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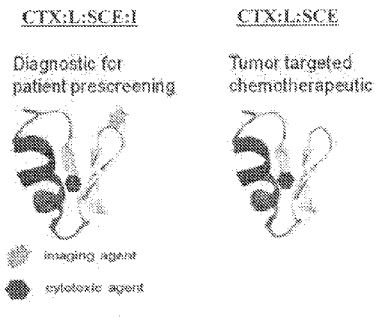

FIG. 1. Schematic diagram of CTX:L:SCE:1 and CTX:L:SCE.

(57) Abstract: The present invention relates to compositions comprising multifunctional agents. More specifically, the present invention provides compositions comprising multifunctional chrolotoxin polypeptide conjugates. Methods for synthesizing such multifunctional agents are also provided.

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MULTIFUNCTIONAL AGENTS

RELATED APPLICATION

[0001] This application claims priority to and the benefit of U.S. Provisional Application No. 61/501,681, filed Jume 27, 2011, the content of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Tumors (benign, pre-malignant or malignant) and unwanted mammalian cell proliferation are a major cause of morbidity and mortality. Malignant tumors (cancer) cause about 13% of all deaths. There is a strong need for the development of improved cancer therapies, and also a need for systems that permit identification of patients likely to respond to therapy.

SUMMARY

[0003] The present invention provides technologies for identifying, characterizing and/or treating cancer patients, and particularly patients likely to respond to therapy. The invention provides, among other things, therapeutic agents, detection agents, and various compositions and methods related thereto.

[0004] For example, the present invention provides agents that comprise a targeting entity, a therapeutic entity, and a detection entity, conjugated to one another. In particular, the present invention provides agents in which the targeting entity is or comprises a chlorotoxin polypeptide. Typically, such a targeting entity polypeptide is about 8-36 amino acids in length. In some embodiments, provided targeting entities comprise a plurality of such polypeptides, for example, as a multimer, conjugate or fusion polypeptide. In accordance with the present invention, provided agents comprise therapeutic and detection entities both conjugated to such a targeting entity. In some embodiments, each of the therapeutic entity and the detection entity is conjugated to the targeting entity. Indeed, the present invention encompasses the surprising finding that such a small targeting entity can preserve its targeting ability even while supporting conjugation to two distinct other entities, each of whose separate activity is also preserved in the conjugate.

[0005] The present invention also provides sets of related multifunctional agents. For example, in some embodiments, the present invention provides sets comprising at least one agent that includes all three of 1) a targeting entity; 2) a therapeutic entity; and 3) a detection entity, and one agent that is lacking one or both of the therapeutic and detection entities. These sets of agents may be used in combination in managing cancer conditions.

[0006] Among other things, the present invention identifies the source of problems that can be encountered with other chlorotoxin conjugates, and provides new conjugates that address a solution to such problems. For example, the present invention recognizes that many prior chlorotoxin conjugates are prepared for specific utility, e.g., as therapeutic agents or as diagnostic agents. According to the present invention, it is recognized that chlorotoxin polypeptides can be conjugated to multiple moieties while maintaining activities of the native counterpart. Furthermore, contemplated chlorotoxin polypeptide conjugates are associated with multiple functions or functionalities, each of which can function independently, that is, without negatively affecting the activity of native chlorotoxin.

[0007] In one aspect, the invention provides multifunctional chlorotoxin polypeptide conjugates, including dual-function chlorotoxin polypeptide conjugates, which show one or more activities of native chlorotoxin. In some embodiments, multifunctional chlorotoxin polypeptide conjugates of the present invention contain two or more moieties conjugated to a single chlorotoxin molecule, wherein the chlorotoxin shows activities (e.g., targeting, binding, detection, etc.) that are identical or significantly similar to those of native chlorotoxin. In some embodiments, each of the two or more moieties conjugated to a single molecule of chlorotoxin polypeptide is of discrete function.

[0008] In some embodiments, the invention provides dual-function chlorotoxin polypeptide conjugates comprising a first functionality that has a therapeutic activity and a second functionality that has a detection activity.

[0009] In some embodiments, the present invention provides multifunctional chlorotoxin polypeptide conjugates that are useful for multiple purposes or combined utilities, including diagnostic and therapeutic use. One advantage of such multifunctional chlorotoxin polypeptide conjugates is that they can be used to visually track or monitor the effect of a therapy over a period of time in patients.

[0010] In certain embodiments, the present invention provides methods of making and of using multifunctional chlorotoxin polypeptide conjugates. In some embodiments, provided multifunctional chlorotoxin polypeptide conjugates may be used in medicine (e.g., in various therapeutic and/or diagnostic contexts).

[0011] One aspect of the invention includes compositions and methods that are useful for prescreening patients who are likely to respond to peptide drug conjugate therapy. Thus, such compositions and methods are useful for determining or identifying a candidate for a specific cancer therapy and/or treatment regimen.

[0012] It is contemplated that described multifunctional chlorotoxin polypeptide conjugates may be used to monitor a patient's response to a cancer therapy by detecting chlorotoxin-labeled tumor cells *in vivo*. Accordingly, the compositions and methods described herein are suitable for testing, evaluating or confirming therapeutic effects of particular cancer therapy.

[0013] In some embodiments, compositions and methods described herein are useful for evaluating the course of treatment regimen to personalize and/or optimize cancer therapy for a particular patient.

[0014] A further aspect of the invention is drawn to methods of synthesizing multifunctional agents contemplated herein. Such methods relate to directed conjugation and involve the formation of at least one intermediate.

BRIEF DESCRIPTION OF THE DRAWING

[0015] **Figure 1** provides a schematic diagram of an exemplary composition of matter comprising: (A) CTX:L:SCE:I; and, (B) CTX:L:SCE.

[0016] **Figure 2** provides images demonstrating tumor-specific uptake of an exemplary multifunctional agent in subjects containing human tumor xenografts.

[0017] **Figure 3** is a graph showing therapeutic activity of CTX:L:SCE and toxicity of conjugated versus non-conjugated SCE in nude mice containing human tumor xenografts.

[0018] **Figure 4** provides a non-limiting embodiment of a reductive amination reaction.

[0019] **Figure 5** provides a non-limiting embodiment of an acid-based hydrolysis reaction.

[0020] **Figure 6** provides a non-limiting embodiment of a carbonate formation reaction.

[0021] **Figure 7** provides a non-limiting embodiment of an acylation reaction.

[0022] **Figure 8** provides a non-limiting embodiment of an O-2-silylation reaction.

Definitions

[0023] As used herein, the terms "about" and "approximately," in reference to a number, is used herein to include numbers that fall within a range of 20%, 10%, 5%, or 1% in either direction (greater than or less than) the number unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0024] As used herein, the term "characteristic sequence element" or "sequence element" refers to a stretch of contiguous amino acids, typically at least 5 amino acids, e.g., at least 5-50, 5-25, 5-15 or 5-10 amino acids, that shows at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with another polypeptide. In some embodiments, a characteristic sequence element participates in or confers function on a polypeptide. In some embodiments, reduced lysine chlorotoxin polypeptides comprise a characteristic sequence element. In some such embodiments, reduced lysine chlorotoxin polypeptides comprise a characteristic sequence element that is TTDHQMAR (SEQ ID NO: 27).

[0025] The terms "chemotherapeutic," "anti-cancer agent" and "anti-cancer drug" are used herein interchangeably. They refer to medications that are used to treat cancer or cancerous conditions. Anti-cancer drugs are conventionally classified in one of the following group: radioisotopes (e.g., Iodine-131, Lutetium-177, Rhenium-188, Yttrium-90), toxins (e.g., diphtheria, pseudomonas, ricin, gelonin), enzymes, enzymes to activate prodrugs, radio-sensitizing drugs, interfering RNAs, superantigens, anti-angiogenic agents, alkylating agents, purine antagonists, pyrimidine antagonists, plant alkaloids, intercalating antibiotics, aromatase inhibitors, anti-metabolites, mitotic inhibitors, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones and anti-androgens. Examples of anti-cancer agents include, but are not limited to, BCNU, cisplatin, gemcitabine, hydroxyurea, paclitaxel, temozolomide, topotecan, fluorouracil, vincristine,

vinblastine, procarbazine, decarbazine, altretamine, methotrexate, mercaptopurine, thioguanine, fludarabine phosphate, cladribine, pentostatin, cytarabine, azacitidine, etoposide, teniposide, irinotecan, docetaxel, doxorubicin, daunorubicin, dactinomycin, idarubicin, plicamycin, mitomycin, bleomysin, tamoxifen, flutamide, leuprolide, goserelin, aminogluthimide, anastrozole, amsacrine, asparaginase, mitoxantrone, mitotane, and amifostine.

[0026] The term "cytotoxic agent" is a compound that can elicit cell killing or reduced viability.

[0027] The term "cytostatic agent" is a compound that can inhibit or suppress cellular proliferation (i.e, growth and multiplication of the cells) without compromising the cell's viability and functionality.

[0028] As used herein, the term "chlorotoxin polypeptide" broadly embraces a peptide corresponding to an amino acid sequence as set forth in SEQ ID NO: 1, and variants thereof.

[0029] As used herein, "native chlorotoxin" refers to a peptide of 36 amino acids in length, having an amino acid sequence (SEQ ID NO: 1), and the term encompasses chlorotoxin that is isolated from venom of scorpion *Leiurius quinquestriatus* or other organisms in which chlorotoxin may be found, as well as recombinant and synthetic chlorotoxin.

[0030] In some embodiments, a chlorotoxin polypeptide has a sequence that differs from that of SEQ ID NO: 1. In some embodiments, such chlorotoxin polypeptides are referred to herein as "chlorotoxin variants." Chlorotoxin variants typically show at least 45% overall sequence identity with chlorotoxin (SEQ ID NO: 1), and having a length of between eight and forty amino acids, inclusive. In some embodiments, the chlorotoxin variant has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% overall sequence identity with SEQ ID NO: 1. In some embodiments, a chlorotoxin polypeptides has at least 65% overall sequence identity with SEQ ID NO: 1. In some embodiments, a chlorotoxin polypeptides has at least 91% overall sequence identity with SEQ ID NO: 1. In some embodiments, a chlorotoxin polypeptides has at least 94% overall sequence identity with SEQ ID NO: 1. In some embodiments, a chlorotoxin polypeptides has at least

97% overall sequence identity with SEQ ID NO: 1. In some embodiments, a chlorotoxin polypeptides shares at least one characteristic sequence element with SEQ ID NO: 1. In some embodiments, the characteristic sequence element is TTDHQMAR (SEQ ID NO: 27).

[0031] In some embodiments, a chlorotoxin polypeptides has a length between eight and forty amino acids inclusive. In some embodiments, a chlorotoxin polypeptide has a length between twenty-four and forty amino acids inclusive. In some specific embodiments, a chlorotoxin polypeptide has a length of about 36 amino acids.

[0032] In some embodiments, a "chlorotoxin polypeptide" includes one or more additional stretch(es) of amino acids, typically at the C- and/or N-terminus and/or as discrete block inserted within a sequence. Typically such additional stretches are about 3 to about 1000 amino acids long. In some embodiments, additional stretches are about 3-100, 3-90, 3-80, 3-70, 3-60, 3-50, 3-40, 3-30 or 3-20 amino acids long. In some embodiments, additional stretches are about or less than 20 amino acids long, about or less than 15 amino acids long, or about or less than 10 amino acids long. In some embodiments, the additional stretch comprises one or more known tags. In some embodiments, the additional stretch comprises a cytotoxic agent, a cytostatic agent, or a combination thereof.

[0033] The phrase "corresponding to," when used to describe positions or sites within amino acid or nucleotide sequences, is used herein as it is understood in the art. As is well known in the art, two or more amino acid or nucleotide sequences can be aligned using standard bioinformatic tools, including programs such as BLAST, ClustalX, Sequencher, and etc. Even though the two or more sequences may not match exactly and/or do not have the same length, an alignment of the sequences can still be performed and, if desirable, a "consensus" sequence generated. Indeed, programs and algorithms used for alignments typically tolerate definable levels of differences, including insertions, deletions, inversions, polymorphisms, point mutations, etc. Such alignments can aid in the determination of which positions in one nucleotide sequence correspond to which positions in other nucleotide sequences.

[0034] As used herein, a "detection moiety" in the context of provided multifunctional agents refers to a molecular structure or module that allows visualization/imaging, measurements (localization, quantification, etc.) and/or monitoring of an agent in vitro and/or

in vivo using one or more detection techniques including but not limited to spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, chemical or other means..

[0035] As used herein, the phrase "dosing regimen" refers to a set of unit doses (typically more than one) that are administered individually separated by periods of time. The recommended set of doses (i.e., amounts, timing, route of administration, etc.) for a particular pharmaceutical agent or composition constitutes its dosing regimen. In some embodiments, a particular dosing regimen is correlated with a certain biologics or therapeutic effect or result.

[0036] As used herein, the terms "effective amount" and "effective dose" refer to any amount or dose of a compound or composition that is sufficient to fulfill its intended purpose(s), i.e., a desired biological or medicinal response in a tissue or subject at an acceptable benefit/risk ratio. For example, in certain embodiments of the present invention, the purpose(s) may be: to inhibit angiogenesis, cause regression of neovasculature, interfere with activity of another bioactive molecule, cause regression of a tumor, inhibit metastases, reduce extent of metastases, etc. The relevant intended purpose may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). In some embodiments, a therapeutically effective amount is an amount that, when administered to a population of subjects that meet certain clinical criteria for a disease or disorder (for example, as determined by symptoms manifested, disease progression/stage, genetic profile, etc.), a statistically significant therapeutic response is obtained among the population. A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular pharmaceutical agent, a therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. In some embodiments, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific pharmaceutical agent employed; the duration of the treatment; and like factors as is well known in the medical arts. Those of ordinary skill in the art will appreciate that in some embodiments of the invention, a unit

dosage may be considered to contain an effective amount if it contains an amount appropriate for administration in the context of a dosage regimen correlated with a positive outcome.

[0037] As used herein, "essentially consisting of" means a subject composition is virtually free of other constituents and does not preclude presence of a trace amount or residual level of such constituents in the composition.

[0038] As used herein, terms "fluorophore," "fluorescent moiety," "fluorescent label," "fluorescent dye" and "fluorescent labeling moiety" are used herein interchangeably. They refer to a molecule that, in solution and upon excitation with light of appropriate wavelength, emits light back. Numerous fluorescent dyes of a wide variety of structures and characteristics are suitable for use in the practice of this invention. Similarly, methods and materials are known for fluorescently labeling nucleic acids (see, for example, R.P. Haugland, *"Molecular Probes: Handbook of Fluorescent Probes and Research Chemicals 1992-1994"*, 5^{th} *Ed., a 1994, Molecular Probes, Inc.*). In choosing a fluorophore, it is often desirable that the fluorescent molecule absorbs light and emits fluorescence with high efficiency (i.e., high molar absorption coefficient and fluorescence quantum yield, respectively) and is photostable (i.e., it does not undergo significant degradation upon light excitation within the time necessary to perform the analysis). Flourophores useful in the disclosed embodiments may be selected for advantageous diagnostic features such as wavelength (e.g., near infared or infared) that produces a high signal to noise ratio when used *in vivo*.

[0039] As used herein, the term "inhibit" means to prevent something from happening, to delay occurrence of something happening, and/or to reduce the extent or likelihood of something happening.

[0040] The terms "labeled" and "labeled with a detectable agent or moiety" are used herein interchangeably to specify that an entity (e.g., a reduced lysine chlorotoxin polypeptide or chlorotoxin polypeptide conjugate) can be visualized, for example following binding to another entity (e.g., a neoplastic tumor tissue) or localization at the site of interest. The detectable agent or moiety may be selected such that it generates a signal that can be measured and whose intensity is related to (e.g., proportional to) the amount of bound entity. A wide variety of systems for labeling and/or detecting proteins and peptides are known in the art. Labeled proteins and peptides can be prepared by incorporation of, or conjugation to, a label that is detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical,

optical, chemical, or other means. A label or labeling moiety may be directly detectable (i.e., it does not require any further reaction or manipulation to be detectable, e.g., a fluorophore is directly detectable) or it may be indirectly detectable (i.e., it is made detectable through reaction or binding with another entity that is detectable, e.g., a hapten is detectable by immunostaining after reaction with an appropriate antibody comprising a reporter such as a fluorophore). Suitable detectable agents include, but are not limited to, radionuclides, fluorophores, chemiluminescent agents, microparticles, enzymes, colorimetric labels, magnetic labels, haptens, molecular beacons, aptamer beacons, and the like.

[0041] A "pharmaceutical composition" is herein defined as a composition that comprises an effective amount of at least one active agent; such a composition typically also contains at least one pharmaceutically acceptable carrier.

[0042] As used herein, the term "preventing" when used to refer to the action of an agent to a process (e.g., angiogenesis, metastasis, cancer progression, etc.) means reducing extent of and/or delaying onset of such a process when the agent (e.g., a therapeutic agent such as a chlorotoxin polypeptide conjugate) is administered prior to development of one or more symptoms or attributes associated with the process.

[0043] As used herein, the term "primary tumor" refers to a tumor that is at the original site where the tumor first arose, as opposed to having spread there.

[0044] The term "prodrug" refers to a compound that, after *in vivo* administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. A prodrug may be designed to alter the metabolic stability or the transport characteristics of a compound, to mask side effects or toxicity, to improve the flavor of a compound and/or to alter other characteristics or properties of a compound. By virtue of knowledge of pharmacodynamic processes and drug metabolisms *in vivo,* once a pharmaceutically active compound is identified, those of skill in the pharmaceutical art generally can design prodrugs of the compound (Nogrady, "Medicinal Chemistry A Biochemical Approach", 1985, Oxford University Press: N.Y., pages 388-392). Procedures for the selection and preparation of suitable prodrugs are also known in the art. In some embodiments, a prodrug is a compound whose conversion to its active form (after *in vivo* administration) involves enzymatic catalysis.

[0045] The terms "protein," "polypeptide" and "peptide" are used herein to refer to polymers of amino acids, either in their neutral (uncharged) forms or as salts, and either unmodified or modified by glycosylation, side chain oxidation, or phosphorylation. In certain embodiments, the amino acid sequence is that of a full-length (e.g., native) protein. In some embodiments, the amino acid sequence is a smaller fragment of a full-length (e.g., native) protein. In some embodiments, a polypeptide contains one or more additional substituents attached to one or more amino acid side chains. Non-limiting examples of such substituents include: glycosyl units, lipids, polymers or inorganic ions such as phosphates, as well as modifications relating to chemical conversion of the chains, such as oxidation of sulfhydryl groups. In particular, the term "protein" encompasses protein isoforms, i.e., variants that are encoded by the same gene, but that differ in their pI or MW, or both. Such isoforms can differ in some features of amino acid sequence (e.g., as a result of alternative slicing or limited proteolysis), or in the alternative, may arise from differential post-translational modification (e.g., glycosylation, acylation or phosphorylation).

[0046] As used herein, the phrase "reduced lysine chlorotoxin polypeptide" refers to a chlorotoxin polypeptide that has fewer lysine residues than chlorotoxin (SEQ ID NO: 1) has and/or has fewer lysine residues that are available as a site for conjugation than chlorotoxin has. In certain embodiments, a reduced lysine chlorotoxin polypeptide has not more than one lysine residue. In some embodiments, a reduced lysine chlorotoxin polypeptide has only one lysine residue. In certain embodiments, a reduced lysine chlorotoxin polypeptide has not more than one lysine residue available as a site for conjugation. In some embodiments, all but one lysine residue in a reduced lysine chlorotoxin polypeptide have been modified such that they are not available as a site for conjugation. In some embodiments, all lysine residues in a reduced lysine chlorotoxin polypeptide have been modified such that they are not available as a site for conjugation. In some embodiments, a reduced lysine chlorotoxin polypeptide contains a single site available for conjugation.

[0047] The term "regress," when used to refer to blood vessels and/or vasculature (including neovasculature and/or neovessels), is used herein to mean to retract or shrink.

[0048] As used herein, the terms "small molecule" and "small chemical entity" include any small chemical or other moiety that can act to affect biological processes. Small molecules can include any number of therapeutic agents presently known and used, or can be small

molecules synthesized in a library of such molecules for the purpose of screening for biological function(s). Small molecules are distinguished from macromolecules by size. Small molecules suitable for use in the present invention usually have molecular weight less than about 5,000 daltons (Da), less than about 2,500 Da, less than 1,000 Da, or less than about 500 Da. Small molecules include synthetic molecules as well as naturally occurring molecules. Small molecules include, without limitation, small chemical entities, such as chemotoxins and cytostatic drugs, which may be referred to as "SCEs." Typically, SCEs are non-peptide, non-nucleic acid molecules.

[0049] The terms "subject" and "individual" are used herein interchangeably. They refer to a vertebrate, preferably human or another mammal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate) that can be afflicted with or is susceptible to a disease or disorder (e.g., cancer, macular degeneration, etc.) but may or may not have the disease or disorder. In many embodiments, the subject is a human subject. In many embodiments, the subject is a patient. Unless otherwise stated, the terms "individual" and "subject" do not denote a particular age, and thus encompass adults, children, and newborn.

[0050] As used herein, the term "susceptible" means having an increased risk for and/or a propensity for (typically based on genetic predisposition, environmental factors, personal history, or combinations thereof) something, i.e., a disease, disorder, or condition (such as, for example, cancer, metastatic cancer, macular degeneration, rheumatoid arthritis, etc.) than is observed in the general population. The term takes into account that an individual "susceptible" for a condition may never be diagnosed with the condition.

[0051] As used herein, the term "systemic administration" refers to administration of an agent such that the agent becomes widely distributed in the body in significant amounts and has a biological effect, e.g., its desired effect, in the blood and/or reaches its desired site of action via the vascular system. Typical systemic routes of administration include administration by (1) introducing the agent directly into the vascular system or (2) oral, pulmonary, or intramuscular administration wherein the agent is adsorbed, enters the vascular system, and is carried to one or more desired site(s) of action via the blood.

[0052] As used herein, a "targeting moiety" in the context of provided multifunctional agents refers to a molecular structure or module that affects or controls the site of action by

specifically interacting with, or has affinity for, a target of interest. In some embodiments, a targeting moiety useful for the present invention is a polypeptide, including a chlorotoxin polypeptide. Various chlorotoxin polypeptides which can be used as a targeting moiety for provided multifunctional agents are described herein. In some embodiments, where a targeting moiety is a polypeptide, the targeting moiety may be abbreviated as ***P*** (for **p**olypeptide). In some embodiments, where a targeting moiety is a chlorotoxin polypeptide, the targeting moiety may be abbreviated as CTX (for chlorotoxin).

[0053] As used herein, a "therapeutic moiety" in the context of provided multifunctional agents refers to a molecular structure or module that confers a therapeutic effect. In some embodiments, therapeutic effecs conferred by a therapeutic moiety of a multifunctional agent of the present invention include anti-cancer effects. Accoridngly, a therapeutic moiety may be an anti-cancer agent (e.g., chemotherapeutic agent). In some embodiments, anti-cancer agents useful for the present invention are agents that inhibit tumor growth, agents that inhibit proliferation of cancer cells, agents that preferentially kill cancer cells, agents that inhibit angiogenesis, etc. In some embodiments, such agents are small molecules. Small molecules include, without limitation, small chemical-based entities, such as chemotoxins and cytostatic drugs, which may be referred to as "SCEs." Typically, SCEs are non-peptide, non-nucleic acid molecules. More generally, a therapeutic moiety of multifunctional agents may be abbreviated as ***T*** (for a **t**herapeutic entity). In those embodiments that include both a therapeutic entity and a diagnostic entity, the therapeutic entity and the target entity are not the same entity.

[0054] As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. For example, "treating" a cancer may refer to inhibiting survival, growth, and/or spread of tumor cells; preventing, delaying, and/or reducing the likelihood of occurrence of metastases and/or recurrences; and/or reducing the number, growth rate, size, etc., of metastases. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some embodiments, treatment comprises delivery of a pharmaceutical composition to a subject.

[0055] As used herein, the phrase "unit dose" refers to a discrete amount of a pharmaceutical composition comprising a predetermined amount of an active ingredient (e.g., a therapeutic agent). The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0056] The invention encompasses the recognition that certain polypeptides, such as chlorotoxin polypeptides, can be used to generate conjugate agents having multiple functions, e.g., associated with multiple functionalities, e.g., multiple conjugated entities, while retaining one or more activities of native chlorotoxin. Accordingly, the present invention provides, *inter alia*, multifunctional peptide conjugates, such as dual-function peptide conjugates.

[0057] As described herein, a multifunctional agent of the invention comprises multiple entities that are conjugated together to form an agent, which offers a broad range of utility or application. The term "function" as applied to "multifunctional agents" of the instant invention broadly refers to a functionally discernable molecular structure associated with certain utility. Examples of functionalities in the context of the present disclosure include, without limitation, utility associated with targeting, utility associated with therapeutic effects (e.g., cytotoxic and/or cytostatic effects, anti-proliferative effects, anti-angiogenic effects, etc.), utility associated with detection or labeling, etc., each of which is discussed in more detail below. Functionality associated with specific diagnostic purposes based on detection method is generally referred to as modality.

[0058] In a broad sense, an "entity" is a molecular structure or module having at least one function. An entity when attached to an agent may be generally referred to as a moiety. For example, a targeting entity is a molecular structure which can be contained in an agent which affects or controls the site of action by specifically interacting with, or has affinity for, a target of interest. As an example, a target may be a molecule or molecular complex present on a cell surface, e.g., certain cell types, tissues, etc. Thus, a corresponding targeting entity can, by virtue of affinity, specifically or preferentially interact with such a target. Use of targeting moieties for agents such as therapeutic agents is known in the art. The nature of a target-

targeting entity interaction varies depending on the binding pair, and is within the knowledge of the art. In the context of the present application, many of the embodiments involve a target which is a tumor or tumor cells. That is, at the molecular level, a target is a molecule or cellular constituent that is present (e.g., preferentially expressed) on a tumor cell, such that it can specifically or preferentially bind to a targeting entity/moiety upon contact.

[0059] Various components (e.g., entities) of the multifunctional agent of the present invention are discussed in more detail below. In the context of the present application, each moiety of a multifunctional agent is a separate entity. As discussed further below, multifunctional agents provided herein contain separate molecular entities, each of which confers separate function (e.g., targeting function, therapeutic function, detection function, etc.). For example, a radioactive agent may not perform the diagnostic and therapeutic function in a single embodiment of the disclosed multifunctional agents.

Targeting entities

[0060] Thus, useful targeting entities can be any molecules that have specificity for at least one target of interest. In some embodiments, a target is a tumor cell or tumor cells. More specifically, at the molecular level, a target is a molecule or molecular complex present on tumor cells. Thus, contemplated targeting entities exert specificity for such target (e.g., tumor cells) and are able to localize to and/or bind to the target. In some embodiments if the present invention, a target is a tumor specific antigen. In some embodiments, a target may be Annexin A2 or related family member thereof. In some embodiments, contemplated targeting entities localize to tumor cells and retain the association with tumor cells over a period of time. In some embodiments, contemplated targeting entities bind to at least one receptor present on the surface of tumor cells and are subsequently internalized.

[0061] Targeting entities useful for contemplated compositions and methods described herein include chlorotoxin polypeptides. Thus, such agents comprise a chlorotoxin polypeptide as a targeting moiety. Chlorotoxin polypeptides useful for the present invention include a polypeptide corresponding to native chlorotoxin (e.g., polypeptide as set forth in SEQ ID NO: 1), and polypeptides with one or more modifications as compared with SEQ ID NO: 1, which retain at least one activity of the native chlorotoxin.

[0062] Thus, in certain embodiments, the term "chlorotoxin" refers to the full-length, 36 amino acid polypeptide naturally derived from *Leiurus quinquestriatus* scorpion venom (DeBin *et al.*, Am. J. Physiol., 1993, 264: C361-369), which comprises the amino acid sequence of native chlorotoxin as set forth in SEQ ID NO: 1 of International Application No. WO 2003/101474, the contents of which are incorporated herein by reference. The term "chlorotoxin" includes polypeptides comprising SEQ ID NO: 1 which have been synthetically or recombinantly produced, such as those disclosed in U.S. Pat. No. 6,319,891 (which is incorporated herein by reference in its entirety).

[0063] The terms variant and derivative, as in chlorotoxin variant and chlorotoxin derivative, are used interchangeably herein. Thus, various chlorotoxin polypeptides can be used as targeting moieties to practice the invention described herein, to the extent that they are biologically active.

[0064] As used herein, the term "biologically active chlorotoxin polypeptides" refers to any of a wide variety of derivatives, analogs, variants, polypeptide fragments and mimetics of chlorotoxin and related peptides which retain at least one property or function of chlorotoxin (as described herein). In some embodiments, chlorotoxin polypeptides suitable for use according to the present invention are represented by the formula: $TTX_1X_2X_3MX_4X_5X_6$ (SEQ ID NO: 67), which corresponds to amino acid residues 7-15 of SEQ ID NO: 1, wherein X_1 is typically an acidic amino acid (e.g., aspartic acid and glutamic acid); X_2 is any amino acid (e.g., alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine); X_3 is typically an amide amino acid (e.g., asparagine and glutamine); X_4 is an any amino acid (e.g., serine, threonine and alanine); and X_5 is a basic amino acid (e.g., histidine, lysine and arginine); and, X_6 is any amino acid (e.g., lysine, arginine and alanine). In some embodiments, X_1 is aspartic acid, X_2 is histidine or proline, X_3 is glutamine, X_4 is alanine and X_5 is arginine or lysine, and X_6 is lysine, alanine or arginine. Various variants, analogs, and derivatives of such polypeptides (as described herein) are also embraced by this invention.

[0065] Examples of chlorotoxin derivatives include, but are not limited to, peptide variants of chlorotoxin, peptide fragments of chlorotoxin, for example, fragments comprising or consisting of contiguous 10-mer peptides of any one of the following sequences:

HHHHHHMCMPCFTTDHQMARKCDDCCGGKGRGKCYGPQCLCR SEQ ID NO: 69),
YMCMPCFTTDHQMARKCDDCCGGKGRGKCYGPQCLCR (SEQ ID NO: 70),
YSYMCMPCFTTDHQMARKCDDCCGGKGRGKCYGPQCLCR (SEQ ID NO: 71),
MCMPCFTTDHQMARKCDDCCGGKGRGKCFGPQCLCR (SEQ ID NO: 72),
RCKPCFTTDPQMSKKCADCCGGKGKGKCYGPQCLC (SEQ ID NO: 73),
RCSPCFTTDQQMTKKCYDCCGGKGKGKCYGPQCICAPY (SEQ ID NO: 74)

(which correspond to SEQ ID NOs: 2, 3, 4, 5, 6, and 7 as set forth in International Application No. WO 2003/101474 or sequence comprising residues 10-18 or 21-30 of SEQ ID NO: 1).

[0066] Accordingly, a "biologically active chlorotoxin polypeptide" may comprise fewer than the 36 amino acids of chlorotoxin and which retains at least one property or function of chlorotoxin. As used herein, a "property or function" of chlorotoxin includes, but is not limited to, its ability to ability to suppress formation of and/or cause regression of neovessels; ability to interfere with activity of its binding partner(s) (which may include, for example, Annexin A2); ability to arrest abnormal cell growth; ability to specifically bind to a tumor/cancer cell compared to a normal cell; ability to specifically bind to a metastasizing tumor/cancer cell or a tumor/cancer cell in a metastasis compared to a normal cell; ability to be internalized into a tumor/cancer cell; ability to kill a tumor/cancer cell. The tumor/cancer cell may be in vitro, ex vivo, in vitro, part of a metastasis, a primary isolate from a subject, a cultured cell, or a cell line.

[0067] Examples of chlorotoxin derivatives include peptides having a fragment of the amino acid sequence set forth in SEQ ID NO: 1, having at least about 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, or 35 contiguous amino acid residues, associated with the activity of chlorotoxin. Such fragments may contain functional regions of the chlorotoxin polypeptide, identified as regions of the amino acid sequence that correspond to known peptide domains, as well as regions of pronounced hydrophilicity. Such fragments may also include two core sequences linked to one another, in any order, with intervening amino acid removed or replaced by a linker.

[0068] Derivatives of chlorotoxin include polypeptides comprising a conservative or non- conservative substitution of at least one amino acid residue when the derivative sequence and the chlorotoxin sequence are maximally aligned. The substitution may be one that

enhances at least one property or function of chlorotoxin, inhibits at least one property or function of chlorotoxin, or is neutral to at least one property or function of chlorotoxin.

[0069] Examples of derivatives of chlorotoxin suitable for use in the practice of the present invention are described in International Application No. WO 2003/101474 (which is incorporated herein by reference in its entirety). Particular examples include polypeptides that comprise or consist of KGRGKSY (SEQ ID NO: 65; corresponding to amino acid residues 23-29 of SEQ ID NO: 1 with a C→S substitution) or KGRGKCY (SEQ ID NO: 66; corresponding to amino acid residues 23-29 of SEQ ID NO: 1). In some embodiments, chlorotoxin polypeptides suitable for use according to the present invention are represented by the formula: $TTX_1X_2X_3MX_4X_5X_6$ (SEQ ID NO: 67), which corresponds to amino acid residues 7-15 of SEQ ID NO: 1, wherein X_1 is an acidic amino acid selected from the group consisting of aspartic acid and glutamic acid; X_2 is an amino acid selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine; X_3 is an amide amino acid selected from the group consisting of asparagine and glutamine; X_4 is an any amino acid but in a preferred embodiment is selected from the group consisting of serine, threonine and alanine; and X_5 is a basic amino acid selected from the group consisting of histidine, lysine and arginine; X_6 is an any amino acid but in a preferred embodiment is selected from the group consisting of lysine, arginine and alanine. In some embodiments, X_1 is aspartic acid, X_2 is histidine or proline, X_3 is glutamine, X_4 is alanine and X_5 is arginine or lysine, and X_6 is lysine, alanine or arginine. Various variants, analogs, and derivatives of such polypeptides are also embraced by this invention.

[0070] Other examples of chlorotoxin derivatives include those polypeptides containing predetermined mutations by, e.g., homologous recombination, site-directed or PCR mutagenesis, and the alleles or other naturally-occurring variants of the family of peptides; and derivatives wherein the peptide has been covalently modified by substitution, chemical, enzymatic or other appropriate means with a moiety other than a naturally-occurring amino acid (for example a detectable moiety such as enzyme or a radioisotope).

[0071] Particular examples include chlorotoxin polypeptides disclosed in WO/2003/101474, which are incorporated herein by reference.

Mono-lysine chlorotoxin polypeptides

[0072] As mentioned, in some embodiments, a biologically active chlorotoxin derivative which may be used as a targeting entity of a multifunctional chlorotoxin agent contains at least one point mutation. For example, useful chlorotoxin derivatives of the present disclosure may contain at least one amino acid substitution (relative to native chlorotoxin). In some embodiments of the invention, such derivatives contain a substitution of at least one of the lysine residues to another amino acid. In some embodiments, chlorotoxin derivatives contain no more than one lysine residue. These derivatives having fewer lysine residues than the native counterpart are herein referred to as reduced lysine chlorotoxin polypeptides, or reduced lysine chlorotoxin derivatives/variants.

[0073] In certain embodiments, a reduced lysine chlorotoxin polypeptide has an amino acid sequence corresponding to that of SEQ ID NO: 1 in that the reduced lysine chlorotoxin polypeptide has at least 45% overall sequence identity with SEQ ID NO: 1 and a length of between twenty-four and forty amino acid residues inclusive. In some embodiments, a reduced lysine chlorotoxin polypeptide has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% overall sequence identity with SEQ ID NO: 1. In some embodiments, a reduced lysine chlorotoxin polypeptide has at least 65% overall sequence identity with SEQ ID NO: 1. In some embodiments, a chlorotoxin polypeptide has at least 91% overall sequence identity with SEQ ID NO: 1. For example, a reduced lysine chlorotoxin polypeptide may be identical in amino acid sequence to chlorotoxin at 33 out of 36 amino acid residues (i.e., ~91.7% sequence identity). In some embodiments, a chlorotoxin polypeptide has at least 94% overall sequence identity with SEQ ID NO: 1. For example, a reduced lysine chlorotoxin polypeptide may be identical in amino acid sequence to chlorotoxin at 34 out of 36 amino acid residues (i.e., ~94.4% sequence identity). In some embodiments, a reduced lysine chlorotoxin polypeptide has at least 97% overall sequence identity with SEQ ID NO: 1. For example, a reduced lysine chlorotoxin polypeptide may be identical in amino acid sequence to chlorotoxin at 35 out of 36 amino acid residues (i.e., ~97.2% sequence identity). In some embodiments, a reduced lysine chlorotoxin polypeptide is and/or contains a stretch of 33, 34, 35, 36, 37, or 38 amino acids whose sequence corresponds to or shows at least 45%,

at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% overall sequence identity with the sequence of chlorotoxin

[0074] In certain embodiments, reduced lysine chlorotoxin polypeptides have not more than one lysine available as a site for conjugation. In some such embodiments, one lysine is available and it is at a position within the chlorotoxin polypeptide that corresponds to a position where a lysine is present in chlorotoxin (i.e., position 15, 23 or 27 of SEQ ID NO: 1). In some embodiments, the single lysine that is available is at position 15 of SEQ ID NO: l. In some embodiments, the single lysine that is available is at position 23 of SEQ ID NO: l. In some embodiments, the single lysine that is available is at position 27 of SEQ ID NO: l. In some embodiments, a single lysine is present in a reduced lysine chlorotoxin polypeptide of the present invention at a position corresponding to a site in chlorotoxin that does not contain a lysine residue (i.e., not at a position corresponding to any of positions 15, 23 or 27 of SEQ ID NO: 1).

[0075] In certain embodiments, a reduced lysine chlorotoxin polypeptide lacks at least one amino acid residue corresponding to position 15, 23, or 27 of SEQ ID NO: 1.

[0076] In certain embodiments, a reduced lysine chlorotoxin polypeptide lacks lysine residues entirely. (See, e.g., SEQ ID NOs: 2, 5, 6, 24, 25 and 26). In some embodiments, an amino acid is missing where a lysine residue is normally found in chlorotoxin. In some embodiments, one or more lysine residues normally found in chlorotoxin is/are replaced by another amino acid residue and/or by an amino acid derivative. In other words, at least one amino acid residue in the polypeptide corresponding to positions 15, 23 or 27 of SEQ ID NO: 1 is not a lysine. In addition to the nineteen other naturally occurring amino acids of which polypeptides are typically comprised (alanine, arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine), a variety of substitutes may be used. Non-limiting examples of substitutes include other naturally occurring amino acids, non-naturally occurring amino acids such as D-amino acids, and amino acid derivatives. Non-limiting examples of other naturally occurring amino acids include beta-

alanine, carnitine, citrulline, cystine, gamma-aminobutyric acid, hydroxyproline, ornithine, and taurine. (For additional examples of naturally occurring amino acids and of amino acid derivatives, see, e.g., Wagner and Musso (1983), "New Naturally Occurring Amino Acids," *Agnew. Chem. Int. Ed. Engl.,* 22:816-828, the entire of contents of which are herein incorporated by reference.) In some embodiments, one or more lysine residues is/are replaced by arginine and/or alanine.

[0077] In some embodiments in which a reduced lysine chlorotoxin polypeptide lacks lysine residues entirely, one terminus or both termini (i.e., the N- and/or C-terminus) of the reduced lysine chlorotoxin polypeptide can serve as a site for chemical conjugation. Availability of the terminus or termini may depend on the particular conjugation chemistry employed. In some embodiments in which a reduced lysine chlorotoxin polypeptide lacks lysine residues entirely, only the alpha amino group at the N-terminus is available as a site for conjugation.

[0078] In embodiments in which more than one lysine residue is replaced, the same or different amino acid residue(s) or amino acid derivative(s) may be used to replace the lysine residues. See, e.g., SEQ ID NOs: 17-22 for non-limiting examples in which the same amino acid residue has been used to replace lysine residues and SEQ ID NO: 23 for a non-limiting example in which different amino acid residues have been used to replace lysine residues.

[0079] In some embodiments, a reduced lysine chlorotoxin polypeptide has only one lysine in the sequence (a "monolysine chlorotoxin polypeptide"). In some embodiments, such a chlorotoxin polypeptide has a lysine residue where a lysine residue is normally present in chlorotoxin, e.g., at a position corresponding to position 15, 23, or 27 of SEQ ID NO: 1 (See, e.g., SEQ ID NOs: 14-23 for non-limiting examples). In some embodiments, a reduced lysine chlorotoxin polypeptide does not have any lysine residues where a lysine residue is normally present in native chlorotoxin (i.e., positions 15, 23, and 27 of SEQ ID NO: 1), but has a lysine residue at a position that does not correspond to any of positions 15, 23, and 27 of SEQ ID NO: 1. As with chlorotoxin polypeptides having no lysines at all, monolysine chlorotoxin polypeptides may be missing amino acids at one or more positions corresponding to positions 15, 23, or and 27 of SEQ ID NO: 1, and/or may have an amino acid or amino

acid derivative substitution at one or more positions corresponding to positions 15, 23, or 27 of SEQ ID NO: 1.

[0080] Exemplary chlorotoxin polypeptides useful for the present invention are provided in the following tables.

Table 1: Sequences of Chlorotoxin and of Exemplary Chlorotoxin Polypeptides.

Chlorotoxin		
SEQ ID NO:	**Comment**	**Sequence (N-terminus to C-terminus)**
1	Full length chlorotoxin	MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC R

Exemplary chlorotoxin polypeptides		
SEQ ID NO:	**Comment**	**Sequence (N-terminus to C-terminus)**
2	No lysines	MCMPC FTTDH QMARC DDCCG GGRGC YGPQC LCR
3	No lysines at positions 15, 23, or 27 of SEQ ID NO: 1; lysine at N-terminus	KMCMP CFTTD HQMAR CDDCC GGGRG CYGPQ CLCR
4	No lysines at positions 15, 23 or 27 of SEQ ID NO: 1; lysine at C-terminus	MCMPC FTTDH QMARC DDCCG GGRGC YGPQC LCRK
5	Lysines at positions 15, 23, and 27 of SEQ ID NO: 1 replaced by alanine	MCMPC FTTDH QMARA CDDCC GGAGR GACYG PQCLC R
6	Lysines at positions 15, 23, and 27 of SEQ	MCMPC FTTDH QMARR CDDCC GGRGR GRCYG PQCLC R

	ID NO: 1 replaced by arginine	
7	Lysines at positions 15, 23, and 27 of SEQ ID NO: 1 replaced by alanine; lysine at N-terminus	KMCMP CFTTD HQMAR ACDDC CGGAG RGACY GPQCL CR
8	Lysines at positions 15, 23, and 27 of SEQ ID NO: 1 replaced by arginine; lysine at N-terminus	KMCMP CFTTD HQMAR RCDDC CGGRG RGRCY GPQCL CR
9	Lysines at positions 15, 23, and 27 of SEQ ID NO: 1 replaced by alanine; lysine at C-terminus	MCMPC FTTDH QMARA CDDCC GGAGR GACYG PQCLC RK
10	Lysines at positions 15, 23, and 27 of SEQ ID NO: 1 replaced by arginine; lysine at C-terminus	MCMPC FTTDH QMARR CDDCC GGRGR GRCYG PQCLC RK
11	No lysine at position 15 of SEQ ID NO: 1	MCMPC FTTDH QMARC DDCCG GKGRG KCYGP QCLCR
12	No lysine at position 23 of SEQ ID NO: 1	MCMPC FTTDH QMARK CDDCC GGGRG KCYGP QCLCR
13	No lysine at position 27 of SEQ ID NO: 1	MCMPC FTTDH QMARK CDDCC GGKGR GCYGP QCLCR
14	No lysines at positions 15 and 23 of SEQ ID	MCMPC FTTDH QMARC DDCCG GGRGK CYGPQ CLCR

	NO: 1	
15	No lysines at positions 15 and 27 of SEQ ID NO: 1	MCMPC FTTDH QMARC DDCCG GKGRG CYGPQ CLCR
16	No lysines at positions 23 and 27 of SEQ ID NO: 1	MCMPC FTTDH QMARK CDDCC GGGRG CYGPQ CLCR
17	Lysines at positions 15 and 23 of SEQ ID NO: 1 replaced by alanine	MCMPC FTTDH QMARA CDDCC GGAGR GKCYG PQCLC R
18	Lysines at positions 15 and 27 of SEQ ID NO: 1 replaced by alanine	MCMPC FTTDH QMARA CDDCC GGKGR GACYG PQCLC R
19	Lysines at positions 23 and 27 of SEQ ID NO: 1 replaced by alanine	MCMPC FTTDH QMARK CDDCC GGAGR GACYG PQCLC R
20	Lysines at positions 15 and 23 of SEQ ID NO: 1 replaced by arginine	MCMPC FTTDH QMARR CDDCC GGRGR GKCYG PQCLC R
21	Lysines at positions 15 and 27 of SEQ ID NO: 1 replaced by arginine	MCMPC FTTDH QMARR CDDCC GGKGR GRCYG PQCLC R
22	Lysines at positions 23 and 27 of SEQ ID NO: 1 replaced by arginine	MCMPC FTTDH QMARK CDDCC GGRGR GRCYG PQCLC R
23	Lysine at position 15 of SEQ ID NO: 1	MCMPC FTTDH QMARR CDDCC GGKGR GACYG PQCLC R

	replaced by arginine; lysine at position 27 of SEQ ID NO: 1 replaced by alanine	
24	No lysine at position 15 of SEQ ID NO: 1; lysines at positions 23 and 27 of SEQ ID NO: 1 replaced by arginine	MCMPC FTTDH QMARC DDCCG GAGRG ACYGP QCLCR
25	No lysine at position 23 of SEQ ID NO: 1; lysines at positions 15 and 27 replaced by arginine	MCMPC FTTDH QMARA CDDCC GGGRG ACYGP QCLCR
26	No lysine at position 27 of SEQ ID NO: 1; lysines at positions 15 and 23 replaced by arginine	MCMPC FTTDH QMARR CDDCC GGRGR GCYGP QCLCR
27	Residues 7-14 of SEQ ID NO: 1	TTDHQ MAR
28	C→S of SEQ ID NO: 1	MSMPS FTTDH QMARK SDDSS GGKGR GKSYG PQSLS R
29	Residues 1-10 of SEQ ID NO: 28	MSMPS FTTDH
30	Residues 2-11 of SEQ ID NO: 28	SMPSF TTDHQ
31	Residues 3-12 of SEQ ID NO: 28	MPSFT TDHQM

32	Residues 4-13 of SEQ ID NO: 28	PSFTT DHQMA
33	Residues 5-14 of SEQ ID NO: 28	SFTTD HQMAR
34	Residues 6-15 of SEQ ID NO: 28	FTTDH QMARK
35	Residues 7-16 of SEQ ID NO: 28	TTDHQ MARKS
36	Residues 8-17 of SEQ ID NO: 28	TDHQM ARKSD
37	Residues 9-18 of SEQ ID NO: 28	DHQMA RKSDD
38	Residues 10-19 of SEQ ID NO: 28	HQMAR KSDDS
39	Residues 11-20 of SEQ ID NO: 28	QMARK SDDSS
40	Residues 12-21 of SEQ ID NO: 28	MARKS DDSSG
41	Residues 13-22 of SEQ ID NO: 28	ARKSD DSSGG
42	Residues 14-23 of SEQ ID NO: 28	RKSDD SSGGK
43	Residues 15-24 of SEQ ID NO: 28	KSDDS SGGKG
44	Residues 16-25 of SEQ ID NO: 28	SDDSS GGKGR

45	Residues 17-26 of SEQ ID NO: 28	DDSSG GKGRG
46	Residues 18-27 of SEQ ID NO: 28	DSSGG KGRGK
47	Residues 19-28 of SEQ ID NO: 28	SSGGK GRGKS
48	Residues 20-29 of SEQ ID NO: 28	SGGKG RGKSY
49	Residues 21-30 of SEQ ID NO: 28	GGKGR GKSYG
50	Residues 22-31 of SEQ ID NO: 28	GKGRG KSYGP
51	Residues 23-32 of SEQ ID NO: 28	KGRGK SYGPQ
52	Residues 24-33 of SEQ ID NO: 28	GRGKS YGPQS
53	Residues 25-34 of SEQ ID NO: 28	RGKSY GPQSL
54	Residues 26-35 of SEQ ID NO: 28	GKSYG PQSLS
55	Residues 27-36 of SEQ ID NO: 28	KSYGP QSLSR
56	Residues 7-16 of SEQ ID NO: 1	TTDHQ MARKC
57	Residues 2-11 of SEQ ID NO: 28; Cysteine at	CMPSF TTDHQ

	N-term	
58	Residues 5-14 of SEQ ID NO: 28; Cysteine at N-term	CFTTD HQMAR
59	Residues 8-17 of SEQ ID NO: 28; Cysteine at position 9	TDHQM ARKCD
60	Residues 17-26 of SEQ ID NO: 28; Cysteine at position 2	DCSGG KGRGG
61	Residues 18-27 of SEQ ID NO: 28; Cysteine at N-term	CSGGK GRGKS
62	Residues 19-28 of SEQ ID NO: 28; Cysteine at N-term	CGGKG RGKSY
63	Residues 18-27 of SEQ ID NO: 28; Cysteine at C-term	SSGGK GRGKC
64	Residues 19-28 of SEQ ID NO: 28; Cysteine at position 9	SGGKG RGKCY
65	Residues 23-29 of SEQ ID NO: 1; with C→S	KGRGK SY
66	Residues 23-29 of SEQ ID NO: 1	KGRGK CY
69	SEQ ID NO: 2 of WO 2003/101474	HHHHH HMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR
70	SEQ ID NO: 3 of WO	YMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR

	2003/101474	
71	SEQ ID NO: 4 of WO 2003/101474	YSYMC MPCFT TDHQM ARKCD DCCGG KGRGK CYGPQ CLCR
72	SEQ ID NO: 5 of WO 2003/101474	MCMPC FTTDH QMARK CDDCC GGKGR GKCFG PQCLC R
73	SEQ ID NO: 6 of WO 2003/101474	RCKPC FTTDP QMSKK CADCC GGKGK GKCYG PQCLC
74	SEQ ID NO: 7 of WO 2003/101474	RCSPC FTTDQ QMTKK CYDCC GGKGK GKCYG PQCIC APY

[0081] In any of the embodiments contemplated herein, (such as the chlorotoxin polypeptides shown in Table 1 above), a polypeptide (e.g., a targeting entity) may comprise one or more amino acid modifications. In some embodiments, one or more amino acids of a polypeptide may be substituted with another constituent. For example, in some embodiments, a polypeptide described herein may include one or more amino acid analogs or non-canonical or unnatural (i.g., non-natural) amino acids. In some embodiments, such substituent may be chemically synthesized, or expressed recombinantly using known protein engineering techniques. A number of amino acid analogs useful for practicing the invention are known in the art In addition, inventive fusion proteins can be derivatized by well-known organic chemistry techniques.

[0082] Thus, in certain embodiments of the present invention, amino acid substitutions encompass, non-canonical amino acid residues, which include naturally rare (in peptides or proteins) amino acid residues or unnatural amino acid residues. Non-canonical amino acid residues can be incorporated into a polypeptide by chemical peptide synthesis rather than by synthesis in biological systems, such as recombinantly expressing cells, or alternatively the skilled artisan can employ known techniques of protein engineering that use recombinantly expressing cells. (See, e.g., Link et al., Non-canonical amino acids in protein engineering, Current Opinion in Biotechnology, 14(6):603-609 (2003)). The term "non-canonical amino acid residue" refers to amino acid residues in D- or L-form that are not among the 20 canonical amino acids generally incorporated into naturally occurring proteins, for example, β-amino

acids, homoamino acids, cyclic amino acids and amino acids with derivatized side chains. Examples include (in the L-form or D-form; abbreviated as in parentheses): citrulline (Cit), homocitrulline (hCit), N^{α}-methylcitrulline (NMeCit), N^{α}-methylhomocitrulline (N^{α}-MeHoCit), ornithine (Orn), N^{α}-Methylornithine (N^{α}-MeOrn or NMeOrn), sarcosine (Sar), homolysine (hLys or hK), homoarginine (hArg or hR), homoglutamine (hQ), N^{α}-methylarginine (NMeR), N^{α}-methylleucine (N^{α}-MeL or NMeL), N-methylhomolysine (NMeHoK), N^{α}-methylglutamine (NMeQ), norleucine (Nle), norvaline (Nva), 1,2,3,4-tetrahydroisoquinoline (Tic), Octahydroindole-2-carboxylic acid (Oic), 3-(1-naphthyl)alanine (1-Nal), 3-(2-naphthyl)alanine (2-Nal), 1,2,3,4-tetrahydroisoquinoline (Tic), 2-indanylglycine (Igl), para-iodophenylalanine (pI-Phe), para-aminophenylalanine (4AmP or 4-Amino-Phe), 4-guanidino phenylalanine (Guf), glycyllysine (abbreviated herein "K(N^{ε}-glycyl)" or "K(glycyl)" or "K(gly)"), nitrophenylalanine (nitrophe), aminophenylalanine (aminophe or Amino-Phe), benzylphenylalanine (benzylphe), γ-carboxyglutamic acid (γ-carboxyglu), hydroxyproline (hydroxypro), p-carboxyl-phenylalanine (Cpa), α-aminoadipic acid (Aad), Nα-methyl valine (NMeVal), N-α-methyl leucine (NMeLeu), Nα-methylnorleucine (NMeNle), cyclopentylglycine (Cpg), cyclohexylglycine (Chg), acetylarginine (acetylarg), α,β-diaminopropionoic acid (Dpr), α,γ-diaminobutyric acid (Dab), diaminopropionic acid (Dap), cyclohexylalanine (Cha), 4-methyl-phenylalanine (MePhe), β,β-diphenyl-alanine (BiPhA), aminobutyric acid (Abu), 4-phenyl-phenylalanine (or biphenylalanine; 4Bip), α-amino-isobutyric acid (Aib), β-alanine, β-aminopropionic acid, piperidinic acid, aminocaproic acid, aminoheptanoic acid, aminopimelic acid, desmosine, diaminopimelic acid, N-ethylglycine, N-ethylaspargine, hydroxylysine, allo-hydroxylysine, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, 4-hydroxyproline (Hyp), γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, ω-methylarginine, 4-Amino-O-Phthalic Acid (4APA), allyl glycine (allyl-Gly), and other similar amino acids, and derivatized forms of any of these as described herein.

[0083] Polypeptide portions of the inventive compositions, such as a targeting polypeptide, can also be chemically derivatized at one or more amino acid residues. Polypeptides that contain derivatized amino acid residues can be synthesized by known organic chemistry techniques. "Chemical derivative" or "chemically derivatized" in the context of a peptide refers to a subject peptide having one or more residues chemically derivatized by

reaction of a functional side group. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty canonical amino acids, whether in L- or D-form. For example, 4-hydroxyproline may be substituted for proline; 5-hydroxylysine maybe substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

[0084] Useful derivatizations include, in some embodiments, those in which the amino terminal of a polypeptide, such as but not limited to a chlorotoxin polypeptide, is chemically blocked so that conjugation with the vehicle will be prevented from taking place at an N-terminal free amino group. There may also be other beneficial effects of such a modification, for example a reduction in the polypeptide's susceptibility to enzymatic proteolysis. The N-terminus of a polypeptide, e.g., a chlorotoxin polypeptide, can be acylated or modified to a substituted amine, or derivatized with another functional group, such as an aromatic or aryl moiety (e.g., an indole acid, benzyl (Bzl or Bn), dibenzyl (DiBzl or Bn_2), benzoyl, or benzyloxycarbonyl (Cbz or Z), N,N-dimethylglycine or creatine. For example, in some embodiments, an acyl moiety, such as, but not limited to, a formyl, acetyl (Ac), propanoyl, butanyl, heptanyl, hexanoyl, octanoyl, or nonanoyl, can be covalently linked to the N-terminal end of the polypeptide, which can prevent undesired side reactions during conjugation of the vehicle to the peptide. Alternatively, a fatty acid (e.g. butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic or the like) or polyethylene glycol moiety can be covalently linked to the N-terminal end of the polypeptide. Other exemplary N-terminal derivative groups include --NRR^1 (other than --NH_2), --$NRC(O)R^1$, --$NRC(O)OR^1$, --$NRS(O)_{2R}{}^1$, --$NHC(O)NHR^1$, succinimide, or benzyloxycarbonyl-NH-(Cbz-NH--), wherein R and R^1 are each independently hydrogen or lower alkyl and wherein the phenyl ring may be substituted with 1 to 3 substituents selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, chloro, and bromo.

[0085] Use of mulitmeric entities is also contemplated for practicing the present invention. The term "multimer" or "multimeric" (as opposed to "monomer" or "monomeric")

as applied to an entity of a multifunctional agent as described herein refers to molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions to contribute to functionality associated with the entity. Mutimers include monomers, dimers, trimers, tetramers, pentamers, hexamers, etc. In some embodiments, multifunctional agents of the invention include various multimeric targeting entities. Accordingly, in some embodiments of the invention, a multimeric chlorotoxin polypeptide may be used as a targeting entity of a multifunctional conjugate. For example, such a multimeric targeting entity may comprise dimeric chlorotoxin polypeptides, trimeric chlorotoxin polypeptides, etc. In some embodiments, each polypeptide monomer unit of a multimeric complex comprises a chlorotoxin polypeptide, such as those provided in Table 1. In some embodiments, at least one of the polypeptides of a multimeric chlorotoxin targeting entity is a full length chlorotoxin polypeptide. These include native chlorotoxin as set forth in SEQ ID NO: 1, as well as any variants, such as reduced lysine chlorotoxin polypeptides, which are also described herein. In some embodiments, contemplated chlorotoxin polypeptides correspond to fragments of chlorotoxin. Non-limiting examples of such fragments are provided in Table 1, as set forth in SEQ ID NOs: 27 and 29-66.

[0086] In some embodiments, contemplated multimeric chlorotoxin targeting entity is a homomeric complex, while in other embodiments, contemplated multimeric chlorotoxin targeting entity is a heteromeric complex. For example, any suitable chlorotoxin polypeptides (e.g., those provided in Table 1) may be used in any combination to form a multimeric targeting entity contemplated herein. Accordingly, in some embodiments, a targeting entity of a multifunctional conjugate comprises a dimeric chlorotoxin polypeptides, each of which may be about 36 amino acids in length, e.g., having about 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30 residues. In some embodiments, each monomer of a dimetic chlorotoxin polypeptides may contain shorter fragment, such as about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 residues.

[0087] Multimeric targeting polypeptides embraced by the present invention may be produced by any suitable methods known in the art. In some embodiments, to facilitate multimeric formation, monomers may be adopted either chemically or recombinantly to include a modified or substitution residue which can be used to attach a linker. The modified monomers can then be multimerized to form a multimer via such a linker. Additionally or alternatively, one or more cysteine residues may be used strategically to link the monomer

units via one or more disulfide bonds, to form a multimeric targeting entity. In some embodiments, a multimeric targeting entity may be recombinantly produced, e.g., as a fusion protein.

[0088] The present invention also includes linear polypeptides. In particular, in some embodiments, provided linear polypeptides are useful as a targeting entity. In some embodiments, provided linear polypeptides are chlorotoxin polypeptides comprising one or more modifications to render the polypeptide linear. In some embodiments, one or more cysteine residues of a chlorotoxin polypeptide may be absent, modified, or replaced with another residue or analog, so as to prevent disulfide bonds from forming, either intramolarcularly or intermolecularly. In some embodiments, each of the cysteine residues present in a chlorotoxin polypeptide may be absent, modified, or replaced with another residue or analog. For example, in some embodiments, each of the naturally occurring cysteine residues of chlorotoxin (i.e., amino acid positions at 2, 5, 16, 19, 20, 28, 33, and 35) is absent, modified, or replaced with another residue or analog. In some embodiments, all of the cysteine residues are replaced with amino acid analogs. In some embodiments, some or all of the cysteine residues are replaced with glycine analogs, such as allyl glycine (allyl-Gly). Accordingly, the invention includes a linear chlorotoxin polypeptide having allyl-Gly residues in lieu of cysteine residues. In some embodiments, cysteine residues have been replaced with (S)-allyl-Gly. In some embodiments, provided linear chlorotoxin polypeptides are also reduced lysine chlorotoxin polypeptides. For example, in some embodiments, provided linear reduced lysine chlorotoxin polypeptides contain no more than one lysine residue available for conjugation. The present inevntion thus contemplates that any one of the exemplary chlorotoxin polypeptides provided herein (including those provided in Table 1 and Table 2) may be modified to render it linear.

Preparation of chlorotoxin polypeptides

[0089] Chlorotoxin polypeptides useful for contemplated compositions and methods of the present invention can be prepared using any of a wide variety of methods, including standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the nucleic acids encoding these peptides may be synthesized using commercially

available oligonucleotide synthesis instrumentation and the proteins may be produced recombinantly using standard recombinant production systems.

[0090] Other suitable chlorotoxin polypeptides include peptide mimetics that mimic the three- dimensional structure of chlorotoxin. Such peptide mimetics may have significant advantages over naturally occurring peptides including, for example, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc), altered specificity (e.g., broad-spectrum biological activities, reduced antigenicity and others).

[0091] In certain embodiments, mimetics are molecules that mimic elements of chlorotoxin polypeptide secondary structure. Peptide backbones of proteins exist mainly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimetic is expected to permit molecular interactions similar to the natural molecule. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of compounds are also referred to as peptide mimetics or peptidomimetics (see, for example, Fauchere, Adv. Drug Res., 1986, 15: 29-69; Veber & Freidinger, 1985, Trends Neurosci., 1985, 8: 392-396; Evans *et al.*, J. Med. Chem., 1987, 30: 1229-1239) and are usually developed with the aid of computerized molecular modeling.

[0092] Generally, peptide mimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), but have one or more peptide linkages optionally replaced by a non-peptide linkage. The use of peptide mimetics can be enhanced through the use of combinatorial chemistry to create drug libraries. The design of peptide mimetics can be aided by identifying amino acid mutations that increase or decrease the binding of a peptide to, for example, a tumor cell. Approaches that can be used include the yeast two hybrid method (see, for example, Chien *et al.*, Proc. Natl. Acad. Sci. USA, 1991, 88: 9578- 9582) and using the phase display method. The two-hybrid method detects protein-protein interactions in yeast (Field *et al.*, Nature, 1989, 340: 245-246). The phage display method detects the interaction between an immobilized protein and a protein that is expressed on the surface of phages such as lambda and M 13 (Amberg *et al.*, Strategies, 1993, 6: 2-4; Hogrefe *et al.*, Gene, 1993, 128: 119-126). These methods allow positive and

negative selection of peptide - protein interactions and the identification of the sequences that determine these interactions.

[0093] In certain embodiments, conjugate agents as described herein comprise a polypeptide toxin of another scorpion species that displays similar or related activity to chlorotoxin described above. As used herein, the term "similar or related activity to chlorotoxin" refers, in particular, to the selective/specific binding to tumor/cancer cells. Examples of suitable related scorpion toxins include, but are not limited to toxins or related peptides of scorpion origin that display amino acid and/or nucleotide sequence identity to chlorotoxin. Examples of related scorpion toxins include, but are not limited to, CT neurotoxin from Mesobuthus martenssi (GenBank Accession No.AAD473730), Neurotoxin BmK 41-2 from Buthus martensii karsch (GenBank Accession No. A59356), Neurotoxin Bml2-b from Buthus martensii (GenBank Accession No. AAKl 6444), Probable Toxin LGH 8/6 from Leiurus quinquestriatus hebraeu (GenBank Accession No. P55966), and Small toxin from Mesubutus tamulus sindicus (GenBank Accession No. P15229).

[0094] Related scorpion toxins suitable for use in the present invention comprise polypeptides that have an amino acid sequence of at least about 75%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% sequence identity with the entire chlorotoxin sequence as set forth in SEQ ID NO: 1 of International Application No. WO 2003/101474 (which is incorporated herein by reference in its entirety). In certain embodiments, related scorpion toxins include those scorpion toxins that have a sequence homologous to KGRGKSY (SEQ ID NO: 65) (corresponding to SEQ ID NO: 8 of chlorotoxin, as set forth in International Application No. WO 2003/101474) or $TTX_1X_2X_3MX_4X_5K$ (SEQ ID NO: 68) (corresponding to SEQ ID NO: 13 of chlorotoxin, as set forth in International Application No. WO 2003/101474). $TTX_1X_2X_3MX_4X_5K$ (SEQ ID NO: 68) corresponds to amino acid residues 7-15 of SEQ ID NO: 1, wherein X_1 is typically an acidic amino acid (e.g., aspartic acid and glutamic acid); X_2 is any amino acid (e.g., alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine); X_3 is typically an amide amino acid (e.g., asparagine and glutamine); X_4 is an any amino acid (e.g., serine, threonine and alanine); and X_5 is a basic amino acid (e.g., histidine, lysine and arginine). In some embodiments, X_1 is aspartic acid, X_2 is histidine or proline, X_3 is

glutamine, X_4 is alanine and X_5 is arginine or lysine. Various variants, analogs, and derivatives of such polypeptides (as described herein) are also embraced by this invention.

[0095] In some embodiments, provided reduced lysine chlorotoxin polypeptides have an amino acid sequence that includes one or more than one lysine residues but nonetheless have a reduced number of lysines available for conjugation when compared with chlorotoxin. In some embodiments, one or more lysine residues in a reduced lysine chlorotoxin polypeptide provided herein is/are made unavailable as a site for conjugation though they are present in the chlorotoxin polypeptide. For example, one or more lysine residue(s) can be covalently or non-covalently modified such that the one or more lysine residue(s) is/are blocked from participating in a chemical conjugation reaction, leaving fewer than 3, 2 or 1 (i.e., "reduced" lysine) lysine residue(s) available as a site for conjugation. Non-limiting examples of covalent modifications to lysine residues that could be employed in this manner include pegylation (i.e., modification by attachment of a polyethylene glycol polymer), methylation (including di- and tri- methylation), and attachment of other alkyl group(s). In certain embodiments, one or more lysine residues is/are modified at the epsilon NH_2 group. For example, if a given R group (e.g., butyl, propyl, or ethyl group) is used to covalently modify a lysine residue, the epsilon NH_2 group can be modified to an NR_2 or NR_3 group.

[0096] In certain embodiments, blocking of particular lysine residue is achieved by incorporating a modified lysine (in which sites available for conjugation are already blocked) during the appropriate step during synthesis of the reduced lysine chlorotoxin polypeptide. Modified lysines are readily available commercially and can be synthesized by routine methods known in the art. Non-limiting examples of modified lysines that can be used in this manner include, but are not limited to, di-substituted lysine or trisubstituted lysines (e.g., N,N-R_2-lysine or N,N,N-R_3-lysine, where R is the blocking group) and lysines with short PEG molecules attached to them. R can be any group that when covalently attached to the lysine would serve to block the lysine residue from participating in a chemical conjugation reaction. For example, alkyl groups (e.g., butyl, methyl, and ethyl) may serve as blocking groups. For example, N,N-dimethyl-lysine and/or N,N,N-trimethyl-lysine be used during synthesis.

[0097] In certain embodiments, one terminus or both termini (i.e., the N- and/or C-terminus) of the reduced lysine chlorotoxin polypeptide is blocked so as to prevent the terminus/ termini from participating in a chemical conjugation reaction. For example, in some embodiments, a conjugation chemistry is used in which at least one terminus would participate in the conjugation reaction if it were not blocked. A variety of methods of blocking N- and/or C-termini of polypeptides are known in the art, including, but not limited to, covalent modification by the addition of alkyl groups (e.g., methylation) at amines.

[0098] Methods of synthesizing reduced lysine chlorotoxin polypeptides as described herein are known in the art. In some peptide synthesis methods, an amino group of one amino acid (or amino acid derivative) is linked to a carboxyl group of another amino acid (or amino acid derivative) that has been activated by reacting it with a reagent such as dicyclohexylcarbodiimide (DCC). When the free amino group attacks the activated carboxyl group, a peptide bond is formed and dicyclohexylurea is released. In such methods, other potentially reactive groups (such as the α-amino group of the N-terminal amino acid or amino acid derivative and the carboxyl group of the C-terminal amino acid or amino acid derivative) may be blocked ("protected") from participating in the chemical reaction. Thus, only particular active groups react such that the desired product is formed. Blocking groups useful for this purpose include without limitation *tertbutoxycarbonyl* groups (t-Boc) and benzoyloxycarbonyl groups to protect amine groups; and simple esters (such as methyl and ethyl groups) and benzyl esters to protect carboxyl groups. Blocking groups can typically be subsequently removed with a treatment that leaves peptide bonds intact (for example, treatment with dilute acid). This process of protecting reacting groups that should not react, coupling to form a peptide bond, and deprotecting reactive groups may be repeated. A peptide may be synthesized by sequentially adding amino acids to a growing peptide chain. Both liquid-phase and solid phase peptide synthesis methods are suitable for use in accordance with the invention. In solid-phase peptide synthesis methods, the growing peptide chain is typically linked to an insoluble matrix (such as, for example, polystyrene beads) by linking the carboxy-terminal amino acid to the matrix. At the end of synthesis, the peptide can be released from the matrix using a cleaving reagent that does not disrupt peptide bonds, such as hydrofluoric acid (HF). Protecting groups are also typically removed at this time. Automated, high throughput, and/or parallel peptide synthesis methods may also be used in accordance with the invention. For more information about peptide synthesis methods, see,

e.g., Merrifield (1969) "Solid-phase peptide synthesis," Adv Enzymol Relat Areas Mol Biol., 32:221-96; Fridkin *et al.,* (1974) *Annu Rev Biochem.,* 43 (0):419-43 ; Merrifield (1997) "Concept and Early Development of Solid Phase Peptide Synthesis," *Methods in Enzymology,* 289:3-13; Sabatino *et al.,* (2009) "Advances in automatic, manual and microwave-assisted solid-phase peptide synthesis," *Curr Opin Drug Discov Devel.,* 11(6):762-70, the entire contents of each of which are herein incorporated by reference.

[0099] In some embodiments, modifications to lysine residues are used in combination with other means as described herein (e.g., replacement of a lysine residue with another amino acid or amino acid derivative and/or lack of a lysine residue where one is normally found in chlorotoxin).

[00100] In some embodiments, the protecting group in the N-terminus is not removed at the end of synthesis. Leaving the protecting group on may, for example, serve to generate a reduced lysine chlorotoxin polypeptide with a blocked N-terminus, thus limiting the sites available for conjugation in a particular chemical conjugation scheme.

Therapeutic entities

[00101] Multifunctional agents described herein in many embodiments comprise at least one therapeutic entity, in addition to a targeting entity (e.g., chlorotoxin polypeptides) described above. Contemplated therapeutic entities include, without limitation, anti-cancer agents (e.g., agents that inhibit tumor growth, agents that inhibit proliferation of cancer cells, agents that preferentially kill cancer cells, agents that inhibit angiogenesis, etc.), agents that attenuate any adverse effects (e.g., antiemetics, etc.) and/or with other approved chemotherapeutic drugs, as well as adjuvants (e.g., agents that elicit adjuvant effects).

[00102] Suitable therapeutic entities include anti-cancer agents can belong to any of various classes of compounds including, but not limited to, small molecules, peptides, saccharides, steroids, antibodies, fusion proteins, antisense polynucleotides, ribozymes, small interfering RNAs, peptidomimetics, and the like. Similarly, suitable anti-cancer agents can be found among any of a variety of classes of anti-cancer agents including, but not limited to, alkylating agents, anti-metabolite drugs, anti-mitotic antibiotics, alkaloidal anti-tumor agents,

hormones and anti-hormones, interferons, non-steroidal anti-inflammatory drugs, and various other anti-tumor agents.

[00103] Examples of chemotherapeutics include, but are not limited to, anti-mitotic agents, alkylating drugs (e.g., mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide, etc.), antimetabolites (e.g., methotrexate, etc.), purine antagonists and pyrimidine antagonists (e.g., 6-mercaptopurine, 5-fluorouracil, cytarabine, gemcitabine, etc.), spindle poisons (e.g., vinblastine, vincristine, vinorelbine, paclitaxel, etc.), podophyllotoxins (e.g., etoposide, irinotecan, topotecan, etc.), antibiotics (e.g., doxorubicin, bleomycin, mitomycin, etc.), nitrosureas (e.g., carmustine, lomustine, nomustine, etc.), inorganic ions (e.g., cisp latin, carboplatin, etc.), enzymes (e.g., asparaginase, etc.), and hormones (e.g., tamoxifen, leuprolide, flutamide, megestrol, etc.), to name a few. For a more comprehensive discussion of updated cancer therapies see, www.cancer.gov/, a list of the FDA approved oncology drugs at http://www.fda.gov/cder/cancer/druglistframe.htm, and The Merck Manual, Seventeenth Ed. 1999, the entire contents of which are hereby incorporated by reference.

[00104] Non-limiting examples of cytotoxic agents which can be employed as a therapeutic entity for any of the multifunctional agents contemplated in the present disclosure may be selected from: CHOPP (cyclophosphamide, doxorubicin, vincristine, prednisone, and procarbazine); CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); COP (cyclophosphamide, vincristine, and prednisone); CAP-BOP (cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone); m- BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and leucovorin); ProMACE-MOPP (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, mechloethamine, vincristine, prednisone, and procarbazine); ProMACE- CytaBOM (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, cytarabine, bleomycin, and vincristine); MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin, and leucovorin); MOPP (mechloethamine, vincristine, prednisone, and procarbazine); ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine); MOPP (mechloethamine, vincristine, prednisone and procarbazine) alternating with ABV (adriamycin/doxorubicin, bleomycin, and vinblastine); MOPP (mechloethamine, vincristine, prednisone, and procarbazine) alternating with ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine); ChIVPP (chlorambucil, vinblastine, procarbazine, and prednisone); IMVP- 16 (ifosfamide, methotrexate, and etoposide); MIME (methyl-gag,

ifosfamide, methotrexate, and etoposide); DHAP (dexamethasone, high- dose cytaribine, and cisplatin); ESHAP (etoposide, methylpredisolone, high-dose cytarabine, and cisplatin); CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin); CAMP (lomustine, mitoxantrone, cytarabine, and prednisone); CVP-I (cyclophosphamide, vincristine, and prednisone), ESHOP (etoposide, methylpredisolone, high-dose cytarabine, vincristine and cisplatin); EPOCH (etoposide, vincristine, and doxorubicin for 96 hours with bolus doses of cyclophosphamide and oral prednisone), ICE (ifosfamide, cyclophosphamide, and etoposide), CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin), CHOP-B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin), CEPP-B (cyclophosphamide, etoposide, procarbazine, and bleomycin), and P/DOCE (epirubicin or doxorubicin, vincristine, cyclophosphamide, and prednisone).

[00105] In some embodiments, chemotherapeutic drugs prescribed for brain tumors may be employed as a therapeutic entity in accordance with the invention. These include, but are not limited to, temozolomide (Temodar®), procarbazine (Matulane®), and lomustine (CCNU), which are taken orally; vincristine (Oncovin® or Vincasar PFS®), cisplatin (Platinol®), carmustine (BCNU, BiCNU), and carboplatin (Paraplatin®), which are administered intravenously; and mexotrexate (Rheumatrex® or Trexall®), which can be administered orally, intravenously or intrathecally (i.e., injected directly into spinal fluid). BCNU is also given under the form of a polymer wafer implant during surgery (Giadel® wafers). One of the most commonly prescribed combination therapy for brain tumors is PCV (procarbazine, CCNU, and vincristine) which is usually given every six weeks.

[00106] In embodiments where the tumor to be treated is a brain tumor of neuroectodermal origin, a composition or method of the present invention may employ agents for the management of symptoms such as seizures and cerebral edema. Examples of anticonvulsants successfully administered to control seizures associated with brain tumors include, but are not limited to, phenytoin (Dilantin®), Carbamazepine (Tegretol®) and divalproex sodium (Depakote®). Swelling of the brain may be treated with steroids (e.g., dexamethasone (Decadron®).

Certain embodiments of chlorotoxin polypeptide conjugate agents

[00107] In a number of embodiments, the invention provides multifunctional agents comprising a target entity which essentially consists of a chlorotoxin polypeptide (e.g., chlorotoxin or its derivative). In such embodiments, therefore, the multifunctional agents according to the present invention are chlorotoxin polypeptide conjugates. Non-limiting embodiments of useful chlorotoxin polypeptide conjugates are provided below.

[00108] For example, provided chlorotoxin polypeptide conjugates comprise a reduced lysine chlorotoxin polypeptide and a nucleic acid molecule that is useful as a therapeutic (e.g., anti-cancer) agent. A variety of chemical types and structural forms of nucleic acid can be suitable for such strategies. These include, by way of non-limiting example, DNA, including single-stranded (ssDNA) and double-stranded (dsDNA); RNA, including, but not limited to ssRNA, dsRNA, tRNA, mRNA, rRNA, enzymatic RNA; RNA:DNA hybrids, triplexed DNA (e.g., dsDNA in association with a short oligonucleotide), and the like.

[00109] In some embodiments, the nucleic acid agent is between about 5 and 2000 nucleotides long. In some embodiments, the nucleic acid agent is at least about 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, 50 or more nucleotides long. In some embodiments, the nucleic acid agent is less than about 2000, 1900, 1800, 1700, 1600, 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 45, 40, 35, 30, 25, 20 or fewer nucleotides long.

[00110] In some embodiments, the nucleic acid agent comprises a promoter and/or other sequences that regulate transcription. In some embodiments, the nucleic acid agent comprises an origin of replication and/or other sequences that regulate replication. In some embodiments, the nucleic acid agent does not include a promoter and/or an origin of replication.

[00111] Nucleic acid anti-cancer agents suitable for use in the practice of the present invention include those agents that target genes associated with tumorigenesis and cell growth or cell transformation (e.g., proto-oncogenes, which code for proteins that stimulate cell division), angiogenic/anti-angiogenic genes, tumor suppressor genes (which code for proteins that suppress cell division), genes encoding proteins associated with tumor growth

and/or tumor migration, and suicide genes (which induce apoptosis or other forms of cell death), especially suicide genes that are most active in rapidly dividing cells.

[00112] Examples of genes associated with tumorigenesis and/or cell transformation include MLL fusion genes, BCR-ABL, TEL-AML1, EWS-FLI1, TLS-FUS, PAX3- FKHR, Bc1-2, AML1-ETO, AML1-MTG8, Ras, Fos PDGF, RET, APC, NF-1, Rb, p53, MDM2 and the like; overexpressed genes such as multidrug resistance genes; cyclins; beta-Catenin; telomerase genes; c-myc, n-myc, Bc1-2, Erb-B1 and Erb-B2; and mutated genes such as Ras, Mos, Raf, and Met. Examples of tumor suppressor genes include, but are not limited to, p53, p21, RB1, WT1, NF1, VHL, APC, DAP kinase, p16, ARF, Neurofibromin, and PTEN. Examples of genes that can be targeted by nucleic acid agents useful in anti-cancer therapy include genes encoding proteins associated with tumor migration such as integrins, selectins, and metalloproteinases; anti-angiogenic genes encoding proteins that promote formation of new vessels such as Vascular Endothelial Growth Factor (VEGF) or VEGFr; anti-angiogenic genes encoding proteins that inhibit neovascularization such as endostatin, angiostatin, and VEGF-R2; and genes encoding proteins such as interleukins, interferon, fibroblast growth factor (α-FGF and(β-FGF), insulin-like growth factor (e.g., IGF-1 and IGF-2), Platelet-derived growth factor (PDGF), tumor necrosis factor (TNF), Transforming Growth Factor (e.g., TGF-α and TGF-β, Epidermal growth factor (EGF), Keratinocyte Growth Factor (KGF), stem cell factor and its receptor c-Kit (SCF/c-Kit) ligand, CD40L/CD40, VLA-4 VCAM-1, ICAM-1/LFA-1, hyalurin/CD44, and the like. As will be recognized by one skilled in the art, the foregoing examples are not exclusive.

[00113] Nucleic acid agents suitable for use in the invention may have any of a variety of uses including, for example, use as anti-cancer or other therapeutic agents, probes, primers, etc. Nucleic acid agents may have enzymatic activity (e.g., ribozyme activity), gene expression inhibitory activity (e.g., as antisense or siRNA agents, etc), and/or other activities. Nucleic acids agents may be active themselves or may be vectors that deliver active nucleic acid agents (e.g., through replication and/or transcription of a delivered nucleic acid). For purposes of the present specification, such vector nucleic acids are considered "therapeutic agents" if they encode or otherwise deliver a therapeutically active agent, even if they do not themselves have therapeutic activity.

[00114] In certain embodiments, chlorotoxin polypeptide conjugates comprise a nucleic acid therapeutic agent that comprises or encodes an antisense compound. The terms "antisense compound or agent," "antisense oligomer," "antisense oligonucleotide," and "antisense oligonucleotide analog" are used herein interchangeably, and refer to a sequence of nucleotide bases and a subunit-to-subunit backbone that allows the antisense compound to hybridize to a target sequence in an RNA by Watson-Crick base pairing to form an RNA oligomer heteroduplex within the target sequence. The oligomer may have exact sequence complementarity within the target sequence or near complementarity. Such antisense oligomers may block or inhibit translation of the mRNA containing the target sequence, or inhibit gene transcription. Antisense oligomers may bind to double-stranded or single-stranded sequences.

[00115] Examples of antisense oligonucleotides suitable for use in the practice of the present invention include, for example, those mentioned in the following reviews: R.A Stahel *et al.,* Lung Cancer, 2003, 41: S81-S88; K.F. Pirollo *et al.,* Pharmacol. Ther., 2003, 99: 55-77; A.C. Stephens and R.P. Rivers, Curr. Opin. Mol. Ther., 2003, 5: 118- 122; N.M. Dean and C.F. Bennett, Oncogene, 2003, 22: 9087-9096; N. Schiavone *et al.,* Curr. Pharm. Des., 2004, 10: 769-784; L. Vidal *et al.,* Eur. J. Cancer, 2005, 41: 2812- 2818; T. Aboul-Fadl, Curr. Med. Chem., 2005, 12: 2193-2214; M.E. Gleave and B.P. Monia, Nat. Rev. Cancer, 2005, 5: 468-479; Y.S. Cho-Chung, Curr. Pharm. Des., 2005, 11: 2811-2823; E. Rayburn *et al.,* Lett. Drug Design & Discov., 2005, 2: 1-18; E.R. Rayburn *et al.,* Expert Opin. Emerg. Drugs, 2006, 11: 337-352; I. Tamm and M. Wagner, Mol. Biotechnol., 2006, 33: 221-238 (each of which is incorporated herein by reference in its entirety).

[00116] Examples of suitable antisense oligonucleotides include, for example oblimersen sodium (also known as Genasense™ or G31239, developed by Genta, Inc., Berkeley Heights, NJ), a phosphorothioate oligomer targeted towards the initiation codon region of the bcl-2 mRNA. Bcl-2 is a potent inhibitor of apoptosis and is overexpressed in many cancer including follicular lymphomas, breast cancer, colon cancer, prostate cancer, and intermediate/high-grade lymphomas (C.A. Stein *et al.,* Semin. Oncol., 2005, 32: 563-573; S.R. Frankel, Semin. Oncol., 2003, 30: 300-304). Other suitable antisense oligonucleotides include GEM-231 (HYB0165, Hybridon, Inc., Cambridge, MA), which is a mixed backbone oligonucleotide directed against cAMP-dependent protein kinase A (PKA) (S. Goel *et al.,* Clin. Cancer Res., 203, 9: 4069-4076); Affinitak (ISIS 3521 or aprinocarsen, ISIS pharmaceuticals,

Inc., Carlsbad, CA), an antisense inhibitor of PKCalpha; OGX-011 (Isis 112989, Isis Pharmaceuticals, Inc.), a 2'-methoxyethyl modified antisense oligonucleotide against clusterin, a glycoprotein implicated in the regulation of the cell cycle, tissue remodeling, lipid transport, and cell death and which is overexpressed in cancers of breast, prostate and colon; ISIS 5132 (Isis 112989, Isis Pharmaceuticals, Inc.), a phosphorothioate oligonucleotide complementary to a sequence of the 3'-unstranslated region of the c-raf-1 mRNA (S.P. Henry *et al.,* Anticancer Drug Des., 1997, 12: 409-420; B.P. Monia *et al.,* Proc. Natl. Acad. Sci. USA, 1996, 93: 15481- 15484; C.M. Rudin *et al.,* Clin. Cancer Res., 2001, 7: 1214-1220); ISIS 2503 (Isis Pharmaceuticals, Inc.), a phosphorothioate oligonucleotide antisense inhibitor of human H-ras mRNA expression (J. Kurreck, Eur. J. Biochem., 2003, 270: 1628-1644); oligonucleotides targeting the X-linked inhibitor of apoptosis protein (XIAP), which blocks a substantial portion of the apoptosis pathway, such as GEM 640 (AEG 35156, Aegera Therapeutics Inc. and Hybridon, Inc.) or targeting survivin, an inhibitor of apoptosis protein (IAP), such as ISIS 23722 (Isis Pharmaceuticals, Inc.), a 2'-O-methoxyethyl chimeric oligonucleotide; MG98, which targets DNA methyl transferase; and GTI-2040 (Lorus Therapeutics, Inc. Toronto, Canada), a 20-mer oligonucleotide that is complementary to a coding region in the mRNA of the R2 small subunit component of human ribonucleotide reductase.

[00117] Other suitable antisense oligonucleotides include antisense oligonucleotides that are being developed against Her-2/neu, c-Myb, c-Myc, and c-Raf (see, for example, A. Biroccio *et al.,* Oncogene, 2003, 22: 6579-6588; Y. Lee *et al.,* Cancer Res., 2003, 63: 2802-2811; B. Lu *et al.,* Cancer Res., 2004, 64: 2840-2845; K.F. Pirollo *et al.,* Pharmacol. Ther., 2003, 99: 55-77; and A. Rait *et al.,* Ann. N. Y. Acad. Sci., 2003, 1002: 78-89).

[00118] In certain embodiments, chlorotoxin polypeptide conjugates of the present invention comprise a nucleic acid anti-cancer agent that comprises or encodes an interfering RNA molecule. The terms "interfering RNA" and "interfering RNA molecule" are used herein interchangeably, and refer to an RNA molecule that can inhibit or downregulate gene expression or silence a gene in a sequence-specific manner, for example by mediating RNA interference (RNAi). RNA interference (RNAi) is an evolutionarily conserved, sequence-specific mechanism triggered by double-stranded RNA (dsRNA) that induces degradation of complementary target single-stranded mRNA and "silencing" of the corresponding translated

sequences (McManus and Sharp, 2002, Nature Rev. Genet., 2002, 3: 737). RNAi functions by enzymatic cleavage of longer dsRNA strands into biologically active "short-interfering RNA" (siRNA) sequences of about 21-23 nucleotides in length (Elbashir *et al.,* Genes Dev., 2001, 15: 188). RNA interference has emerged as a promising approach for therapy of cancer.

[00119] An interfering RNA suitable for use in the practice of the present invention can be provided in any of several forms. For example, an interfering RNA can be provided as one or more of an isolated short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), or short hairpin RNA (shRNA).

[00120] Examples of interfering RNA molecules suitable for use in the present invention include, for example, the iRNAs cited in the following reviews: O. Milhavet *et al.,* Pharmacol. Rev., 2003, 55: 629-648; F. Bi *et al.,* Curr. Gene. Ther., 2003, 3: 411- 417; P.Y. Lu *et al.,* Curr. Opin. Mol. Ther., 2003, 5: 225-234; I. Friedrich *et al.,* Semin. Cancer Biol., 2004, 14: 223-230; M. Izquierdo, Cancer Gene Ther., 2005, 12: 217-227; P.Y. Lu *et al.,* Adv. Genet., 2005, 54: 117-142; G.R. Devi, Cancer Gene Ther., 2006, 13: 819-829; M.A. Behlke, Mol. Ther., 2006, 13: 644-670; and L.N. Putral *et al.,* Drug News Perspect., 2006, 19: 317-324 (the contents of each of which are incorporated herein by reference in their entirety).

[00121] Other examples of suitable interfering RNA molecules include, but are not limited to, p53 interfering RNAs (e.g., T.R. Brummelkamp *et al.,* Science, 2002, 296: 550-553; M.T. Hemman *et al.,* Nat. Genet., 2003, 33: 396-400); interfering RNAs that target the bcr-abl fusion, which is associated with development of chronic myeloid leukemia and acute lymphoblastic leukemia (e.g., M. Scherr *et al.,* Blood, 2003, 101: 1566-1569; M.J. Li *et al.,* Oligonucleotides, 2003, 13: 401-409), interfering RNAs that inhibit expression of NPM-ALK, a protein that is found in 75% of anaplastic large cell lymphomas and leads to expression of a constitutively active kinase associated with tumor formation (U. Ritter *et al.,* Oligonucleotides, 2003, 13: 365-373); interfering RNAs that target oncogenes, such as Raf-1 (T.F. Lou *et al.,* Oligonucleotides, 2003, 13: 313- 324), K-Ras (T.R. Brummelkamp *et al.,* Cancer Cell, 2002, 2: 243-247), erbB-2 (G. Yang *et al., J.* Biol. Chem., 2004, 279: 4339-4345); interfering RNAs that target b-catenin protein, whose over-expression leads to transactivation of the T-cell factor target genes, which is thought to be the main

transforming event in colorectal cancer (M. van de Wetering *et al.,* EMBO Rep., 2003, 4: 609-615).

[00122] In certain embodiments, chlorotoxin polypeptide conjugates comprise a nucleic acid therapeutic agent that is a ribozyme. As used herein, the term "ribozyme" refers to a catalytic RNA molecule that can cleave other RNA molecules in a target-specific marmer Ribozymes can be used to downregulate the expression of any undesirable products of genes of interest. Examples of ribozymes that can be used in the practice of the present invention include, but are not limited to, ANGIOZYME™ (RPI.4610, Sima Therapeutics, Boulder, CO), a ribozyme targeting the conserved region of human, mouse, and rat vascular endothelial growth factor receptor (VEGFR)-1 mRNA, and Herzyme (Sima Therapeutics).

[00123] In certain embodiments, entities or moieties within chlorotoxin polypeptide conjugates comprise a photosensitizer used in photodynamic therapy (PDT). In PDT, local or systemic administration of a photosensitizer to a patient is followed by irradiation with light that is absorbed by the photosensitizer in the tissue or organ to be treated. Light absorption by the photosensitizer generates reactive species (e.g., radicals) that are detrimental to cells. For maximal efficacy, a photosensitizer typically is in a form suitable for administration, and also in a form that can readily undergo cellular internalization at the target site, often with some degree of selectivity over normal tissues.

[00124] While some photosensitizers (e.g., Photofrin®, QLT, Inc., Vancouver, BC, Canada) have been delivered successfully as part of a simple aqueous solution, such aqueous solutions may not be suitable for hydrophobic photosensitizer drugs, such as those that have a tetra- or poly-pyrrole-based structure. These drugs have an inherent tendency to aggregate by molecular stacking, which results in a significant reduction in the efficacy of the photosensitization processes (Siggel *et al., J.* Phys. Chem., 1996, 100: 2070-2075). Approaches to minimize aggregation include liposomal formulations (e.g., for benzoporphyrin derivative monoacid A, BPDMA, Verteporfin®, QLT, Inc., Vancouver, Canada; and zinc phthalocyanine, CIBA-Geigy, Ltd., Basel, Switzerland), and conjugation of photosensitizers to biocompatible block copolymers (Peterson *et al.,* Cancer Res., 1996, 56: 3980-3985) and/or antibodies (Omelyanenko *et al.,* Int. J. Cancer, 1998, 75: 600-608).

[00125] Chlorotoxin polypeptide conjugates comprising a reduced lysine chlorotoxin polypeptide associated with a photosensitizer can be used as new delivery systems in PDT. In addition to reducing photosensitizer aggregation, delivery of photosensitizers according to the present invention exhibits other advantages such as increased specificity for target tissues/organ and cellular internalization of the photosensitizer.

[00126] Photosensitizers suitable for use in the present invention include any of a variety of synthetic and naturally occurring molecules that have photosensitizing properties useful in PDT. In certain embodiments, the absorption spectrum of the photosensitizer is in the visible range, typically between 350 nm and 1200 nm, preferably between 400 nm and 900 nm, e.g., between 600 nm and 900 nm. Suitable photosensitizers that can be coupled to toxins according to the present invention include, but are not limited to, porphyrins and porphyrin derivatives (e.g., chlorins, bacteriochlorins, isobacteriochlorins, phthalocyanines, and naphthalocyanines); metalloporphyrins, metallophthalocyanines, angelicins, chalcogenapyrrillium dyes, chlorophylls, coumarins, flavins and related compounds such as alloxazine and riboflavin, fullerenes, pheophorbides, pyropheophorbides, cyanines (e.g., merocyanine 540), pheophytins, sapphyrins, texaphyrins, purpurins, porphycenes, phenothiaziniums, methylene blue derivatives, naphthalimides, nile blue derivatives, quinones, perylenequinones (e.g., hypericins, hypocrellins, and cercosporins), psoralens, quinones, retinoids, rhodamines, thiophenes, verdins, xanthene dyes (e.g., eosins, erythrosins, rose bengals), dimeric and oligomeric forms of porphyrins, and prodrugs such as 5-aminolevulinic acid (R.W. Redmond and J.N. Gamlin, Photochem. Photobiol., 1999, 70: 391-475).

[00127] Exemplary photosensitizers suitable for use in the present invention include those described in U.S. Pat. Nos. 5,171,741; 5,171,749; 5,173,504; 5,308,608; 5,405,957; 5,512,675; 5,726,304; 5,831,088; 5,929,105; and 5,880,145 (the contents of each of which are incorporated herein by reference in their entirety).

[00128] In certain embodiments, chlorotoxin polypeptide conjugates comprise a radiosensitizer. As used herein, the term "radiosensitizer" refers to a molecule, compound or agent that makes tumor cells more sensitive to radiation therapy. Administration of a radiosensitizer to a patient receiving radiation therapy generally results in enhancement of the effects of radiation therapy. Ideally, a radiosensitizer exerts its function only on target

cells. For ease of use, a radiosensitizer should also be able to find target cells even if it is administered systemically. However, currently available radiosensitizers are typically not selective for tumors, and they are distributed by diffusion in a mammalian body. Chlorotoxin polypeptide conjugates of the present invention can be used as a new delivery system for radiosensitizers.

[00129] A variety of radiosensitizers are known in the art. Examples of radiosensitizers suitable for use in the present invention include, but are not limited to, paclitaxel (TAXOL®), carboplatin, cisplatin, and oxaliplatin (Amorino *et al.*, Radiat. Oncol. Investig. 1999; 7: 343-352; Choy, Oncology, 1999, 13: 22-38; Safran *et al.*, Cancer Invest., 2001, 19: 1-7; Dionet *et al.*, Anticancer Res., 2002, 22: 721-725; Cividalli *et al.*, Radiat. Oncol. Biol. Phys., 2002, 52: 1092-1098); gemcitabine (Gemzar®) (Choy, Oncology, 2000, 14: 7-14; Mornex and Girard, Annals of Oncology, 2006, 17: 1743-1747); etanidazole (Nitrolmidazole®) (Inanami *et al.*, Int. J. Radiat. Biol., 2002, 78: 267- 274); misonidazole (Tamulevicius *et al.*, Br. J. Radiology, 1981, 54: 318-324; Palcic *et al.*, Radiat. Res., 1984, 100: 340-347), tirapazamine (Masunaga *et al.*, Br. J. Radiol., 2006, 79: 991-998; Rischin *et al.*, J. Clin. Oncol., 2001, 19: 535-542; Shulman *et al.*, Int. J. Radiat. Oncol. Biol. Phys., 1999, 44: 349-353); and nucleic acid base derivatives, e.g., halogenated purines or pyrimidines, such as 5-fluorodeoxyuridine (Buchholz *et al.*, Int. J. Radiat. Oncol. Biol. Phys., 1995, 32: 1053-1058).

[00130] In certain embodiments, chlorotoxin polypeptide conjugates comprise a radioisotope. Examples of suitable radioisotopes include any α-, β- or γ-emitter, which, when localized at a tumor site, results in cell destruction (S.E. Order, "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", Monoclonal Antibodies for Cancer Detection and Therapy, R.W. Baldwin *et al. (Eds.)*, Academic Press, 1985). Examples of such radioisotopes include, but are not limited to, iodine-131 (^{131}I), iodine-125 (^{125}I), bismuth-212 (^{212}Bi), bismuth-213 (^{213}Bi), astatine-211 (^{211}At), rhenium-186 (^{186}Re), rhenium-188 (^{188}Re), phosphorus-32 (^{32}P), yttrium-90 (^{90}yY), samarium-153 (^{153}Sm), and lutetium-177 (^{177}Lu).

[00131] In certain embodiments, chlorotoxin polypeptide conjugates comprise a superantigen or biologically active portion thereof. Superantigens constitute a group of bacterial and viral proteins that are extremely efficient in activating a large fraction of the T-

cell population. Superantigens bind directly to the major histocompatibility complex (MHC) without being processed. In fact, superantigens bind unprocessed outside the antigenbinding groove on the MHC class II molecules, thereby avoiding most of the polymorphism in the conventional peptide-binding site.

[00132] A superantigen-based tumor therapeutic approach has been developed for the treatment of solid tumors. In this approach, a targeting moiety, for example, an antibody or antibody fragment, is conjugated to a superantigen, providing a targeted superantigen. If the antibody, or antibody fragment, recognizes a tumor-associated antigen, the targeted superantigen, bound to tumors cells, can trigger superantigen-activated cytotoxic T-cells to kill the tumor cells directly by superantigen-dependent cell mediated cytotoxicity. (See, e.g., Søgaard *et al.,* (1996) "Antibody-targeted superantigens in cancer immunotherapy," *Immunotechnology, 2(3):* 151-162, the entire contents of which are herein incorporated by reference.)

[00133] Superantigen-based tumor therapeutics have had some success. For example, fusion proteins with wild-type staphylococcal enterotoxin A (SEA) have been investigated in clinical trials of colorectal and pancreatic cancer (Giantonio *et al., J.* Clin. Oncol., 1997, 15: 1994-2007; Alpaugh *et a.,* Clin. Cancer Res., 1998, 4: 1903-1914; Cheng *et al., J.* Clin. Oncol., 2004, 22: 602-609; the entire contents of each of which are herein incorporated by reference); staphylococcal superantigens of the enterotoxin gene cluster (egc) have been studied for the treatment of non-small cell lung cancer (Terman *et al.,* Clin. Chest Med., 2006, 27: 321-324, the entire contents of which are herein incorporated by reference), and staphylococcal enterotoxin B has been evaluated for the intravesical immunotherapy of superficial bladder cancer (Perabo *et al.,* Int. J. Cancer, 2005, 115: 591-598, the entire contents of which are herein incorporated by reference).

[00134] A superantigen, or a biologically active portion thereof, can be associated to a reduced lysine chlorotoxin polypeptide to form a chlorotoxin polypeptide conjugate according to the present invention and used in a therapy, e.g., an anti-cancer therapy, as described herein.

[00135] Examples of superantigens suitable for use in the present invention include, but are not limited to, staphylococcal enterotoxin (SE) (e.g., staphylococcal enterotoxin A (SEA) or staphylococcal enterotoxin E (SEE)), Streptococcus pyogenes exotoxin (SPE), Staphylococcus aureus toxic shock-syndrome toxin (TSST-1), streptococcal mitogenic

exotoxin (SME), streptococcal superantigen (SSA), and staphylococcal superantigens of the enterotoxin gene cluster. As known to one skilled in the art, the three-dimensional structures of the above listed superantigens can be obtained from the Protein Data Bank. Similarly, the nucleic acid sequences and the amino acid sequences of the above listed superantigens and other superantigens can be obtained from GenBank.

[00136] In certain embodiments, a chlorotoxin polypeptide conjugate of the present invention may be used in directed enzyme prodrug therapy. In a directed enzyme prodrug therapy approach, a directed/targeted enzyme and a prodrug are administered to a subject, wherein the targeted enzyme is specifically localized to a portion of the subject's body where it converts the prodrug into an active drug. The prodrug can be converted to an active drug in one step (by the targeted enzyme) or in more than one step. For example, the prodrug can be converted to a precursor of an active drug by the targeted enzyme. The precursor can then be converted into the active drug by, for example, the catalytic activity of one or more additional targeted enzymes, one or more non-targeted enzymes administered to the subject, one or more enzymes naturally present in the subject or at the target site in the subject (e.g., a protease, phosphatase, kinase or polymerase), by an agent that is administered to the subject, and/or by a chemical process that is not enzymatically catalyzed (e.g., oxidation, hydrolysis, isomerization, epimerization, etc.).

[00137] Different approaches have been used to direct/target the enzyme to the site of interest. For example, in ADEPT (antibody-directed enzyme prodrug therapy), an antibody designed/developed against a tumor antigen is linked to an enzyme and injected in a subject, resulting in selective binding of the enzyme to the tumor. When the discrimination between tumor and normal tissue enzyme levels is sufficient, a prodrug is administered to the subject. The prodrug is converted to its active form by the enzyme only within the tumor. Selectivity is achieved by the tumor specificity of the antibody and by delaying prodrug administration until there is a large differential between tumor and normal tissue enzyme levels. Early clinical trials are promising and indicate that ADEPT may become an effective treatment for all solid cancers for which tumor-associated or tumor-specific antibodies are known. Tumors have also been targeted with the genes encoding for prodrug activating enzymes. This approach has been called virus-directed enzyme prodrug therapy (VDEPT) or more generally GDEPT (gene-directed enzyme prodrug therapy, and has shown good results in laboratory systems. Other versions of directed enzyme prodrug

therapy include PDEPT (polymer-directed enzyme prodrug therapy), LEAPT (lectin-directed enzyme-activated prodrug therapy), and CDEPT (clostridial-directed enzyme prodrug therapy). A conjugate according to the present invention, which comprises a prodrug activating enzyme associated with a reduced lysine chlorotoxin polypeptide, can be used in a similar way.

[00138] Nonlimiting examples of enzyme/prodrug/active drug combinations suitable for use in the present invention are described, for example, in Bagshawe *et al.*, Current Opinions in Immunology, 1999, 11: 579-583; Wilman, "Prodrugs in Cancer Therapy", Biochemical Society Transactions, 14: 375-382, 615th Meeting, Belfast, 1986; Stella *et al.*, *"Prodrugs: A Chemical Approach To Targeted Drug Delivery"*, in *"Directed Drug Delivery"*, Borchardt *et al.*, (Eds), pp. 247-267 (Humana Press, 1985). Nonlimiting examples of enzyme/prodrug/active anti-cancer drug combinations are described, for example, in Rooseboom *et al.*, Pharmacol. Reviews, 2004, 56: 53-102.

[00139] Examples of prodrug activating enzymes include, but are not limited to, nitroreductase, cytochrome P450, purine-nucleoside phosphorylase, thymidine kinase, alkaline phosphatase, β-glucuronidase, carboxypeptidase, penicillin amidase, β-lactamase, cytosine deaminase, and methionine γ-lyase.

[00140] Examples of anti-cancer drugs that can be formed *in vivo* by activation of a prodrug by a prodrug activating enzyme include, but are not limited to, 5-(aziridin-1-y1)- 4-hydroxyl-amino-2-nitro-benzamide, isophosphoramide mustard, phosphoramide mustard, 2-fluoroadenine, 6-methylpurine, ganciclovir-triphosphate nucleotide, etoposide, mitomycin C, p-[*N,N*-bis(2-chloroethyl)amino]phenol (POM), doxorubicin, oxazolidinone, 9-aminocamptothecin, mustard, methotrexate, benzoic acid mustard, doxorubicin, adriamycin, daunomycin, carminomycin, bleomycins, esperamicins, melphalan, palytoxin, 4-desacetylvinblastine-3-carboxylic acid hydrazide, phenylenediamine mustard, 4'-carboxyphthalato(1,2-cyclohexane-diamine) platinum, taxol, 5-fluorouracil, methylselenol, and carbonothionic difluoride.

[00141] In certain embodiments, a therapeutic (e.g., anti-cancer) agent within a chlorotoxin polypeptide conjugate of the present invention comprises an anti-angiogenic agent. Antiangiogenic agents suitable for use in the present invention include any molecule, compound, or factor that blocks, inhibits, slows down, or reduces the process of

angiogenesis, or the process by which new blood vessels form by developing from preexisting vessels. Such a molecule, compound, or factor can block angiogenesis by blocking, inhibiting, slowing down, or reducing any of the steps involved in angiogenesis, including (but not limited to) steps of (1) dissolution of the membrane of the originating vessel, (2) migration and proliferation of endothelial cells, and (3) formation of new vasculature by migrating cells.

[00142] Examples of anti-angiogenic agents include, but are not limited to, bevacizumab (AVASTIN®), celecoxib (CELEBREX®), endostatin, thalidomide, EMD121974 (Cilengitide), TNP-470, squalamine, combretastatin A4, interferon-α, anti-VEGF antibody, SU5416, SU6668, PTK787/2K 22584, Marimistal, AG3340, COL-3, Neovastat, and BMS-275291.

[00143] Anti-angiogenic agents may be used in a variety of therapeutic contexts, including, but not limited to, anti-cancer therapies and therapies for macular degeneration.

[00144] As will be recognized by one skilled in the art, the specific examples of therapeutic agents cited herein represent only a very small number of the therapeutic agents that are suitable for use in the practice of the present invention.

Detection entities

[00145] Multifunctional agents described herein in many embodiments comprise at least one detection entity, in addition to a targeting entity described above.

[00146] A detection entity may be any entity that allows detection of a chlorotoxin agent after binding to a tissue or localization at a system of interest. Any of a wide variety of detectable agents can be used as detection entity (e.g., labeling moieties) in multifunctional chlorotoxin agents of the present invention. A detection entity may be directly detectable or indirectly detectable. Examples of detection entity include, but are not limited to: various ligands, radionuclides (e.g., ^{3}H, ^{14}C, ^{18}F, ^{19}F, ^{32}P, ^{35}S, ^{135}I, ^{125}I, ^{123}I, ^{64}Cu, ^{187}Re, ^{111}In, ^{90}Y, ^{99m}Tc, ^{177}Lu, etc.), fluorescent dyes (for specific exemplary fluorescent dyes, see below), chemiluminescent agents (such as, for example, acridinum esters, stabilized dioxetanes, and

the like), bioluminescent agents, spectrally resolvable inorganic fluorescent semiconductors nanocrystals (i.e., quantum dots), metal nanoparticles (e.g., gold, silver, copper, platinum, etc.) nanoclusters, paramagnetic metal ions, enzymes (for specific examples of enzymes, see below), colorimetric labels (such as, for example, dyes, colloidal gold, and the like), biotin, dioxigenin, haptens, and proteins for which antisera or monoclonal antibodies are available.

[00147] In certain embodiments, a detection entity comprises a fluorescent label. Numerous known fluorescent labeling moieties of a wide variety of chemical structures and physical characteristics are suitable for use in the practice of methods of diagnosis of the present invention. Suitable fluorescent dyes include, but are not limited to, fluorescein and fluorescein dyes (e.g., fluorescein isothiocyanine or FITC, naphthofluorescein, 4',5'-dichloro-2',7'- dimethoxyfluorescein, β carboxyfluorescein or FAM, etc.), carbocyanine, merocyanine, styryl dyes, oxonol dyes, phycoerythrin, erythrosin, eosin, rhodamine dyes (e.g., carboxytetramethyl- rhodamine or TAMRA, carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), lissamine rhodamine B, rhodamine 6G, rhodamine Green, rhodamine Red, tetramethylrhodamine (TMR), etc.), coumarin and coumarin dyes (e.g. , methoxycoumarin, dialkylaminocoumarin, hydroxycoumarin, aminomethylcoumarin (AMCA), etc.), Oregon Green Dyes (e.g., Oregon Green 488, Oregon Green 500, Oregon Green 514., etc.), Texas Red, Texas Red-X, Spectrum Red™, Spectrum Green™, cyanine dyes (e.g., Cy-3™, Cy-5™, Cy-3.5™, Cy-5.5™ etc.), Alexa Fluor dyes (e.g., Alexa Fluor 350, Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660, Alexa Fluor 680, etc.), BODIPY dyes (e.g., BODIPY FL, BODIPY R6G, BODIPY TMR, BODIPY TR, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665, etc.), IRDyes (e.g., IRD40, IRD 700, IRD 800, etc.), and the like. For more examples of suitable fluorescent dyes and methods for coupling fluorescent dyes to other chemical entities such as proteins and peptides, see, for example, "The Handbook of Fluorescent Probes and Research Products", 9^{111}Ed., Molecular Probes, Inc., Eugene, OR.

[00148] Favorable properties of fluorescent labeling agents include high molar absorption coefficient, high fluorescence quantum yield, and photostability. In certain embodiments, labeling fluorophores desirably exhibit absorption and emission wavelengths in the visible (i.e., between 400 and 750 nm) rather than in the ultraviolet range of the spectrum (i.e., lower than 400 nm).

[00149] In certain embodiments, a detection entity comprises an enzyme. Examples of suitable enzymes include, but are not limited to, those used in an ELISA, e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, etc. Other examples include beta-glucuronidase, beta-D-glucosidase, urease, glucose oxidase, etc. An enzyme may be conjugated to a targeting entity (e.g., chlorotoxin moiety) using a linker group such as a carbodiimide, a diisocyanate, a glutaraldehyde, and the like. More detailed description of suitable linkers is provided elsewhere herein.

[00150] In certain embodiments, a detection entity comprises a radioisotope that is detectable by Single Photon Emission Computed Tomography (SPECT) or Position Emission Tomography (PET). Examples of such radionuclides include, but are not limited to, iodine-131 (^{131}I), iodine-125 (^{125}I), bismuth-212 (^{212}Bi), bismuth-213 (^{213}Bi), astatine-221 (^{211}At), copper-67 (^{67}Cu), copper-64 (^{64}Cu), rhenium-186 (^{186}Re), rhenium-186 (^{188}Re), phosphorus-32 (^{32}P), samarium- 153 (^{153}Sm), lutetium-177 (^{117}Lu), technetium-99m (^{99m}Tc), gallium-67 (^{67}Ga), indium-I 11 (^{111}In), and thallium-201 (^{201}Tl).

[00151] In certain embodiments, a labeling moiety comprises a radioisotope that is detectable by Gamma camera. Examples of such radioisotopes include, but are not limited to, iodine-131 (^{131}I), and technetium-99m (^{99m}Tc).

[00152] In certain embodiments, a detection entity comprises a paramagnetic metal ion that is a good contrast enhancer in Magnetic Resonance Imaging (MRI). Examples of such paramagnetic metal ions include, but are not limited to, gadolinium III (Gd^{3+}), chromium III (Cr^{3+}), dysprosium III (Dy^{3+}), iron III (Fe^{3+}), manganese II (Mn^{2+}), and ytterbium III (Yb^{3+}). In certain embodiments, the detection entity comprises gadolinium III (Gd^{3+}). Gadolinium is an FDA-approved contrast agent for MRI, which accumulates in abnormal tissues causing these abnormal areas to become very bright (enhanced) on the magnetic resonance image. Gadolinium is known to provide great contrast between normal and abnormal tissues in different areas of the body, in particular in the brain.

[00153] In certain embodiments, a labeling moiety comprises a stable paramagnetic isotope detectable by nuclear magnetic resonance spectroscopy (MRS). Examples of suitable stable paramagnetic isotopes include, but are not limited to, carbon- 13 (^{13}C) and fluorine- 19 (^{19}F).

Conjugation

[00154] As stated above, multifunctional agents described herein comprise multiple entities, each having at least one function. As already noted, certain embodiments of contemplated multifunctional agents comprise a targeting entity and at least one of the following entities: a detection entity and a therapeutic entity. In some embodiments, a multifunctional agent of the invention contains a targeting entity and a therapeutic entity; but not a detection entity. In some embodiments, a multifunctional agent of the invention contains a targeting entity; a detection entity; but not a therapeutic entity. In some embodiments, a multifunctional agent of the invention contains a targeting entity; a therapeutic entity; and a detection entity. In any of contemplated embodiments, the entities of an agent are conjugated to one another. Conjugation of various entities to form a multifunctional agent is not limited to particular modes of conjugation. For example, two entities may be covalently conjugated directly to each other. Alternatively, two entities may be indirectly conjugated to each other, such as via a linker entity. In some embodiments, a multifunctional agent may include different types of conjugation within the agent, such that some entities of the agent are conjugated via direct conjugation while other entities of the agent are indirectly conjugated via one or more linkers. In some embodiments, a multifunctional agent of the invention comprises a single type of a linker entity. In other embodiments, a multifunctional agent of the invention comprises more than one types of a linker entities. In some embodiments, a multifunctional agent includes a single type of linker entities but of varying length.

[00155] In many of the embodiments described herein, association between or amongst entities contained in a multifunctional agent is covalent. As will be appreciated by one skilled in the art, the moieties may be attached to each other either directly or indirectly (e.g., through a linker, as described above).

[00156] In certain embodiments, where one entity (such as a targeting entity) and a second entity of a multifunctional agent are directly covalently linked to each other, such direct covalent conjugation can be through a linkage (e.g., a linker or linking entity) such as an amide, ester, carbon-carbon, disulfide, carbamate, ether, thioether, urea, amine, or carbonate linkage. Covalent conjugation can be achieved by taking advantage of functional groups present on the first entity and/or the second entity of the multifunctional agent. Alternatively, a non-critical amino acid may be replaced by another amino acid that will introduce a useful

group (such as amino, carboxy or sulfhydryl) for coupling purposes. Alternatively, an additional amino acid may be added to at least one of the entities of the multifunctional agent to introduce a useful group (such as amino, carboxy or sulfhydryl) for coupling purposes. Suitable functional groups that can be used to attach moieties together include, but are not limited to, amines, anhydrides, hydroxyl groups, carboxy groups, thiols, and the like. An activating agent, such as a carbodiimide, can be used to form a direct linkage. A wide variety of activating agents are known in the art and are suitable for conjugating one entity to a second entity.

[00157] In other embodiments, entities of a multifunctional agent embraced by the present invention are indirectly covalently linked to each other via a linker group. Such a linker group may also be referred to as a linker or a linking entity. This can be accomplished by using any number of stable bifunctional agents well known in the art, including homofunctional and heterofunctional agents (for examples of such agents, see, e.g., Pierce Catalog and Handbook). The use of a bifunctional linker differs from the use of an activating agent in that the former results in a linking moiety being present in the resulting conjugate (agent), whereas the latter results in a direct coupling between the two moieties involved in the reaction. The role of a bifunctional linker may be to allow reaction between two otherwise inert moieties. Alternatively or additionally, the bifunctional linker that becomes part of the reaction product may be selected such that it confers some degree of conformational flexibility to the chlorotoxin agent (e.g., the bifunctional linker comprises a straight alkyl chain containing several atoms, for example, the straight alkyl chain contains between 2 and 10 carbon atoms). Alternatively or additionally, the bifunctional linker may be selected such that the linkage formed between a chlorotoxin agent and therapeutic agent is cleavable, e.g. hydrolysable (for examples of such linkers, see e.g. U.S. Pat. Nos. 5,773,001; 5,739,116 and 5,877,296, each of which is incorporated herein by reference in its entirety). Such linkers, for example, may be used when higher activity of certain entities, such as a targeting agent (e.g., the chlorotoxin polypeptide) and/or of a therapeutic entity is observed after hydrolysis of the conjugate. Exemplary mechanisms by which an entity may be cleaved from a multifunctional agent include hydrolysis in the acidic pH of the lysosomes (hydrazones, acetals, and cis-aconitate-like amides), peptide cleavage by lysosomal enzymes (the capthepsins and other lysosomal enzymes), and reduction of disulfides). Another mechanism by which such an entity is cleaved from the multifunctional agent includes hydrolysis at physiological pH extra-

or intra-cellularly. This mechanism applies when the crosslinker used to couple one entity to another entity is a biodegradable/bioerodible component, such as polydextran and the like.

[00158] For example, hydrazone-containing multifunctional agents can be made with introduced carbonyl groups that provide the desired release properties. Multifunctional agents can also be made with a linker that comprise an alkyl chain with a disulfide group at one end and a hydrazine derivative at the other end. Linkers containing functional groups other than hydrazones also have the potential to be cleaved in the acidic milieu of lysosomes. For example, multifunctional agents can be made from thiol-reactive linkers that contain a group other than a hydrazone that is cleavable intracellularly, such as esters, amides, and acetals/ketals.

[00159] Another example of class of pH sensitive linkers are the cis-aconitates, which have a carboxylic acid group juxtaposed to an amide group. The carboxylic acid accelerates amide hydrolysis in the acidic lysosomes. Linkers that achieve a similar type of hydrolysis rate acceleration with several other types of structures can also be used.

[00160] Another potential release method for chlorotoxin agents is the enzymatic hydrolysis of peptides by the lysosomal enzymes. In one example, a peptidic toxin is attached via an amide bond to para-aminobenzyl alcohol and then a carbamate or carbonate is made between the benzyl alcohol and the therapeutic agent. Cleavage of the peptide leads to collapse of the amino benzyl carbamate or carbonate, and release of the therapeutic agent. In another example, a phenol can be cleaved by collapse of the linker instead of the carbamate. In another variation, disulfide reduction is used to initiate the collapse of a para-mercaptobenzyl carbamate or carbonate.

[00161] Useful linkers which may be used as a linking entity of a multifunctional agent provided herein include, without limitation: polyethylene glycol, a copolymer of ethylene glycol, a polypropylene glycol, a copolymer of propylene glycol, a carboxymethylcellulose, a polyvinyl pyrrolidone, a poly-1,3-dioxolane, a poly-1,3,6-trioxane, an ethylene/maleic anhydride copolymer, a polyaminoacid, a dextran n-vinyl pyrrolidone, a poly n-vinyl pyrrolidone, a propylene glycol homopolymer, a propylene oxide polymer, an ethylene oxide polymer, a polyoxyethylated polyol, a polyvinyl alcohol, a linear or branched glycosylated chain, a polyacetal, a long chain fatty acid, a long chain hydrophobic aliphatic group.

[00162] Embraced also herein are multifunctional agents that include at least one entity which involves non-covalent association. Examples of non-covalent interactions include, but are not limited to, hydrophobic interactions, electrostatic interactions, dipole interactions, van der Waals interactions, and hydrogen bonding. Irrespective of the nature of the binding, interaction, or coupling, the association between a first entity and a second entity is, in some embodiments, selective, specific and strong enough so that the second entity contained in the agent does not dissociate from the first entity before or during transport/delivery to and into the target. Thus, Association amongst multiple entities of a multifunctional agent may be achieved using any chemical, biochemical, enzymatic, or genetic coupling known to one skilled in the art.

Synthesis of multifunctional agents

[00163] While various methodologies and processes for the chemical synthesis of multifunctional agents of the present invention are known in the art, the nature of multi-component conjugates poses a particular challenge in synthesizing and/or isolating an essentially homogeneous product. The ability to produce a substantially single conjugate species with reasonable efficiency utilizing chemistry that will not disrupt relevant activities of conjugate entities can present particular difficulties in the context of clinical and pharmaceutical applications. A number of approaches typically taken in the chemical synthesis of multi-component molecules (such as labeled antibody and targeted drug) ultimately yield a heterogeneous mixture of reaction products due in part to the fact that certain steps in the synthesis involve at least a degree of random conjugation reactions which result in mixed product species. As a result, it would be necessary to carry out a subsequent purification step in order to isolate a single species from the reaction products. This is particularly true for almost any therapeutic agents.

[00164] Among other things, the present inevntion provides improved methods for the chemical synthesis of multifunctional agents. Accordingly, the invention also includes such methods, as provided in more detail below.

[00165] In one aspect, the methods are drawn to the synthesis of multifunctional agents by directed conjugation. As used herein, "directed conjugation" refers to a chemical synthesis

process aimed to reduce random or non-specific conjugation and typically involves the formation of at least one intermediate species. The invention encompasses the recognition that a series of conjugation reaction schemes can be utilized to control specific conjugation products. In its most useful form, the directed conjugation approach will allow synthesis of a single species of product and intermediate thereof. For example, a coupling reaction may produce a single conjugate species comprising multiple entities attached at the same chemical constituents such that the resulting product represents a homogeneous mixture.

[00166] Accordingly, in some embodiments, described methods involve sequential conjugation reactions that allow non-random (e.g., directed) conjugation of entities to form a multifunctional conjugate. As mentioned, directed conjugation synthesis is carried out in at least two phases. In a first phase of directed conjugation-based synthesis, in some embodiments, a therapeutic entity (such as a cytotoxic moiety) is first coupled to a linking moiety in one or more steps to form an intermadiate. In some embodiments, additional reaction step(s) may be necessary to form a suitable intermediate. Additional reaction steps required for producing an intermediate will depend on the nature of the entities/molecules being conjugated. In some embodiments, additional reaction steps for the formation of an intermediate include carborate formation reaction, acylation reaction and silylation reaction. In the second phase of directed conjugation-based synthesis, the intermediate formed from the first phase is first hydrolized then subjected to reductive amination, whereby the intermediate species becomes conjugated to a peptide targeting entity to form a multifunctional conjugate. The resulting product is chracterized by having its N-terminus protected by an attached linker. As such, the N-terminus of the polypeptide is no longer available for further conjugation in a subsequent conjugation step in which additional entity is attached. In this way, in the subsequent conjugation step, for example, a detection entity (such as a label) may be readily attached at a suitable site on the molecule. In some embodiments, a lysine residue may be used to conjugate the additional entity. Such a lysine residue available for conjugation of an entity may or may not be present in a naturally ocurring polypeptide used as a targeting entity. Various embodiments of chlorotoxin polypeptides having reduced lysine residues are provided in more detail elsewhere herein.

[00167] In some embodiments, a targeting entity is a chlorotoxin polypeptide. A variety of useful therapeutic entities that may be conjugated to a chlorotoxin polypeptide are described herein.

[00168] To illustrate directed conjugation in the context of the present disclosure, chlorotoxin (SEQ ID NO: 1) and commercial paclitaxel are used to generate a multifunctional conjugate, in which the drug is conjugated to chlorotoxin via a linker. In this non-limiting example of a multifunctional agent, a chlorotoxin polypeptide (CTX) is a targeting moiety, and paclitaxel, which is a small chemical-based entity (SCE), is a therapeutic moiety. Details of the chemical synthesis are provided in the Examples below. Paclitaxel and the NHS cross linker can be conjugated following the vendor's (Pierce) recommendation to generate a water soluble paclitaxel-NHS intermediate. The intermediate may then be incubated at a molar excess in the presence of chlorotoxin and in dimethylformamide at pH 8.5 for ~4 hours to generate a chlorotoxin-paclitaxel conjugate, **P-L-T**, where ***P*** represents polypeptide (in this case chlorotoxin); ***L*** represents a linker; and ***T*** represents a therapeutic entity (in this case Paclitaxel). This reagent may be then purified as a single peak using HPLC and prepared for labeling using a suitable dye, e.g., NHS-IRDye 800CW (LiCor) dye, as the **D** component. In addition, a multifunctional conjugate, **P-L-T-D**, where ***P*** represents polypeptide (in this case chlorotoxin); ***L*** represents a linker; ***T*** represents a therapeutic entity (in this case Paclitaxel) and ***D*** represents a detection entity (in this case a dye), may then be generated by diluting the HPLC-purified intermediate (**P-L-T**) above 0.25 mg/ml in 1X phosphate-buffered saline (pH 7.4). To this, the ***L*** component, such as NHS-IRDye 800CW, may be added (e.g., from a 100% DMSO stock solution) to a final molar ratio of about 1:1.2 **P-L-T** to **L** ratio and mixed thoroughly. The conjugation may then allowed to proceed for a duration of time, e.g., 4 hours at suitable temperature, e.g., room temperature.

[00169] Conjugation efficiency may be monitored by any suitable means, such as RP-HPLC (214 nm and 750 nm), LC-MS, and SDS-PAGE. For each **P-L-T**, an average of one dye may be added per molecule, with no evidence of remaining free dye and little or no doubly-labeled peptide. Following conjugation, **P-L-T-D** is diluted to appropriate concentration for injection in a suitable buffer, such as 1X PBS without further modifications. The art is familiar with parameters of relevant reaction conditions exemplified above. The skilled artisan may readily optimize certain reaction conditions depending on the particular molecules being conjugated and/or other factors involved in the particular case.

[00170] The final agent may be then used for various methods contemplated herein. In some embodiments, such agents are used for diagnosis in detecting human xenograft tumors in sutiable animal models (e.g., nude mice) and the **P-L-T** agent may be used to test for

pharmacologic activity. A schematic diagram illustrating the **P-L-T-D** and **P-L-T** is provided in **Figure 1**.

[00171] One exemplary embodiment of such synthesis scheme is provided in Example 1. As shown, an initial phase of the synthesis results in the formation of an intermediate. The intermediate may then be conjugated to a peptide targeting entity by amination. This may be carried out by NHS acylation chemistry. Thus, in some embodiments, the conjugation chemistry is based on NHS (N-hydroxysuccinimide)/EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) chemistry. The resulting amide intermediate can then be used to conjugate to the N-terminus of chlorotoxin. Unlike the standard reductive amination, which results in multiple (e.g., heterogeneous) reaction products by random conjugation to various available sites on chlorotoxin, the approach taken here provides protection of the N-terminus of the polypeptide, rendering it unavailable for subsequent conjugation reaction(s). As a result, it is possible to direct certain degree of conjugation to optimize synthesis of substantially single species product.

[00172] A number of suitable linkers are available in the art. Many therapeutic moieties, in particular anti-cancer agents, have limited solubility in water, which limits drug loading on a conjugate due to aggregation of the therapeutic moiety. One approach to overcoming this is to add solubilizing groups to the linker conjugates made with a linker consisting of PEG (polyethylene glycol) and a dipeptide can be used, including, for example, those having a PEG di-acid thiol-acid, or maleimide-acid attached to the reduced lysine chlorotoxin polypeptide conjugate, a dipeptide spacer, and an amide bound to the entity or moiety. Approaches that incorporate PEG groups may be beneficial in overcoming aggregation and limits in drug loading. Accordingly, in some embodiments of the present invention, suitable linkers for linking a therapeutic entity to a targeting entity contain an aldehyde, e.g., PEGylated, alkyl aldehyde, etc. Without wishing to be bound by a particular theory, it is believed that use of such linker can enhance solubility of the reaction intermediate. As exemplified herein, the method involving the formation of a derivative (e.g., intermediate) to protect the reactive N-terminus of the entity represent novel tactics for synthesizing multifunctional agents.

[00173] In certain embodiments, an anti-cancer agent within an inventive conjugate is a poorly water soluble compound. As will be recognized by one skilled in the art, a wide

variety of poorly water soluble anti-cancer agents are suitable for use in the present invention.

[00174] For example, an anti-cancer agent may be selected among taxanes, which are recognized as effective agents in the treatment of many solid tumors that are refractory to other anti-neoplastic agents. Two currently approved taxanes are paclitaxel (TAXOL™) and docetaxel (TAXOTERE™). Paclitaxel, docetaxel, and other taxanes act by enhancing the polymerization of tubulin, an essential protein in the formation of spindle microtubules. Polymerization of tubulin results in the formation of very stable, nonfunctional tubules, which inhibits cell replication and leads to cell death.

[00175] Paclitaxel is very poorly water soluble, and therefore, cannot be practically formulated with water for intravenous administration. Some formulations of TAXOL™ for injection or intravenous infusion have been developed using CREMOPHOR EL™ (polyoxyethylated castor oil) as a drug carrier. However, CREMOPHOR™ EL is itself toxic, and is considered to be, at least in part, responsible for the hypersensitivity reactions (severe skin rashes, hives, flushing, dyspnea, tacchycardia and others) associated with administration of such preparations. To avoid such side effects, pre-medication is often prescribed along with paclitaxel formulations containing CREMOPHOR™. Docetaxel, which is an analog of paclitaxel, is like paclitaxel poorly soluble in water. The currently most preferred solvent used to dissolve docetaxel for pharmaceutical use is polysorbate 80 (TWEEN 80). In addition to causing hypersensitivity reactions in patients, TWEEN 80 cannot be used with PVC delivery apparatus, because of its tendency to leach diethylhexyl phthalate, which is highly toxic.

[00176] A conjugate according to the present invention comprising a taxane and chlorotoxin polyeptide can be used as an improved delivery method to avoid the use of solvents and carriers that induce adverse reactions in patients.

[00177] In some embodiments, an anti-cancer agent within a chlorotoxin polypeptide conjugate may belong to the enediyne family of antibiotics. As a family, the enediyne antibiotics are particularly potent anti-tumor agents. Some members are 1000 times more potent than adriamycin, one of the most effective, clinically used anti-tumor antibiotics (Y.S. Zhen *et al.*, J. Antibiot., 1989, 42: 1294-1298). For example, an anti-cancer agent within an inventive conjugate may be a member of the enediyne family of

calicheamicins. Originally isolated from a broth extract of the soil microorganism Micromonospora echinospora ssp. calichensis, the calicheamicins were detected in a screen for potent DNA damaging agents (M.D. Lee *et al.,* J. Am. Chem. Soc., 1987, 109: 3464-3466; M.D. Lee *et al.,* J. Am. Chem. Soc., 1987, 109: 3466-3468; W.M. Maiese *et al.,* J. Antibiot., 1989, 42: 558-563; M.D. Lee *et al.,* J. Antibiot., 1989, 42: 1070-1087).

[00178] Calicheamicins are characterized by a complex, rigid bicyclic enediyne allylic trisulfide core structure linked through glycosyl bonds to an oligosaccharide chain. The oligosaccharide portion contains a number of substituted sugar derivatives, and a substituted tetrahydropyran ring. The enediyne containing core (or aglycone) and carbohydrate portions of calicheamicins have been reported to carry out different roles in the biological activity of these molecules. It is generally believed that the core portion cleaves DNA, whereas the oligosaccharide portion of the calicheamicins serves as a recognition and delivery system and guides the drug to a double-stranded DNA minor groove in which the drug anchors itself ("Enediyne Antibiotics as Antitumor Agents", Doyle and Borders, 1995, Marcel-Dekker: New York;). Double-stranded DNA cleavage is a type of damage that is usually non-repairable or non-easily repairable for the cell and is most often lethal.

[00179] Because of their chemical and biological properties, several analogues of the calicheamicins have been tested in preclinical models as potential anti-tumor agents. Their development as single agent therapies has not been pursued because of delayed toxicities that limit the therapeutic dose range for treatment. However, their potency makes them particularly useful for targeted chemotherapy.

[00180] Other examples of suitable poorly water soluble anti-cancer agents include tamoxifen and BCNU. Tamoxifen has been used with varying degrees of success to treat a variety of estrogen receptor positive carcinomas such as breast cancer, endometrial carcinoma, prostate carcinoma, ovarian carcinoma, renal carcinoma, melanoma, colorectal tumors, desmoid tumors, pancreatic carcinoma, and pituitary tumors. In addition to being limited by poor water solubility, chemotherapy using tamoxifen can cause side effects such as cellular drug resistance. BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) is well known for its anti-tumor properties and, since 1972, it has been charted by the National Cancer Institute for use against brain tumors, colon cancer,

Hodgkin's Disease, lung cancer and multiple myeloma. However, the efficient use of this anti-cancer drug is also compromised by its low solubility.

[00181] According to the invention, optionally, the next phase of the synthesis of a multifunctional agent may include attaching a detection entity to the agent in certain utilities. This may be accomplished by utilizing one or more lysine residues present on a targeting entity. Chlorotoxin in its native form contains three lysine residues, which are at amino acid residues 15, 23 and 27. These lysine residues provide convenient means of conjugation, such as for a detection moiety (e.g., label). In some embodiments, the one or more entities or moieties is/are associated with reduced lysine chlorotoxin polypeptides via a lysine residue and/or via a terminus of the reduced lysine chlorotoxin polypeptide. In some such embodiments, the position(s) where entities or moieties can be attached to a reduced lysine chlorotoxin polypeptide is limited by the number of lysine residues available as a site for conjugation. For example, entities or moieties can be attached at the single available lysine residue in monolysine chlorotoxin polypeptides.

[00182] In certain embodiments, entities or moieties are associated at the N-terminus of or at the C-terminus of the reduced lysine chlorotoxin polypeptide. In some such embodiments, the reduced lysine chlorotoxin polypeptide does not have any lysine residues available for conjugation at any of the "native" positions within chlorotoxin (e.g., positions corresponding to positions 15, 23 and 27).

[00183] Table 2 below presents some non-limiting examples of modification schemes that could be used to produce reduced lysine chlorotoxin polypeptides.

Table 2: Exemplary modification schemes.

SEQ ID NO:	Core sequence (N-terminus to C-terminus)	Position(s) of lysine residue(s)
1	MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC R 5 10 15 20 25 30 35	15, 23 and 27
		15 and 23
		15 and 27
		23 and 27
11	MCMPC FTTDH QMARC DDCCG GKGRG KCYGP QCLCR 5 10 15 20 25 30 35	22 and 26
		22
		26

12	MCMPC FTTDH QMARK CDDCC GGGRG KCYGP QCLCR 5 10 15 20 25 30 35	15 and 26 15 26
13	MCMPC FTTDH QMARK CDDCC GGKGR GCYGP QCLCR 5 10 15 20 25 30 35	15 and 23 15 23
75 (lysine added to N-term)	KMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR 5 10 15 20 25 30 35	16, 24 and 28
76 (lysine added to C-term)	MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC RK 5 10 15 20 25 30 35	15, 23 and 27

[00184] As can readily be appreciated by those skilled in the art, a conjugate of the present invention can comprise any number of chlorotoxin polypeptides and any number of entities or moieties, associated to one another by any number of different ways. The design of a conjugate will be influenced by its intended purpose(s) and the properties that are desirable in the particular context of its use. In some embodiments, multifunctional agents are constructed in the following configurations, where: ***P*** ("peptide") denotes a targeting entity; ***T*** denotes a therapeutic entity; ***D*** denotes a detection entity; and ***L*** denotes a linking entity.

[00185] T-L-P-D

[00186] T-L-P-L-D

[00187] T-P-D

[00188] D-P-T

[00189] T-P

[00190] D-L-P

[00191] P-D

[00192] P-L-D

[00193] However, one of ordinary skill in the art will appreciate that a number of other configurations are possible in accordance with the present disclosure.

Pharmaceutical compositions

[00194] Multifunctional agents provided herein can be formulated into suitable pharmaceutical compositions. A pharmaceutical composition will generally comprise an effective amount of at least one targeting entity (e.g., chlorotoxin polypeptides) conjugated to at least one therapeutic entity and at least one pharmaceutically acceptable carrier or excipient. In some embodiments, such pharmaceutical composition may further comprise at least one detection entity. In any of these embodiments, such pharmaceutical composition may additionally comprise one or more linking entities that link two or more of the entities contained in the composition.

[00195] Useful embodiments include a pharmaceutical composition comprising a chlorotoxin polypeptide as a targeting entity, directly or indirectly conjugated to a therapeutic agent, such as a cytotoxic agent, formulated in a pharmaceutically acceptable carrier. In some embodiments, a therapeutic agent conjugated to a chlorotoxin polypeptide is a small molecule. For example, such a therapeutic agent may be conjugated to the chlorotoxin polypeptide via a linker. In some embodiments, such conjugation occurs at the N-terminus of the chlorotoxin polypeptide, or at one of the lysine residues present in the chlorotoxin polypeptide. Exemplary embodiments are provided in the Examples below.

[00196] As already noted, some embodiments of pharmaceutical compositions also include a detection entity/moiety in addition to targeting and therapeutic entities. While it is not limiting, in many embodiments, a detection entity may be conjugated to a site on a targeting moiety. For example, where a pharmaceutical composition comprises a chlorotoxin polypeptide as a targeting entity of the multifunctional agent, a detection entity may be conjugated directly or indirectly to the chlorotoxin polypeptide. In some embodiments, for example, at least one lysine residues present on chlorotoxin polypeptides can be utilized for attaching a detection moiety to form the multifunctional agent. It is also possible to attach a detection moiety to other components of the agent, including to a linker. Thus, according to the present invention, a multifunctional agent may constitute both a therapeutic multifunctional agent and a detection multifunctional agent.

[00197] Pharmaceutical compositions may be formulated using conventional methods well- known in the art. The optimal pharmaceutical formulation can be varied depending upon the route and/or mode of administration and desired dosage. Such formulations may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the administered compounds. Formulation may produce solid, liquid or semi-liquid pharmaceutical compositions.

[00198] Pharmaceutical compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "unit dosage form", as used herein, refers to a physically discrete unit of a pharmaceutical agent for the patient to be treated. Each unit contains a predetermined quantity of active material calculated to produce the desired therapeutic and/or diagnostic effect. It will be understood, however, that the total dosage of the composition will be decided by the attending physician within the scope of sound medical judgment.

[00199] As mentioned above, in certain embodiments, the multifunctional agent is administered intravenously through injection or infusion. Pharmaceutical compositions suitable for administration by injection or infusion may be formulated according to the known art using suitable dispersing or wetting agents, and suspending agents. The pharmaceutical composition may also be a sterile injectable solution, suspension or emulsion in a non-toxic diluent or solvent, for example, as a solution in 2,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S. P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solution or suspension medium. For this purpose, any bland fixed oil can be used including synthetic mono- or di-glycerides. Fatty acids such as oleic acid may also be used in the preparation of injectable formulations.

[00200] Multifunctional agents, including chlorotoxin polypeptide conjugates described herein, may be administered *per se* and/or in the form of a pharmaceutical composition. In some embodiments, provided are pharmaceutical compositions comprising an effective amount of at least one chlorotoxin polypeptide conjugate and at least one pharmaceutically acceptable carrier.

[00201] A multifunctional agent, such as a chlorotoxin polypeptide conjugate, or a pharmaceutical composition thereof, may be administered according to the present invention in

such amounts and for such a time as is necessary or sufficient to achieve at least one desired result. For example, an inventive pharmaceutical composition can be administered in such amounts and for such a time that it kills cancer cells, reduces tumor size, inhibits tumor growth or metastasis, treats various leukemias, and/or prolongs the survival time of mammals (including humans) with those diseases, or otherwise yields clinical benefit.

[00202] Pharmaceutical compositions of the present invention may be administered using any amount and any route of administration effective for achieving the desired therapeutic effect.

[00203] The exact amount of pharmaceutical composition to be administered will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition, and the like (see below).

[00204] The optimal pharmaceutical formulation can be varied depending upon the route of administration and desired dosage. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered compounds.

[00205] After formulation with one or more appropriate physiologically acceptable carrier(s) or excipient(s) in a desired dosage, pharmaceutical compositions of the present invention can be administered to humans or other mammals by any suitable route. Various delivery systems are known and can be used to administer such compositions, including, tablets, capsules, injectable solutions, etc. Methods of administration include, but are not limited to, dermal, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, pulmonary, epidural, ocular, and oral routes. A composition may be administered by any convenient or otherwise appropriate route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral, mucosa, rectal and intestinal mucosa, etc) and may be administered together with other biologically active agents. Administration can be systemic and/or local.

[00206] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents, and suspending agents. A sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a non-toxic parenterally acceptable

diluent or solvent, for example, as a solution in 2,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solution or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid may also be used in the preparation of injectable formulations. Sterile liquid carriers are useful in sterile liquid from compositions for parenteral administration.

[00207] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use. Liquid pharmaceutical compositions which are sterile solutions or suspensions can be administered by, for example, intravenous, intramuscular, intraperitoneal or subcutaneous injection. Injection may be via single push or by gradual infusion (e.g., 30 minute intravenous infusion). Where necessary, the composition may include a local anesthetic to ease pain at the site of injection.

[00208] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming micro-encapsuled matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations can also be prepared by entrapping the drug in liposomes (also known as lipid vesicles) or microemulsions that are compatible with body tissues.

[00209] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, elixirs, and pressurized compositions. In addition to the active ingredient (i.e.,

conjugate), the liquid dosage form may contain inert diluents commonly used in the art such as, for example, water or other solvent, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cotton seed, ground nut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, suspending agents, preservatives, sweetening, flavoring, and perfuming agents, thickening agents, colors, viscosity regulators, stabilizers or osmoregulators. Suitable examples of liquid carriers for oral administration include water (partially containing additives as above; e.g., cellulose derivatives, such as sodium caboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols such as glycols) and their derivatives, and oils (e.g., fractionated coconut oil and arachis oil)).

[00210] Solid dosage forms for oral administration include, for example, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active ingredient is mixed with at least one inert, physiologically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and one or more of: (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; (c) humectants such as glycerol; (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (e) solution retarding agents such as paraffin; (f) absorption accelerators such as quaternary ammonium compounds; (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate; (h) absorbents such as kaolin and bentonite clay; and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. Additional or alternative excipients suitable for solid formulations include surface modifying agents such as non-ionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents. The

amount of solid carrier per solid dosage form will vary widely. In some embodiments, the amount of solid carrier per solid dosage form is from about 25 mg to about 1 g.

[00211] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition such that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

[00212] In certain embodiments, it may be desirable to administer an inventive composition locally to an area in need of treatment. This may be achieved, for example, by local infusion during surgery, topically application, by injection, by means of a catheter, by means of suppository, or by means of a skin patch or stent or other implant, among other ways.

[00213] Some compositions for topical adminstration may be formulated as a gel, an ointment, a lotion, or a cream which can include carriers such as water, glycerol, alcohol, propylene glycol, fatty alcohols, triglycerides, fatty acid esters, or mineral oil. Other topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylenemonolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as antioxidants, humectants, viscosity stabilizers, and similar agents may be added as necessary. Percutaneous penetration enhancers such as Azone may also be included.

[00214] In addition, in certain instances, compositions may be disposed within transdermal devices placed upon, in, or under the skin. Such devices include patches, implants, and injections which release the compound onto the skin, by either passive or active release mechanisms. Transdermal administrations include all administrations across the surface of the body and the inner linings of bodily passage including epithelial and mucosal tissues.

Such administrations may be carried out using the present compositions in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

[00215] Transdermal administration may be accomplished, for example, through use of a transdermal patch containing active ingredient(s) and a carrier that is non-toxic to the skin, and allows the delivery of at least some of the active ingredient(s) for systemic absorption into the bloodstream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. Creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing active ingredient(s) may also be suitable. A variety of occlusive devices may be used to release active ingredient(s) into the bloodstream such as a semipermeable membrane covering a reservoir containing the active ingredient(s) with or without a carrier, or a matrix containing the active ingredient.

[00216] Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

[00217] Materials and methods for producing various formulations are known in the art and may be adapted for practicing the subject invention.

[00218] In some embodiments, compositions provided by the present invention include one or more encapsulating agents. In general, an encapsulating agent can be any physiologically tolerable agent that can be used to entrap an entity such as a conjugate or a moiety. By *"entrapped"* it is meant that the encapsulating agent may encircle or enclose the entity, or an "entrapped" entity may be embedded partially or wholly within the material comprising the encapsulating agent.

[00219] In some embodiments, the encapsulating agent is part of the moiety (such as therapeutic moiety), and the targeting entity of the inventive composition is conjugated to the encapsulating agent. In some such embodiments, the targeting entity, such as chlorotoxin polypeptide is conjugated to the outer surface of the encapsulating agent. In some such embodiments, the targeting entity is exposed on the environment external to the

encapsulating agent. The targeting entity may be conjugated to the encapsulating agent by a direct interaction (which may be non-covalent or covalent), or it may be conjugated to the encapsulating agent via a linker.

[00220] In some embodiments, the multifunctional agent (e.g., conjugate comprising the reduced lysine chlorotoxin polypeptide and the moiety) is enclosed by the encapsulating agent. The conjugate may be enclosed partially or wholly within a space or environment (for example, an aqueous environment) defined and/or created by the encapsulating agent. In some embodiments, the conjugate is at least partially embedded within the encapsulating agent. For example, if the encapsulating agent comprises lipid membranes, the conjugate may be at least partially embedded within or among lipid molecules in the membrane. In some embodiments, the conjugate is wholly embedded within the encapsulating agent.

[00221] A variety of types of encapsulating agents are known in the art, as are methods of using such agents to entrap drugs, biomolecules, and the like. In certain embodiments, the encapsulating agent comprises a small particle having a core and a surface. Such encapsulating agents include, but are not limited to, liposomes, micelles, microparticles, nanoparticles, etc.

[00222] Liposomes are typically approximately spherically shaped bilayer structures or vesicles and comprised of natural or synthetic phospholipid membranes. Liposomes may further comprise other membrane components such as cholesterol and protein. The interior core of liposomes typically contain an aqueous solution. Therapeutic agents and/or conjugates described herein may be dissolved in the aqueous solution. As previously mentioned, therapeutic agents and conjugates may be embedded within the membrane of the liposome. Liposomes may be especially useful for delivering agents such as nucleic acid agents (such as those described above), including inhibitory RNAs such as siRNAs.

[00223] Micelles are similar to liposomes, except they generally form from a single layer of phospholipids and lack an internal aqueous solution. Reverse micelles that are made to include internal aqueous solution may also be used in accordance with the present invention.

[00224] In some embodiments, the particle is a microparticle, at least one dimension of which averages to be smaller than about 1 μm. For example, the smallest dimension of the particles can average about 100 nm, about 120 nm, about 140 nm, about 160 nm, about 180 nm, about 200 nm, about 220 nm, about 240 nm, about 260 nm, about 280 nm, about 300 nm, about 320 nm, about 340 nm, about 360 nm, about 380 nm, about 400 nm, about 420 nm, about 440 nm, about 460 nm, about 480 nm, about 500 nm, about 550 nm, about 600 nm, about 650 nm, about 700 nm, about 750 nm, about 800 nm, about 850 nm, about 900 nm, or about 950 nm.

[00225] In some embodiments, the particle is a nanoparticle, at least one dimension of which averages to be smaller than about 100 μm. For example, the smallest dimension of the particles can average about 1 nm, about 2 nm, about 3 nm, about 4 nm, about 5 nm, about 6 nm, about 7 nm, about 8 nm, about 9 nm, about 10 nm, about 11 nm, about 12 nm, about 13nm, about 14 nm, about 15 nm, about 16 nm, about 17 nm, about 18 nm, about 19 nm, about 20 nm, about 22 nm, about 24 nm, about 26 nm, about 28 nm, about 30 nm, about 32 nm, about 34 nm, about 36 nm, about 38 nm, about 40 nm, about 42 nm, about 44 nm, about 46 nm, about 48 nm, about 50 nm, about 55 nm, about 60 nm, about 65 nm, about 70 nm, about 75 nm, about 80 nm, about 85 nm, about 90 nm, about 95 nm, or about 99 nm.

[00226] In some embodiments, the core of the particle comprises a material having magnetic resonance activity, which may advantageous in diagnostic and/or therapeutic applications. Materials having magnetic resonance activity include metals and their oxides, such as aluminum- cobalt-, indium-, iron-, copper-, germanium-, manganese-, nickel-, tin-, titanium-, palladium-, platinum-, selenium-, silicon-, silver-, zinc-, etc. containing metals.

[00227] In some embodiments, therapeutic agents provided herein comprise nucleic acids. Nucleic acids may be enclosed wholly within the encapsulating agent. In some embodiments, nucleic acid agents are embedded within the encapsulating agent. For example, the encapsulating agent may be a liposome and the nucleic agent may be enclosed within the liposome. The nucleic acid agent may be at least partially embedded within the lipid molecules of the liposome.

Kits

[00228] In another aspect, the present invention provides a pharmaceutical pack or kit comprising one or more containers (e.g., vials, ampoules, test tubes, flasks or bottles) containing one or more ingredients of a pharmaceutical composition as described herein, allowing administration of a chlorotoxin polypeptide conjugate of the present invention.

[00229] In some embodiments, kits provide multifunctional detection agents described herein. In some embodiments, kits may include therapeutic multifunctional agents of the invention, as well as corresponding multifunctional detection agents as a set.

Methods of using multifunctional agents (e.g., chlorotoxin polypeptide conjugates)

[00230] In certain embodiments, provided are methods comprising the step of administering a composition comprising a multifunctional agents as described herein to an individual/subject having or suspected of having a tumor, such that the agent binds specifically to the tumor. In some embodiments, such methods are useful in treatment and/or diagnosis of cancer. In some embodiments, such methods are useful in reducing the likelihood that the individual/subject will develop a tumor, that one or more tumors in the individual will increase in size, that one or more tumors in the individual will metastasize, and/or that the cancer will progress by any other measure (such as clinical stage).

[00231] In some embodiments, a subject is susceptible to or at risk of developing a disease or disorder. In some embodiments, a subject has a disease or disorder, such as that associated with abnormal cell proliferation. In some embodiments, a subject is suspected of having a disease or disorder, such as that associated with abnormal cell proliferation. In some embodiments, a subject has been diagnosed with a disease or disorder, such as that associated with abnormal cell proliferation. In some embodiments, a subject has received and/or is receiving one or more therapies for a disease or disorder associated with abnormal cell proliferation. In some embodiments, a subject has received then stopped receiving one or more therapies. In some embodiments, a subject is in remission. In many embodiments, a subject has a form of cancer. In some embodiments, a subject has a tumor, such as solid tumor. In some embodiments, a tumor has metastasized.

[00232] In certain embodiments, provided are methods comprising the step of administering a composition comprising a multifunctional agent as described herein to an individual having or suspected of having a disease or condition characterized by aberrant angiogenesis, such that multifunctional agent reduces extent of angiogenesis. In some embodiments, the multifunctional agent prevents the formation of neovasculature. In some embodiments, the multifunctional agent causes existing neovasculature to regress.

Dosages and administration

[00233] Compositions according to the present invention may be administered according to a regimen consisting of a single dose or a plurality of doses over a period of time. As an example, a subject with a tumor may receive a first (e.g., exploratory) round of a multifunctional agent, such as a chlorotoxin polypeptide conjugate, comprising a detection entity and a therapeutic entity. The therapeutic entity represents a drug which may be effective for the tumor. Such an exploratory step provides a preliminary opportunity to determine whether the subject is a candidate for the particular treatment or therapy, without commiting the subject to a full treatment regimen, which may cause unwanted side effects. Use of such multifunctional agents which comprise a targeting entity such as chlorotoxin, a detection entity such as a label useful for imaging, and a therapeutic entity such as a drug to be tried on a preliminary basis, allows simultaneous confirmation of localizing the tumor, and evaluation of effects of the conjugated therapeutic on the particular subject. Moreover, depending on the relative sensitivity of an attached detection moiety, effects of a therapy or particular treatment regimen may be monitored visually (e.g., by imaging means) over a period of time. In some embodiments, a second or subsequent round of administration may involve a multifunctional agent comprising the same targeting entity and the therapeutic entity but without a detection entity. In some embodiments, after a certain period of time or following a treatment regimen, a subject may receive a "follow-up" round of the multifunctional agent comprising the same targeting entity, detection entity, and the therapeutic entity, in order to confirm that the treatment has been effective.

[00234] Described multifunctional agents, such as chlorotoxin conjugates, or pharmaceutical compositions thereof, may be administered using any administration route effective for achieving the desired effect (e.g., therapeutic, diagnostic, etc.). In certain

embodiments of the invention, the inventive agents are delivered systemically. Typical systemic routes of administration include, but are not limited to, intramuscular, intravenous, pulmonary, and oral routes. Systemic administration may also be performed, for example, by infusion or bolus injection, or by absorption through epithelial or mucocutaneous linings (e.g., oral, mucosa, rectal and intestinal mucosa, etc). In certain embodiments, the agent is administered intravenously.

[00235] Alternatively or additionally, other routes of administration may also be used. In certain embodiments, the inventive agent is administered by a route selected from the group consisting of intravenous, intracranial (including intracavitary), intramuscular, intratumoral, subcutaneous, intraocular, periocular, topical application, or by combinations thereof.

[00236] As discussed below, it may be desirable to reduce extent of angiogenesis in ocular neovascularization diseases. In some embodiments, a multifunctional agent of the invention is a chlorotoxin polypeptide conjugate, which may be delivered to the eye. Delivery to the eye may be achieved, for example, using intraocular and/or periocular routes such as intravitreal injection, subjunctival injection, etc. Topical application of chlorotoxin agents to the eye may also be achieved, for example, using eye drops.

[00237] Ocular routes of adminstration may be particularly useful for treatment of ocular neovascularization diseases such as macular degeneration.

[00238] Administration may be one or multiple times daily, weekly (or at some other multiple day interval) or on an intermittent schedule. For example, a composition may be administered one or more times per day on a weekly basis for a period of weeks (e.g., 4- 10 weeks). Alternatively, a composition may be administered daily for a period of days (e.g., 1-10 days) following by a period of days (e.g., 1-30 days) without administration, with that cycle repeated a given number of times (e.g., 2-10 cycles). In some embodiments, at least two, at least three, at least four, at least five, or at least six doses are administered. In some embodiments, the composition is administered weekly for at least two weeks, three weeks, four weeks, five weeks, or six weeks.

[00239] Administration may be carried out in any convenient manner, or in any combination of manners, such as by injection (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like), oral administration, and/or intracavitary admininstration.

[00240] Depending on the route of administration, effective doses may be calculated according to the organ function, body weight, or body surface area of the subject to be treated. Optimization of the appropriate dosages can readily be made by one skilled in the art in light of pharmacokinetic data observed in human clinical trials. Final dosage regimen may be determined by the attending physician, considering various factors that modify the action of the drugs, e.g., the drug's specific activity, the severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any present infection, time of administration, the use (or not) of concomitant therapies, and other clinical factors.

[00241] Typical dosages range from about 1.0 pg/kg body weight to about 100 mg/kg body weight. (Dosages are presented herein in terms of the weight of the reduced lysine chlorotoxin polypeptide part of the conjugate as a non-limiting example.)

[00242] For example, for systemic administration of any of the inventive agent described herein, typical dosages range from about 100.0 ng/kg body weight to about 10.0 mg/kg body weight. For example, in certain embodiments where a chlorotoxin polypeptide conjugate is administered intravenously, dosing of the agent may comprise administration of one or more doses comprising about 0.001 mg/kg to about 5 mg/kg, e.g., from about 0.001 mg/kg to about 5 mg/kg, from about 0.01 mg/kg to about 4 mg/kg, from about 0.02 mg/kg to about 3 mg/kg, from about 0.03 mg/kg to about 2 mg/kg or from about 0.03 mg/kg to about 1.5 mg/kg of chlorotoxin. For example, in some embodiments, one or more doses of chlorotoxin polypeptide conjugate may be administered that each contains about 0.002 mg/kg, about 0.004 mg/kg, about 0.006 mg/kg, about 0.008 mg/kg, about 0.009 mg/kg, about 0.01 mg/kg, about 0.02 mg/kg or more than 0.02 mg/kg of chlorotoxin. In some embodiments, one or more doses of chlorotoxin polypeptide conjugate may be administered that each contains about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.09 mg/kg, about 1.0 mg/kg or more than 1.0 mg/kg of chlorotoxin. In some embodiments, one or more doses of chlorotoxin polypeptide conjugate may be administered that each contains about 0.05 mg/kg, about 0.10 mg/kg, about 0.15

mg/kg, about 0.20 mg/kg, about 0.25 mg/kg, about 0.30 mg/kg, about 0.35 mg/kg, about 0.40 mg/kg, about 0.45 mg/kg, about 0.50 mg/kg, about 0.55 mg/kg, about 0.60 mg/kg, about 0.65 mg/kg, about 0.70 mg/kg, about 0.75 mg/kg, about 0.80 mg/kg, about 0.85 mg/kg, about 0.90 mg/kg, about 0.95 mg/kg, about 1.0 mg/kg, or more than about 1 mg/kg of chlorotoxin. In yet other embodiments, one or more doses of chlorotoxin polypeptide conjugate may be administered that each contains about 1.0 mg/kg, about 1.05 mg/kg, about 1.10 mg/kg, about 1.15 mg/kg, about 1.20 mg/kg, about 1.25 mg/kg, about 1.3 mg/kg, about 1.35 mg/kg, about 1.40 mg/kg, about 1.45 mg/kg, about 1.50 mg/kg, or more than about 1.50 mg/kg of chlorotoxin. In such embodiments, at treatment may comprise administration of a single dose of chlorotoxin polypeptide conjugate or administration of 2 doses, 3 doses, 4 doses, 5 doses, 6 doses or more than 6 doses. Two consecutive doses may be administered at 1 day interval, 2 days interval, 3 days interval, 4 days interval, 5 days interval, 6 days interval, 7 days interval, or more than 7 days interval (e.g., 10 days, 2 weeks, or more than 2 weeks).

[00243] For direct administration to the site via microinfusion, typical dosages range from about 1 ng/kg body weight to about 1 mg/kg body weight.

[00244] In certain embodiments where the chlorotoxin polypeptide conjugate is administered locally, in particular in cases of intracavitary administration to the brain, dosing of the conjugate may comprise administration of one or more doses comprising about 0.01 mg to about 100 mg of chlorotoxin polypeptide, e.g., from about 0.05 to about 50 mg, from about 0.1 mg to about 25 mg, from about 0.1 mg to about 10 mg, from about 0.1 mg to about 5 mg, or from about 0.1 mg to about 1.0 mg. For example, in certain embodiments, one or more doses of chlorotoxin polypeptide conjugate may be administered that each contains about 1 mg, about 1.5 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg or about 5 mg of reduced lysine chlorotoxin polypeptide. In some embodiments, one or more doses of chlorotoxin polypeptide conjugate may be administered that each contains about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.55 mg, about 0.6 mg, about 0.65 mg, about 0.7 mg, about 0.75 mg, about 0.8 mg, about 0.85 mg, about 0.9 mg, about 0.95 mg or about 1 mg of reduced lysine chlorotoxin polypeptide. In some embodiments, a treatment may comprise administration of a single dose of chlorotoxin polypeptide conjugate or administration of 2 doses, 3 doses, 4 doses, 5 doses, 6 doses or more than 6 doses. Two consecutive doses may be administered at 1 day interval, 2 days interval, 3 days interval, 4

days interval, 5 days interval, 6 days interval, 7 days interval, or more than 7 days interval (e.g., 10 days, 2 weeks, or more than 2 weeks). In some embodiments, multiple doses are administered, and the amount of reduced lysine chlorotoxin polypeptide administered is not the same for every dose. For example, in some embodiments, doses may be adjusted (e.g., escalated or reduced) from one dose to another as determined by the attending clinician.

[00245] It will be appreciated that pharmaceutical combinations of the present invention can be employed in combination with additional therapies (i.e., a treatment according to the present invention can be administered concurrently with, prior to, or subsequently to one or more desired therapeutics or medical procedures). The particular combination of therapies (therapeutics and/or procedures) to employ in such a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved.

Indications

[00246] Compositions and methods of the present invention can be used in a variety of antiproliferative and/or antiangiogenic contexts to treat and/or diagnose diseases or conditions.

Anti-proliferative contexts

[00247] In certain embodiments, compositions and methods of the present invention are used to treat and/or diagnose conditions involving uncontrolled cell proliferation, such as primary and/or metastatic cancers, and other cancerous conditions. For example, compositions and methods of the present invention should be useful for reducing size of solid tumors, inhibiting tumor growth or metastasis, treating various lymphatic cancers, and/or prolonging the survival time of mammals (including humans) suffering from these diseases.

[00248] Examples of cancers and cancer conditions that can be treated and/or diagnosed according to the present invention include, but are not limited to, tumors of the brain and central nervous system (e.g., tumors of the meninges, brain, spinal cord, cranial nerves and other parts of the CNS, such as glioblastomas or medulloblastomas); head and/or neck cancer, breast tumors, tumors of the circulatory system (e.g., heart, mediastinum

and pleura, and other intrathoracic organs, vascular tumors, and tumor-associated vascular tissue); tumors of the blood and lymphatic system (e.g., Hodgkin's disease, Non-Hodgkin's disease lymphoma, Burkitt's lymphoma, AIDS-related lymphomas, malignant immunoproliferative diseases, multiple myeloma, and malignant plasma cell neoplasms, lymphoid leukemia, myeloid leukemia, acute or chronic lymphocytic leukemia, monocytic leukemia, other leukemias of specific cell type, leukemia of unspecified cell type, unspecified malignant neoplasms of lymphoid, haematopoietic and related tissues, such as diffuse large cell lymphoma, T-cell lymphoma or cutaneous T-cell lymphoma); tumors of the excretory system (e.g., kidney, renal pelvis, ureter, bladder, and other urinary organs); tumors of the gastrointestinal tract (e.g., esophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus, and anal canal); tumors involving the liver and intrahepatic bile ducts, gall bladder, and other parts of the biliary tract, pancreas, and other digestive organs; tumors of the oral cavity (e.g., lip, tongue, gum, floor of mouth, palate, parotid gland, salivary glands, tonsil, oropharynx, nasopharynx, puriform sinus, hypopharynx, and other sites of the oral cavity); tumors of the reproductive system (e.g., vulva, vagina, Cervix uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); tumors of the respiratory tract (e.g., nasal cavity, middle ear, accessory sinuses, larynx, trachea, bronchus and lung, such as small cell lung cancer and non-small cell lung cancer); tumors of the skeletal system (e.g., bone and articular cartilage of limbs, bone articular cartilage and other sites); tumors of the skin (e.g., malignant melanoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues including peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneoum and peritoneum, eye and adnexa, thyroid, adrenal gland, and other endocrine glands and related structures, secondary and unspecified malignant neoplasms of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasms of other sites.

[00249] In some embodiments, the tumor is cutaneous or intraocular melanoma. In some embodiments, the tumor is metastatic melanoma. In some embodiments, the tumor is non-small cell lung cancer. In some embodimetns, the tumor is colon or colorectal cancer.

[00250] In some embodiments, compositions and methods are useful in the treatment and/or diagnosis of neuroectodermal tumors. (See, e.g., U.S. Pat. No. 6,667,156; the entire contents of which are herein incorporated by reference.) In some embodiments, the neuroectodermal tumor is glioma. (See, e.g., U.S. Pat. Nos. 5,905,027; 6,028,174; 6,319,891; 6,429,187; and 6,870,029; and International Patent Application publications WO03/101475A2, WO09/021136A1, and WO 2009/140599; the entire contents of each of which are herein incorporated by reference.) Types of glioma for which compositions and methods of the invention are useful include, but are not limited to, glioblastoma multiformes (WHO grad IV), anaplastic astrocytomas (WHO grade III), low grade gliomas (WHO grade II), pliocytic astrocytomas (WHO grade I), oligodendrogliomas, gangliomas, meningiomas, and ependymomas. In some embodiments, the neuroectodermal tumor is selected from the group consisting of medulloblastomas, neuroblastomas, pheochromocytomas, melanomas, peripheral primitive neuroectodermal tumors, small cell carcinoma of the lung, Ewing's sarcoma, and metastatic tumors in the brain.

[00251] In certain embodiments of the present invention, compositions and methods are used in the treatment and/or diagnosis of sarcomas. In some embodiments, compositions and methods of the present invention are used in the treatment and/or diagnosis of bladder cancer, breast cancer, chronic lymphoma leukemia, head and neck cancer, endometrial cancer, Non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, and prostate cancer. In some embodiments, the sarcoma is selected from the group consisting of prostate cancer or breast cancer. (See, e.g., International Patent Application publications W003/101474A1, W003/10475A2, and WO 2009/140599, the entire contents of each of which are herein incorporated by reference.) In some embodiments, the sarcome is pancreatic cancer.

[00252] In certain embodiments of the present invention, compositions and methods are useful in the treatment and/or diagnosis of myeloproliferative disorders (e.g., tumors of myeloid origin) and/or lymphoproliferative disorders (e.g., tumors of lymphoid origin) (See, e.g., International Patent Application publication W005/099774, the entire contents of which are herein incorporated by reference).

[00253] Types of myeloproliferative disorders for which compositions and methods of the present invention are useful include, but are not limited to, polycythemia vera (PV), essential

thrombocythemia (ET), agnogenic myeloid metaplasia (AMM) (also referred to as idiopathic myelofibrosis (IMF)), and chronic myelogenous leukemia (CML).

[00254] In some embodiments, compositions and methods of the present invention are used to detect, treat, diagnose and/or confirm a lymphoproliferative disorder. In some embodiments, the lymphoproliferative disorder is a non-Hodgkin's lymphoma. In some embodiments, the lymphoproliferative disorder is a B cell neoplasm, such as, for example, a precursor B-cell lymphoblastic leukemia/lymphoma or a mature B cell neoplasm. Non-limiting types of mature B cell neoplasms include B cell chronic lymphocytic leukemia/small lymphocytic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone B cell lymphoma, hairy cell leukemia, extranodal marginal zone B cell lymphoma, mantle cell lymphoma, follicular lymphoma, nodal marginal zone lymphoma, diffuse large B cell lymphoma, Burkitt's lymphoma, plasmacytoma, and plasma cell myeloma.

[00255] In some embodiments, compositions and methods of the present invention are used to treat a T cell neoplasm. Non-limiting types of T cell neoplasms include T cell prolymphocytic leukemia, T cell large granular lymphocytic leukemia, NK cell leukemia, extranodal NK/T cell lymphoma, mycosis fungoides, primary cutaneous anaplastic large cell lymphoma, subcutaneous panniculitis-like T cell lymphoma, enteropathy-type intestinal T cell lymphoma, hepatosplenic gamma-delta T cell lymphoma, angioimmunoblastic T cell lymphoma, peripheral T cell lymphoma, anaplastic large cell lymphoma, and adult T cell lymphoma.

[00256] Tumors that can be detected and/or treated using compositions and methods of the present invention may be refractory to treatment with other chemotherapeutics. The term "refractory", when used herein in reference to a tumor means that the tumor (and/or metastases thereof), upon treatment with at least one chemotherapeutic other than an inventive composition, shows no or only weak anti-proliferative response (i.e., no or only weak inhibition of tumor growth) after the treatment of such a chemotherapeutic agent — that is, a tumor that cannot be treated at all or only with unsatisfying results with other (preferably standard) chemotherapeutics. The present invention, where treatment of refractory tumors and the like is mentioned, is to be understood to encompass not only (i) tumors where one or more chemotherapeutics have already failed during treatment of a patient, but also (ii)

tumors that can be shown to be refractory by other means, e.g., biopsy and culture in the presence of chemotherapeutics.

Anti-angiogenic contexts

[00257] Chlorotoxin has been shown to exert anti-angiogenic properties. See, e.g., International Patent Application publication W02009/117018, the entire contents of which are herein incorporated by reference. In certain embodiments, compositions and methods of the present invention are used to treating, diagnose, and/or ameliorate a disease or condition such as, for example cancer (including metastatic cancer, as described above), ocular neovascularization (such as macular degeneration), inflammatory diseases (such as arthritis), etc. In some embodiments, the condition or disease is characterized by choroidal neovascularization. Examples of such conditions or diseases include, but are not limited to, macular degeneration (including wet macular degeneration, age-related macular degeneration, etc.), myopia, ocular trauma, pseudoxanthoma elasticum, and combinations thereof.

[00258] Macular degeneration is the leading cause of vision loss and blindness in Americans aged 65 and older. Macular dengeration typically occurs in the age-related form (often called AMD or ARMD), though juvenile macular degneration occurs as well. In AMD/ARMD, the macula - the part of the retina that is responsible for sharp, central vision- degenerates. Macular degeneration is typically diagnosed as either dry (non-neovascular) or wet In dry macular degeneration, yellowish spots knwn as drusen begin to accumulate from deposits or debris from deteriorating tissue from mostly around the macula. Central vision less usually occurs gradually and is not as severe as vision loss in wet macular degeneration.

[00259] Wet macular degeneration, as the "neovascular" designation suggests, is characterized by new blood vessels growing aberrantly, e.g., on the macula. Such new blood vessels may grow beneath the retina, leaking blood and fluid. Such leakage causes permanent damage to light-sensitive retinal cells, which die and create blind spots in central vision. Wet macular degeneation may be further grouped into two categories. In the occult form of wet macular degeneration, new blood vessel growth beneath the retina is not as pronounced and leakage is less evident, typically resulting in less severe vision less. In the classic form of wet macular degeneration, blood vessel growth and scarring have very clear,

delineated outlines that are observable beneath the retina. Classic wet macular degeneration is also known as classic choroidal neovascularization and usually results in more severe vision loss.

[00260] Given the role of angiogenesis in wet macular degeneration, which comprises many AMD/ARMD cases, inventive compositions and methods may be useful in treating, diagnosing, and/or ameliorating such disorders. Current therapies for wet macular degeneration involve angiogenesis inhibitors such as Lucentis™, Macugen™, and/or Visudyne™, optionally combined with photodynamic therapy (PDT) to target drugs to specific cells. Photocoagulation, in which a high energy laser beam is used to create small burns in areas of the retina with abnormal blood vessels, is also used to treat wet macular degeneration.

[00261] In some embodiments, chlorotoxin polypeptide conjugates (or a pharmaceutical composition thereof) are administered to a subject suffering from wet macular degeneration and/or age-related macular degeneration. Among subjects suffering from wet macular degeneration, subjects may suffer from the occult or the classic form. In some embodiments, chlorotoxin polypeptide conjugates cause regression of existing neovasculature. In some embodiments, chlorotoxin polypeptide conjugates prevent sprouting of new vessels. In certain embodiments, chlorotoxin polypeptide conjugates are combined with other treatments for wet macular degeneration, such as photocoagulation, treatment with other angiogenesis inhibitors, photodynamic therapy, etc.

[00262] In some embodiments, chlorotoxin agents as described herein are administered in combination with or as part of a therapeutic regimen with one or more therapeutic regimens recommended for treatment of a disease, disorder, or condition associated with angiogenesis. To give but a few examples, recommended regimens for treatment of cancer can be found at the web site have a URL of www.cancer.gov, the website of the National Cancer Institute. Recommended regimens for treatment of macular degeneration can be found at the web site having URL www.mayoclinic org/macular-degeneration/treatment.html. Treatment regimens may include chemotherapy, surgery, and/or radiation therapy.

EXAMPLES

[00263] The following Examples are provided for illustration purposes only and are not to be construed to be limiting. The multifunctional agents exemplified below comprise a targeting moiety, a therapeutic moiety, a detection moiety, and/or a linker. A chlorotoxin polypeptide used as a targeting moiety of the multifunctional agent is abbreviated as "CTX." A small chemical-based entity used as a therapeutic moiety of the multifunctional agent is abbreviated as "SCE." An imaging agent used as a detection entity of the multifunctional agent is abbreviated as "I." A linker or linkers used to conjugate these entities of the multifunctional agent are shown as "L."

Example 1: Development of CTX:L:SCE and CTX:L:SCE:I.

[00264] The directed conjugation approach described herein was used to synthesize a test multifunctional agent comprising Taxol as a therapeutic entity and a chlorotoxin polypeptide as a targeting agent. CTX:L:SCE:I compounds for diagnosing patients whose disease tissue may uptake a conjugated targeted deliverable pharmacologic agent CTX:L:SCE. For this example we describe a targeted chemotherapeutic to be used to treat tumors. Detailed methods for the synthesis are provided below.

[00265] **Figure 4** shows a reductive amination reaction. Compound ER-880932-00 (8.0 mg, 0.0020 mmol) was added to a solution of compound ER-894954-00 (2.57 mg, 2.30E-6 mol; EISAI; Sample ID UM - 721(a)) in acetonitrile (0.4 uL, 0.008 mmol) and 0.1 M of Sodium acetate in Water (0.4 mL) at 0 °C, followed by the addition of sodium cyanoborohydride (3.0 mg, 0.048 mmol). The reaction mixture was stirred for 3 h at 0°C and 2h at 23°C, while progress of the reaction was monitored by uPLC-MS. After 3 h, the crude reaction mixture was filtered to remove the resin and concentrated. The residue was dissolved in water (3 mL) and purified on the HPLC (method Prep1040P2lo, C-Ph column), using standard solvent system (CH_3CN/H_2O/FA) to isolate compound ER-895970-20-01 (3.5 mg, 39%) as a white solid.

[00266] **Figure 5** provides an acid-based hydrolysis reaction. 1 M of Hydrogen chloride in Water (600 uL) was added to a solution of compound ER-894925-00 (14 mg, 0.011 mmol; EISAI; Sample ID UM - 720(a)) in Tetrahydrofuran (0.8 mL, 0.01 mol) at 0°C. After stirring for 48 h at 23°C LC/MS and TLC (silica, CH_2Cl_2:MeOH, 20:1) showed that a new a more polar spot had formed. The reaction was neutralize with solid $NaHCO_3$ (300 mg) and extracted with methylene chloride (3 x 10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*.

Purification by preparative TLC (CH_2Cl_2:MeOH, 20:1) gave compound ER-895954-00-01 (9.3 mg, 78%). ^1H NMR and uPLC-MS data confirmed the purity and identity of the product.

[00267] **Figure 6** provides a carbonate formation reaction. Compound ER-885474-00 (12 mg, 0.038 mmol; EISAI; Sample ID UM - 717(a)) in methylene chloride (0.6 mL, 0.009 mol) was added to a solution of compound ER-894922-00 (20 mg, 0.02 mmol; EISAI; Sample ID UM - 718(a)) in isopropyl alcohol (0.8 mL, 0.01 mol) at 23°C and the reaction vessel was sealed. After stirring for 60h at 60°C TLC showed that a more polar spot had formed. The solvent was removed under vacuum and the residue was purified by flash chromatography over silica gel using 30% EtOAc-Heptane to 100% EtOAc to afford compound ER-894925 (14 mg 60%). ^1H NMR and uPLC-MS data confirmed the purity and identity of the product.

[00268] **Figure 7** provides an acylation reaction. *N,N*-Carbonyldiimidazole (36.8 mg, 0.227 mmol) was added to a solution of compound ER-894888-00 (110.0 mg, 0.1136 mmol; EISAI; Sample ID MHP - 1599(a)) in methylene chloride (3 mL, 0.05 mol) at 23°C followed by 4-dimethylaminopyridine (3.5 mg, 0.028 mmol). After stirring for 18h at 23°C TLC showed that a more polar spot had formed. Water (2 mL) was added, and the layers extracted with EtOAc (3 x 10 mL), washed with water (2 x 10 mL), brine (1 x 10 mL), dried and concentrated under vacuum. The crude material was purified by flash chromatography 30% EtOAc-Heptane to 100% EtOAc to isolate compound ER-894922 (86.6 mg, 71%). NMR and uPLC-MS data confirmed the purity and identity of the product.

[00269] **Figure 8** provides an O-2-silylation reaction, in which tert-Butyldimethylsilyl chloride (771 mg, 5.12 mmol) was added to a mixture of compound ER-517446-00 (874.0 mg, 1.024 mmol) (LC Labs) and 1H-Imidazole (696.8 mg, 10.24 mol) in *N,N*-Dimethylformamide (10.0 mL, 0.129 mol) the resulting solution stirred at 60°C for 14 h. After 16h uPLC-MS showed a major peak for product (4.31 min, m/z = 968.5), but some starting Taxol remained (3.04 min, m/z = 854.39). The solution was concentrated *in vacuo* and taken up in EtOAc (40 mL) and washed with saturated NH_4Cl (1 x 20 mL), brine (1 x 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to leave a white solid. Flash chromatography of the residue on silica gel using 40% to 50% to 60% to 80% EtOAc-heptane gave the product, compound ER-894888 (874 mg, 88%) as a white solid. ^1H and ^{13}C NMR data along with mass spectral (LC-MS) analysis supported the identity and purity of the product as shown.

[00270] CTX:L:SCE:I was generated by diluting the mass spec purified CTX:L:SCE above 0.25 mg/ml in 1X phosphate-buffered saline (pH 7.4). To this, the L component, NHS-IRDye 800CW (LiCor, cat# 929-70021), was added from a 100% DMSO stock solution to a

final molar ratio of 1:1.2 CTX:L:SCE to L ration and mixed thoroughly. The conjugation was allowed to proceed for 4 hours at room temperature. Conjugation efficiency was monitored by RP-HPLC (214 nm and 750 nm), LC-MS, and SDS-PAGE. For each CTX:L:SCE, an average of one dye was added per molecule, with no evidence of remaining free dye and little or no doubly-labeled peptide. Following conjugation, CTX:L:SCE:I were diluted to appropriate concentrations for injection in 1X PBS without further modifications. The final agent was then used for diagnosis in detecting human xenograft tumors in nude mice and the CTX:L:SCE agent was used to test for pharmacologic activity. A schematic diagram for the CTX:L:SCE:I and CTX:L:SCE is provided in **Figure 1**.

Example 2: Disease tissue uptake of CTX:L:SCE:I.

[00271] Nude mice containing human xenograft tumors were used to test the ability of the chlorotoxin-NHS-taxol-IRDye (CTX:L:SCE:I; P:L:T:D) agent to target these tumors *in vivo*. The mice were then imaged for tumor specific targeting. As shown a gradual increase of tumor specific uptake was observed over time demonstrating the suitability of using this diagnostic CTX:L:SCE:I for the potential of the CTX:L:SCE to have tumor-specific activity. Chlorotoxin labeled with a fluorescent dye (e.g. Cye5.5) have been shown to be capable of localizing to tumor in similar models (not shown) as chlorotoxin has the ability to target these tumor types via its targeting activities.

[00272] Animals were placed into a warming chamber under a heat lamp. While the animal is warmed to vaso-dilate the tail vein, 250 uL of the chlorotoxin-NHS-taxol-IRDye (CTX:L:SCE:I) agent was brought up in a sterile 1mL syringe with a sterile 27G1/2 needle. The volume was adjusted to the injection volume of 250 uL.

[00273] The animal was then removed from the warming chamber and restrained using a restrainer (vetequipt). With the tail extended through the slot and held firmly, the tail was rotated 90 degrees clockwise to expose the left vein. The syringe containing the chlorotoxin-NHS-taxol-IRDye (CTX:L:SCE:I) agent with needle, was placed, bevel up, directly over the tail vein and inserted gently into the vein. 250 uL of the solution in the syringe were then dispensed into the animal at a rate of approximately 10 uL/sec. Direct finger pressure was applied to the injection site upon removal of the needle and held for approximately 5 seconds.

[00274] To image the ability of the chlorotoxin-NHS-taxol-IRDye (CTX:L:SCE:I) agent's ability to localize to the tumor, animals were placed into an anesthesia chamber for approximately 3-4 minutes using isofluorane until the animals are unconscious. Animals were then imaged using a (IVIS Lumina kinetic, Caliper) Living Imaging System with the fluorescence setting of 745 Excitation and 720 emission. Images were taken of the dorsal side, the right side, ventral side and left side at 0, 1, 24, 48 and 96 hrs. After each successive image the animal was allowed to regain consciousness in a recovery chamber receiving 100% oxygen flush then normal air.

[00275] As shown in **Figure 2** a gradual accumulation of the CTX:L:SCE:I agent was observed in the tumor while accumulation in other organs outside of the primary collection of the kidney after intravenous administration was observed, thus demonstrating ability of this agent to localize to tumor for cytotoxic drug delivery and therapy.

Example 3: In vivo activity of CTX:L:SCE.

[00276] To test the pharmacologic activity of the CTX:L:SCE which consist of chlorotoxin-NHS-taxol, on tumor xenografts, nude mice injected with human tumor cell line were employed. Mice xenografts were treated with the conjugated taxol (here called TM601-TAXOL), parent paclitaxel (TAXOL) and saline as a negative control at taxol equivalent doses of 3.7 mg/kg three times per week for three weeks. Briefly, six mice per group were used to determine the effects of saline, 3.7 mg/kg paclitaxel and chlorotoxin-NHS-taxol (CTX:L:SCE) at a dose equivalent to having 3.7 mg/kg of paclitaxel on tumor growth. Animals were treated Monday, Wednesday and Friday for 3 consecutive weeks and tumor growth was monitored using caliper measurements three times per week for up to 50 days. Tumors were then analyzed for growth by caliper measurements a various time points during and after the completion of treatment. As shown in **Figure 3**, the CTX:L:SCE, chlorotoxin-NHS-taxol conjugate had a statically improved anti-tumor response as compared to the paclitaxel parental compound when dosed at a subpharmacologic dose demonstrating the improved pharmacologic activity when formatted in a disease targeting format of CTX:L:SCE. The CTX:L:SCE (TM601-taxol) had a robust anti-tumor response and a longer duration of response as compared to the parental compound. Moreover, acute toxicity studies showed the CTX:L:SCE compound to be safe while the parental compound showed 100% lethality when both administered at a 20 mg/kg dose (not shown).

[00277] These data along with those shown in Example 2, **Figure 2** demonstrate the utility of the invention in developing a companion set of CTX:L:SCE:I and CTX:L:SCE for identifying disease tissues that can uptake a drug:disease targeting agent (CTX:L:SCE) for enhanced therapeutic activity will minimal unwanted off target side effects.

Example 4: Clinical applications.

[00278] A peptide-small chemical entity-and-an imaging agent conjugate is useful for prescreening patients capable of uptaking the peptide-small chemical entity for diagnosis and possible treatment of the disease cell. The invention comprises of peptides that can bind disease cells when two or more agents are coupled to the peptide via direct or linker-mediated attachment.

[00279] The invention is useful, for example, for identifying patients who may clinically benefit from treatment of a targeted agent conjugated or fused to pharmaceutical agent, comprising administering to a patient, who has been diagnosed with a disease cell expressing a target for the said agent, an effective amount of a composition of matter consisting of a formula CTX:L:SCE:I whereby CTX consists of chlorotoxin residues capable of binding chlorotoxin target on disease (e.g., MCMPCFTTDHQMARACDDCCGGAGRGKCYGPQCLCR and MCMPCFTTDHQMARACDDCCGGAGRGKCYGPQCLCR; or variants/fragments, such as TTDHQMARKS (SEQ ID NO: 35) and TTDHQMARKC (SEQ ID NO: 56)); L consists of a linker from a group of agents including polyethylene glycol, a copolymer of ethylene glycol, a polypropylene glycol, a copolymer of propylene glycol, a carboxymethylcellulose, a polyvinyl pyrrolidone, a poly-1,3-dioxolane, a poly-1,3,6-trioxane, an ethylene/maleic anhydride copolymer, a polyaminoacid, a dextran n-vinyl pyrrolidone, a poly n-vinyl pyrrolidone, a propylene glycol homopolymer, a propylene oxide polymer, an ethylene oxide polymer, a polyoxyethylated polyol, a polyvinyl alcohol, a linear or branched glycosylated chain, a polyacetal, a long chain fatty acid, a long chain hydrophobic aliphatic group or a direct aminoacid linkage between CTX and SCE; SCE consist of a toxin peptide derived from pseudomonas or a cytotoxic agent capable of damaging DNA, suppressing microtubule function, tyrosine kinase signaling, pI3K signaling or RNA processing/translation; and I is an

imaging agent consisting of radionuclides, heavy metals, infrared dyes or other substrates capable of being detected in vivo by those skilled in the art.

Equivalents

[00280] It should be understood that the preceding is merely a detailed description of certain preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. It is intended that the invention encompass all such modifications within the scope of the appended claims. All references, patents and patent applications and publications that are cited or referred to in this application are incorporated in their entirety herein by reference.

What is claimed is:

CLAIMS

1. A multifunctional agent comprising:

a targeting entity that comprises a chlorotoxin polypeptide;

a detection entity; and

a therapeutic entity,

wherein the targeting entity, detection entity, and therapeutic entity are conjugated to one another via covalent conjugation.

2. The multifunctional agent of claim 1, wherein each of the targeting entity and the detection entity are different entities.

3. The multifunctional agent of claim 1 or 2, wherein each of the detection entity and the therapeutic entity is conjugated to the targeting entity via covalent conjugation.

4. The multifunctional agent of claim 3, wherein at least one of the covalent conjugations is a direct conjugation.

5. The multifunctional agent of claim 3, wherein at least one of the detection entity and the therapeutic entity is covalently conjugated to the targeting entity by a linker entity.

6. The multifunctional agent of claim 5, wherein each of the detection entity and the therapeutic entity is covalently conjugated to the targeting entity by a linker entity.

7. The multifunctional agent of any one of claims 1-6, wherein the linker entity is selected from a group consisting of:

polyethylene glycol, a copolymer of ethylene glycol, a polypropylene glycol, a copolymer of propylene glycol, a carboxymethylcellulose, a polyvinyl pyrrolidone, a poly-1,3-dioxolane, a poly-1,3,6-trioxane, an ethylene/maleic anhydride copolymer, a polyaminoacid, a dextran n-vinyl pyrrolidone, a poly n-vinyl pyrrolidone, a propylene glycol homopolymer, a propylene oxide polymer, an ethylene oxide polymer, a

polyoxyethylated polyol, a polyvinyl alcohol, a linear or branched glycosylated chain, a polyacetal, a long chain fatty acid, and a long chain hydrophobic aliphatic group.

8. The multifunctional agent of any one of claims 1-7, wherein the chlorotoxin polypeptide is not more than 40 amino acids long.

9. The multifunctional agent of any one of claims 1-8, wherein the chlorotoxin polypeptide has an amino acid sequence that shows at least 85% overall identity with that of SEQ ID NO: 1.

10. The multifunctional agent of claim 9, wherein the chlorotoxin polypeptide has an amino acid sequence that differs from that of SEQ ID NO: 1 at least in that the chlorotoxin polypeptide sequence has not more than one lysine residues.

11. The multifunctional agent of any one of claims 1-10 wherein the detection entity is selected from the group consisting of:

ligands, radionuclides, fluorescent dyes, chemiluminescent agents, bioluminescent agents, spectrally resolvable inorganic fluorescent semiconductor nanocrystals, quantum dots, microparticles, metal nanoparticles, nanoclusters, magnetic particles, paramagnetic metal ions, enzymes, colorimetric labels, biotin, dioxigenin, haptens and any combination thereof.

12. The multifunctional agent of any one of claims 1-11, wherein the targeting entity comprises a chlorotoxin polypeptide of formula: $TTX_1X_2X_3MX_4X_5X_6$ (SEQ ID NO: 67),

wherein X_1 is an acidic amino acid selected from the group consisting of aspartic acid and glutamic acid;

wherein X_2 is an amino acid selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine;

wherein X_3 is an amide amino acid selected from the group consisting of asparagine and glutamine;

wherein X_4 is an any amino acid selected from the group consisting of serine, threonine and alanine;

wherein X_5 is a basic amino acid selected from the group consisting of histidine, lysine and arginine; and,

wherein X_6 is an any amino acid selected from the group consisting of lysine, arginine and alanine.

13 The multifunctional agent of claim 12, wherein the chlorotoxin polypeptide is:

MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC R (SEQ ID NO: 1);

MCMPC FTTDH QMARC DDCCG GGRGC YGPQC LCR (SEQ ID NO: 2);

KMCMP CFTTD HQMAR CDDCC GGGRG CYGPQ CLCR (SEQ ID NO: 3);

MCMPC FTTDH QMARC DDCCG GGRGC YGPQC LCRK (SEQ ID NO: 4);

MCMPC FTTDH QMARA CDDCC GGAGR GACYG PQCLC R (SEQ ID NO: 5);

MCMPC FTTDH QMARR CDDCC GGRGR GRCYG PQCLC R (SEQ ID NO: 6);

KMCMP CFTTD HQMAR ACDDC CGGAG RGACY GPQCL CR (SEQ ID NO: 7);

KMCMP CFTTD HQMAR RCDDC CGGRG RGRCY GPQCL CR (SEQ ID NO: 8);

MCMPC FTTDH QMARA CDDCC GGAGR GACYG PQCLC RK (SEQ ID NO: 9);

MCMPC FTTDH QMARR CDDCC GGRGR GRCYG PQCLC RK (SEQ ID NO: 10);

MCMPC FTTDH QMARC DDCCG GKGRG KCYGP QCLCR (SEQ ID NO: 11);

MCMPC FTTDH QMARK CDDCC GGGRG KCYGP QCLCR (SEQ ID NO: 12);

MCMPC FTTDH QMARK CDDCC GGKGR GCYGP QCLCR (SEQ ID NO: 13);

MCMPC FTTDH QMARC DDCCG GGRGK CYGPQ CLCR (SEQ ID NO: 14);

MCMPC FTTDH QMARC DDCCG GKGRG CYGPQ CLCR (SEQ ID NO: 15);

MCMPC FTTDH QMARK CDDCC GGGRG CYGPQ CLCR (SEQ ID NO: 16);

MCMPC FTTDH QMARA CDDCC GGAGR GKCYG PQCLC R (SEQ ID NO: 17);

MCMPC FTTDH QMARA CDDCC GGKGR GACYG PQCLC R (SEQ ID NO: 18);

MCMPC FTTDH QMARK CDDCC GGAGR GACYG PQCLC R (SEQ ID NO: 19);

MCMPC FTTDH QMARR CDDCC GGRGR GKCYG PQCLC R (SEQ ID NO: 20);

MCMPC FTTDH QMARR CDDCC GGKGR GRCYG PQCLC R (SEQ ID NO: 21);

MCMPC FTTDH QMARK CDDCC GGRGR GRCYG PQCLC R (SEQ ID NO: 22);

MCMPC FTTDH QMARR CDDCC GGKGR GACYG PQCLC R (SEQ ID NO: 23;

MCMPC FTTDH QMARC DDCCG GAGRG ACYGP QCLCR (SEQ ID NO: 24);

MCMPC FTTDH QMARA CDDCC GGGRG ACYGP QCLCR (SEQ ID NO: 25);

MCMPC FTTDH QMARR CDDCC GGRGR GCYGP QCLCR (SEQ ID NO: 26);

TTDHQ MAR (SEQ ID NO: 27);

MSMPS FTTDH QMARK SDDSS GGKGR GKSYG PQSLS R (SEQ ID NO: 28);

MSMPS FTTDH (SEQ ID NO: 29);

SMPSF TTDHQ (SEQ ID NO: 30);

MPSFT TDHQM (SEQ ID NO: 31);

PSFTT DHQMA (SEQ ID NO: 32);

SFTTD HQMAR (SEQ ID NO: 33);

FTTDH QMARK (SEQ ID NO: 34);

TTDHQ MARKS (SEQ ID NO: 35);

TDHQM ARKSD (SEQ ID NO: 36);

DHQMA RKSDD (SEQ ID NO: 37);

HQMAR KSDDS (SEQ ID NO: 38);

QMARK SDDSS (SEQ ID NO: 39);

MARKS DDSSG (SEQ ID NO: 40);

ARKSD DSSGG (SEQ ID NO: 41);

RKSDD SSGGK (SEQ ID NO: 42);

KSDDS SGGKG (SEQ ID NO: 43);

SDDSS GGKGR (SEQ ID NO: 44);

DDSSG GKGRG (SEQ ID NO: 45);

DSSGG KGRGK (SEQ ID NO: 46);

SSGGK GRGKS (SEQ ID NO: 47);

SGGKG RGKSY (SEQ ID NO: 48);

GGKGR GKSYG (SEQ ID NO: 49);

GKGRG KSYGP (SEQ ID NO: 50);

KGRGK SYGPQ (SEQ ID NO: 51);

GRGKS YGPQS (SEQ ID NO: 52);

RGKSY GPQSL (SEQ ID NO: 53);

GKSYG PQSLS (SEQ ID NO: 54);

KSYGP QSLSR (SEQ ID NO: 55);

TTDHQ MARKC (SEQ ID NO: 56);

CMPSF TTDHQ (SEQ ID NO: 57);

CFTTD HQMAR (SEQ ID NO: 58);

TDHQM ARKCD (SEQ ID NO: 59);

DCSGG KGRGG (SEQ ID NO: 60);

CSGGK GRGKS (SEQ ID NO: 61);

CGGKG RGKSY (SEQ ID NO: 62);

SSGGK GRGKC (SEQ ID NO: 63);

SGGKG RGKCY (SEQ ID NO: 64);

KGRGK SY (SEQ ID NO: 65);

KGRGK CY (SEQ ID NO: 66);

HHHHH HMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR (SEQ ID NO: 69);

YMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR (SEQ ID NO: 70);

YSYMC MPCFT TDHQM ARKCD DCCGG KGRGK CYGPQ CLCR (SEQ ID NO: 71);

MCMPC FTTDH QMARK CDDCC GGKGR GKCFG PQCLC R (SEQ ID NO: 72);

RCKPC FTTDP QMSKK CADCC GGKGK GKCYG PQCLC (SEQ ID NO: 73);

RCSPC FTTDQ QMTKK CYDCC GGKGK GKCYG PQCIC APY (SEQ ID NO: 74);

KMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR (SEQ ID NO: 75); or,

MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC RK (SEQ ID NO: 76).

14. A set of multifunctional agents comprising:

(1) a first multifunctional agent comprising:

(a) a targeting entity,

(b) a detection entity, and

(c) a therapeutic entity,

wherein the targeting entity, the detection entity and the therapeutic entity are conjugated to one another;

(2) a second multifunctional agent comprising:

(a) a targeting entity and

(b) a therapeutic entity,

wherein the targeting entity and the therapeutic entity are conjugated to each other.

15. The set of multifunctional agents of claim 14, wherein each of the first multifunctional agent and the second multifunctional agent further comprises a linker.

16. The set of multifunctional agents of claim 15, wherein the linker is selected from the group consisting of:

polyethylene glycol, a copolymer of ethylene glycol, a polypropylene glycol, a copolymer of propylene glycol, a carboxymethylcellulose, a polyvinyl pyrrolidone, a poly-1,3-dioxolane, a poly-1,3,6-trioxane, an ethylene/maleic anhydride copolymer, a polyaminoacid, a dextran n-vinyl pyrrolidone, a poly n-vinyl pyrrolidone, a propylene glycol homopolymer, a propylene oxide polymer, an ethylene oxide polymer, a polyoxyethylated polyol, a polyvinyl alcohol, a linear or branched glycosylated chain, a polyacetal, a long chain fatty acid, and a long chain hydrophobic aliphatic group.

17. A method comprising a step of:

administering to an individual having a tumor or susceptible to developing a tumor a composition comprising an effective amount of a detection multifunctional agent,

wherein the detection multifunctional agent comprises a targeting entity and a detection entity, and,

wherein the amount is effective to bind to a tumor in an organism containing a tumor.

18. The method of claim 17, wherein the detection multifunctional agent further comprises a therapeutic entity.

19. The method of claim 17, further comprising a step of:

detecting binding of the agent to one or more tumors in the individual.

20. The method of claim 19, further comprising a step of:

identifying the individual as having a tumor.

21. The method of claim 19 or 20, further comprising a step of:

identifying the individual as a candidate for therapy with a therapeutic multifunctional agent comprising:

a targeting entity and

a therapeutic entity.

22. The method of claim 21, wherein the a therapeutic multifunctional agent further comprises a detection entity.

23. The method of any one of claims 17-22, further comprising a step of:

administering to the individual the therapeutic multifunctional agent.

24. The method of claim 23, wherein the step of administering the therapeutic multifunctional agent comprises administering according to a regimen correlated with effective therapy.

25. A method of synthesizing a multifunctional agent, the method comprising steps of:

Carrying out a first phase of conjugation, wherein a therapeutic entity and a linker entity with an aldehyde group are conjugated to form an intermediate; and,

Carrying out a second phase of conjugation, wherein the intermediate and a polypeptide targeting entity are conjugated,

wherein the second phase comprises a reductive amination reaction and a hydrolysis reaction.

26. A conjugate produced by the method of claim 25.

27. A pharmaceutical composition comprising:

a multifunctional agent comprising:

a chlorotoxin polypeptide not more than 40 amino acids long, which chlorotoxin polypeptide has an amino acid sequence that shows at least 85% identity with SEQ ID NO: 1;

a detection entity;

a therapeutic entity; and

a pharmaceutically acceptable carrier.

28. The pharmaceutical composition of claim 27, wherein the therapeutic agent is an anti-cancer agent.

29. The pharmaceutical composition of claim 28, wherein the anti-cancer agent is a small molecule.

30. The pharmaceutical composition of claim 28 or 29, wherein the anti-cancer agent is selected from the group consisting of:

anti-mitotic agents, adjuvants, alkylating drugs (mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide), antimetabolites (methotrexate), purine antagonists and pyrimidine antagonists (6-mercaptopurine, 5-fluorouracil, cytarabine, gemcitabine), spindle poisons (vinblastine, vincristine, vinorelbine, paclitaxel), podophyllotoxins (etoposide, irinotecan, topotecan), antibiotics (doxorubicin, bleomycin, mitomycin), nitrosureas (carmustine, lomustine, nomustine), inorganic ions (cisp latin, carboplatin), enzymes (asparaginase), and hormones (tamoxifen, leuprolide, flutamide, megestrol), radioisotopes (Iodine-131, Lutetium-177, Rhenium-188, Yttrium-90), toxins (diphtheria, pseudomonas, ricin, gelonin), enzymes, enzymes to activate prodrugs, radio-sensitizing drugs, interfering RNAs, superantigens, anti-angiogenic agents, alkylating agents, purine antagonists, pyrimidine antagonists, plant alkaloids, intercalating antibiotics, aromatase inhibitors, anti-metabolites, mitotic inhibitors, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones and anti-androgens, BCNU, cisplatin, gemcitabine, hydroxyurea, paclitaxel, temozolomide, topotecan, fluorouracil, vincristine, vinblastine, procarbazine, decarbazine, altretamine, methotrexate, mercaptopurine, thioguanine, fludarabine phosphate, cladribine, pentostatin, cytarabine, azacitidine, etoposide, teniposide, irinotecan, docetaxel, doxorubicin, daunorubicin, dactinomycin, idarubicin, plicamycin, mitomycin, bleomysin, tamoxifen, flutamide, leuprolide, goserelin, aminogluthimide, anastrozole, amsacrine, asparaginase, mitoxantrone, mitotane and amifostine.

31. The pharmaceutical composition of claim 27, wherein the detection entity is selected from the group consisting of:

ligands, radionuclides, fluorescent dyes, chemiluminescent agents, bioluminescent agents, spectrally resolvable inorganic fluorescent semiconductor nanocrystals, quantum dots, microparticles, metal nanoparticles, nanoclusters, magnetic particles, paramagnetic metal ions, enzymes, colorimetric labels, biotin, dioxigenin, haptens and any combination thereof.

32. The pharmaceutical composition according to any one of claims 27-31, wherein the chlorotoxin polypeptide contains not more than one lysine residues.

33. The pharmaceutical composition according to any one of claims 27-31, wherein the targeting entity comprises a chlorotoxin polypeptide of formula: $TTX_1X_2X_3MX_4X_5X_6$ (SEQ ID NO: 67),

wherein X_1 is an acidic amino acid selected from the group consisting of aspartic acid and glutamic acid;

X_2 is an amino acid selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine;

X_3 is an amide amino acid selected from the group consisting of asparagine and glutamine;

X_4 is an amino acid selected from the group consisting of serine, threonine and alanine;

X_5 is a basic amino acid selected from the group consisting of histidine, lysine and arginine; and,

X_6 is an amino acid selected from the group consisting of lysine, arginine and alanine.

34 The pharmaceutical composition of any one of claims 27-33, wherein the chlorotoxin polypeptide is:

MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC R (SEQ ID NO: 1);

MCMPC FTTDH QMARC DDCCG GGRGC YGPQC LCR (SEQ ID NO: 2);

KMCMP CFTTD HQMAR CDDCC GGGRG CYGPQ CLCR (SEQ ID NO: 3);

MCMPC FTTDH QMARC DDCCG GGRGC YGPQC LCRK (SEQ ID NO: 4);

MCMPC FTTDH QMARA CDDCC GGAGR GACYG PQCLC R (SEQ ID NO: 5);

MCMPC FTTDH QMARR CDDCC GGRGR GRCYG PQCLC R (SEQ ID NO: 6);

KMCMP CFTTD HQMAR ACDDC CGGAG RGACY GPQCL CR (SEQ ID NO: 7);

KMCMP CFTTD HQMAR RCDDC CGGRG RGRCY GPQCL CR (SEQ ID NO: 8);

MCMPC FTTDH QMARA CDDCC GGAGR GACYG PQCLC RK (SEQ ID NO: 9);

MCMPC FTTDH QMARR CDDCC GGRGR GRCYG PQCLC RK (SEQ ID NO: 10);

MCMPC FTTDH QMARC DDCCG GKGRG KCYGP QCLCR (SEQ ID NO: 11);

MCMPC FTTDH QMARK CDDCC GGGRG KCYGP QCLCR (SEQ ID NO: 12);

MCMPC FTTDH QMARK CDDCC GGKGR GCYGP QCLCR (SEQ ID NO: 13);

MCMPC FTTDH QMARC DDCCG GGRGK CYGPQ CLCR (SEQ ID NO: 14);

MCMPC FTTDH QMARC DDCCG GKGRG CYGPQ CLCR (SEQ ID NO: 15);

MCMPC FTTDH QMARK CDDCC GGGRG CYGPQ CLCR (SEQ ID NO: 16);

MCMPC FTTDH QMARA CDDCC GGAGR GKCYG PQCLC R (SEQ ID NO: 17);

MCMPC FTTDH QMARA CDDCC GGKGR GACYG PQCLC R (SEQ ID NO: 18);

MCMPC FTTDH QMARK CDDCC GGAGR GACYG PQCLC R (SEQ ID NO: 19);

MCMPC FTTDH QMARR CDDCC GGRGR GKCYG PQCLC R (SEQ ID NO: 20);

MCMPC FTTDH QMARR CDDCC GGKGR GRCYG PQCLC R (SEQ ID NO: 21);

MCMPC FTTDH QMARK CDDCC GGRGR GRCYG PQCLC R (SEQ ID NO: 22);

MCMPC FTTDH QMARR CDDCC GGKGR GACYG PQCLC R (SEQ ID NO: 23;

MCMPC FTTDH QMARC DDCCG GAGRG ACYGP QCLCR (SEQ ID NO: 24);

MCMPC FTTDH QMARA CDDCC GGGRG ACYGP QCLCR (SEQ ID NO: 25);

MCMPC FTTDH QMARR CDDCC GGRGR GCYGP QCLCR (SEQ ID NO: 26);

TTDHQ MAR (SEQ ID NO: 27);

MSMPS FTTDH QMARK SDDSS GGKGR GKSYG PQSLS R (SEQ ID NO: 28);

MSMPS FTTDH (SEQ ID NO: 29);

SMPSF TTDHQ (SEQ ID NO: 30);

MPSFT TDHQM (SEQ ID NO: 31);

PSFTT DHQMA (SEQ ID NO: 32);

SFTTD HQMAR (SEQ ID NO: 33);

FTTDH QMARK (SEQ ID NO: 34);

TTDHQ MARKS (SEQ ID NO: 35);

TDHQM ARKSD (SEQ ID NO: 36);

DHQMA RKSDD (SEQ ID NO: 37);

HQMAR KSDDS (SEQ ID NO: 38);

QMARK SDDSS (SEQ ID NO: 39);

MARKS DDSSG (SEQ ID NO: 40);

ARKSD DSSGG (SEQ ID NO: 41);

RKSDD SSGGK (SEQ ID NO: 42);

KSDDS SGGKG (SEQ ID NO: 43);

SDDSS GGKGR (SEQ ID NO: 44);

DDSSG GKGRG (SEQ ID NO: 45);

DSSGG KGRGK (SEQ ID NO: 46);

SSGGK GRGKS (SEQ ID NO: 47);

SGGKG RGKSY (SEQ ID NO: 48);

GGKGR GKSYG (SEQ ID NO: 49);

GKGRG KSYGP (SEQ ID NO: 50);

KGRGK SYGPQ (SEQ ID NO: 51);

GRGKS YGPQS (SEQ ID NO: 52);

RGKSY GPQSL (SEQ ID NO: 53);

GKSYG PQSLS (SEQ ID NO: 54);

KSYGP QSLSR (SEQ ID NO: 55);

TTDHQ MARKC (SEQ ID NO: 56);

CMPSF TTDHQ (SEQ ID NO: 57);

CFTTD HQMAR (SEQ ID NO: 58);

TDHQM ARKCD (SEQ ID NO: 59);

DCSGG KGRGG (SEQ ID NO: 60);

CSGGK GRGKS (SEQ ID NO: 61);

CGGKG RGKSY (SEQ ID NO: 62);

SSGGK GRGKC (SEQ ID NO: 63);

SGGKG RGKCY (SEQ ID NO: 64);

KGRGK SY (SEQ ID NO: 65);

KGRGK CY (SEQ ID NO: 66);

HHHHH HMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR (SEQ ID NO: 69);

YMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR (SEQ ID NO: 70);

YSYMC MPCFT TDHQM ARKCD DCCGG KGRGK CYGPQ CLCR (SEQ ID NO: 71);

MCMPC FTTDH QMARK CDDCC GGKGR GKCFG PQCLC R (SEQ ID NO: 72);

RCKPC FTTDP QMSKK CADCC GGKGK GKCYG PQCLC (SEQ ID NO: 73);

RCSPC FTTDQ QMTKK CYDCC GGKGK GKCYG PQCIC APY (SEQ ID NO: 74);

KMCMP CFTTD HQMAR KCDDC CGGKG RGKCY GPQCL CR (SEQ ID NO: 75); or,

MCMPC FTTDH QMARK CDDCC GGKGR GKCYG PQCLC RK (SEQ ID NO: 76).

35. The pharmaceutical composition according to any one of claims 27-34, wherein the pharmaceutical composition is formulated for parenteral delivery.

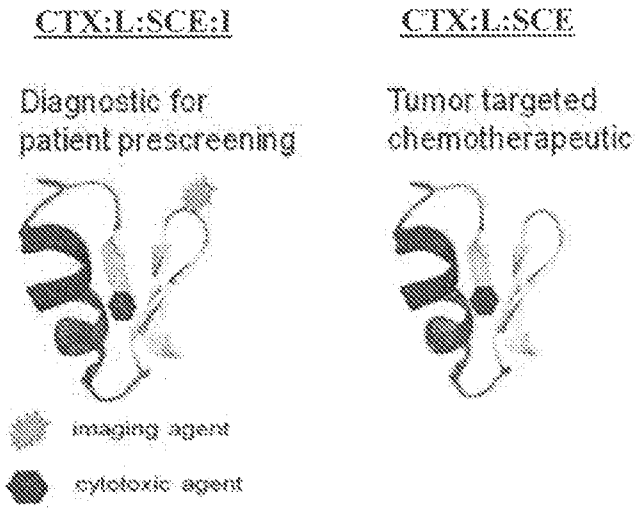

FIG. 1. Schematic diagram of CTX:L:SCE:I and CTX:L:SCE.

K = kidney; T = tumor

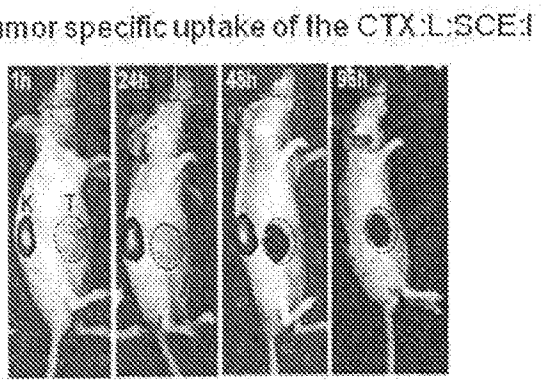

FIG. 2. In vivo imaging of mouse xenografts containing a human tumor.

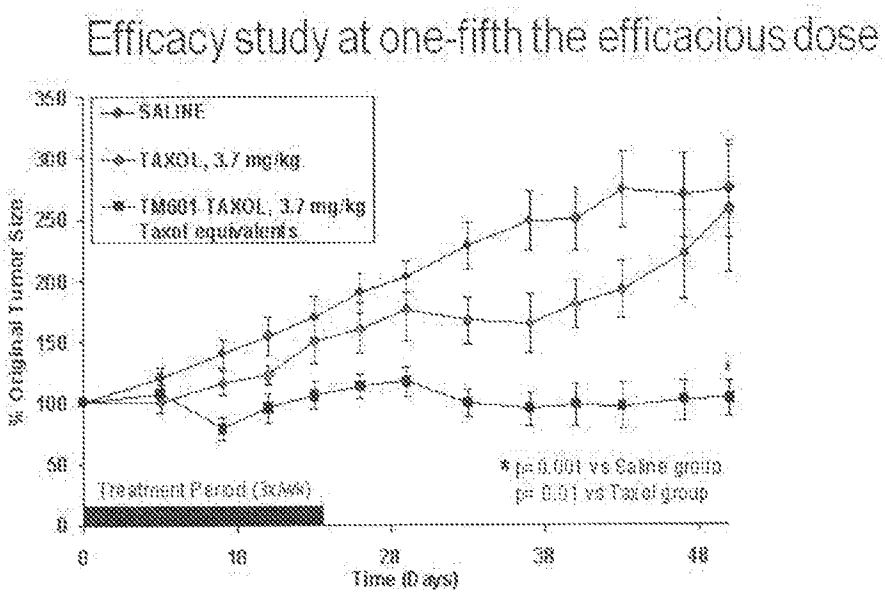

FIG. 3. In vivo activity of the CTX LSCE in treating human tumors in mouse xenografts.

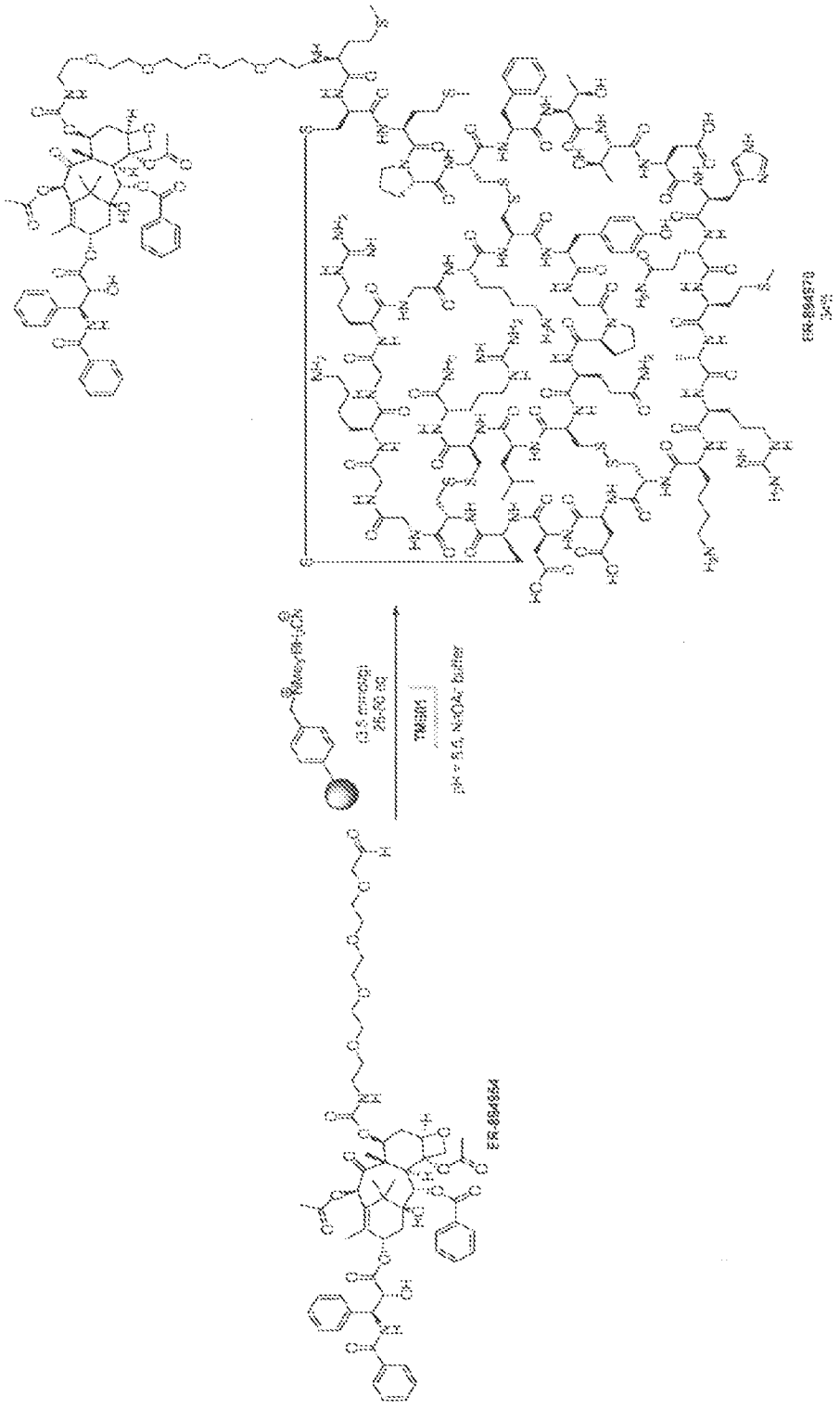

FIG.4

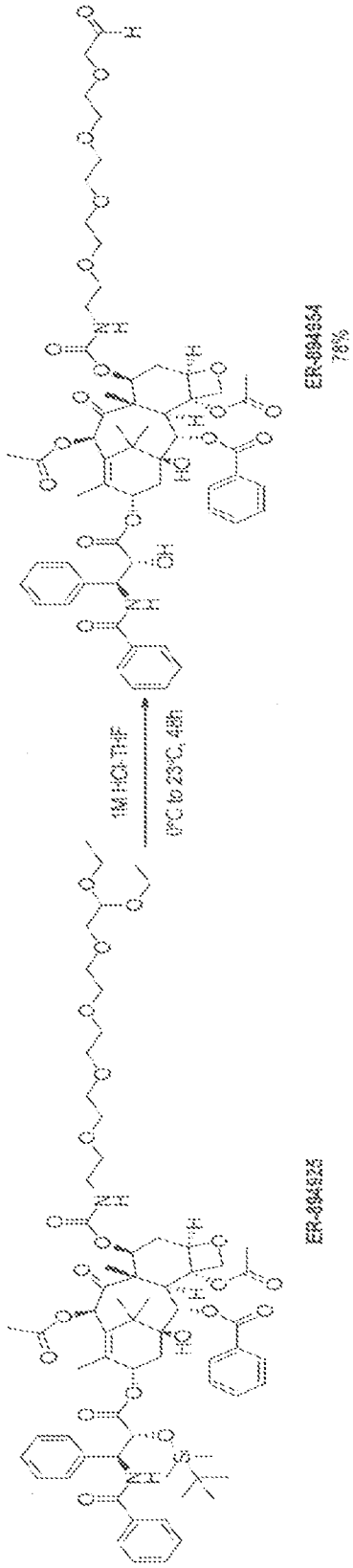

FIG. 5

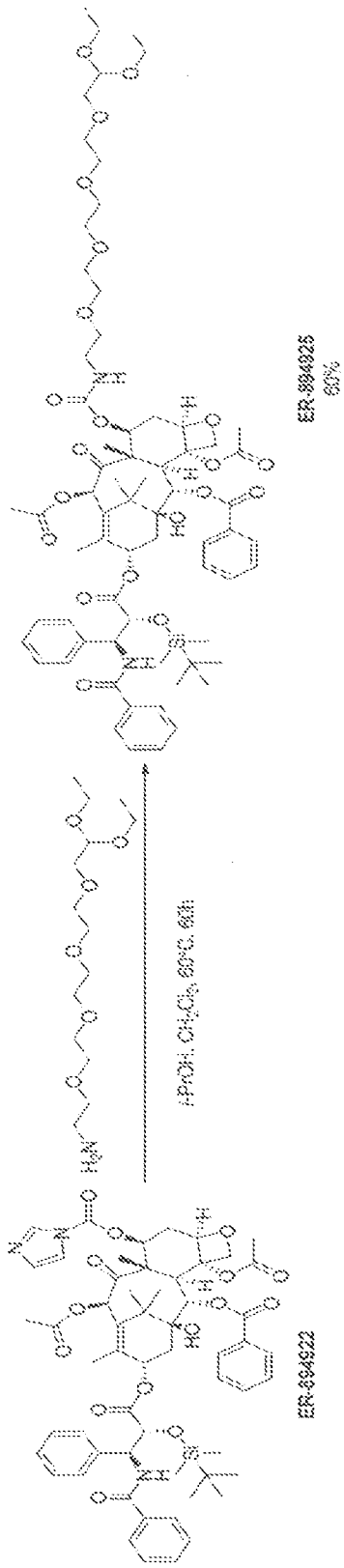

FIG. 6

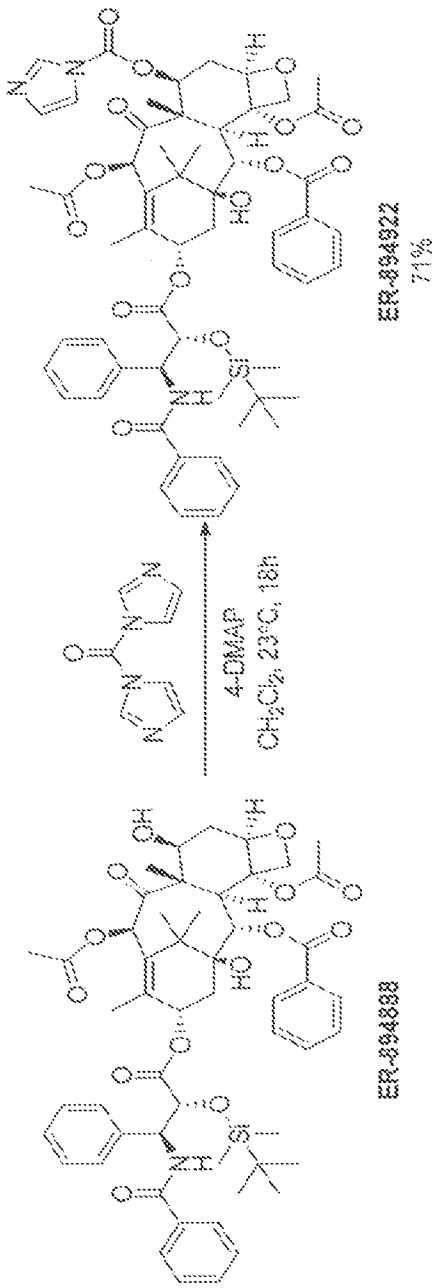

FIG. 7

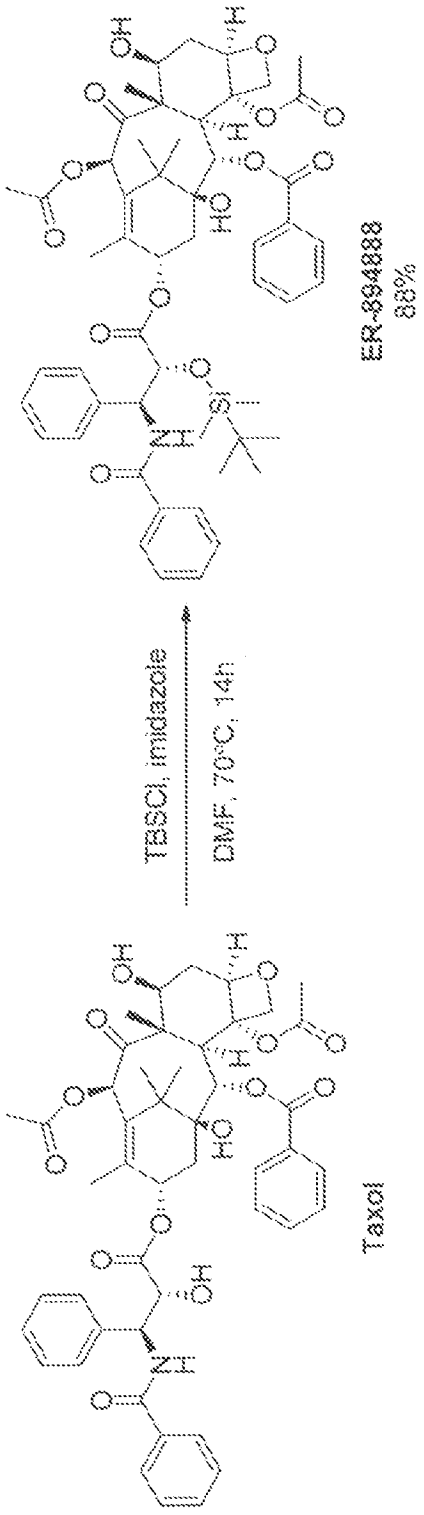

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/044480

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 A61K49/00
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUN C ET AL: "Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles", NIH Public Access Author manuscript NANOMEDICINE, August 2008 (2008-08), pages 1-16, XP002684887, DOI: 10.2217/17435889.3.4.495 Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2890026/ [retrieved on 2012-10-09] the whole document ----- -/--	1-35

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 October 2012	24/10/2012

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

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VEISEH O ET AL: "A ligand-mediated nanovector for targeted gene delivery and transfection in cancer cells", BIOMATERIALS, vol. 30, no. 4, 1 February 2009 (2009-02-01), pages 649-657, XP025693623, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB ISSN: 0142-9612, DOI: 10.1016/J.BIOMATERIALS.2008.10.003 [retrieved on 2008-11-05]	1-9,11, 13-24, 27-31, 34,35
Y	the whole document	10,12, 32,33

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X	WO 2009/021136 A1 (TRANSMOLECULAR INC [US]; JACOBY DOUGLAS [US]; SENTISSI ABDELLAH [US]) 12 February 2009 (2009-02-12) cited in the application claims 1,10	1-9,11, 13-24, 27-31, 34,35

X	WO 97/24619 A1 (UAB RESEARCH FOUNDATION [US]) 10 July 1997 (1997-07-10) example 19	1-24, 27-35

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/044480

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		EP 2431385 A2	21-03-2012
		ES 2341767 T3	28-06-2010
		JP 2005537234 A	08-12-2005
		JP 2011153149 A	11-08-2011
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		WO 03101475 A1	11-12-2003