



(51) International Patent Classification:

A61P 31/12 (2006.01) A61K 38/08 (2006.01)
A61P 35/04 (2006.01) A61K 38/48 (2006.01)
C07K 7/14 (2006.01) A61K 39/395 (2006.01)
C07K 16/22 (2006.01)

(21) International Application Number:

PCT/US2018/030488

(22) International Filing Date:

01 May 2018 (01.05.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/500,456 02 May 2017 (02.05.2017) US
62/518,493 12 June 2017 (12.06.2017) US

(71) Applicants: BETH ISRAEL DEACONESS MEDICAL CENTER, INC. [US/US]; 330 Brookline Avenue, Boston, Massachusetts 02215 (US). **FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT**.

(72) Inventor: BHATT, Rupal; c/o Beth Israel Deaconess Medical Center, Inc., 330 Brookline Avenue, Boston, Massachusetts 02215 (US).

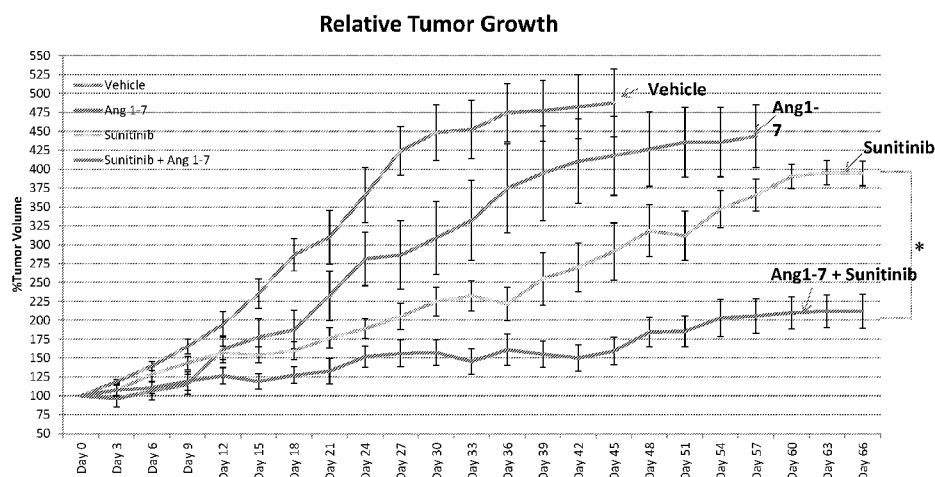
(74) Agent: HUNTER-ENSOR, PH.D., Melissa; Greenberg Traurig, LLP, One International Place, Boston, Massachusetts 02110 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: COMBINATION THERAPY FOR CANCER

FIG. 1 Sunitinib + Ang (1-7) A498 Xenograft



(57) Abstract: The present invention features compositions comprising a tyrosine kinase inhibitor and an agent that enhances Ang1-7 levels, and methods of using such compositions for the treatment of neoplasias (e.g., metastatic renal cell carcinoma).

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

COMBINATION THERAPY FOR CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the following U.S. Provisional Application
5 Nos.: 62/500,456, filed May 2, 2017, and 62/518,493, filed June 12, 2017, the entire contents
of each of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

10 Many patients with metastatic renal cell carcinoma (mRCC) therapy benefit from
treatment with tyrosine kinase inhibitors (TKI) such as sunitinib, which act through
blockade of VEGFR (Vascular Endothelial Growth Factor Receptor). However, responses
are limited and not durable and tumors eventually become resistant to therapy. The
VEGFR tyrosine kinase inhibitors likely inhibit tumor growth through their activity on the
15 tumor endothelium but also induce many changes in the tumor and in the tumor
microenvironment. There is an urgent need for therapies to improve outcomes in patients
with mRCC and related diseases.

SUMMARY OF THE INVENTION

20 As described below, the present invention features compositions comprising a
tyrosine kinase inhibitor and an agent that enhances Ang1-7 levels, and methods of using
such compositions for the treatment of neoplasias (e.g., metastatic renal cell carcinoma).

The invention is based, at least in part, on the discovery that treatment of metastatic
renal cell carcinoma (mRCC) with a VEGF tyrosine kinase inhibitor (e.g., sunitinib) and an
25 agent that increases levels of Ang 1-7 in a murine model of human mRCC reduced tumor
growth in mice. Accordingly, restoration of Ang1-7 levels in conjunction with VEGF
inhibition is an effective treatment for metastatic RCC. VEGFR tyrosine kinase inhibitors
useful in the invention include sunitinib, sorafenib, axitinib, and pazopanib.

In one aspect, the invention features a composition for the treatment of a neoplasia
30 comprising a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels.

In another aspect, the invention features a composition for the treatment of a neoplasia
involving a tyrosine kinase inhibitor and a Mas receptor agonist.

In another aspect, the invention features a composition for the treatment of a neoplasia
comprising sunitinib and an Ang1-7 peptide.

In another aspect, the invention features a method for reducing the survival and/or proliferation of a neoplasia, the method involving contacting the neoplasia with a tyrosine kinase inhibitor and a Mas receptor agonist, thereby reducing the survival and/or proliferation of the neoplasia.

5 In another aspect, the invention features a method for reducing the survival and/or proliferation of a neoplasia, the method comprising contacting the neoplasia with a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels, thereby reducing the survival and/or proliferation of the neoplasia.

10 In another aspect, the invention features a method for treating a neoplasia, the method comprising administering a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels to a subject having a neoplasia, thereby treating the neoplasia.

In another aspect, the invention features a method for treating a neoplasia, the method involving administering a tyrosine kinase inhibitor and a Mas receptor agonist to a subject having a neoplasia, thereby treating the neoplasia.

15 In various embodiments of the above aspects or any other aspect delineated herein, the agent is Ang1-7 or diminazene aceturate (DIZE). In various embodiments of the above aspects, the tyrosine kinase inhibitor is a VEGF tyrosine kinase inhibitor (e.g., sunitinib, sorafenib, axitinib, and pazopanib). In other embodiments of the above-aspects, the agent that increases Ang1-7 levels is a fragment of angiotensin. In other embodiments of the
20 above-aspects, the fragment of angiotensin is a peptide comprising amino acids: Asp-Arg-Val-Ser-Ile-His-Pro. In other embodiments of the above-aspects, the Mas receptor agonist is a small molecule, peptide, or polynucleotide. In other embodiments of the above-aspects, the peptide is Ang-(1-7), alamandine, NorLeu3-Angiotensin (1-7), linear Pancyte, or TXA302. In other embodiments of the above aspects, the small molecule is AVE 0991. In various
25 embodiments of the above aspects, the agent is diminazene aceturate. In other embodiments of the above-aspects, the agent or the Mas receptor agonist is a peptide comprising amino acids: Asp-Arg-Val-Ser-Ile-His-Pro. In other embodiments of the above-aspects, the VEGF tyrosine kinase inhibitor is sunitinib and the agent or the Mas receptor agonist is an Ang1-7 peptide. In other embodiments of the above aspects, the sunitinib and the Ang1-7 peptide or
30 analog thereof are included in a single formulation or are formulated separately.

The invention provides compositions comprising a tyrosine kinase inhibitor and an agent that increases levels of angiotensin 1-7. Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided

below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

5 Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988);
10 The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By "agent that increases Ang1-7 levels" is meant a small molecule, polynucleotide, polypeptide, or fragment thereof, that increases the expression of an Ang1-7 peptide or a
15 polynucleotide encoding said peptide. In one embodiment, the agent is an Angiotensin polypeptide or is a fragment of angiotensin, such as an exogenous Ang1-7 peptide.

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). Functional equivalents and analogs of Ang-(1-7) include, for
20 example, agents that act as Mas receptor agonists or that otherwise increase, such as alamandine (See, Lautner et al., Circ Res. 2013 Apr 12;112(8):1104-11), NorLeu3-Angiotensin (1-7) [DSC127] (See, Rodgers et al., Adv Wound Care (New Rochelle). 2015 Jun 1; 4(6): 339-345), Ave0991 (See, Santos et al., Cardiovasc Drug Rev. 2006), linear Pancyte, which is a linear analogue of A(1-7) with serine at position #4 and cysteine at #7
25 (See, Machado-Silva et al., Expert Opinion on Therapeutic Patents, Vol. 26 (6): 669-678, 2016), diminazene aceturate (DIZE) (See, Qi et al., Hypertension Oct. 62: 746-752, 2013), TXA302 (See, Caggiano et al., Stroke 2018;49:ATMP33, 2018) or any other agents that increase the level or activity of Ang-(1-7). It is noted that linear Pancyte lacks the cyclic structure of conventional Pancyte.

30 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)", "Ang1-7:" and "Ang-(1-7)" are used interchangeably. Naturally-occurring Angiotensin (1-7) (also referred to as Ang-(1-7)) is a seven amino acid peptide: Asp-Arg-Val-Ser-Ile-His-Pro. In other embodiments, an Ang-(1-7) peptide comprises Asp-Arg-Val-Ser-Ile-His-Cys.

5 By "agent" is meant a peptide, nucleic acid molecule, or small compound.

By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

20 In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

"Detect" refers to identifying the presence, absence or amount of the analyte to be detected.

30 By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include metastatic or localized renal carcinoma.

By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s)

used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

5 By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 3, 4, 5, 6, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

10 The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially
15 affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example,
20 polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

25 By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that
30 exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as

well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 7 amino acids, 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid

sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By “hybridize” is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or

by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.

Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of
5 numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "treat," "treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that,
10 although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

15 Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

20 The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of
25 any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing that sunitinib and Ang1-7 treatment reduces the growth of human renal cell carcinoma in murine xenografts.

30

DETAILED DESCRIPTION OF THE INVENTION

The invention features compositions comprising a tyrosine kinase inhibitor and an agent that enhances Ang1-7 levels, and methods of using such compositions for the treatment of neoplasias (e.g., metastatic renal cell carcinoma).

5 The invention is based, at least in part, on the discovery that a tyrosine kinase inhibitor and an agent that enhances Ang1-7 levels (e.g., an Ang1-7 peptide) inhibits tumor growth.

The present invention provides methods of treating metastatic renal cell carcinoma and related diseases and/or disorders or symptoms thereof which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a compound of
10 the formulae herein to a subject (e.g., a mammal such as a human). Thus, one embodiment is a method of treating a subject suffering from or susceptible to a neoplastic disease or disorder or symptom thereof. The method includes the step of administering to the mammal a therapeutic amount of an amount of a compound herein sufficient to treat the disease or disorder or symptom thereof, under conditions such that the disease or disorder is treated.

15

Ang1-7

Ang1-7 is part of the renin-angiotensin system and is converted from a precursor, also known as Angiotensinogen, which is an alpha-2-globulin that is produced constitutively and released into the circulation mainly by the liver. Angiotensinogen is a member of the serpin
20 family and also known as renin substrate. Human angiotensinogen is 452 amino acids long, but other species have angiotensinogen of varying sizes. In one embodiment, the first 12 amino acids are the important for angiotensin activity: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile

Different types of angiotensin may be formed by the action of various enzymes. For
25 example, Angiotensin (1-7) is generated by action of Angiotensin-converting enzyme 2 (ACE 2). 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. As used herein, the term "naturally-occurring Angiotensin (1-7)" includes any Angiotensin (1-7)
30 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) are known in the art, and described, for example, in US Patent No. 9,107,870. 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.

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).

In some embodiments, a functional equivalent of angiotensin (1-7) also encompass any peptide that contain 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.

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, tryptophane, 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.

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 4-25 amino acids (e.g., 4-20, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7 amino acids). In some embodiments, the linear peptide contains 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids.

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.

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 $H_2N-CH(R)-COOH$. 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.

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

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.sub.2NH--, --CH.sub.2S--, --CH.sub.2--CH.sub.2--, --CH.dbd.CH-- (cis and trans), --CH.sub.2SO--, --CH(OH)CH.sub.2--, --COCH.sub.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.

Ang-(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.

Ang-(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. Pat. Nos. 4,305,872 and 4,316, 891, Bodonsky et al. Chem. Ind. (London), 38:1597 (1966); and
5 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. Pat. No. 4,244,946. (Reviewed in Houver-Weyl, Methods of Organic Chemistry. Vol E22a. Synthesis of Peptides and Peptidomimetics, Murray Goodman,
10 Editor-in-Chief, Thieme. Stuttgart. New York 2002).

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
15 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, 3.sup.rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001.

During any process of the preparation of an Ang-(1-7) peptide, it may be desirable to
20 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, Weinheim p. 142. For example, alpha amino protecting groups include acyl type protecting
25 groups (e.g., trifluoroacetyl, formyl, acetyl), aliphatic urethane protecting groups (e.g., t-butylloxycarbonyl (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
30 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.

Further, Ang-(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).

5 Alternatively, Ang-(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.

10 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
15 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.

20 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-
25 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
30 vivo, and thus can have greater therapeutic efficacy than the original peptide (Brady and Dodson, Nature 368:692-693 (1994); ameson 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.

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.

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 .alpha.,.alpha.-disubstituted amino acids, N-alkyl amino acids, C-.alpha.-methyl amino acids, .beta.-amino acids, and .beta.-methyl amino acids. Amino acids analogs useful in the present invention may include, but are not limited to, .beta.-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.

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
5 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

10 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
15 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.
20 Such cyclic bonds and other modifications can also be applied to the cyclic peptides and derivative compounds of this invention.

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
25 the molecule; .alpha.- and .beta.-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
30 alkoxy-carbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (e.g., methylation, benzylation, t-butylation, tosylation, alkoxy-carbonylation, 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, .beta.,.beta.-tetramethylene cysteine, .beta.,.beta.-pentamethylene cysteine, .beta.-

5 mercaptopropionic acid, .beta.,.beta.-pentamethylene-.beta.-mercaptopropionic acid, 2-mercaptopbenzene, 2-mercaptaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, .alpha.-aminoadipic acid, m-aminomethylbenzoic acid and .alpha.,.beta.-diaminopropionic acid.

Following synthesis of a linear peptide, with or without N-acetylation and/or C-

10 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

15 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.sub.2 and K.sub.3Fe(CN).sub.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

20 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-

25 terminal amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), .alpha.-amino adipic acid, m-aminomethylbenzoic acid, .alpha.,.beta.-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

30 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-
 5 containing residue and an appropriately derivatized .alpha.-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
 10 DMF.

Exemplary Angiotensin-(1-7) Peptides

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: (SEQ ID NO:

15 1) Asp.sup.1-Arg.sup.2-Val.sup.3-Tyr.sup.4-Ile.sup.5-His.sup.6-Pro.sup.7

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

(SEQ ID NO: 4) Xaa.sup.1-Xaa.sup.2-Xaa.sup.3-Xaa.sup.4-Xaa.sup.5-Xaa.sup.6-Xaa.sup.7,
 or a pharmaceutically acceptable salt thereof. Xaa.sup.1 is any amino acid or a dicarboxylic
 20 acid. In certain embodiments, Xaa.sup.1 is Asp, Glu, Asn, Acpc (1-aminocyclopentane carboxylic acid), Ala, Me.sub.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.sup.1 is a negatively-charged amino acid, such as Asp or Glu, typically Asp.

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

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

Xaa.sup.4 is Tyr, Tyr(PO.sub.3), Thr, Ser, homoSer (homoserine), azaTyr (aza-.alpha.-sup.1-homo-L-tyrosine) or Ala. In certain embodiments, Xaa.sup.4 is a hydroxyl-substituted amino acid such as Tyr, Ser or Thr, typically Tyr.

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

Xaa.sup.6 is His, Arg or 6-NH.sub.2-Phe (6-aminophenylalanine). In certain embodiments, Xaa.sup.6 is a fully or partially positively-charged amino acid such as Arg or His.

5 Xaa.sup.7 is Cys, Pro or Ala.

In certain embodiments, one or more of Xaa.sup.1-Xaa.sup.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.sup.1-Xaa.sup.1 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa.sup.1-Xaa.sup.6 are
10 identical to the corresponding amino acid in naturally-occurring Ang-(1-7).

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

In certain embodiments, the peptide has the amino acid sequence Asp.sup.1-Arg.sup.2-Val.sup.3-Ser.sup.4-Ile.sup.5-His.sup.6-Cys.sup.7 (SEQ ID NO:2) or Asp.sup.1-Arg.sup.2-Val.sup.3-ser.sup.4-Ile.sup.5-His.sup.6-Cys.sup.7 (SEQ ID NO:6).
20

In some embodiments, a linear angiotensin (1-7) peptide is an Ang (1-9) peptide having a sequence of Asp.sup.1-Arg.sup.2-Val.sup.3-Tyr.sup.4-Ile.sup.5-His.sup.6-Pro.sup.7-Phe-.sup.8-His.sup.9 (SEQ ID NO: 23). In some embodiments, an angiotensin (1-7) peptide
25 is a derivative of Ang (1-9). For exemplary Ang (1-9) peptides, including Ang(1-9) derivatives, see U.S. Patent Publication 2012/0172301, the disclosure of which is hereby incorporated by reference.

In some embodiments, a linear angiotensin (1-7) peptide is Alamandine, or an Alamandine derivative. Alamandine is a naturally occurring peptide with an amino acid
30 sequence of Ala'-Arg.sup.2-Val.sup.3-Tyr.sup.4-Ile.sup.5-His.sup.6-Pro'(SEQ ID NO: 24) that is known to be a component of the Renin-Angiotensin system (see Lautner et al., Discovery and Characterization of Alamandine, 2013, Circ. Res. 112(8): 1104-1111). A discussion of Alamandine and Alamandine derivatives may be found in European Patent

Application 2,264,048, the disclosure of which is hereby incorporated by reference.

Exemplary Cyclic Angiotensin (1-7) Peptides

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
5 corresponding to positions Tyr.sup.4 and Pro.sup.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). One example of such an
10 analog is Asp.sup.1-Arg.sup.2-Val.sup.3-Ser.sup.4-Ile.sup.5-His.sup.6-Cys.sup.7 (SEQ ID NO: 22), wherein a linkage is formed between Ser.sup.4 and Cys.sup.7.

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
15 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.

Ang (1-7) Receptor Agonists

In some embodiments, the present invention provides methods of treating cancer
20 using 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)
25 activity. In some embodiments, an angiotensin-(1-7) receptor agonist directly interacts with an angiotensin-(1-7) receptor (i.e., including but not limited to 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.

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

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.

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.

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, and octyl. A C1-C4 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

An alkenyl group is a straight chained or branched non-aromatic hydrocarbon that 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.

Aromatic (aryl) groups include carbocyclic aromatic groups such as phenyl, naphthyl, and anthracenyl, 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.

An aralkyl group is an alkyl group substituted by an aryl group.

5 **Tyrosine Kinase Inhibitors and VEGF pathway inhibitors**

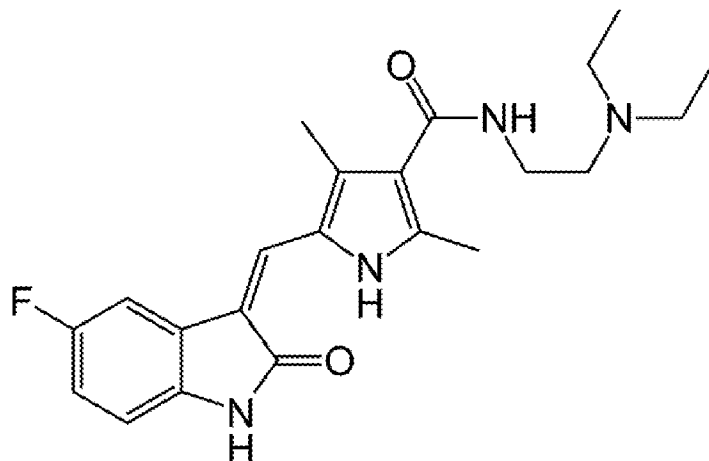
Tyrosine Kinase Inhibitors are a class of molecules that protein tyrosine kinases of which there are two types, receptor tyrosine kinases (RTKs) and cellular tyrosine kinases (CTKs), and serine-threonine kinases (STKs). RTK mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor
10 dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response (e.g., cell division, metabolic effects on the extracellular microenvironment, etc.). See, Schlessinger and Ullrich, 1992,
15 Neuron 9:303 391.

It has been shown that tyrosine phosphorylation sites on growth factor receptors function as high-affinity binding sites for SH2 (src homology) domains of signaling molecules. Fantl et al., 1992, Cell 69:413 423, Songyang et al., 1994, Mol. Cell. Biol. 14:2777 2785), Songyang et al., 1993, Cell 72:767 778, and Koch et al., 1991, Science
20 252:668 678. Several intracellular substrate proteins that associate with RTKs have been identified. They may be divided into two principal groups: (1) substrates that have a catalytic domain, and (2) substrates which lack such domain but which serve as adapters and associate with catalytically active molecules. Songyang et al., 1993, Cell 72:767 778. The specificity of the interactions between receptors and SH2 domains of their substrates is determined by the
25 amino acid residues immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine residues on particular receptors are consistent with the observed differences in their substrate phosphorylation profiles. Songyang et al., 1993, Cell 72:767 778. These observations suggest that the function of each RTK is determined not only
30 by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

STKs, being primarily cytosolic, affect the internal biochemistry of the cell, often as a down-line response to a PTK event. STKs have been implicated in the signaling process which initiates DNA synthesis and subsequent mitosis leading to cell proliferation.

5 Tyrosine kinase inhibitors are useful in combination with agents that increase Ang1-7 levels in a subject. Tyrosine kinase inhibitors are known in the art and described, for example, in US patent No. 7,125,905 and 6573293, each of which is incorporated herein by reference in its entirety. In particular embodiments, the tyrosine kinase inhibitor is a VEGF tyrosine kinase inhibitor, such as sunitinib, sorafenib, axitinib, and pazopanib. VEGF tyrosine kinase inhibitors are combined with an Ang1-7 peptide or other agent that increases
10 endogenous Ang1-7 levels. Ang1-7 peptides are known in the art and described, for example, in US Patent No. 9, 107,870 and in US Patent Publications 2015/0246093 and 2016/0074464, each of which is incorporated herein by reference in its entirety.

In one embodiment, the tyrosine kinase inhibitor is sunitinib. The skeletal formula of sunitinib is provided below:



In one embodiment, an Ang(1-7) peptide is administered concurrently with a VEGF tyrosine kinase inhibitor, such as sunitinib, sorafenib, axitinib, and pazopanib. In another embodiment, the Ang(1-7) peptide is administered within 1, 3, 5, 10, 12, 15, or 24 hours of a VEGF tyrosine kinase inhibitor, such as sunitinib, sorafenib, axitinib, and pazopanib. In another embodiment, the Ang(1-7) peptide is administered within 1, 2, 3, 4, 5, 6, or 7 days of a VEGF tyrosine kinase inhibitor, such as sunitinib, sorafenib, axitinib, and pazopanib.

Formulations

In accordance with the methods of the invention, an Ang (1-7) peptide or angiotensin (1-7) receptor agonist and a tyrosine kinase inhibitor is administered to a subject as described herein. The compositions can be formulated separately or together. In one embodiment, each agent is 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, for example intravenous or subcutaneous 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)).

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 pyrrolidone, 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 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.

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, sustained release formulation, or powder. The composition can also be formulated as a suppository, with traditional binders and carriers such as triglycerides.

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.

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.

An Ang (1-7) peptide or angiotensin (1-7) receptor agonist in combination with a tyrosine kinase inhibitor as described herein can be formulated as neutral or salt forms.

Pharmaceutically acceptable salts include 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.

Oral Formulations

In some embodiments, a suitable pharmaceutical composition is an oral formulation.

5 It is contemplated that any medically-acceptable oral formulation may be used within the scope of the present invention.

In some embodiments, provided compositions include at least one pH-lowering agent.

It is contemplated that a pH-lowering agent suitable for use in some embodiments of the present invention include any pharmaceutically acceptable pH-lowering agent, or
10 combination of pH-lowering agents, that are a) not toxic to the gastrointestinal tract, b) are capable of either delivering hydrogen ions or capable of inducing higher hydrogen ion content from the local environment, and/or c) that are capable of being orally administered in an amount sufficient to lower the local intestinal pH below the pH optima for proteases found there. Various tests may be used to determine if a pH-lowering agent is suitable for the
15 present invention and what amount is appropriate. For example, a pH-lowering agent or combination of pH-lowering agents is suitable for the present invention if a particular amount, when added to a solution of 10 milliliters of 0.1M sodium bicarbonate lowers the pH of the solution to no higher than 5.5, 4.7, or 3.5. In some embodiments, an amount of pH-lowering agent or agents may be added to lower pH, in a solution of 10 milliliters of 0.1M
20 sodium bicarbonate, to no higher than 3.4, 3.2, 3.0, or 2.8.

In some embodiments, a suitable pH-lowering agent or agents include at least one pH-lowering agent that has a pKa no higher than 4.2 (e.g., no higher than 4.0, 3.8, 3.6, 3.4, 3.2, 3.0 or 2.8). Exemplary pH-lowering agents suitable for the present invention include, but are not limited to, carboxylic acids such as acetylsalicylic, acetic, ascorbic, citric, fumaric,
25 glucuronic, glutaric, glyceric, glycolic, glyoxylic, isocitric, isovaleric, lactic, maleic, oxaloacetic, oxalosuccinic, propionic, pyruvic, succinic, tartaric, and valeric; aluminum chloride; zinc chloride; acid salts of amino acids (or derivatives thereof) including acid salts of acetylglutamic acid, alanine, arginine, asparagine, aspartic acid, betaine, carnitine, carnosine, citrulline, creatine, glutamic acid, glycine, histidine, hydroxylysine,
30 hydroxyproline, hypotaurine, isoleucine, leucine, lysine, methylhistidine, norleucine, ornithine, phenylalanine, proline, sarcosine, serine, taurine, threonine, tryptophan, tyrosine, and valine; certain phosphate esters including fructose 1,6 diphosphate and glucose 1,6

diphosphate may also be appropriate pH-lowering agents in certain embodiments. In particular embodiments, citric acid or tartaric acid is used as pH-lowering agent.

The quantity required of any particular pH-lowering agent or combination of pH-lowering agents may vary. Typically, a suitable amount may be determined using various tests known in the art and described herein (for example, using pH-lowering test in a solution of 10 milliliters of 0.1M sodium bicarbonate described above). As non-limiting examples, suitable amount of a pH lowering agent used in a formulation according to the present invention may be an amount of or greater than about 100 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 425 mg, 450 mg, 475 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675, mg, 700 mg, 725 mg, 750 mg, 775 mg, 800 mg, 825 mg, 850 mg, 875 mg, 900 mg, 925 mg, 950 mg, 975 mg, or 1,000 mg. In other embodiments, the amount of citric acid used may exceed 1,000 mg.

In some embodiments, a suitable amount of a pH lowering agent (e.g., citric acid or tartaric acid) used may be measured as a percent of the total weight of a particular dosage form. As non-limiting examples, a suitable amount of a pH lowering agent used may be an amount of or greater than about 10% (e.g., of or greater than 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) of the total weight of a solid dosage form.

In various embodiments, a composition of the invention includes one or more absorption enhancers. As used herein, an absorption enhancer refers to an agent that increase the solubility of other components in either the aqueous or lipophilic environment into which they are released and/or enhance the uptake of an active peptide (e.g., an angiotensin (1-7) peptide) across the intestinal wall. In some embodiments, an absorption enhancer is referred to as a solubility enhancer and/or an uptake enhancer.

In some embodiments, it is possible to have a mixture of absorption enhancers wherein some provide enhanced solubility, some provide enhanced uptake, and some provide both. It is possible to have various numbers of absorption enhancers in a given embodiment including, without limitation, one, two, three, four, five, six, seven, eight, nine, or ten absorption enhancers.

Surface active agents are an example of useful absorption enhancers with properties of both solubility enhancers and uptake enhancers. In some embodiment, when surface active agents are used as absorption enhancers, they may be free flowing powders for facilitating the mixing and loading of capsules during the manufacturing process. In other embodiments

when a surface active agent is used to increase the bioavailability of an angiotensin (1-7) peptide, the surface active agent may be selected from the group consisting of (a) anionic surface active agents such as cholesterol derivatives (e.g. bile acids), (b) cationic surface agents (e.g. acyl carnitines, phospholipids and the like), (c) non-ionic surface active agents, and (d) mixtures of anionic surface active agents and negative charge neutralizers, and combinations thereof. Negative charge neutralizers include but are not limited to acyl carnitines, cetyl pyridinium chloride, and the like.

In some embodiments, an acid soluble bile acid and a cationic surface active agent with be used together as absorption enhancers. Acyl carnitines (such as lauroyl carnitine), phospholipids and bile acids may be particularly effective absorption enhancers in some embodiments.

While a variety of absorption enhancers are suitable for use in various embodiments, the following exemplary list is intended to illustrate some embodiments of the present invention. Without limitation, some suitable absorption enhancers include: (a) salicylates such as sodium salicylate, 3-methoxysalicylate, 5-methoxysalicylate and homovanilate; (b) bile acids such as taurocholic, taurodeoxycholic, deoxycholic, cholic, glycholic, lithocholate, chenodeoxycholic, ursodeoxycholic, ursocholic, dehydrocholic, fusidic, etc.; (c) non-ionic surfactants such as polyoxyethylene ethers (e.g. Brij 36T, Brij 52, Brij 56, Brij 76, Brij 96, Texaphor A6, Texaphor A14, Texaphor A60 etc.), p-t-octyl phenol polyoxyethylenes (Triton X-45, Triton X-100, Triton X-114, Triton X-305 etc.) nonylphenoxypolyoxyethylenes (e.g. Igepal CO series), polyoxyethylene sorbitan esters (e.g. Tween-20, Tween-80 etc.); (d) anionic surfactants such as dioctyl sodium sulfosuccinate; (e) lyso-phospholipids such as lysolecithin and lysophosphatidylethanolamine; (f) acylcarnitines, acylcholines and acyl amino acids such as lauroylcarnitine, myristoylcarnitine, palmitoylcarnitine, lauroylcholine, myristoylcholine, palmitoylcholine, hexadecyllysine, N-acylphenylalanine, N-acylglycine etc.; (g) water soluble phospholipids such as diheptanoylphosphatidylcholine, dioctylphosphatidylcholine etc.; (h) medium-chain glycerides which are mixtures of mono-, di- and triglycerides containing medium-chain-length fatty acids (caprylic, capric and lauric acids); (i) ethylene-diaminetetraacetic acid; (j) cationic surfactants such as cetylpyridinium chloride; (k) fatty acid derivatives of polyethylene glycol such as Labrasol, Labrafac, etc.; and (l) alkylsaccharides such as lauroyl maltoside, lauroyl sucrose, myristoyl sucrose, palmitoyl sucrose, etc.

In some embodiments, the absorption enhancer(s) will be present in a quantity measured as a percent by weight, relative to the overall weight of the pharmaceutical composition (typically exclusive of enteric coating). By way of additional non-limiting example, the quantity of absorption enhancer present in an embodiment may range from 0.1 to 20 percent by weight; from 0.5 to 20 percent by weight; from 1.0 to 20 percent by weight, from 2.0 to 20 percent by weight, from 3.0 to 20 percent by weight, from 4.0 to 20 percent by weight, from 5.0 to 20 percent by weight, from 5.0 to 15 percent by weight, from 5.0 to 14 percent by weight, from 5.0 to 13 percent by weight, from 5.0 to 12 percent by weight, from 5.0 to 12 percent by weight, from 5.0 to 11 percent by weight, from 5.0 to 10 percent by weight, from 6.0 to 10 percent by weight, from 7.0 to 10 percent by weight, from 8.0 to 10 percent by weight, from 9.0 to 10 percent by weight, from 5.0 to 9.0 percent by weight, from 5.0 to 8.0 percent by weight, from 5.0 to 7.0 percent by weight, and from 5.0 to 6.0 percent by weight.

In some embodiments, the weight ratio of pH-lowering agent(s) to absorption enhancer(s) may be about 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1 or between any two of the foregoing exemplary ratios. The total weight of all pH-lowering agents and the total weight of all absorption enhancers in a given pharmaceutical composition is included in the foregoing exemplary ratios. For example, if a pharmaceutical composition includes two pH-lowering agents and three absorption enhancers, the foregoing ratios will be computed on the total combined weight of both pH-lowering agents and the total combined weight of all three absorption enhancers.

In some embodiments, the absorption enhancer(s) will be soluble at acid pH, such as less than pH 5.5, and in particular, between pH 3.0 and pH 5.0.

In some embodiments, provided compositions comprise one or more protective vehicles. As used herein, a protective vehicle refers to any protective component and/or structure, such as a carrier, a layer, a coating or other vehicle, that protects an active peptide (e.g., an angiotensin (1-7) peptide) from stomach proteases. Typically, a protective vehicle dissolves eventually so that the active and other ingredients in a particular dosage form may be released. A common form of protective vehicle is an enteric coating. In some embodiments, a suitable enteric coating may prevent breakdown of the pharmaceutical composition of the invention in 0.1N HCl for at least two hours, then capable of permitting complete release of all contents of the pharmaceutical composition within thirty minutes after

pH is increased to 6.3 in a dissolution bath in which said composition is rotating at 100 revolutions per minute.

Many enteric coatings are known in the art and are useful in one or more embodiments. Non-limiting examples of enteric coatings include cellulose acetate phthalate, hydroxypropyl methylethylcellulose succinate, hydroxypropyl methylcellulose phthalate, 5 carboxyl methylethylcellulose and methacrylic acid-methyl methacrylate copolymer. In some embodiments, an angiotensin (1-7) peptide, absorption enhancers such as solubility and/or uptake enhancer(s), and pH-lowering agent(s), are included in a sufficiently viscous protective syrup to permit protected passage of the components of the embodiment through 10 the stomach.

Suitable enteric coatings may be applied, for example, to capsules after the active and other components of the invention have been loaded within the capsule. In other embodiments, enteric coating is coated on the outside of a tablet or coated on the outer surface of particles of active components which are then pressed into tablet form, or loaded 15 into a capsule.

In some embodiments it may be desirable that all components of the invention be released from the carrier or vehicle, and solubilized in the intestinal environment as simultaneously as possible. It may also be preferred in some embodiments that the vehicle or carrier release the active components in the small intestine where uptake enhancers that 20 increase transcellular or paracellular transport are less likely to cause undesirable side effects than if the same uptake enhancers were later released in the colon. It will be appreciated, however, that the present invention is believed effective in the colon as well as in the small intestine. Numerous vehicles or carriers, in addition to the ones discussed above, are known in the art.

25 In some embodiments, it may be desirable (especially in optimizing how simultaneously the components of the invention are released) to keep the amount of enteric coating low. In some embodiments, an enteric coating adds no more than 30% to the weight of the remainder of pharmaceutical composition such as a solid dosage form (the "remainder" being the pharmaceutical composition exclusive of enteric coating itself). In other 30 embodiments, an enteric coating adds less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, or less than 10%. In some embodiments, a protective vehicle such as an enteric coating constitutes an amount of or less than approximately 25%, 24%, 23%, 22%, 21%, 20%, 19%,

18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5% of the total weight of a pharmaceutical composition (e.g., a solid dosage form).

Dosages

5 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).
10 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 a disease severity index score by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100% or more.
15 For example, in some embodiments, an appropriate dose or amount is a dose or amount sufficient to reduce a disease severity index score by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100%. 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
20 optimal dosage ranges or amounts to be administered.

In various embodiments, an Ang (1-7) peptide or angiotensin (1-7) receptor agonist is 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
25 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.

30 Therapeutically effective dosage amounts of angiotensin (1-7) peptides or angiotensin (1-7) receptor agonists, as well as tyrosine kinase inhibitors including derivatives, analogs, and/or salts may be present in varying amounts in various embodiments. For example, in some embodiments, a therapeutically effective amount of an angiotensin (1-7) peptide or a

tyrosine kinase inhibitor may be an amount ranging from about 10-1000 mg (e.g., about 20 mg-1,000 mg, 30 mg-1,000 mg, 40 mg-1,000 mg, 50 mg-1,000 mg, 60 mg-1,000 mg, 70 mg-1,000 mg, 80 mg-1,000 mg, 90 mg-1,000 mg, about 10-900 mg, 10-800 mg, 10-700 mg, 10-600 mg, 10-500 mg, 100-1000 mg, 100-900 mg, 100-800 mg, 100-700 mg, 100-600 mg, 100-500 mg, 100-400 mg, 100-300 mg, 200-1000 mg, 200-900 mg, 200-800 mg, 200-700 mg, 200-600 mg, 200-500 mg, 200-400 mg, 300-1000 mg, 300-900 mg, 300-800 mg, 300-700 mg, 300-600 mg, 300-500 mg, 400 mg-1,000 mg, 500 mg-1,000 mg, 100 mg-900 mg, 200 mg-800 mg, 300 mg-700 mg, 400 mg-700 mg, and 500 mg-600 mg). In some embodiments, an angiotensin (1-7) peptide or angiotensin (1-7) receptor agonist is present in an amount of or greater than about 10 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg. In some embodiments, an angiotensin (1-7) peptide or angiotensin (1-7) receptor agonist is present in an amount of or less than about 1000 mg, 950 mg, 900 mg, 850 mg, 800 mg, 750 mg, 700 mg, 650 mg, 600 mg, 550 mg, 500 mg, 450 mg, 400 mg, 350 mg, 300 mg, 250 mg, 200 mg, 150 mg, or 100 mg. 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.

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.

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.

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 angiotensin (1-7) peptide is administered at an effective dose ranging from about 1-1,000 $\mu\text{g/kg/day}$ (e.g., ranging from about 1-900 $\mu\text{g/kg/day}$, 1-800 $\mu\text{g/kg/day}$, 1-700 $\mu\text{g/kg/day}$, 1-600 $\mu\text{g/kg/day}$, 1-500 $\mu\text{g/kg/day}$, 1-400 $\mu\text{g/kg/day}$, 1-300 $\mu\text{g/kg/day}$, 1-200 $\mu\text{g/kg/day}$, 1-100 $\mu\text{g/kg/day}$, 1-90 $\mu\text{g/kg/day}$, 1-80 $\mu\text{g/kg/day}$, 1-70 $\mu\text{g/kg/day}$, 1-60 $\mu\text{g/kg/day}$, 1-50 $\mu\text{g/kg/day}$, 1-40 $\mu\text{g/kg/day}$, 1-30 $\mu\text{g/kg/day}$, 1-20 $\mu\text{g/kg/day}$, 1-10 $\mu\text{g/kg/day}$).

In some embodiments, the angiotensin (1-7) peptide or an analog thereof is administered at an effective dose ranging from about 1-500 $\mu\text{g/kg/day}$. In some embodiments, the angiotensin (1-7) peptide or an analog thereof is administered at an effective dose ranging from about 1-100 $\mu\text{g/kg/day}$. In some embodiments, the angiotensin (1-7) peptide or an analog thereof is administered at an effective dose ranging from about 1-60 $\mu\text{g/kg/day}$. In some embodiments, the angiotensin (1-7) peptide or an analog thereof 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.

In some embodiments, the tyrosine kinase inhibitor is sunitinib. In one embodiment, a composition of the invention comprises sunitinib, which is administered continuously or at
5 a starting dose of 1, 5, 10, 25, 35, 45, or 50 mg orally once daily for four weeks, followed by two weeks off treatment.

Routes of Administration

An angiotensin (1-7) peptide or angiotensin (1-7) receptor agonist and a tyrosine
10 kinase inhibitor (e.g., sunitinib) as described herein (or a composition or medicament containing an angiotensin (1-7) peptide or Angiotensin (1-7) receptor agonist as described herein) may be administered by any appropriate route. In some embodiments, a combination of the invention is administered parenterally. In some embodiments, the parenteral administration is selected from intravenous, intradermal, inhalation, transdermal (topical),
15 intraocular, intramuscular, subcutaneous, intramuscular, and/or transmucosal administration. In some embodiments, an combination of the invention as 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
20 limited to, thigh region, abdominal region, gluteal region, or scapular region. In some embodiments, a combination of the invention as described herein is administered intravenously. In other embodiments, an combination of the invention as described herein is administered by direct administration to a target tissue, such as a tumor (intratumorally). Alternatively combination of the invention as described herein (or a composition or
25 medicament containing an angiotensin (1-7) peptide or angiotensin (1-7) receptor agonist as 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.

In some embodiments, a combination of the invention as described herein is
30 administered orally. In some embodiments, the solid dosage form is a capsule or tablet. Various methods and ingredients for making oral formulations are known in the art and it is expected that one of skill would be able to determine which of these methods and ingredients will be compatible with the invention as described in this specification and/or in U.S.

Provisional Patent Application Ser. No. 61/701,972, filed on Sep. 17, 2012, the disclosure of which is hereby incorporated in its entirety. Such methods and ingredients are also contemplated as within the scope of the present invention.

Various embodiments may include differing dosing regimen. In some embodiments, a combination of the invention is administered via continuous infusion. In some embodiments, the continuous infusion is intravenous. In other embodiments, the continuous infusion is subcutaneous. Alternatively or additionally, in some embodiments, the angiotensin (1-7) peptide or angiotensin (1-7) receptor agonist is administered bimonthly, monthly, twice monthly, triweekly, biweekly, weekly, twice weekly, thrice weekly, daily, twice daily, or on another clinically desirable dosing schedule. The dosing regimen for a single subject need not be at a fixed interval, but can be varied over time, depending on the needs of the subject.

Kits

In some embodiments, the present invention further provides kits or other articles of manufacture which contains an Ang (1-7) peptide, an angiotensin (1-7) receptor agonist, and a tyrosine kinase inhibitor 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, 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.

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 a tyrosine kinase inhibitor and an Ang (1-7) peptide or angiotensin (1-7) receptor agonist. 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.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 1989); “Oligonucleotide Synthesis” (Gait, 1984); “Animal Cell Culture” (Freshney, 1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis, 1994); “Current Protocols in Immunology” (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1: A combination of a tyrosine kinase inhibitor and Ang 1-7

Angiotensin System Inhibitors (ASIs), which include Angiotensin Converting Enzyme Inhibitors (ACEi) and Angiotensin Receptor Blockers (ARBs) can improve the

outcomes of patients with mRCC treated with VEGF pathway inhibitors. To date there is no known mechanism for this combinatorial effect. This effect was observed in murine xenograft system. In that system, tumors exposed to the combination of ASIs and sunitinib showed slower growth than sunitinib alone. Sunitinib treatment lead to loss of ACE2
5 expression. Without wishing to be bound to theory, this is expected to lead to a decrease in the production of Ang(1-7) from Ang-II.

The renin angiotensin system (RAS) is a hormone system which regulates blood pressure and fluid balance. Two classes of antihypertensives are routinely used to treat blood pressure and are often used to treat the hypertension that develops in patients treated
10 with VEGFR TKIs. ACE inhibitors (ACEi) include agents such as captopril and Lisinopril and Angiotensin Receptor Blockers (ARBs) include losartan and candesartan. The main mediator of RAS effects is angiotensin 2 (Ang-II). Ang-II is generated by the activity of ACE1 which converts Ang-I to Ang-II. This conversion is blocked by ACEi such as captopril. Ang-II acts by binding to Ang-II receptors which are blocked by ARBs such as
15 losartan. Ang-II can be converted to the peptide Ang(1-7) by the activity of ACE2 (not inhibited by ACEi).

Ang-II has multiple oncogenic effects on tumors such as induction of tumor cell survival and angiogenesis. Ang(1-7) is a peptide that has opposing effects to Ang-II and among other anti-tumor effects, has a negative impact on angiogenesis.

20 A murine xenograft system to study resistance antiangiogenic agents was developed. To assess the effects of antiangiogenic therapy on the growth of human RCC xenografts, established human VHL deficient RCC cell lines (A498) were implanted subcutaneously (1×10^7 cells) into the flanks of 6-8 week old nude/beige mice. The tumors were measured in two axes and the the largest axis was followed. Treatment with sunitinib or sorafenib was begun
25 when the tumors reached a diameter of 12 mm (roughly 3 weeks after injection) and resistance to this treatment was monitored. Resistance was defined as the time required for a tumor to grow 2mm beyond its pretreatment size. This is roughly analogous to the current clinical standards by which resistance in patients is assessed by radiologic criteria in which 20% growth is considered disease progression (RECIST). For sunitinib the period of relative
30 stabilization was about 10-12 days. This model was used to study the mechanisms by which tumors escape angiogenesis inhibition.

Transcriptome analysis of the untreated tumors vs the resistant tumors was performed. ACE2 was the second most down-modulated gene common to the sorafenib and sunitinib

treated 786-0 model with a 2.4 fold down modulation in the resistant tumors vs the control untreated tumors. Moreover, ACE2 was in the top 3 most down-modulated genes in the A498 tumor model with a 5-fold decrease in resistant tumors vs untreated tumors ($P < 0.001$).

To understand how inhibition of VEGFR signaling and angiotensin signaling slows tumor growth, tumors and plasma were collected from mice from all treatment arms at the time of sacrifice. To determine whether Ang-II receptor (AT2R) and Ang(1-7) are expressed on tumor cells, the receptors were measured by Western analysis in the murine xenograft tumors. Expression of both receptors was detected in tumors with similar levels in the vehicle and sunitinib treated tumors. To further assess whether the tumor cells expressed angiotensin receptors, Western analysis was performed in the RCC tumor lines in vitro and found both receptors expressed in both our VHL-deficient RCC lines (A498 and 786-0). It is likely that there is a direct antitumor response, which is supported by the fact that the Ang(1-7) and AT2 receptors are expressed in RCC in vivo and in vitro.

The effect of sunitinib and Ang(1-7) on the growth of human RCC xenografts was tested. 1×10^7 cells were injected into the flanks of nude-beige mice. Established human VHL deficient RCC cell lines (A498) were implanted subcutaneously (1×10^7 cells) into the flanks of 6-8 week old nude/beige mice. The tumors were measured in two axes and the largest axis was followed. Treatment with sunitinib (30mg/kg daily by gavage) and Ang(1-7) 1mg/kg Ang(1-7) IP three times per week was begun when the tumors reached a diameter of 12 mm (roughly 3 weeks after injection). We have defined resistance as the time required for a tumor to grow 2mm beyond its pretreatment size. This is roughly analogous to the current clinical standards by which resistance in patients is assessed by radiologic criteria in which 20% growth is considered disease progression (RECIST). For sunitinib the period of relative stabilization was about 10-12 days.

Surprising, the combination of sunitinib and Ang(1-7) slowed tumor growth, as shown in Figure 1.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of

listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically
5 and individually indicated to be incorporated by reference.

What is claimed is:

1. A composition for the treatment of a neoplasia comprising a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels.

5

2. The composition of claim 1, wherein the agent is Ang1-7.

3. The composition of claim 1, wherein the agent is diminazene aceturate.

10 4. A composition for the treatment of a neoplasia comprising a tyrosine kinase inhibitor and a Mas receptor agonist.

5. The composition of claim 4, wherein the Mas receptor agonist is a small molecule, peptide, or polynucleotide.

15

6. The composition of claim 4, wherein the peptide is Ang1-7, alamandine, NorLeu3- Ang1-7, linear Pancyte, or TXA302.

7. The composition of claim 4, wherein the small molecule is AVE 0991.

20

8. The composition of claim 1 or 4, wherein the tyrosine kinase inhibitor is a VEGF tyrosine kinase inhibitor.

9. The composition of claim 8, wherein the VEGF tyrosine kinase inhibitor is selected from the group consisting of sunitinib, sorafenib, axitinib, and pazopanib.

25

10. The composition of claim 1, wherein the agent that increases Ang1-7 levels is a fragment of angiotensin.

30 11. The composition of claim 10, wherein the fragment of angiotensin is a peptide comprising amino acids: Asp-Arg-Val-Ser-Ile-His-Pro.

12. A composition for the treatment of a neoplasia comprising sunitinib and an Ang1-7 peptide.

13. The composition of any of claims 1-12, wherein the sunitinib and the Ang1-7 peptide or analog thereof are included in a single formulation or are formulated separately.

14. A method for reducing the survival and/or proliferation of a neoplasia, the method comprising contacting the neoplasia with a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels, thereby reducing the survival and/or proliferation of the neoplasia.

15. The method of claim 14, wherein the agent is Ang1-7.

16. The method of claim 1, wherein the agent is diminazene aceturate.

17. A method for reducing the survival and/or proliferation of a neoplasia, the method comprising contacting the neoplasia with a tyrosine kinase inhibitor and a Mas receptor agonist, thereby reducing the survival and/or proliferation of the neoplasia.

18. The method of claim 17, wherein the Mas receptor agonist is a small molecule, peptide, or polynucleotide.

19. The method of claim 17, wherein the peptide is Ang-(1-7), alamandine, NorLeu3-Angiotensin (1-7), linear Pancyte, or TXA302.

20. The method of claim 17, wherein the small molecule is AVE 0991.

21. The method of claim 17, wherein the tyrosine kinase inhibitor is a VEGF tyrosine kinase inhibitor.

22. The method of any of claims 14-21, wherein the VEGF tyrosine kinase inhibitor is selected from the group consisting of sunitinib, sorafenib, axitinib, and pazopanib.

23. The method of claim 14, wherein the agent that increases Ang1-7 levels is a fragment of angiotensin.

24. A method for treating a neoplasia, the method comprising administering a tyrosine
5 kinase inhibitor and an agent that increases Ang1-7 levels to a subject having a neoplasia, thereby treating the neoplasia.

25. A method for treating a neoplasia, the method comprising administering a tyrosine
10 kinase inhibitor and a Mas receptor agonist to a subject having a neoplasia, thereby treating the neoplasia.

26. The method of claim 24 or 25, wherein the VEGF tyrosine kinase inhibitor is selected from the group consisting of sunitinib, sorafenib, axitinib, and pazopanib.

15 27. The method of claim 24, wherein the agent that increases Ang1-7 levels is a fragment of angiotensin.

28. The method of claim 25, wherein the Mas receptor agonist is a small molecule,
peptide, or polynucleotide.
20

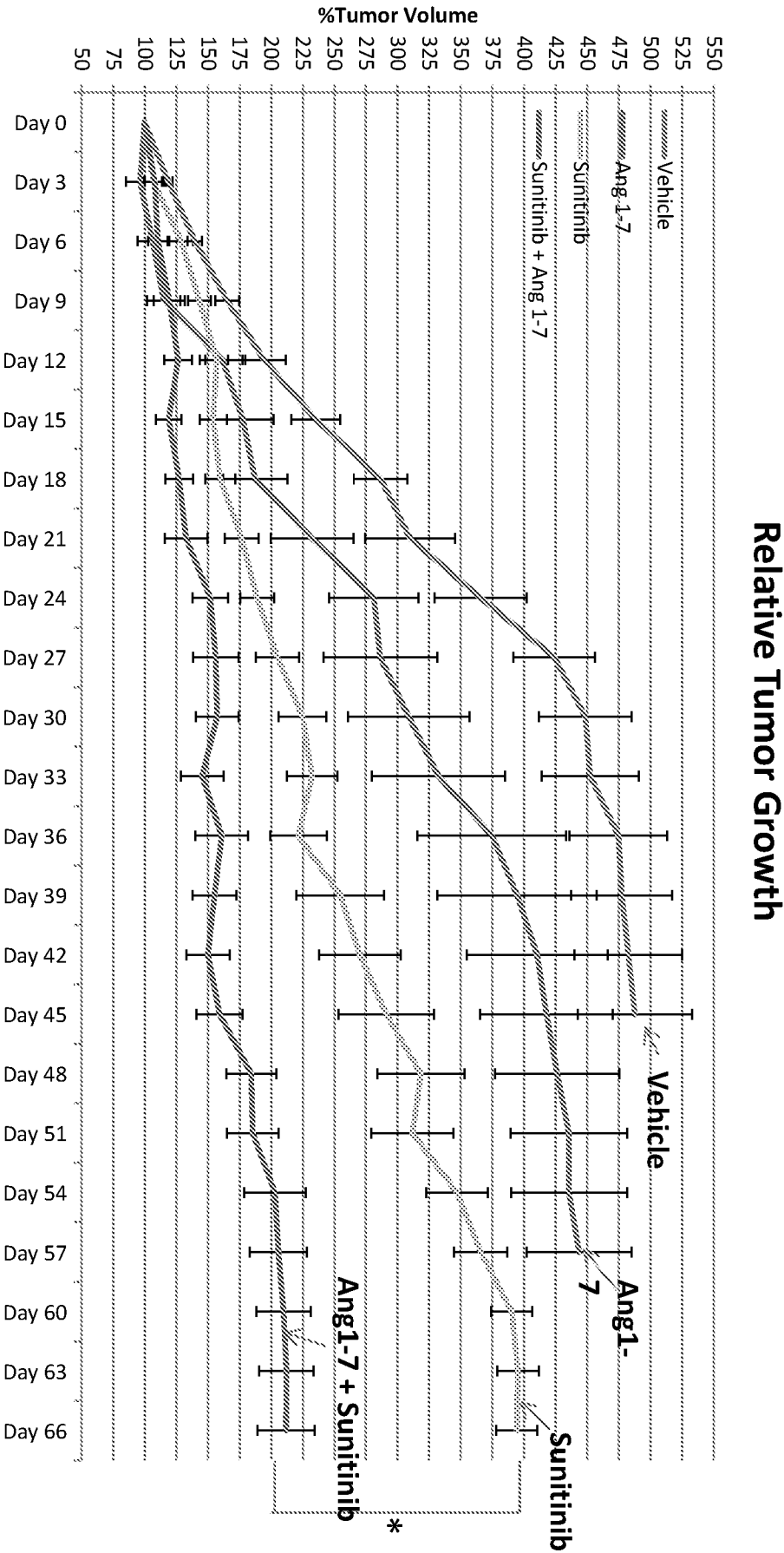
29. The method of claim 28, wherein the peptide is Ang-(1-7), alamandine, NorLeu3-Angiotensin (1-7), linear Pancyte, or TXA302.

30. The method of claim 28, wherein the small molecule is AVE 0991.
25

31. The method of claim 24 or 25, wherein the agent or the Mas receptor agonist is a peptide comprising amino acids: Asp-Arg-Val-Ser-Ile-His-Pro.

32. The method of claim 24 or 25, wherein the VEGF tyrosine kinase inhibitor is
30 sunitinib and the agent or the Mas receptor agonist is an Ang1-7 peptide.

FIG. 1 Sunitinib + Ang (1-7) A498 Xenograft



4 arm ANOVA: P = 4.72E-06
Sunitinib vs Combination Student T test: P = 1.15E-05

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/30488

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61P 31/12, 35/04; C07K 7/14, 16/22; A61K 38/08, 38/48, 39/395 (2018.01)

CPC - A61P 31/12, 35/04; C07K 7/14, 16/22; A61K 38/08, 38/085, 38/48, 39/395

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)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	US 2011/0033524 A1 (JANZEK-HAWLAT, E et al.) 10 February 2011; paragraphs [0013], [0029], [0031], [0034], [0066]-[0067]; claim 1	1-2, 4-6, 10, 14-15, 17-19, 23-25, 27-29
Y	WO 2011/103316 A1 (PRECISION THERAPEUTICS, INC.) 25 August 2011; paragraph [007]	8/1, 8/4, 9/8/1, 9/8/4, 11-12, 21, 22/14-15, 22/17-19, 22/21, 26/24-25, 31/24-25, 32/24-25
Y	US 2011/0281805 A1 (WALTHER, T et al.) 17 November 2011; paragraphs [0016], [0022]	11, 31/24-25
A	(TALLANT, EA et al.) Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. American Journal of Physiology-Heart and Circulatory Physiology. October 2005, Epub 10 June 2005, Vol. 289, No. 4; pages H1560-H1566; abstract; DOI: 10.1152/ajpheart.00941.2004	4-6, 8/4, 9/8/4, 17-19, 21, 22/21, 25, 26/25, 32/25

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

5 September 2018 (05.09.2018)

Date of mailing of the international search report

20 SEP 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/30488

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	◁ (ALKATHERI, A et al.) Renin Angiotensin System and Hepatic Malignancy. Pharmacologia. 2013, Vol. 4, No. 4, pages 327-334; DOI: 10.5567/pharmacologia.2013.327.334	1-2, 4-6, 8/1, 8/4, 9/8/1, 9/8/4, 10-12, 14-15, 17-19, 21, 22/14-15, 22/17-19, 22/21, 23-25, 26/24-25, 27-29, 31/24-25, 32/24-25
A	~ (PETTY, WJ et al.) Phase I and Pharmacokinetic Study of Angiotensin-(1-7), an Endogenous Antiangiogenic Hormone. Clinical Cancer Research. 01 December 2009, Epub 17 November 2009, Vol. 15, No. 23; pages 7398-7404; DOI: 10.1158/1078-0432.CCR-09-1957	1-2, 4-6, 8/1, 8/4, 9/8/1, 9/8/4, 10-12, 14-15, 17-19, 21, 22/14-15, 22/17-19, 22/21, 23-25, 26/24-25, 27-29, 31/24-25, 32/24-25
A	US 2005/0069533 A1 (MILLAN, RD et al.) 31 March 2005; entire document	1-2, 4-6, 8/1, 8/4, 9/8/1, 9/8/4, 10-12, 14-15, 17-19, 21, 22/14-15, 22/17-19, 22/21, 23-25, 26/24-25, 27-29, 31/24-25, 32/24-25
P, X	WO 2017/100776 A1 (WAKE FOREST UNIVERSITY HEALTH SCIENCES) 15 June 2017; entire document	1-2, 4-6, 8/1, 8/4, 9/8/1, 9/8/4, 10-12, 14-15, 17-19, 21, 22/14-15, 22/17-19, 22/21, 23-25, 26/24-25, 27-29, 31/24-25, 32/24-25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/30488

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.: 13
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

-Please See Supplemental Page-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-12, 14-32; Ang1-7 (agent), and sunitinib (tyrosine kinase inhibitor)

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/30488

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

Groups I+, Claims 1-12, 14-32, Ang1-7 (agent), and sunitinib (tyrosine kinase inhibitor) are directed toward compositions and methods for the treatment of neoplasia or reducing the survival or proliferation of a neoplasia.

The compositions and methods will be searched to the extent they encompass Ang1-7 (first exemplary agent), and sunitinib (first exemplary tyrosine kinase inhibitor). Applicant is invited to elect additional agent(s) and/or tyrosine kinase inhibitor(s), to be searched. Additional agent(s) and/or tyrosine kinase inhibitor(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2, 4, 5 (in-part), 6 (in-part), 8, 9 (in-part), 10-12, 14, 15, 17, 18 (in-part), 19 (in-part), 21, 22 (in-part), 23-25, 26 (in-part), 27, 28 (in-part), 29 (in-part), 31, and 32 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass Ang1-7 agent), and sunitinib (tyrosine kinase inhibitor). Applicants must specify the claims that encompass any additionally elected agent(s) and/or tyrosine kinase inhibitor(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an agent encompassing diminazene aceturate (agent).

No technical features are shared between the agents and/or tyrosine kinase inhibitors of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a composition for the treatment of a neoplasia comprising a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels and is a Mas receptor agonist; a method for reducing the survival and/or proliferation of a neoplasia and treating a neoplasia, the method comprising contacting the neoplasia with a tyrosine kinase inhibitor and an agent that increases Ang1-7 levels and is a Mas receptor agonist, thereby reducing the survival and/or proliferation of the neoplasia; these shared technical features are previously disclosed by US 2011/0033524 A1 to Janzek-hawlat et al. (hereinafter 'Janzek') in light of evidence provided by the article 'Cardioprotective Effects of Telmisartan against Heart Failure in rate Induced By Experimental Autoimmune Myocarditis through the Modulation of Angiotensin-Convertin Enzyme-2/Angiotensin 1-7/Mas Receptor Axis' by Sukumaran et al. (hereinafter 'Sukumaran').

Janzek discloses a composition for the treatment of a neoplasia (a medicament (composition) for the treatment of tumors (a neoplasia); paragraph [0013]) comprising a tyrosine kinase inhibitor (comprising a tyrosine kinase inhibitor; paragraph [0029]) and an agent that increases Ang1-7 levels, which is a Mas receptor agonist (and ACE2, which increases the concentration of Ang 1-7; paragraphs [0029], [0034]; wherein Ang 1-7 is a Mas receptor agonist (see Sukumaran, page 1078, first column, second paragraph)); a method for reducing the survival and/or proliferation of a neoplasia (use of ACE2 for treatment of tumors, including producing antiproliferative effects (a method of reducing proliferation and treating a neoplasia); paragraphs [0013]), [0021], Claim 1) the method comprising contacting the neoplasia with a tyrosine kinase inhibitor (the method comprising administering to a subject (contacting the neoplasia with) a tyrosine kinase inhibitor; paragraphs [0013], [0029], Claim 1) and an agent that increases Ang1-7 levels and is a Mas receptor agonist (and ACE2, which increases the concentration of Ang 1-7; paragraphs [0029], [0034]; wherein Ang 1-7 is a Mas receptor agonist (see Sukumaran, page 1078, first column, second paragraph)), thereby reducing the survival and/or proliferation of the neoplasia (thereby treating the cancer, including producing antiproliferative effects (reducing the survival and/or proliferation of the neoplasia); paragraphs [0013], [0021], Claim 1).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Janzek reference, unity of invention is lacking.