapeutically effective amount described herein is provided in one day.

[0189] In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose ranging from about 1-1,000 μg/kg/day (e.g., ranging from about 1-900 μg/kg/day, 1-800 μg/kg/day, 1-700 μg/kg/day, 1-600 μg/kg/day, 1-500 μg/kg/day, 1-400 μg/kg/day, 1-300 μg/kg/day, 1-200 μg/kg/day, 1-100 μg/kg/day, 1-90 μg/kg/day, 1-80 μg/kg/day, 1-70 μg/kg/day, 1-60 μg/kg/day, 1-50 μg/kg/day, 1-40 μg/kg/day, 1-30 μg/kg/day, 1-20 μg/kg/day, 1-10 μg/kg/day). In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose ranging from about 1-500 μg/kg/day. In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose ranging from about 50-500 μg/kg/day. In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose ranging from about 1-100 μg/kg/day. In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose ranging from about 1-60 μg/kg/day. In some embodiments, the angiotensin (1-7) peptide is administered at an effective dose selected from about 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1,000 ug/kg/day.

[0190] In some embodiments, a provided composition is provided as a pharmaceutical formulation. In some embodiments, a pharmaceutical formulation is or comprises a unit dose amount for administration in accordance with a dosing regimen correlated with achievement of the reduced incidence or risk of GVHD.

[0191] In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein administered as a single dose. In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered at regular intervals. Administration at an "interval," as used herein, indicates that the therapeutically effective amount is administered periodically (as distinguished from a one-time dose). The interval can be determined by standard clinical techniques. In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered bimonthly, monthly, twice monthly, triweekly, biweekly, weekly, twice weekly, thrice weekly, daily, twice daily, or every six hours. The administration interval for a single individual need not be a fixed interval, but can be varied over time, depending on the needs of the individual.

[0192] As used herein, the term "bimonthly" means administration once per two months (*i.e.,* once every two months); the term "monthly" means administration once per month; the term "triweekly" means administration once per three weeks (*i.e.*, once every three weeks); the term "biweekly" means administration once per two weeks (*i.e.*, once every two weeks); the term "weekly" means administration once per week; and the term "daily" means administration once per day.

[0193] In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered at regular intervals indefinitely. In some embodiments, a formulation comprising angiotensin (1-7) peptide described herein is administered at regular intervals for a defined period. In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered at regular intervals for 5 years, 4, years, 3, years, 2, years, 1 year, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, a month, 3 weeks, 2, weeks, a week, 6 days, 5 days, 4 days, 3 days, 2 days or a day.

[0194] In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered prior to transplantation. In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered for at least 1 year, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, a month, 3 weeks, 2, weeks, a week, 6 days, 5 days, 4 days, 3 days, 2 days or a day prior to transplantation. Alternatively or additionally, in some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered after transplantation. In some embodiments, a formulation comprising an angiotensin (1-7) peptide described herein is administered for at least 1 year, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, a month, 3 weeks, 2, weeks, a week, 6 days, 5 days, 4 days, 3 days, 2 days or a day after transplantation. In some embodiments, an angiotensin (1-7) peptide described herein is administered concurrently with (i.e., at about the same time as) a transplantation. For example, an angiotensin (1-7) peptide can be administered on the same day as a transplantation.

Combination Therapy

[0195] In some embodiments, an angiotensin (1-7) peptide is administered in combination with one or more known therapeutic agents (e.g., immunosuppressive agents, anti-proliferatives (e.g., antibiotics), anti-inflammatories, pain relievers, etc) currently used for GVHD prophylaxis and treatment (e.g., cyclosporine, tacrolimus, sirolimus, methotrexate, prednisone, Campath, anti-thymocyte globulin [ATG], MMF, etc.). In some embodiments, the known therapeutic agent(s) is/are administered according to its standard or approved dosing regimen and/or schedule. In some embodiments, the known therapeutic agent(s) is/are administered according to a regimen that is altered as compared with its standard or approved dosing regimen and/or schedule. In some embodiments, such an altered regimen differs from the standard or approved dosing regimen in that one or more unit doses is altered (e.g., reduced or increased) in amount, and/or in that dosing is altered in frequency (e.g., in that one or more intervals between unit doses is expanded, resulting in lower frequency, or is reduced, resulting in higher frequency).

[0196] In some embodiments, an angiotensin (1-7) peptide is administered in combination with one or more immunosuppressants. An "immunosuppressant" is any molecule that reduces or eliminates an immune response in a host when the host is challenged with an immunogenic molecule, including immunogenic molecules present on transplanted organs, tissues or cells. Examples of immunosuppressants include but are not limited to antithymocyte globulin (ATG), anti-TNF agents, azathioprine (or other inosine 5'-monophosphate dehydrogenase inhibitors), azodiacarbonide, bisindolyl maleimide VIII, brequinar, chlorambucil, CTLA4-Ig, corticosteroids, cyclophosphamide, cyclosporine A, deoxyspergualin, dexamethasone, glucocorticoids, IL-2 antagonists (e.g., daclizumab and basiliximab), leflunomide, mercaptopurine, 6-mercaptopurine (6-MP), methotrexate, methylprednisolone, mizoribine, mizoribine monophosphate, muromonab CD3, mycophenolate mofetil, OKT3, prednisone, sirolimus, rapamycin, rho (D) immune globin, tacrolimus (FK506), vitamin D analogs (e.g., MC1288), *etc.*

[0197] In some embodiments, provided compositions comprise growth factors (e.g., filgrastim). In some embodiments, provided compositions comprise DNA nucleoside analogs (e.g., fludarabine). In some embodiments, provided compositions comprise antineoplastic alkylating agents (e.g., melphalan, thiotepa).

[0198] In some embodiments provided compositions are administered in combination with one or more conventional treatments for GVHD. Conventional treatments for GVHD includes steroid (e.g., methylprednisolone) therapy. Chronic GVHD is treated with a combination of steroids and cyclosporin A.

Treatment of Mucositis

[0199] The present invention also provides methods and compositions for treatment of mucositis. In particular, the present invention can be used to treat mucositis caused by transplantation (e.g., allogeneic transplantation). By treating mucositis, it is meant that the compositions of the present invention may reduce the intensity, severity, duration or frequency, or delay the onset of one or more symptom of mucositis. For example, treatment with an angiotensin (1-7) peptide described herein can reduce the severity of mucositis, such as by reducing a stage or grade of severity of mucositis. In one embodiment, treatment with an

angiotensin (1-7) peptide described herein reduces mucositis by one, two, three, or four stages (e.g., stage 4 to stage 3, 2, 1, or 0) or grades as compared to a control. In some embodiments, treatment with an angiotensin (1-7) peptide results in substantially free of Stage or Grade 4, 3, 2, or 1 mucositis in the subject following transplantation. In some embodiments, treatment with an angiotensin (1-7) peptide results in substantially free of Stage or Grade 4, 3 or 2 mucositis in the subject following transplantation. In some embodiments, treatment with an angiotensin (1-7) peptide results in substantially free of Stage or Grade 4 or 3 mucositis in the subject following transplantation. In some embodiments, treatment with an angiotensin (1-7) peptide results in substantially free of mucositis following transplantation.

[0200] Various dosages, formulations, administration modes, and combination therapies described above in connection with GVHD treatment may be applied to the treatment of mucositis.

[0201] The terms, "improve," "increase" or "reduce," as used herein, indicate values that are relative to a control. In some embodiments, a suitable control is a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with GVHD, mucositis or a recipient of transplant, who is about the same age and/or gender as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable). The individual (also referred to as "patient" or "subject") being treated is an individual (fetus, infant, child, adolescent, or adult human) suffering from or at risk of developing GVHD or mucositis.

Kits

[0202] The present invention further provides kits or other articles of manufacture which contains an angiotensin (1-7) peptide or a formulation containing the same and provides instructions for its reconstitution (if lyophilized) and/or use. Kits or other articles of manufacture may include a container, a syringe, vial and any other articles, devices or equipment useful in administration (e.g., subcutaneous, oral, by inhalation). Suitable containers include, for example, bottles, vials, syringes (e.g., pre-filled syringes), ampules, cartridges, reservoirs, or lyo-jects.

The container may be formed from a variety of materials such as glass or plastic. In some embodiments, a container is a pre-filled syringe. Suitable pre-filled syringes include, but are not limited to, borosilicate glass syringes with baked silicone coating, borosilicate glass syringes with sprayed silicone, or plastic resin syringes without silicone.

[0203] Typically, the container may holds formulations and a label on, or associated with, the container that may indicate directions for reconstitution and/or use. For example, the label may indicate that the formulation is reconstituted to concentrations as described above. The label may further indicate that the formulation is useful or intended for, for example, subcutaneous administration. In some embodiments, a container may contain a single dose of a stable formulation containing an angiotensin (1-7) peptide. In various embodiments, a single dose of the stable formulation is present in a volume of less than about 15 ml, 10 ml, 5.0 ml, 4.0 ml, 3.5 ml, 3.0 ml, 2.5 ml, 2.0 ml, 1.5 ml, 1.0 ml, or 0.5 ml. Alternatively, a container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g., from 2-6 administrations) of the formulation. Kits or other articles of manufacture may further include a second container comprising a suitable diluent (e.g., BWFI, saline, buffered saline). Upon mixing of the diluent and the formulation, the final protein concentration in the reconstituted formulation will generally be at least 1 mg/ml (e.g., at least 5 mg/ml, at least 10 mg/ml, at least 20 mg/ml, at least 30 mg/ml, at least 40 mg/ml, at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml). Kits or other articles of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, kits or other articles of manufacture may include an instruction for self-administration.

[0204] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXEMPLIFICATION

Example 1: Clinical Trial of Angiotensin-(1-7) treated double cord blood transplant recipients

Subject selection criteria

[0205] Human subjects were selected from subjects undergoing double cord blood transplantation for the treatment of a variety of malignancies. Malignancies included: having ≤10% bone marrow blasts; having Acute Myelogenous Leukemia (AML) past first remission, in first or subsequent relapse, induction failure, or in first remission with high-risk for relapse (with high-risk cytogenetics or presence of flt3 mutation or with secondary leukemia from prior chemotherapy); having Myelodysplastic Syndrome or Myelofibrosis of intermediate or high-risk; having Acute Lymphoblastic Leukemia (ALL) with induction failure, in first complete remission with Philadelphia chromosome or translocation (4:11), hypodiploidy and or evidence of minimal residual disease by flow cytometry, in second or third complete remission or second relapse; having Chronic Myelocytic leukemia (CML) in second chronic phase or accelerated phase; having Non-Hodgkin's Lymphoma (NHL) with induction failure, in second or third complete remission or relapse; or having Chronic Lymphocytic leukemia (CLL) with progressive disease following standard therapy.

[0206] Subjects were selected of either gender ranging in age from 18-60 years with adequate major organ function and with an Eastern Cooperative Oncology Group (ECOG) status of ≤ 2. Adequate major organ function was demonstrated by: left ventricular ejection fraction of at least 40%: pulmonary function test (PFT) demonstrating a diffusion capacity of at least 50% predicted: creatinine < 1.6 mg/dL; Serum Glutamic Pyruvic Transaminase (SGPT)/ Serum Glutamic Oxaloacetic Transaminase (SGOT), alkaline phosphatase, and LDH ≤ 3.0 times the upper limit of normal (ULN); and bilirubin ≤ 1.5 times ULN. Female subjects capable of reproduction, and male subjects with partners capable of reproduction, were required to agree to use of an effective contraceptive method during the course of the study and until 2 months following the last administration of Ang-(1-7).

[0207] Female subjects capable of reproduction were required to have a negative beta human chorionic gonadotropin (βHCG) serum or urine pregnancy test result prior to starting the conditioning regimen. Female subjects who were surgically sterilized or who had not experienced menses for > 2 years were not required to have a pregnancy test.

[0208] Subjects were selected for whom a 7/8 or 8/8 HLA-match related or 8/8 HLA-match unrelated bone marrow donor was not available, or if the progress of the subject's disease dictated that it was not in the subject's best interest to wait for an unrelated marrow donor to be procured. Subjects were selected with two cord blood units matched with the subject at 4/6, 5/6, or 6/6 HLA class I (serological) and II (molecular) antigens available, with each unit containing at least 1 x 10^7 total nucleated cells/kg recipient body weight (pre-thaw).

[0209] Individuals who had received antineoplastic treatment including chemotherapy, immunotherapy and radiation therapy ≤ 2 weeks prior to the screening period, had undergone prior total body irradiation, who had received prior autologous or allogeneic hematopoietic cell transplants, who were seropositive for HIV, Hepatitis B or Hepatitis C, who had received an investigational drug within 30 days of projected first administration of study drug, who were pregnant or breastfeeding, who had a known hypersensitivity to Ang-(1-7) peptide, or with a willing and appropriate HLA-matched related marrow donor were excluded from the study. Individuals with current alcohol use, illicit drug use, or any other condition (e.g., psychiatric disorder) that, in the opinion of the Investigator, may interfere with the subject's ability to comply with the study requirements or visit schedule were excluded from the study. Individuals with an uncontrolled serious medical condition such as persistent septicemia despite adequate antibiotic therapy, decompensated congestive heart failure despite cardiac medications or pulmonary insufficiency requiring intubation (excluding primary disease for which CB transplantation is proposed), or psychiatric condition that would limit informed consent were excluded from the study.

Conditioning Regimen

[0210] The conditioning regimen was administered starting on Day -8 or Day -6 and was completed on Day -1. All subjects received one of the following two conditioning regimens shown in tables 1-2.

Table 1: 8 Day Conditioning Regimen

Day	Conditioning Regimen
-8	Melphalan 140 mg/m^2
-7	Thiotepa 5 mg/kg

-6	Fludarabine 40 mg/m^2
-5	Fludarabine 40 mg/m^2
-4	Fludarabine 40 mg/m^2 and ATG 1.25 mg/kg
-3	Fludarabine 40 mg/m^2 and ATG 1.75 mg/kg
-2	Rest
-1	Rest

Table 2: 6 Day Conditioning Regimen

Day	Conditioning Regimen
-6	Admit/Hydration
-5	Fludarabine 40 mg/m^2
-4	Fludarabine 40 mg/m^2
-3	Fludarabine 40 mg/m^2, rabbit ATG 1.25 mg/kg
-2	Fludarabine 40 mg/m^2, Melphalan 140 mg/m^2 IV and rabbit ATG 1.75 mg/kg
-1	Rest

Acute GVHD Prophylaxis and Hematopoietic Cytokine Support

[0211] Acute GVHD Prophylaxis and Hematopoietic Cytokine Support are considered standard practice in subjects receiving allogeneic transplant.

[0212] Tacrolimus was administered in a starting dose of 0.03 mg/kg (ideal body weight) daily starting on Day -2, and was changed to oral dosing when tolerated. Tacrolimus was tapered around Day 180, if no acute GVHD was present. Mycophenolate mofetil (MMF) was administrated at 1 gram twice daily. If the subject was < 50 kg, they were given 15 mg/kg orally, twice daily, adjusted to tablet size; intravenous administration at the same dosage was used if oral administration was not tolerated. MMF was started on day –3. If renal failure was present and glomerular filtration rate (GFR) was < 25 mL/min, 1 gram MMF twice daily was not exceeded. No dose adjustment was made for liver disease. MMF was stopped at day 100 if no acute GVHD was present. If the subject had acute GVHD requiring systemic therapy, MMF was optionally stopped 7 days after control of acute GVHD.

[0213] G-CSF was administered at a dose of 5 μg/kg/day (rounded up to the nearest vial) subcutaneously beginning on Day 0, and continuing until the absolute neutrophil count (ANC) was > 2.5 x 10^9/L.

Treatment

[0214] For the study, subjects undergoing double cord blood transplantation were injected with Ang-(1-7). Ang-(1-7) was supplied as a sterile, non-pyrogenic solution for injection at concentrations of 30 mg/mL. The parenteral formulation was produced as a pH-buffered solution, adjusted for the proper osmolality with the addition of 4% mannitol to provide a final osmolality of 295-415 mOsm. The product was packaged in a 2-mL, single-use, stoppered vial with a 1.0-mL fill.

[0215] 300 μg/kg/day or 1000 μg/kg/day of Ang-(1-7) was administered to ten subjects each once daily for 28 days. The first dose of Ang-(1-7) was given within 8 hours of completion of a transfusion of two units of cord blood.

[0216] Ang-(1-7) concentrations were standardized so that a 10-μL volume of solution was dispensed per kilogram of subject body mass for subjects receiving 300 μg/kg, and 33 μL was dispensed per kilogram of subject body mass for subjects receiving 1000 μg/kg. The dose was calculated for each subject based on the subject's mass.

[0217] For administration, Ang-(1-7) was aseptically drawn into a syringe prior to injection. Qualified site personnel administered Ang-(1-7) at the investigational site. A new, unused vial or vials was used for each dose. Ang-(1-7) was administered once daily with a subcutaneous needle into sites located in the abdomen or thigh. Given the potential discomfort associated with a larger volume administered subcutaneously to subjects treated at the 1000 μg/kg/day dose, the full dose was, at the Investigator's discretion, given as two injections into different sites.

Clinical assessment

[0218] Prior to enrollment, subjects were evaluated during the Screening Period (Day -22 to Day-9 relative to the cord blood transplant) to determine eligibility. A variety of standard

clinical and laboratory assessments were used to evaluate subjects prior to study entry, including: physical exam including height, skin, medical history including diagnosis; vital signs; severity grading of oral mucositis; adverse events (baseline) and concomitant therapies, bone marrow sample for cellularity; complete blood count with differential and platelet count; chemistry panel; amylase and Lactate Dehydrogenase (LDH); Beta Human Chorionic Gonadotropin (βHCG) serum or urine pregnancy test for women of childbearing potential; infectious disease screening; HLA class I and II typing (may have been done prior to the screening period); and an immune reconstitution panel.

[0219] During the conditioning regimen (Day -8 or -6 to Day-1 relative to the cord blood transplant), medical history, vital signs (blood pressure, heart rate, respirations, and temperature), adverse events (baseline) and concomitant therapies (including blood transfusion), CBC with differential and platelet count, and a Chemistry panel (sodium, chloride, potassium, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, total bilirubin, ALT, AST, total protein, albumin, and alkaline phosphatase) of subjects was assessed.

[0220] The treatment period begins on day 0, the day of the cord blood transplant. The following assessments are performed on day 0: physical exam including weight (the Day 0 weight measurement will determine the dose of Ang-(1-7) to be administered throughout the treatment period); vital signs (blood pressure, heart rate, respirations, and temperature); severity grading of oral mucositis; acute GVHD assessment at engraftment; adverse events and concomitant therapies (including blood transfusion and hematopoietic growth factors) within 30 days of first Ang-(1-7) dose; pregnancy test for women of childbearing potential; CBC with differential and platelet count; chemistry panel (sodium, chloride, potassium, magnesium, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, total bilirubin, ALT, AST, total protein, albumin, and alkaline phosphatase); and amylase and LDH. The following assessments were performed daily during the treatment period: vital signs (blood pressure, heart rate, respirations, and temperature); severity grading of oral mucositis; adverse events and concomitant medications (including blood transfusion and hematopoietic growth factors); and CBC with differential and platelet count. The following assessments were performed weekly at days 6, 13, 20 and 27 of the treatment period: a symptom-directed physical exam; weight; chemistry panel (sodium, chloride, potassium, magnesium, bicarbonate, BUN, creatinine,

glucose, calcium, phosphorus, total bilirubin, ALT, AST, total protein, albumin, and alkaline phosphatase); amylase and LDH; PT/PTT and INR (day 6 only); and chest X-Ray (Day 27 only). For all weekly assessments during the treatment period, there was a +/- 1 day window.

[0221] The follow-up period begins one week after the point at which a subject completes the treatment period (i.e., one week after Day 27) and lasts up to day 100 after transplantation. The initial follow-up visit was performed +/- 2 days following subject treatment period completion. There was no allowable window at Engraftment. All other assessments during the follow-up period were performed in a +/- 3 day window. The follow-up period assessment timepoints are days 34 (Month 1), 41, 48, 55, 62 (Month 2), 69, 76, 83, and 90. Weekly assessments included: a physical exam including weight; adverse events and concomitant medications (including blood transfusion and hematopoietic growth factors); severity grading of oral mucositis; acute GVHD assessment; vital signs (blood pressure, heart rate, respiration, and temperature); and CBC with differential and platelet count (daily until engraftment, then as clinically indicated until day 100). Monthly assessments include: bone marrow sample for cellularity and chimerism (day 62 only); peripheral blood for chimerism at engraftment and then Days 62 and 100 (if bone marrow sampling not performed); immune reconstitution panel (peripheral blood concentrations of CD3+, CD4+, CD8+, CD19+, and CD56+ cells (at engraftment, Day 34 and Day 62); amylase and LDH; and ECOG performance status.

GVHD assessment

[0222] GVHD was assessed using the criteria shown in tables 3 and 4. The skin, liver and gut were staged from 1-4 according to degree of involvement. Exemplary criteria for staging are shown in table 3. Skin staging was assessed by extent of maculopapular rash, generalized erythroderma, generalized exfoliative dermatitis, ulcerative dermatitis, and/or bullous formation. Liver staging was assessed by bilirubin level. Gut staging was assessed by volume of diarrhea and/or presence of nausea, abdominal cramping, abdominal pain and/or ileus. A clinical grade from 0 to IV was determined based on skin stage, liver stage, gut stage, and/or functional impairment. Exemplary criteria for clinical grading are shown in table 4.

Table 3: Clinical Staging of Acute GVHD

Stage	Skin Findings	Liver Findings (Bilirubin level, mg/dL)	Gut Findings
1	Maculopapular rash on <25% of body surface	2-3	Diarrhea 500-1000 mL/day or persistent nausea
2	Maculopapular rash on 25-50% of body surface	3.1-6	Diarrhea 1000-1500 mL/day
3	Rash covering >50% of the body or generalized erythroderma	6.1-15	Diarrhea >1500 mL/day
4	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation	>15	Diarrhea >1500 mL/day plus severe abdominal cramping/pain with/without ileus

Table 4: Clinical Grading of Acute GVHD

Overall Grade	Stage			
	Skin	Liver	Gut	Functional Impairment
0 (None)	0	0	0	0
I (Mild)	1 to 2	0	0	0
II (Moderate)	1 to 3	1	1	1
III (Severe)	2 to 3	2 to 3	2 to 3	2
IV (Life-threatening)	2 to 4	2 to 4	2 to 4	3

[0223] Table 5 shows clinical trial data for 9 subjects treated with 300 μg/kg/day Ang-(1-7). Two of nine subjects showed symptoms of GVHD. Previous studies of patients receiving double cord blood transplants have shown the rate of GVHD to be approximately 60% (MacMillan, M. et al., "Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors," Blood, 2009, 113(11): 2410-2415). The results presented herein suggest that administration of Ang-(1-7) peptide reduces the risk of GVHD.

Table 5: Effect of Ang-(1-7) Treatment on GVHD and Mucositis

Subject	Mucositis (Grade)	GVHD	100 Day survival
1	1	none	yes
2	none	none	yes
3	1	none	no
4	1	Gr 1 (GI)	yes
5	1	none	no
6	1	Gr 3 (GI)	yes
7	1	none	yes
8	2	none	yes
9	x	x	ongoing (relapsed)

Mucositis assessment

[0224] Incidence of oral mucositis was assessed using the World Health Organization (WHO) Oral Toxicity Scale shown in table 6. Grading ranges from 0 to 4 based on the presence of oral soreness, erythema, ulcers, and/or capability to swallow food.

Table 6: WHO Oral Toxicity Scale

Grade	Findings
0	No mucositis
1	Soreness with or without erythema
2	Erythema and ulcers and patient can swallow solid food
3	Ulcers with extensive erythema and patient cannot swallow solid food; requires liquid diet
4	Mucositis to the extent that alimentation is not possible

[0225] Incidence of mucositis is defined by the occurrence of least one adverse event with Medical Dictionary for Regulatory Activities (MedDRA) preferred term that includes "mucositis" or "stomatitis". Separate analyses were reported for events with severity defined as follows: Grade 3 (severe), Grade 4 (lifethreatening), and Grades 3-4 (severe or life-threatening). The severity grade was determined by NCI-CTCAE. The incidence of Grade 3 mucositis will be summarized based on the proportion of subjects with at least one episode of Grade 3 mucositis. Similar analyses will be performed for Grade 4 and Grade 3-4 events.

[0226] As shown in Table 5, one subject showed no symptoms of mucositis, six subjects presented with grade 1 mucositis, and one subject presented with grade 2 mucositis. As the majority of subjects had only minor symptoms, the results presented herein suggest that administration of Ang-(1-7) peptide reduces the risk of mucositis.

EQUIVALENTS AND SCOPE

[0227] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

CLAIMS

We claim:

1. A method of treating or reducing risk for Graft-Versus-Host Disease comprising administering to a subject who is suffering from or susceptible to Graft-Versus-Host Disease (GVHD) an angiotensin (1-7) peptide.

2. The method of claim 1, wherein the angiotensin (1-7) peptide is administered at an effective dose periodically at an administration interval such that at least one symptom or feature of GVHD is reduced in intensity, severity, duration, or frequency or has delayed in onset.

3. The method of claim 1 or 2, wherein the GVHD is acute GVHD.

4. The method of claim 1 or 2, wherein the GVHD is chronic GVHD.

5. The method of any one of the preceding claims, wherein the GVHD is associated with cord blood transplantation.

6. The method of claim 5, wherein the cord blood transplantation is selected from the group consisting of single cord blood transplantation, double cord blood transplantation, manipulated cord blood transplantation, and combination thereof.

7. The method of claim 6, wherein the manipulated cord blood transplantation comprises *ex vivo* expanded cord blood transplantation.

8. The method of claim 6, wherein the manipulated cord blood transplantation comprises treatment of the cord blood with prostaglandins prior to transplant.

9. The method of claim 6, wherein the manipulated cord blood transplantation comprises depleting T-cells from the cord blood prior to transplant.

10. The method of claim 1 or 2, wherein the GVHD is associated with bone marrow transplantation.

11. The method of claim 1 or 2, wherein the GVHD is associated with adult stem cell transplantation.

12. The method of claim 11, wherein the adult stem cell transplantation is allogeneic adult stem cell transplantation.

13. The method of claim 1 or 2, wherein the GVHD is associated with embryonic stem cell transplantation

14. The method of claim 1 or 2, wherein the GVHD is associated with organ transplantation.

15. The method of claim 1 or 2, wherein the GVHD is associated with blood transfusion.

16. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered concurrently with transplantation.

17. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered periodically subsequent to transplantation.

18. The method of claim 17, wherein the angiotensin (1-7) peptide is administered for at least 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 15 weeks, 18 weeks, 21 weeks, 24 weeks, 6 months, 1 year, 2 years, or longer subsequent to the transplantation.

19. The method of claim 18, wherein the angiotensin (1-7) peptide is administered daily, twice a week, weekly, once every two weeks, once every three weeks, monthly, or at a variable interval.

20. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered periodically prior to the transplantation.

21. The method of claim 20, wherein the angiotensin (1-7) peptide is administered for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks prior to the transplantation.

22. The method of claim 20, wherein the angiotensin (1-7) peptide is administered daily, once every two days, twice a week, weekly, or at a variable interval.

23. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered intravenously, intradermally, orally, by inhalation, transdermally (topical), subcutaneously, and/or transmucosally.

24. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered at the effective dose of 5-1,500 μg/kg/day.

25. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered at the effective dose of 100-1,000 μg/kg/day.

26. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered at the effective dose of 300-1,000 μg/kg/day.

27. The method of any one of the preceding claims, wherein the administration of the angiotensin (1-7) peptide results in substantially free of Stage 4, 3, 2, or 1 acute GVHD in the subject 100 days following transplantation.

28. The method of any one of the preceding claims, wherein the administration of the angiotensin (1-7) peptide results in substantially free of Grade IV, III, II, or I acute GVHD in the subject 100 days following transplantation.

29. The method of any one of the preceding claims, wherein the administration of the angiotensin (1-7) peptide results in substantially free of acute GVHD symptoms in the subject 100 days following transplantation.

30. The method of any one of the preceding claims, wherein the administration of the angiotensin (1-7) peptide results in substantially free of chronic GVHD symptoms in the subject 6 months following transplantation.

31. A method of transplantation comprising

administering an angiotensin (1-7) peptide in conjunction with introducing an allogeneic tissue or cell into a subject.

32. The method of claim 31, wherein the allogeneic tissue or cell comprises cord blood.

33. The method of claim 31, wherein the allogeneic tissue or cell comprises bone marrow.

34. The method of claim 31, wherein the allogeneic tissue or cell comprises adult stem cell.

35. The method of claim 31, wherein the allogeneic tissue or cell comprises embryonic stem cell.

36. The method of claim 31, wherein the allogeneic tissue or cell comprises a solid organ.

37. The method of any one of claims 31-36, wherein the angiotensin (1-7) peptide is administered prior to introducing the allogeneic tissue or cell.

38. The method of any one of claims 31-36, wherein the angiotensin (1-7) peptide is administered concurrently with introducing the allogeneic tissue or cell.

39. The method of any one of claims 31-36, wherein the angiotensin (1-7) peptide is administered subsequent to introducing the allogeneic tissue or cell.

40. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide is administered in combination with an immunosuppressant.

41. The method of claim 40, wherein the immunosuppressant is selected from the group consisting of antithymocyte globulin, anti-TNF agents, azathioprine or other inosine 5'-monophosphate dehydrogenase inhibitors, azodiacarbonide, bisindolyl maleimide VIII, brequinar, chlorambucil, CTLA4-Ig, corticosteroids, cyclophosphamide, cyclosporine A, deoxyspergualin, dexamethasone, FK506, glucocorticoids, IL-2 antagonists, leflunomide, mercaptopurine, 6-mercaptopurine, methotrexate, methylprednisolone, mizoribine, mizoribine monophosphate, muromonab CD3, mycophenolate mofetil, OKT3, prednisone, sirolimus, rapamycin, rho (D) immune globin, vitamin D analogs, and combination thereof.

42. The method of any one of claims 31-41, wherein the administration of the angiotensin (1-7) peptide results in reduced intensity, severity, duration, or frequency or delayed onset of at least one symptom or feature of Graft-Versus-Host Disease.

43. The method of any one of claims 31-41, wherein the administration of the angiotensin (1-7) peptide results in reduced intensity, severity, duration, or frequency or delayed onset of at least one symptom or feature of mucositis.

44. The method of any one of the preceding claims, wherein the angiotensin (1-7) peptide comprises the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:1).

45. The method of any one of claims 1-43, wherein the angiotensin (1-7) peptide is a functional equivalent of SEQ ID NO:1.

46. The method of claim 45, wherein the functional equivalent is a linear peptide.

47. The method of claim 46, wherein the linear peptide comprises a sequence that includes at least four amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least four amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7).

48. The method of claim 46, wherein the linear peptide comprises a sequence that includes at least five amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least five amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7).

49. The method of claim 46, wherein the linear peptide comprises a sequence that includes at least six amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least six amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7).

50. The method of any one of claim 47-49, wherein the at least four, five or six amino acids, respectively, further maintain their relative spacing as they appear in the naturally-occurring Angiotensin (1-7).

51. The method of any one of claims 46-50, wherein the linear peptide contains 4-25 amino acids.

52. The method of any one of claims 46-51, wherein the linear peptide is a fragment of the naturally-occurring Angiotensin (1-7).

53. The method of any one of claims 46-52, wherein the linear peptide contains amino acid substitutions, deletions and/or insertions in the naturally-occurring Angiotensin (1-7).

54. The method of claim 53, wherein the linear peptide has an amino acid sequence of Asp^{1}-Arg^{2}-Nle^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:4).

55. The method of claim 53, wherein the linear peptide has an amino acid sequence of Asp^1-Arg^2-Val^3-Ser^4-Ile^5-His^6-Cys^7 (SEQ ID NO:6).

56. The method of claim 45, wherein the functional equivalent is a cyclic peptide.

57. The method of claim 56, wherein the cyclic peptide comprises a linkage between amino acids.

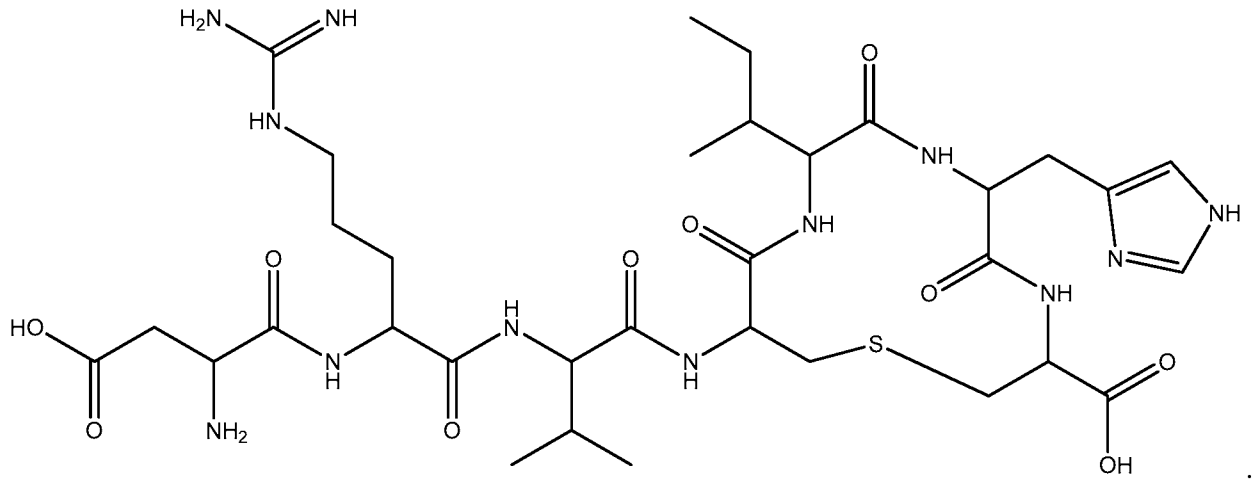
58. The method of claim 57, wherein the linkage is located at residues corresponding to positions Tyr^4 and Pro^7 in naturally-occurring Angiotensin (1-7).

59. The method of claim 56 or 57, wherein the linkage is a thioether bridge.

60. The method of any one of claims 56-59, wherein the cyclic peptide comprises an amino acid sequence otherwise identical to the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:1).

61. The method of any one of claims 56-59, wherein the cyclic peptide comprises a norleucine (Nle) replacing position Val^3 in naturally-occurring Angiotensin (1-7).

62. The method of claim 61, wherein the cyclic peptide is a 4,7-cyclized angiotensin (1-7) with the following formula:

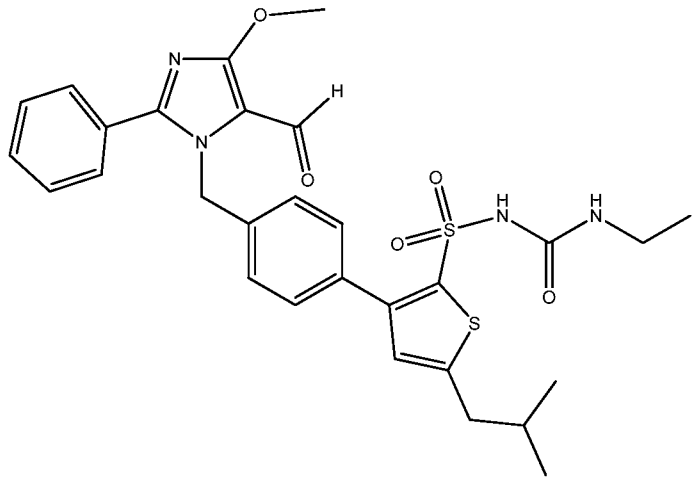


63. The method of any one of claims 44-62, wherein the angiotensin (1-7) peptide comprises one or more chemical modifications to increase protease resistance, serum stability and/or bioavailability.

64. The method of claim 63, wherein the one or more chemical modifications comprise pegylation.

65. A method of treating GVHD comprising administering to a subject who is suffering from or susceptible to GVHD a non-peptidic angiotensin (1-7) receptor agonist.

66. The method of claim 65, wherein the non-peptidic angiotensin (1-7) receptor agonist is a compound with the following structure:



or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/037308

A. CLASSIFICATION OF SUBJECT MATTER

C07K 7/14 *(2006.01)*
A61K 38/08 *(2006.01)*
A61K 38/12 *(2006.01)*
A61K 38/22 *(2006.01)*
A61P 37/06 *(2006.01)*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 7/14, A61K 38/08, 38/12, 38/22, A61P 37/06

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)

PubMed, ROSPATENT, Patents (PCT), DEPATISnet, K-PION, Pat FT, Esp@cenet, SNAPT, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2011088920 A (CBIO LTD) 06.05.2011, claims	1-66
A	US 20070213307 A1 (CELMED ONCOLOGY, INC.) 13.09.2007, claims	1-66
A	EP 739212 A1 (GENETICS INSTITUTE, INC.) 30.10.1996, claims	1-66

☐ 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 document 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	Date of mailing of the international search report
01 July 2013 (01.07.2013)	08 August 2013 (08.08.2013)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer M. Khudyaev Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/037308

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:

According to Rule 13 of the Regulations under the PCT the claimed inventions does not meet the requirement of unity of invention *a priory*, therefore independent claims 1, 31 and 65 form several groups of inventions, which are not so linked as to form a single general inventive concept.

The special technical feature of independent claims 1 and 31 is administering an angiotensin (1-7) peptide.

This special technical feature is absent in claim 65.

The special technical feature of independent claim 65 is administering a non-peptidic angiotensin (1-7) receptor agonist.

This special technical feature is absent in claims 1 and 31.

Hence, claims comprise 2 groups of inventions, namely:

1 invention – claims 1- 64

2 invention – claims 65-66

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.:

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.