HSP90-BINDING CONJUGATES AND COMBINATION THERAPIES THEREOF

REFERENCED TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 62/866,140, filed June 25, 2019, entitled HSP90-BINDING CONJUGATES AND COMBINATION THERAPIES THEREOF, U.S. Provisional Patent Application No. 62/899,777, filed September 13, 2019, entitled TARGETED THERAPEUTICS, and U.S. Provisional Patent Application No. 63/035,048, filed June 5, 2020, entitled HSP90-BINDING CONJUGATES AND COMBINATION THERAPIES THEREOF, the contents of each of which are herein incorporated by reference in their entirety.

FIELD OF THE DISCLOSURE

[0002] The disclosure generally relates to therapies for treating cancer.

BACKGROUND

[0003] Although tremendous advances have been made in chemotherapy, currently available therapeutics and therapies remain unsatisfactory and the prognosis for the majority of patients diagnosed with chemotherapeutically treated diseases (*e.g.*, cancer) remains poor. Often, the applicability and/or effectiveness of chemotherapy, as well as other therapies and diagnostics employing potentially toxic moieties, is limited by undesired side effects.

[0004] Many disease and disorders are characterized by the presence of high levels of certain proteins in specific types of cells. In some cases, the presence of these high levels of protein is caused by overexpression. Historically, some of these proteins have been useful targets for therapeutic molecules or used as biomarkers for the detection of disease. One class of overexpressed intracellular protein that has been recognized as a useful therapeutic target is known as the heat shock proteins.

[0005] Heat shock proteins (HSPs) are a class of proteins that are up-regulated in response to elevated temperature and other environmental stresses, such as ultraviolet light, nutrient deprivation, and oxygen deprivation. HSPs have many known functions, including acting as chaperones to other cellular proteins (called client proteins) to facilitate their proper folding and repair, and to aid in the refolding of misfolded client proteins. There are several known families of HSPs, each having its own set of client proteins. Hsp90 is one of the most abundant HSP families, accounting for about 1-2% of proteins in a cell that is not under stress and increasing to about 4-6% in a cell under stress.

[0006] Inhibition of Hsp90 results in degradation of its client proteins via the ubiquitin proteasome pathway. Unlike other chaperone proteins, the client proteins of Hsp90 are mostly protein kinases or transcription factors involved in signal transduction, and a number of its client proteins have been shown to be involved in the progression of cancer. Hsp90 has been shown by mutational analysis to be necessary for the survival of normal eukaryotic cells. However, Hsp90 is overexpressed in many tumor types, indicating that it may play a significant role in the survival of cancer cells and that cancer cells may be more sensitive to inhibition of Hsp90 than normal cells. For example, cancer cells typically have a large number of mutated and overexpressed oncoproteins that are dependent on Hsp90 for folding. In addition, because the environment of a tumor is typically hostile due to hypoxia, nutrient deprivation, acidosis, etc., tumor cells may be especially dependent on Hsp90 for survival. Moreover, inhibition of Hsp90 causes simultaneous inhibition of a number of oncoproteins, as well as hormone receptors and transcription factors, making it an attractive target for an anti-cancer agent. In view of the above, Hsp90 has been an attractive target of drug development, including such Hsp90 inhibitor (Hsp90i) compounds as ganetespib, AUY-922, and IPI-504. At the same time, the advancement of certain of these compounds which showed early promise, *e.g.*, geldanamycin, has been slowed by those compounds' toxicity profile. Hsp90i compounds developed to date are believed to show great promise as cancer drugs, but other ways the ubiquity of Hsp90 in cancer cells might be leveraged have heretofore remained unexplored until now. Accordingly, the need exists for therapeutic molecules that selectively target proteins, such as Hsp90, that are overexpressed in cells associated with particular diseases or disorders.

SUMMARY

[0007] The present application provides a method of treating cancer in a subject in need thereof, comprising administering an effective amount of Conjugate **1** (SDC-TRAP-0063) or a pharmaceutically acceptable salt thereof to the subject at a dose of 150 mg/m^2, 175 mg/m^2, or 200 mg/m^2 body surface area. Conjugate **1** or a pharmaceutically acceptable salt thereof may be administered once a week for 3 weeks on Day 1, Day 8, and Day 15 followed with one week of no treatment. The cancer may be a cancer of anus, breast, cholangiocarcinoma, colon, Ewing sarcoma, liver, lung, neuroendocrine (unknown primary), ovary, pancreas, salivary gland, or sarcoma. In some embodiments, the cancer is pancreatic adenocarcinoma,

endometrial adenocarcinoma, or squamous cell carcinoma of the anus, cervix, or head and neck.

[0008] The present application also provides method of treating cancer in a subject in need thereof, comprising administering an effective amount of Conjugate **1** or a pharmaceutically acceptable salt thereof to the subject, wherein the subject has received at least one previous anticancer therapy.

[0009] The present application provides a method of treating cancer in a subject in need thereof, comprising administering an effective amount of Conjugate **1** or a pharmaceutically acceptable salt thereof to the subject, wherein the subject shows partial response or stable disease after the treatment.

[0010] In some embodiments, after treatment with Conjugate **1** or a pharmaceutically acceptable salt thereof to the subject, the ratio of the tumor Conjugate **1** level to the plasma Conjugate **1** level is more than 5; the tumor Conjugate **1** level is more than 300 nM; the ratio of the tumor SN-38 level to the plasma SN-38 level is more than 3; and/or the tumor SN-38 level is more than 80 nM.

[0011] The present disclosure also relates to a method of treating a patient with a hyperproliferative disorder such as cancer, comprising administering to the patient: (A) a first component which comprises, as an active agent, Conjugate **1**, or a pharmaceutically-acceptable salt thereof; and optionally (B) a second component which comprises, as an active agent, Component II, or a pharmaceutically-acceptable salt thereof; the amounts of said active agents being such that the combination thereof is therapeutically-effective in the treatment of said hyperproliferative disorder. Component I may comprise a conjugate that targets heat shock protein 90 (HSP90).

[0012] The present disclosure further relates to a composition comprising: (A) a first component which comprises, as an active agent, Conjugate **1**, or a pharmaceutically-acceptable salt thereof; and (B) a second component which comprises, as an active agent, Component I, or a pharmaceutically-acceptable salt thereof.

[0013] The present disclosure also relates to a kit comprising: (A) a first component which comprises, as an active agent, Conjugate **1**, or a pharmaceutically-acceptable salt thereof; and (B) a second component which comprises, as an active agent, Component II, or a pharmaceutically-acceptable salt thereof.

[0014] In addition, the present disclosure relates to the use of Conjugate **1**, or a pharmaceutically-acceptable salt thereof, and Component II, or a pharmaceutically-acceptable salt thereof, for the treatment of a hyperproliferative disorder.

[0015] A yet further aspect of the present disclosure is the use of Conjugate **1**, or a pharmaceutically-acceptable salt thereof, and Component II, or a pharmaceutically-acceptable salt thereof, for the preparation of a medicament for the treatment of a hyperproliferative disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1A shows efficacies in the H1975 model at Conjugate **1** weekly doses of 72 and 100 mg/kg.

[0017] Fig. 1B shows efficacies in the H1975 model at Conjugate **1** weekly doses of 150 mg/kg.

[0018] Fig. 2 shows an estimate of plasma exposures of Conjugate **1** and niraparib, and tumor xenograft exposures of Conjugate **1**.

[0019] Fig. 3A shows average tumor volumes in mice bearing SKOV3 tumors after treatments with vehicle, talazoparib, Conjugate **1**, and Conjugate **1** / talazoparib combination therapy.

[0020] Fig. 3B shows average tumor volumes in mice bearing NCI-H460 tumors after treatments with vehicle, talazoparib, Conjugate **1**, and Conjugate **1** / talazoparib combination therapy.

[0021] Fig. 3C shows pH2AX quantity in NCI-H460 NSCLC xenograft tumors after treatments with talazoparib, Conjugate **1**, and Conjugate **1** / talazoparib combination therapy.

DETAILED DESCRIPTION

[0022] The present disclosure relates to a combination therapy of at least two distinct therapeutic agents for treating a hyperproliferative disorder such as cancer. Each distinct therapeutic agent is referred to as a "component" of the combination therapy. The combination therapy of the disclosure is highly effective in treating various types of cancer and shows enhanced effect compared to the activity of each of the components administered alone. The terms "combination therapy" or "combined treatment" or "in combination" as used herein refers to any form of concurrent or parallel treatment with at least two distinct therapeutic agents. A hyperproliferative disorder embraces any disease or malady characterized by uncontrolled cell proliferation.

[0023] The components of the combination therapy may be administered simultaneously, sequentially, or at any order. The components may be administered at different dosages, with different dosing frequencies, or via different routes, whichever is suitable.

[0024] The term "administered simultaneously" as used herein is not specifically restricted and means that the components of the combination therapy are substantially administered at the same time, e.g. as a mixture or in immediate subsequent sequence.

[0025] The term "administered sequentially" as used herein is not specifically restricted and means that the components of the combination therapy are not administered at the same time but one after the other, or in groups, with a specific time interval between administrations. The time interval may be the same or different between the respective administrations of the components of the combination therapy and may be selected, for example, from the range of 2 minutes to 96 hours, 1 to 7 days or one, two or three weeks. Generally, the time interval between the administrations may be in the range of a few minutes to hours, such as in the range of 2 minutes to 72 hours, 30 minutes to 24 hours, or 1 to 12 hours. Further examples include time intervals in the range of 24 to 96 hours, 12 to 36 hours, 8 to 24 hours, and 6 to 12 hours. In some embodiments, Component I is administered before Component II. In some embodiments, Component II is administered before Component I.

[0026] The molar ratio of the components is not particularly restricted. For example, when two components are combined in a composition, the molar ratio between the two components may be in the range of 1:500 to 500:1, or of 1:100 to 100:1, or of 1:50 to 50:1, or of 1:20 to 20:1, or of 1:5 to 5:1, or 1:1. Similar molar ratios apply when more than two components are combined in a composition. Each component may comprise, independently, a predetermined molar weight percentage from about 1% to 10%, or about 10% to about 20%, or about 20% to about 30%, or about 30% to 40%, or about 40% to 50%, or about 50% to 60%, or about 60% to 70%, or about 70% to 80%, or about 80% to 90%, or about 90% to 99% of the composition.

I. Components in the Combination Therapy

[0027] One aspect of the present disclosure provides a combination therapy of treating a subject with a hyperproliferative disorder such as cancer, comprising administering to the patient: (A) a first component which comprises, as an active agent, Component I (or Compound I), or a prodrug, derivative, or pharmaceutically-acceptable salt thereof; and (B) a second component which comprises, as an active agent, Component II (or Compound II), or a

prodrug, derivative, or a pharmaceutically-acceptable salt thereof; the amounts of said active agents being such that the combination thereof is therapeutically-effective in the treatment of said hyperproliferative disorder.

[0028] In some embodiments, Component I is a small molecule conjugate comprising an active agent or prodrug thereof attached to a targeting moiety, wherein the targeting moiety binds to heat shock protein 90 (HSP90).

[0029] Component II is different from Component I. In some embodiments, Component II comprises a therapeutic agent that treats cancer, such as a checkpoint inhibitor. A checkpoint inhibitor, as used herein, refers to an active agent that blocks immunosuppressive signals in the tumor microenvironment. In some embodiments, the active agent may be an antagonistic agent specific to a coinhibitory checkpoint molecule (e.g., CTLA-4, PD1, PD-L1) that can antagonize or reduce the inhibitory signal to effector immune cells. In some embodiments, the active agent may be an inhibitor that can inhibits and reduces the activity of immune suppressive enzymes (e.g. ARG and IDO) and cytokines (e.g. IL-10), chemokines and other soluble factors (e.g., TGF-β and VEGF) in the tumor microenvironment.

[0030] The term “small molecule” as used herein refers to an organic molecule that is less than 2000 g/mol in molecular weight, less than 1500 g/mol, less than 1000 g/mol, less than 800 g/mol, or less than 500 g/mol. Small molecules are non-polymeric and/or non-oligomeric.

[0031] The term "targeting moiety" as used herein refers to a moiety that binds to or localizes to a specific locale. The moiety may be, for example, a protein, nucleic acid, nucleic acid analog, carbohydrate, or small molecule. The locale may be a tissue, a particular cell type, or a subcellular compartment. In some embodiments, a targeting moiety can specifically bind to a selected molecule such as a protein.

[0032] In some instances, a conjugate may have a molecular weight of less than about 50,000 Da, less than about 40,000 Da, less than about 30,000 Da, less than about 20,000 Da, less than about 15,000 Da, less than about 10,000 Da, less than about 8,000 Da, less than about 5,000 Da, or less than about 3,000 Da. In some cases, the conjugate may have a molecular weight of between about 1,000 Da and about 50,000 Da, between about 1,000 Da and about 40,000 Da, in some embodiments between about 1,000 Da and about 30,000 Da, in some embodiments bout 1,000 Da and about 50,000 Da, between about 1,000 Da and about 20,000 Da, in some embodiments between about 1,000 Da and about 15,000 Da, in some

embodiments between about 1,000 Da and about 10,000 Da, in some embodiments between about 1,000 Da and about 8,000 Da, in some embodiments between about 1,000 Da and about 5,000 Da, and in some embodiments between about 1,000 Da and about 3,000 Da. The molecular weight of the conjugate may be calculated as the sum of the atomic weight of each atom in the formula of the conjugate multiplied by the number of each atom. It may also be measured by mass spectrometry, NMR, chromatography, light scattering, viscosity, and/or any other methods known in the art. It is known in the art that the unit of molecular weight may be g/mol, Dalton (Da), or atomic mass unit (amu), wherein 1 g/mol = 1 Da = 1 amu.

[0033] Component I and Component II may be administered simultaneously, sequentially, or at any order. They may be administered at different dosages, with different dosing frequencies, or via different routes, whichever is suitable.

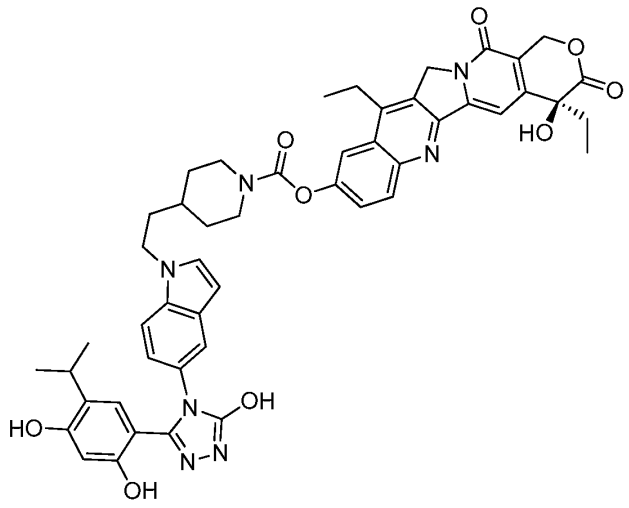
Component I

[0034] In some embodiments, Component I is a conjugate comprising an active agent or prodrug thereof attached to a targeting moiety, wherein the targeting moiety binds to a heat shock protein, such as HSP90. The targeting moiety may be selected from ganetespib, geldanamycin (tanespimycin), IPI-493, macbecins, tripterins, tanespimycins, 17-AAG (alvespimycin), KF-55823, radicicols, KF-58333, KF-58332, 17-DMAG, IPI-504, BIIB-021, BIIB-028, PU-H64, PU-H71, PU-DZ8, PU-HZ151, SNX-2112, SNX-2321, SNX-5422, SNX-7081, SNX-8891, SNX-0723, SAR-567530, ABI-287, ABI-328, AT-13387, NSC-113497, PF-3823863, PF-4470296, EC-102, EC-154, ARQ-250-RP, BC-274, VER-50589, KW-2478, BHI-001, AUY-922, EMD-614684, EMD-683671, XL-888, VER-51047, KOS-2484, KOS-2539, CUDC-305, MPC-3100, CH-5164840, PU-DZ13, PU-HZ151, PU-DZ13, VER-82576, VER-82160, VER-82576, VER-82160, NXD-30001, NVP-HSP990, SST-0201CL1, SST-0115AA1, SST-0221AA1, SST-0223AA1, novobiocin, herbinmycin A, radicicol, CCT018059, PU-H71, celastrol, or a tautomer, analog, or derivative thereof.

[0035] In some examples, Component I comprises SN-38 or irinotecan, lenalidomide, vorinostat, 5-Fluorouracil (5-FU), abiraterone, bendamustine, crizotinib, doxorubicin, pemetrexed, fulvestrant, topotecan, Vascular Disrupting Agent (VDA), or a fragment, derivative, or analog thereof as an active agent.

[0036] In some examples, Component I may be any conjugate of PCT Application No. PCT/US13/36783 (WO2013/158644) filed on April 16, 2013, the contents of which are incorporated herein by reference.

[0037] In one example, Component I is a conjugate comprising ganetespib or its tautomer as a targeting moiety and SN-38 as an active agent. Component I may be Conjugate **1** (also referred to as SDC-TRAP-0063) having a structure of



1 (SDC-TRAP-0063).

[0038] ((*S*)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl 4-(2-(5-(3-(2,4-dihydroxy-5-isopropylphenyl)-5-hydroxy-4H-1,2,4-triazol-4-yl)-1H-indol-1-yl)ethyl)piperidine-1-carboxylate) or its tautomer.

[0039] Conjugate **1** is an injectable, synthetic small molecule drug conjugate comprised of ganetespib attached through a cleavable linker to SN-38, the active metabolite of the marketed topoisomerase I inhibitor, irinotecan. This conjugate leverages the enhanced tumor targeting and preferential tumor retention properties of HSP90 to deliver SN-38 resulting in broad preclinical antitumor activity.

[0040] In some embodiments, Conjugate **1** may be used to treat cancers such as sarcoma, pancreatic cancer, cholangiocarcinoma, ovarian cancer, small cell lung cancer (SCLC), colon cancer, oral cancer, endometrial cancer, cervical cancer, head and neck cancer, squamous cell cancer, or anal cancer. In some embodiments, Conjugate **1** may be used to treat cancers caused by viruses, such as cancers caused by Hepatitis B virus (HBV), Hepatitis C virus (HCV), Epstein-Barr virus (EBV), Human T-cell Leukemia virus (HTLV), Kaposi's sarcoma-associated herpesvirus (KSHV), or Human papillomavirus (HPV). In some embodiments, Conjugate **1** is administered to patients who are HPV positive. In some embodiments, Conjugate **1** is administered to patients who have cancer and are HPV positive,

wherein the cancer may be selected from oral cancer, cervical cancer, head and neck cancer, or squamous cell cancer.

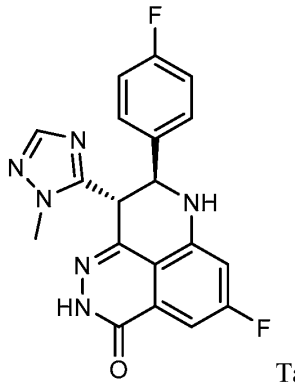
Component II

[0041] In some embodiments, Component II comprises a therapeutic agent that treats cancer, which is different from Component I.

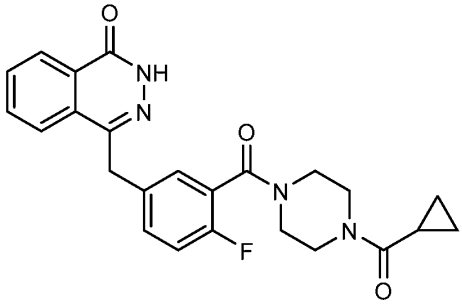
[0042] In some embodiments, Component II may be a chemotherapeutic agent. In some embodiments, Component II may be a chemotherapeutic agent that is used to treat prostate cancer, breast cancer, non-small cell lung cancer (large cell lung cancer), small cell lung cancer, or ovarian cancer.

[0043] In some embodiments, Component I and Component II together cause synthetic lethality and can be used to treat cancers. Cancers are caused by mutations in multiple genes and abnormalities in multiple signaling pathways. Two targets (e.g., genes) are synthetic lethal if the mutations in any one of them will not change the viability of a cell or an organism, but simultaneous mutations in both targets will result in a lethal phenotype. Synthetic lethality anticancer therapeutics may include poly ADP-ribose polymerase inhibitors for *BRCA1*- and *BRCA2*-mutant cancers, checkpoint inhibitors for p53 mutant cancers, and small molecule agents targeting *RAS* gene mutant cancers.

[0044] In some examples, Component II may be a poly ADP ribose polymerase (PARP) inhibitor. PARP is a group of ADP-ribose transferase enzymes that catalyze polyADP-ribosylation of proteins by transferring ADP-ribose groups from the donor substrate nicotinamide adenine dinucleotide (NAD^+) to glutamic acid, aspartic acid, and lysine residues in the acceptor proteins, thereby regulating the functions of those proteins. PARP inhibitors can inhibit the repair of DNA damage and have been approved, primarily, in tumors with defects in DNA repair due to mutations in BRCA1/2. It is challenging to combine PARP inhibitors with other drugs due to overlapping toxicities. Some cancers have high BRCA1 levels and are insensitive to PARP inhibition. The HSP90-binding Component I may have DNA damaging effect and may sensitize the cells to PARP inhibition. Non-limiting examples of PARP inhibitors may include talazoparib (BMN-673), olaparib (AZD-2281), niraparib (MK-4827), iniparib (BSI 201), veliparib (ABT-888), rucaparib (AG014699, PF-01367338), CEP 9722, Fluzoparib (SHR3162), Senaparib (IMP4297), KU0058684, KU0058948, NU1025, or AG14361.



Talazoparib (CAS No. 1207456-01-6)



Olaparib (CAS No. 763113-22-0)

[0045] In some embodiments, Component II may provide supportive care for cancer patients and/or reduce the side effects of Component I. In some embodiments, Component II is a cancer symptom relief drug. The symptom relief drug may reduce diarrhea or the side effects of chemotherapy or radiation therapy. Non-limiting examples of symptom relief drugs include: octreotide or lanreotide; interferon, cypoheptadine or any other antihistamines; and/or a symptom relief drug for carcinoid symdrome, such as telotristat or telotristat etiprate (LX1032, Lexicon®).

[0046] In some embodiments, Component II may be 5-fluorouracil (5-FU), leucovorin (folinic acid), irinotecan, or oxaliplatin, or a derivative or any combination thereof. In some embodiments, Component II is a combination of 5-FU and leucovorin.

[0047] In some embodiments, Component II may be sirtuin modulators such as substituted bridged urea analogs as disclosed in US10590135, bromodomain inhibitors such as benzimidazole derivatives as disclosed in US10442786, bromodomain inhibitors such as pyridinone dicarboxamide as disclosed in US10428026, PAD4 inhibitors such as benzoimidazole derivatives as disclosed in US10407407, NRF2 regulators such as biaryl pyrazoles as disclosed in US10364256, CXCR2 inhibitors such as 1-(cyclopent-2-en-1-yl)-3-(2-hydroxy-3-(arylsulfonyl)phenyl)urea derivatives as disclosed in US10336719, Prolyl

hydroxylase inhibitors as disclosed in US10336711, P13 kinase inhibitors such as benzimidazole derivatives as disclosed in US8435988, LSD1 inhibitors such as cyclopropylamines as disclosed in US8853408, bromodomain inhibitors such as tetrahydroquinoline derivatives as disclosed in US10059699, estrogen receptor inhibitors such as benzothiophene derivatives as disclosed in US9988376, fatty acid synthase inhibitors such as triazolones as disclosed in US8802864, PI3 kinase inhibitors such as quinoline derivatives as disclosed in US8138347, aurora kinase inhibitors as disclosed in US8138338, IGF-1R inhibitors such as 2-[2-{phenylamino}-1H-pyrrolo[2,3-D]pyrimidin-4-yl)amino] benzamide derivatives as disclosed in US7981903, ErbB kinase inhibitors such as 2-pyrimidinyl pyrazolopyridine as disclosed in US7807673, p38 inhibitors such as nicotinamide derivatives as disclosed in US7709506, kinase inhibitors such as heterocyclic amides as disclosed in US20200062735, bromodomain inhibitors such as pyridyl derivatives as disclosed in US20200009140, PERK inhibitors such as isoquinoline derivatives as disclosed in US20190241573, DNMT1 inhibitors such as substituted pyridines as disclosed in US20190194166, protein tyrosine kinase inhibitors such as bicyclic heteroaromatic compounds as disclosed in US20150065527, imidazopyridine Kinase Inhibitors such as the ones disclosed in US20100216779, androgen receptor modulators such as aniline derivatives disclosed in EP1725522, PLK modulators such as benzimidazole thiophene compounds as disclosed in EP1922316, dipeptidyl peptidase inhibitors such as fluoropyrrolidines as disclosed in EP1862457, or tumor necrosis factor inhibitors such as thienodibenzoazulene compounds as disclosed in EP1284977.

[0048] In some embodiments, Component II may be a checkpoint inhibitor. Tumor cells can induce an immunosuppressive microenvironment to help them escape the immune surveillance. The immune suppression in the tumor microenvironment is either induced by intrinsic immune suppression mechanisms, or directly by tumors. Component II of the combination therapy comprises a checkpoint inhibitor that blocks such immunosuppressive signals in the tumor microenvironment.

[0049] In some embodiments, Component II may be an antagonistic agent specific to a coinhibitory checkpoint molecule that can antagonize or reduce the inhibitory signal to effector immune cells (e.g. cytotoxic T cells and natural killer cells).

[0050] In some embodiments, Component II may be an inhibitor that can inhibits and reduces the activity of immune suppressive enzymes (e.g. ARG and IDO) and cytokines (e.g.

IL-10), chemokines and other soluble factors (e.g., TGF-β and VEGF) in the tumor microenvironment.

The Tumor Microenvironment

[0051] In adaptive immune responses for eliminating tumor cells, cytotoxic T cell activation needs both a primary signal from a specific antigen (i.e. first signal) and one or more co-stimulatory signals (i.e. secondary signal). Antigen presenting cells (APCs, e.g., dendritic cells (DCs)) process tumor associated antigens (TAAs) and present antigenic peptides derived from TAAs (i.e. epitopes) on the cell surface as peptide/MHC molecule (class I/II) (p/MHC) complexes and T cells engage APCs loaded with TAAs via their T cell receptors (TCRs) which recognize the p/MHC complexes. This ligation is the primary signal to activate cancer specific cytotoxic T cells. Additionally, a secondary co-stimulating signal is provided by co-stimulatory receptors on the T cells and their ligands (or coreceptors) on the APCs. The interaction between co-stimulatory receptors and their ligands can regulate T cell activation and enhance its activity. CD28, 4-1BB (CD137), and OX40 are well studied co-stimulatory receptors on T cells, which bind to B7-1/2 (CD80/CD86), 4-1BB (CD137L) and OX-40L, respectively on APCs. In normal circumstance, to prevent excessive T-cell proliferation and balance the immunity, a co-inhibitory signal, e.g., CTLA-4, can be induced and expressed by activated T cells and competes with CD28 in binding to B7 ligands on APCs. This can mitigate a T cell response in a normal circumstance. However, in some cancers, tumor cells and regulatory T cells infiltrating the tumor microenvironment can constitutively express CTLA-4. This co-inhibitory signal suppresses the co-stimulatory signal, therefore, depleting an anti-cancer immune response. This immune suppressing mechanism by tumor cells is referred to as an immune checkpoint or checkpoint pathway.

[0052] In addition to CTLA-4 signal, activated T cells can also be induced to express another inhibitory receptor, PD-1 (programed death 1). In normal situation, as an immune response progresses, CD4+ and CD8+ T lymphocytes upregulate the expression of these inhibitory checkpoint receptors (e.g., PD-1). Inflammatory conditions prompt IFN release, which will upregulate the expression of PD-1 ligands: PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-DC) in peripheral tissues, to maintain immune tolerance to prevent autoimmunity. Many human cancer types have been demonstrated to express PD-L1 in the tumor microenvironment (e.g., Zou and Chen, inhibitory B7-family in the tumor

microenvironment. 2008, *Nat Rev Immunol*, 8: 467-477). The PD-1/PD-L1 interaction is highly active with the tumor microenvironment, inhibiting T cell activation.

[0053] Other identified co-inhibitory signals in the tumor microenvironment include TIM-3, LAG-3, BTLA, CD160, CD200R, TIGIT, KLRG-1, KIR, CD244/2B4, VISTA and Ara2R.

[0054] In addition, the tumor microenvironment contains suppressive elements including regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC) and tumor-associated macrophage (TAM); soluble factors such as interleukin 6 (IL-6), IL-10, vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF-β). An important mechanism by which IL-10, TGF-β, and VEGF counteract the development of an anti-cancer immune response is through inhibition of dendritic cell (DC) differentiation, maturation, trafficking, and antigen presentation (Gabrilovich D: Mechanisms and functional significance of tumour-induced dendritic-cell defects, *Nat Rev Immunol*, 2004, 4: 941-952).

[0055] *Regulatory T cells (Treg)*: CD4+CD25+ Treg cells represent a unique population of lymphocytes that are thymus-derived. CD4+CD25+ Treg cells, which were marked by forkhead box transcription factor (Foxp3), play a critical role in maintaining self-tolerance, suppress autoimmunity and regulate immune responses in organ transplantation and tumor immunity. Tumor development often attracts CD4+CD25+ FoxP3+ Treg cells to the tumor area. Tumor infiltrating regulatory T cells secret inhibitory cytokines such as IL-10 and TGFβ to inhibit autoimmune and chronic inflammatory responses and to maintain immune tolerance in tumors (Unitt et al., Compromised lymphocytes infiltrate hepatocellular carcinoma: the role of T-regulatory cells. *Hepatology*. 2005; 41(4):722–730).

[0056] *Myeloid derived suppressor cells (MDSCs):* MDSCs are a group of heterogeneous cells, which could be seen as hallmark of malignancy-associated inflammation and a major mediator for the induction of T cell suppression in cancers. MDSCs are found in many malignant areas and divided phenotypically into granulocytic (G-MDSC) and monocytic (Mo-MDSC) subgroups. MDSCs can induce T regulatory cells, and produce T cell tolerance. Additionally, MDSCs secrete TFG-β and IL-10 and produce nitric oxide (NO) in the presence of IFN-γ or activated T cells.

[0057] *Tumor associated macrophage (TAM)*: TAMs derived from peripheral blood monocytes are multi-functional cells which exhibit different functions to different signals from the tumor microenvironment. Among cell types associated with tumor microenvironment, TAMs are the most influential for tumor progression. In response to

microenvironmental stimuli, such as tumor extracellular matrix, anoxic environment and cytokines secreted by tumor cells, macrophages undergo M1 (classical) or M2 (alternative) activation. In most malignant tumors, TAMs have the phenotype of M2 macrophages.

[0058] Another immune suppressive mechanism relates to tryptophan catabolism by the enzyme indoleamine-2,3-dioxygenase (IDO). Local immune suppression is an active process induced by the malignant cells within the tumor microenvironment and within the sentinel lymph nodes (SLN). (Gajewski et al., Immune suppression in the tumor microenvironment. *J Immunother*, 2006; 29(3):233-240; and Zou W., Immunosuppressive networks in the tumor environment and their therapeutic relevance, *Nat Rev Cancer*, 2005; 5(4):263-274). Studies show that T-cell receptor zeta subunit (TCR) is downregulated and Indoleamine 2,3-dioxygenase (IDO) is upregulated within the tumor draining lymph nodes as part of the elements involved in the regional immune suppression.

[0059] In addition to the suppressive effects medicated by infiltrating regulatory immune cells, tumor cells themselves can secret many molecules to actively inhibit cytotoxic T cell activation and function.

[0060] In some tumors, T cell intrinsic anergy and exhaustion is common, resulting from TCR ligation in the absence of engagement of co-stimulatory receptors on T cells such as CD28.

[0061] In the present disclosure, Component II of the combination therapy inhibits one or more immunosuppressive mechanisms and enhances a cancer specific immune response for eliminating tumor cells.

Checkpoint Inhibitors

[0062] In some embodiments, Component II comprises a checkpoint inhibitor, such as an active agent that block the checkpoint pathway.

[0063] During adaptive immune response, activation of cytotoxic T cells is mediated by a primary signal between antigenic peptide/MHC molecule complexes on antigen presenting cells and the T cell receptor (TCR) on T cells. A secondary co-stimulatory signal is also important to active T cells. Antigen presentation in the absence of the secondary signal is not sufficient to activate T cells, for example CD4+ T helper cells. The well-known co-stimulatory signal involves co-stimulatory receptor CD28 on T cells and its ligands B7-1/CD80 and B7-2/CD86 on antigen presenting cells (APCs). The B7-1/2 and CD28 interaction can augment antigen specific T cell proliferation and cytokine production. To

tightly regulate an immune response, T cells also express CTLA-4 (anti-cytotoxic T-lymphocyte antigen 4), a co-inhibitory competitor of CD80 and CD86 mediated co-stimulation through the receptor CD28 on T cells, which can effectively inhibit T cell activation and function. CTLA-4 expression is often induced when CD28 interacts with B7-1/2 on the surface of an APC. CTLA-4 has higher binding affinity to the co-stimulatory ligand B7-1/2 (CD80/CD86) than the co-stimulatory receptor CD28, and therefore tips the balance from the T cell activating interaction between CD28 and B7-1/2 to inhibitory signaling between CTLA-4 and B7-1/2, leading to suppression of T cell activation. CTLA-4 upregulation is predominantly during the initial activation of T cells in the lymph node.

[0064] Antibodies that specifically bind to CTLA-4 have been used to inhibit this inhibitory checkpoint. The anti CTLA-4 IgG1 humanized antibody: ipilimumab binds to CTLA-4 and prevents the inhibition of CD28/B7 stimulatory signaling. They can lower the threshold for activation of T cells in lymphoid organs, also can deplete T regulatory cells within the tumor microenvironment (Simpson et al., Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp. Med.*, 2013, 210: 1695-1710). Ipilimumab was recently approved by the U.S. Food and Drug Administration for the treatment of patients with metastatic melanoma.

[0065] In some embodiments, Component II of the combination therapy of the present disclosure may comprise an antagonist agent against CTLA-4 such as an antibody, a functional fragment of the antibody, a polypeptide, or a functional fragment of the polypeptide, or a peptide, which can bind to CTLA-4 with high affinity and prevent the interaction of B7-1/2 (CD80/86) with CTLA-4. In one example. The CTLA-4 antagonist is an antagonistic antibody, or a functional fragment thereof. Suitable anti-CTLA-4 antagonistic antibody include, without limitation, anti-CTLA-4 antibodies, human anti-CTLA-4 antibodies, mammalian anti-CTLA-4 antibodies, humanized anti-CTLA-4 antibodies, monoclonal anti-CTLA-4 antibodies, polyclonal anti-CTLA-4 antibodies, chimeric anti-CTLA-4 antibodies, MDX-010 (ipilimumab), tremelimumab (fully humanized), anti-CD28 antibodies, anti-CTLA-4 adnectins, anti-CTLA-4 domain antibodies, single chain anti-CTLA-4 antibody fragments, heavy chain anti-CTLA-4 fragments, light chain anti-CTLA-4 fragments, and the antibodies disclosed in U.S. Pat. Nos.: 8,748, 815; 8, 529, 902; 8, 318, 916; 8,017, 114; 7,744, 875; 7, 605, 238; 7, 465, 446; 7,109,003; 7,132,281; 6, 984,720; 6,682,736; 6, 207,156; 5,977,318; and European Patent No. EP1212422B1; and U.S.

Publication Nos. US 2002/0039581 and US 2002/086014; and Hurwitz et al., *Proc. Natl. Acad. Sci. USA,* 1998, 95(17):10067-10071; the contents of each of which are incorporated by reference herein in their entirety.

[0066] Additional anti-CTLA-4 antagonist agents include, but are not limited to, any inhibitors that are capable of disrupting the ability of CTLA-4 to bind to the ligands CD80/86.

[0067] The inhibitory checkpoint receptor PD-1 (programmed death-1) is expressed on activated T cells and can induce inhibition and apoptosis of T cells following ligation by programmed death ligands 1 and 2 (PD-L1, also known as B7-H1, CD274), and PD-L2 (also known as B7-DC, CD273), which are normally expressed on epithelial cells and endothelial cells and immune cells (e.g., DCs, macrophages and B cells). PD-1 modulates T cell function mainly during the effector phase in peripheral tissues including tumor tissues. PD-1 is expressed on B cells and myeloid cells, in addition to activated T cells. Many human tumor cells can express PD-L1 and hijack this regulatory function to evade immune recognition and destruction by cytotoxic T lymphocytes. Tumor-associated PD-L1 has been shown to induce apoptosis of effector T cells and is thought to contribute to immune evasion by cancers.

[0068] The PD-1/PD-L1 immune checkpoint appears to be involved in multiple tumor types, for example, melanoma. PD-L1 not only provides immune escape for tumor cells but also turns on the apoptosis switch on activated T cells. Therapies that block this interaction have demonstrated promising clinical activity in several tumor types.

[0069] Component II comprises an active agent that blocks the PD-1 pathway include antagonistic peptides/antibodies and soluble PD-L1/2 ligands. Non-limiting examples of such an active agent that blocks the PD-1 and PD-L1/2 checkpoint pathway are listed below.

Agent	Description	Target
Nivolumab (BMS-936558, ONO-4538, MDX-1106	Human IgG	PD-1
Pembrolizumab (MK-3475, lambrolizumab)	Humanized IgG4	PD-1
Pidilizumab (CT-011)	Humanized anti-PD-1 IgG1kappa	PD-1
AMP-224	B7-DC/IgG1 fusion protein	PD-1
MSB0010718 (EMD-Serono)	Human IgG1	PD-L1
MEDI4736	Engineered human IgG 1kappa	PD-L1
MPDL3280A	Engineered IgG1	PD-L1

AUNP-12	branched 29-amino acid peptide	PD-1

[0070] In accordance with the present disclosure, Component II comprises an antagonist agent against PD-1 and PD-L1/2 inhibitory checkpoint pathway. In one embodiment, the antagonist agent may be an antagonistic antibody that specifically binds to PD-l or PD-L1/L2 with high affinity, or a functional fragment thereof. The PD-1 antibodies may be antibodies taught in US Pat. Nos: 8,779,105; 8, 168, 757; 8, 008, 449; 7, 488, 802; 6, 808, 710; and PCT publication No.: WO 2012/145493; the contents of which are incorporated by references herein in their entirety. Antibodies that can specifically bind to PD-L1 with high affinity may be those disclosed in US Pat. Nos.: 8, 552, 154; 8, 217, 149; 7, 943, 743; 7, 635, 757; U.S. Publication No. 2009/0317368, and PCT Publication Nos. WO 2011/066389 and WO 2012/145493; the contents of which are incorporated herein by references in their entirety. In some examples, Component II comprises an antibody selected from 17D8, 2D3, 4H1, 5C4 (also known as nivolumab or BMS-936558), 4A11, 7D3 and 5F4 disclosed in US Pat. NO.: 8,008, 449; AMP-224, Pidilizumab (CT-011), and Pembrolizumab. In other examples, the anti-PD-1 antibody may be a variant of a human monoclonal anti-PD-1 antibody, for example a "mixed and matched" antibody variant in which a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence, or a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence, as disclosed in US publication NO.: 2015/125463; the contents of which are incorporated by reference herein in its entirety.

[0071] In some embodiments, Component II comprises an antagonistic antibody that binds to PD-L1 with high affinity and disrupts the interaction between PD-1/PD-L1/2. Such antibodies may include, without limitation, 3G10, 12A4 (also referred to as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 disclosed in US Pat. NO.: 7,943, 743 (the contents of which are incorporated by reference in its entirety), MPDL3280A, MEDI4736, and MSB0010718. In another example, the anti-PD-L1 antibody may be a variant of a human monoclonal anti-PD-L1 antibody, for example a "mixed and matched" antibody variant in which a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence, or a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence, as disclosed in US publication NO.: 2015/125463; the contents of which are incorporated by reference in its entirety.

[0072] In some embodiments, Component II comprises an antagonistic antibody that binds to PD-L2 with high affinity and disrupts the interaction between PD-1/PD-L1/2. Exemplary anti-PD-L2 antibodies may include, without limitation, antibodies taught by Rozali et al (Rozali et al., Programmed Death Ligand 2 in Cancer-Induced Immune Suppression, *Clinical and Developmental Immunology*, 2012, Volume 2012 (2012), Article ID 656340), and human anti-PD-L2 antibodies disclosed in US Pat. No.: 8, 552, 154 (the contents of which are incorporated herein by reference in their entirety).

[0073] In some embodiments, Component II comprises compounds that inhibit immunosuppressive signal induced due to PD-1, PD-L1 and/or PD-L2 such as cyclic peptidomimetic compounds disclosed in US9233940 to Sasikumar et al. (Aurigene Discovery Tech.), WO2015033303 to Sasikumar et al.; immunomodulating peptidomimetic compounds disclosed in WO2015036927 to Sasikumar et al.; 1,2,4-oxadiazole derivatives disclosed in US2015007302 to Govindan et al.; 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds disclosed in WO2015033301 to Sasikumar et al.; or therapeutic immunomodulating compounds and derivatives or pharmaceutical salts of a peptide derivative of formula (I) or a stereoisomer of a peptide derivative of formula (I) disclosed in WO2015044900 to Sasikumar et al., the contents of each of which are incorporated herein by reference in their entirety.

[0074] In other embodiments, Component II comprises an antibody having binding affinity to both PD-L1 and PD-L2 ligands, for example the single agent of anti-PD-L1 and PD-L2 antibodies disclosed in PCT publication NO.: WO2014/022758; the contents of which are incorporated by reference in its entirety.

[0075] In some embodiments, Component II comprises two or more antibodies selected from anti-PD-1 antibodies, PD-L1 antibodies and PD-L2 antibodies. In one example, an anti-PD-L1 antibody and an anti-PD-L2 antibody may be included in a single conjugate through a linker.

[0076] In some embodiments, Component II comprises a modulatory agent that can simultaneously block the PD-1 and PD-L1/2 mediated negative signal transduction. This modulatory agent may be a non-antibody agent. In some aspects, the non-antibody agents may be PD-L1 proteins, soluble PD-L1 fragments, variants and fusion proteins thereof. The non-antibody agents may be PD-L2 proteins, soluble PD-L2 fragments, variants and fusion proteins thereof. PD-L1 and PD-L2 polypeptides, fusion proteins, and soluble fragments can inhibit or reduce the inhibitory signal transduction that occurs through PD-1 in T cells by

preventing endogenous ligands (i.e. endogenous PD-L1 and PD-L2) of PD-1 from interacting with PD-1. Additionally, the non-antibody agent may be soluble PD-1 fragments, PD-1 fusion proteins which bind to ligands of PD-1 and prevent binding to the endogenous PD-1 receptor on T cells. In one example, the PD-L2 fusion protein is B7-DC-Ig and the PD-1 fusion protein is PD-1-Ig. In another example, the PD-L1, PD-L2 soluble fragments are the extracellular domains of PD-L1 and PD-L2, respectively. In one embodiment, Component II comprises a non-antibody agent disclosed in US publication No.: 2013/017199; the contents of which are incorporated by reference herein in its entirety.

[0077] In addition to CTLA-4 and PD-1, other known immune inhibitory checkpoints include TIM-3 (T cell immunoglobulin and mucin domain-containing molecule 3), LAG-3 (lymphocyte activation gene-3, also known as CD223), BTLA (B and T lymphocyte attenuator), CD200R, KRLG-1, 2B4 (CD244), CD160, KIR (killer immunoglobulin receptor), TIGIT (T-cell immune-receptor with immunoglobulin and ITIM domains), VISTA (V-domain immunoglobulin suppressor of T-cell activation) and A2aR (A2a adenosine receptor) (Ngiow et al., Prospects for TIM3 targeted antitumor immunotherapy, *Cancer Res.*, 2011, 71(21): 6567-6571; Liu et al., Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses, *PNAS*, 2015, 112(21): 6682-6687; and Baitsch et al., Extended Co-Expression of Inhibitory Receptors by Human CD8 T-Cells Depending on Differentiation, Antigen-Specificity and Anatomical Localization.2012, *Plos One*, 7(2): e30852). These molecules that similarly regulate T-cell activation are being assessed as targets of cancer immunotherapy.

[0078] TIM-3 is a transmembrane protein constitutively expressed on IFN-γ–secreting T-helper 1 (Th1/Tc1) cells (Monney et al., Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature*. 2002, 415:536-541), DCs, monocytes, $CD8^+$ T cells, and other lymphocyte subsets as well. TIM-3 is an inhibitory molecule that down-regulates effector Th1/Tc1 cell responses and induces cell death in Th1 cells by binding to its ligand Galectin-9, and also induces peripheral tolerance (Fourcade et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. *J experimental medicine*. 2010; 207:2175-2186). Blocking TIM-3 can enhance cancer vaccine efficacy (Lee et al., The inhibition of the T cell immunoglobulin and mucin domain 3(Tim-3) pathway enhances the efficacy of tumor vaccine. *Biochem. Biophys. Res Commun*, 2010, 402: 88-93).

[0079] It has been shown that extracellular adenosine generated from hypoxia in the tumor microenvironment binds to A2a receptor which is expressed on a variety of immune cells and endothelial cells. The activation of A2aR on immune cells induces increased production of immunosuppressive cytokines (e.g., TGF-β, IL-10), upregulation of alternate immune checkpoint pathway receptors (e.g., PD-1, LAG-3), increased FOXP3 expression in CD4+ T cells driving a regulatory T cell phenotype, and induction of effector T cell anergy. Beavis et al demonstrated that A2aR blockade can improve effector T cell function and suppress metastasis (Beavis et al., Blockade of A2A receptors potently suppresses the metastasis of CD73 + tumors. *Proc Natl Acad Sci USA*, 2013, 110: 14711–14716). Some A2aR inhibitors are used to block A2aR inhibitory signal, including, without limitation, SCH58261, SYN115, ZM241365 and FSPTP (Leone et al., A2aR antagonists: Next generation checkpoint blockade for cancer immunotherapy, *Comput Struct Biotechnol. J* 2015, 13: 265-272).

[0080] LAG-3 is a type I transmembrane protein expressed on activated $CD4^+$ and $CD8^+$ T cells, a subset of γδ T cells, NK cells and regulatory T cells (Tregs), and can negatively regulate immune response (Jha et al., Lymphocyte Activation Gene-3 (LAG-3) Negatively Regulates Environmentally-Induced Autoimmunity, *PLos One*, 2014, 9(8): e104484). LAG-3 negatively regulates T-cell expansion by inhibiting T cell receptor–induced calcium fluxes, thus controlling the size of the memory T-cell pool. LAG-3 signaling is important for $CD4^+$ regulatory T-cell suppression of autoimmune responses, and LAG-3 maintains tolerance to self and tumor antigens via direct effects on $CD8^+$ T cells. A recent study showed that blockade of both PD-1 and LAG-3 could provoke immune cell activation in a mouse model of autoimmunity, supporting that LAG-3 may be another important potential target for checkpoint blockade.

[0081] BTLA, a member of the Ig superfamily, binds to HVEM (herpesvirus entry mediator; also known as TNFRSF14 or CD270), a member of the tumor necrosis factor receptor superfamily (TNFRSF) (Watanabe et al., BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1 Nat Immunol, 2003, 4670–679. HVEM is expressed on T cells (e.g. CD8+ T cells). The HVEM-BTLA pathway plays an inhibitory role in regulating T cell proliferation (Wang et al., *The role of herpesvirus entry mediator as a negative regulator of T cell-mediated responses, J Clin Invest., 2005, 115: 74-77). CD160 is another ligand of HVEM. The co-inhibitory signal of CD160/HVEM can inhibit the*

activation of CD4+ helper T cell (Cai et al., CD160 inhibits activation of human $CD4^+$ T cells through interaction with herpesvirus entry mediator. *Nat Immunol.* 2008; 9:176–185).

[0082] *CD200R is a receptor of CD200 that is expressed on* myeloid cells. CD200 (OX2) is a highly expressed membrane glycoprotein on many cells. Studies indicated that CD200 and CD200R interaction can expand the myeloid-derived suppressor cell (MDSC) population (Holmannova et al., CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; Part I: CD200/CD200R structure, activation, and function. *Acta Medica (Hradec Kralove)* 2012, 55(1):12–17; and Gorczynski, CD200 and its receptors as targets of immunoregulation, *Curr Opin Investig Drug*, 2005, 6(5): 483-488).

[0083] TIGIT is a co-inhibitory receptor that is highly expressed tumor-infiltrating T cells. In the tumor microenvironment, TIGIT can interact with CD226, a costimulatory molecule on T cells in cis, therefore disrupt CD226 dimerization. This inhibitory effect can critically limit antitumor and other CD8+ T cell-dependent responses (Johnston et al., The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function, Cancer cell, 2014, 26(6):923-937).

[0084] KIRs are a family of cell surface proteins expressed on natural killer cells (NKs). They regulate the killing function of these cells by interacting with MHC class I molecules expressed on any cell types, allowing the detection of virally infected cells or tumor cells. Most KIRs are inhibitory, meaning that their recognition of MHC molecules suppresses the cytotoxic activity of their NK cell (Ivarsson et al., Activating killer cell Ig-like receptor in health and disease, *Frontier in Immu.*, 2014, 5: 1-9).

[0085] Additional coinhibitory signals that affect T cell activation include, but are not limited to KLRG-1, 2B4 (also called CD244), and VISTA (Lines et al., VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy, *Cancer Immunol Res.*, 2014, 2(6): 510-517).

[0086] In accordance with the present disclosure, Component II comprises an antagonist or inhibitor of a co-inhibitory molecule selected from CTLA-4, PD-1, PD-L1, PD-L2, TIM-3, LAG-3(CD223), BTLA, CD160, CD200R, TIGIT, KRLG-1, KIR, 2B4 (CD244), VISTA, A2aR and other immune checkpoints. In some aspects, the antagonist agent may be an antagonistic antibody, or a functional fragment thereof, against a coinhibitory checkpoint molecule selected from CTLA-4, PD-1, PD-L1, PD-L2, TIM-3, LAG-3(CD223), BTLA, CD160, CD200R, TIGIT, KRLG-1, KIR, 2B4 (CD244), VISTA and A2aR.

[0087] In some embodiments, Component II comprises an antagonistic antibody, and/or a functional fragment thereof, specific to LAG-3(CD223). Such antagonistic antibodies can specifically bind to LAG-3(CD223) and inhibit regulatory T cells in tumors. In one example, it may be an antagonistic anti-LAG-3(CD223) antibody disclosed in US Pat NOs. 9, 005, 629 and 8,551,481. Component II may also comprise any inhibitor that binds to the amino acid motif KIEELE in the LAG-3(CD223) cytoplasmic domain which is essential for CD223 function, as identified using the methods disclosed in US Pat. NOs. 9,005,629 and 8, 551, 481; the contents each of which are incorporated herein by reference in their entirety. Other antagonistic antibodies specific to LAG-3(CD223) may include antibodies disclosed in US publication NO.20130052642; the contents of which is incorporated herein by reference in its entirety.

[0088] In some embodiments, Component II comprises an antagonistic antibody, and/ or a functional fragment thereof, specific to TIM-3. Such antagonistic antibodies specifically bind to TIM-3 and can be internalized into TIM-3 expressed cells such as tumor cells to kill tumor cells. In other aspects, TIM-3 specific antibodies that specifically bind to the extracellular domain of TIM-3 can inhibit proliferation of TIM-3 expressing cells upon binding, e.g., compared to proliferation in the absence of the antibody and promote T-cell activation, effector function, or trafficking to a tumor site. In one example, the antagonistic anti-TIM-3 antibody may be selected from any antibody disclosed in US Pat. NOs. 8,841,418; 8,709, 412; 8,697,069; 8,647,623; 8,586,038; and 8,552,156; the contents of each of which are incorporated herein by reference in their entirety.

[0089] In addition, the antagonistic TIM-3 specific antibody may be monoclonal antibodies 8B.2C12, 25F.1D6 as disclosed in US Pat. NO. 8, 697,069; 8, 101,176; and 7, 470, 428; the contents of each of which are incorporated herein by reference in their entirety.

[0090] In other embodiments, Component II comprises an agent that can specifically bind to galectin-9 and neutralize its binding to TIM-3, including neutralizing antibodies disclosed in PCT publication NO. 2015/013389; the contents of which are incorporated by reference in its entirety.

[0091] In some embodiments, Component II comprises an antagonistic antibody, and/or a functional fragment thereof, specific to BTLA, including but not limited to antibodies and antigen binding portion of antibodies disclosed in US Pat. NOs. 8, 247, 537; 8, 580, 259; fully human monoclonal antibodies in US Pat. NO.: 8,563,694; and BTLA blocking

antibodies in US Pat. NO.: 8,188, 232; the contents of each of which are incorporated herein by reference in their entirety.

[0092] Other additional antagonist agents that can inhibit BTLA and its receptor HVEM may include agents disclosed in PCT publication NOs.: 2014/184360; 2014/183885; 2010/006071 and 2007/010692; the contents of each of which are incorporated herein by reference in their entirety.

[0093] In certain embodiments, Component II comprises an antagonistic antibody, and/or or a functional fragment thereof, specific to KIR, for example IPH2101 taught by Benson et al., (A phase I trial of the anti-KIR antibody IPH2101 and lenalidomide in patients with relapsed/refractory multiple myeloma, *Clin Cancer Res*., 2015, May 21. pii: clincanres.0304.2015); the contents of which are incorporated by reference in its entirety.

[0094] In other embodiments, the antagonist agent may be any compound that can inhibit the inhibitory function of a coinhibitory checkpoint molecule selected from CTLA-4, PD-1, PD-L1, PD-L2, TIM-3, LAG-3(CD223), BTLA, CD160, CD200R, TIGIT, KRLG-1, KIR, 2B4 (CD244), VISTA and A2aR.

[0095] In some examples, the antagonist agent may be a non-antibody inhibitor such as LAG-3-Ig fusion protein (IMP321) (Romano et al., *J transl. Medicine*, 2014, 12:97), and herpes simplex virus (HSV)-1 glycoprotein D (gD), an antagonist of BTLA)/CD160-HVEM) pathways (Lasaro et al., *Mol Ther*. 2011; 19(9): 1727–1736).

[0096] In some embodiments, Component II comprises an agent that is bispecific or multiple specific. As used herein, the terms “bispecific agent” and “multiple specific agent” refer to any agent that can bind to two targets or multiple targets simultaneously. In some aspects, the bispecific agent may be a bispecific peptide agent that has a first peptide sequence that binds a first target and a second peptide sequence that binds a second different target. The two different targets may be two different inhibitory checkpoint molecules selected from CTLA-4, PD-1 PD-L1, PD-L2, TIM-3, LAG-3(CD223), BTLA, CD160, CD200R, TIGIT, KRLG-1, KIR, 2B4 (CD244), VISTA and A2aR. A non-limiting example of bispecific peptide agents is a bispecific antibody or antigen-binding fragment thereof. Similarly, a multiple specific agent may be a multiple peptide specific agent that has more than one specific binding sequence domain for binding to more than one target. For example, a multiple specific polypeptide can bind at least two, at least three, at least four, at least five,

at least six, or more targets. A non-limiting example of multiple-specific peptide agents is a multiple-specific antibody or antigen-binding fragment thereof.

[0097] In one example, such bispecific agent is the bispecific polypeptide antibody variants for targeting TIM-3 and PD-1, as disclosed in US publication NO.: 2013/0156774; the content of which is incorporated herein by reference in its entirety.

[0098] In some embodiments, Component II comprises a conjugate that has one, two or multiple checkpoint antagonists/inhibitors connected via linkers in one conjugate.

[0099] In some embodiments, Component II comprises any agent that binds to and inhibits a checkpoint receptor. The checkpoint receptor is selected from the group consisting of CTLA-4, PD-1, CD28, inducible T cell co-stimulator (ICOS), B and T lymphocyte attenuator (BTLA), killer cell immunoglobulin-like receptor (KIR), lymphocyte activation gene 3 (LAG3), CD137, OX40, CD27, CD40L, T cell membrane protein 3 (TIM3), and adenosine A2a receptor (A2aR).

[0100] In one example, Component II comprises a CTLA-4 antagonist.

[0101] In another example, Component II comprises a PD-1 antagonist.

[0102] In yet another example, Component II comprises a PD-L1 antagonist.

Enhancer of Zeste Homolog (EZH) Inhibitors

[0103] Schlafen Family Member 11 (SLFN11) is a protein involved in DNA repair deficiency and has been shown to interact with DNA repair proteins. It is a potential marker of sensitivity to DNA damaging agents including irinotecan based on preclinical data. Loss of SLFN11 can occur via epigenetic silencing and this silencing has the potential to cause resistance to chemotherapeutics that cause DNA damage. In ovarian, non-small cell lung (NSCLC) and breast cancer cell lines resistant to carboplatin/cisplatin, the SLFN11 locus is silenced via methylation. It is also found that when SLFN11 is knocked down in cells that express the protein, it increases the resistance of cells that were previously sensitive to platinum drugs. In the clinical setting, some NSCLC and ovarian cancer patients that have poorer survival on platinum drugs showed silencing of the SLFN11 locus. It is desirable to increase and/or restore SLFN11 expression for cancer patients with chemotherapy resistance.

[0104] Enhancer of Zeste Homolog (EZH) proteins have been shown to be involved in SLFN11 silencing. EZH is a histone methylase and represses transcription of genes and can be overexpressed and/or overactive in cancer cells. SCLC preclinical models developed to be resistant to cisplatin/etoposide demonstrated downregulation of SLFN11 as compared to the

sensitive models and treatment with an EZH inhibitor in chemo-resistant cell lines can restore sensitivity *in vitro* and *in vivo*. Chemotherapeutic agents combined with EZH inhibitors may prevent chemotherapy resistance of cancer cells.

[0105] In some embodiments, Component I of the combination therapy is Conjugate **1** and Component II of the combination therapy is an EZH inhibitor. Any EZH inhibitor, such as EZH 1 and 2 inhibitors as well as dual inhibitors, may be used as Component II. Non-limiting examples of EZH inhibitors include EPZ011989 (free base CAS No. 1598383-40-4), EPZ005687 (CAS No. 1396772-26-1), GSK126 (CAS No. 1346574-57-9), GSK343 (CAS No. 1346704-33-3), GSK503 (CAS No. 1346572-63-1), tazemetostat (EPZ-6438, CAS No. 1403254-99-8), 3-deazaneplanocin A (DZNeP, HCl salt CAS No. 120964-45-6), EI1 (CAS No. 1418308-27-6), CPI-360 (CAS No. 1802175-06-9), CPI-169 (CAS No. 1450655-76-1), JQ-EZ-05 (JQEZ5, CAS No. 1913252-04-6), PF-06726304 (CAS No. 1616287-82-1), UNC1999 (CAS No. 1431612-23-5), and UNC2400 (CAS No. 1433200-49-7).

Formulations and Administration

[0106] Each component in the combination therapy of the present disclosure can be formulated using one or more pharmaceutically acceptable excipients to: (1) increase stability; (2) permit the sustained or delayed release (e.g., from a depot formulation of the monomaleimide); (3) alter the biodistribution (e.g., target the monomaleimide compounds to specific tissues or cell types); (4) alter the release profile of the monomaleimide compounds *in vivo*. Component I and Component II can each be administered in different compositions.

[0107] Non-limiting examples of the excipients include any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, and preservatives. Excipients may also include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, hyaluronidase, nanoparticle mimics and combinations thereof. Accordingly, the formulations of each component may include one or more excipients, each in an amount that together increases the stability of the active agents.

[0108] Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference in its entirety) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing

any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of the present disclosure.

[0109] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, e.g., between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[0110] In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0111] In some embodiments, Conjugate **1** is administered to the patient in a pharmaceutical composition, wherein the pharmaceutical composition has a pH of about 4.0 to about 5.0. In some embodiments, the pharmaceutical composition comprises acetate buffer (sodium acetate and acetic acid) having a pH of about 4.0 to about 4.8. In some embodiments, the pharmaceutical composition further comprises mannitol and polyoxyl 15 hydroxystearate.

[0112] In one embodiment, a composition for solution for injection is provided for administering Conjugate **1**. The solution comprises Conjugate **1**, mannitol, Polyoxyl 15 Hydroxystearate, and aqueous acetate buffer. The composition may be infused intravenously (IV).

Administration

[0113] The components of the combination therapy may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to enteral, gastroenteral, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal

administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops. In specific embodiments, compositions may be administered in a way which allows them to cross the blood-brain barrier, vascular barrier, or other epithelial barrier.

[0114] The formulations described herein contain an effective amount of the components in a pharmaceutical carrier appropriate for administration to a patient in need thereof. The formulations may be administered parenterally (e.g., by injection or infusion). The formulations or variations thereof may be administered in any manner including enterally, topically (e.g., to the eye), or via pulmonary administration. In some embodiments, the formulations are administered topically.

Dosing

[0115] The exact amount required by the patient for each component will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like.

[0116] Components of the combination therapy are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present disclosure may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound 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 rate of excretion of the specific compound

employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[0117] In some embodiments, the components of the combination therapy in accordance with the present disclosure may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect.

[0118] The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In some embodiments, the desired dosage may be delivered using multiple administrations (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used.

[0119] The concentration of the components may be between about 0.01 mg/mL to about 50 mg/mL, about 0.1 mg/mL to about 25 mg/mL, about 0.5 mg/mL to about 10 mg/mL, or about 1 mg/mL to about 5 mg/mL in the pharmaceutical composition.

[0120] As used herein, a “split dose” is the division of single unit dose or total daily dose into two or more doses, e.g, two or more administrations of the single unit dose. As used herein, a “single unit dose” is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a “total daily dose” is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose. In one embodiment, the monomaleimide compounds of the present disclosure are administered to a subject in split doses. The monomaleimide compounds may be formulated in buffer only or in a formulation described herein.

[0121] A subject may receive the combination therapy for any suitable length, such as a week, 2 weeks, 3 weeks, 4 weeks, a month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, a year, or until a predetermined milestone is reached (e.g., a TGI% of above 90%, 95%, or 99%).

[0122] In some embodiments, Component I is Conjugate **1** and is administered to a subject in need thereof at a dose of about 30 mg, 60 mg, 120 mg, 240 mg, or 360 mg. Optionally, the dosing of Conjugate **1** administered to a subject in need thereof is based on the body surface area (BSA) of the subject. BSA (meters squared), as used herein, is the square root of height in centimeters times weight in kilograms divided by 3600. For example, the dosing of Conjugate **1** may be at least about 150 mg/m^2, such as about 175 mg/m^2, 200 mg/m^2, 225 mg/m^2, 250 mg/m^2, 275 mg/m^2 or 300 mg/m^2. Conjugate **1** may be administered on Days 1, 8, and 15 of a 28-day cycle.

[0123] In some embodiments, Component II is a PARP inhibitor. The PARP inhibitor may be administered on Days 2, 3, 4, 5 and 6 of a 7-day cycle.

[0124] As discussed in the Examples, scheduling the dosing of Conjugate **1** and a PARP inhibitor in preclinical mouse models achieved efficacy while mitigating potential overlapping toxicities. Translating the optimal schedule to humans is dependent on the pharmacokinetics and toxicities of Conjugate **1** and the specific PARP inhibitor. Estimates on PK and number of dosing days are dependent on the PARPi being considered. Fig. 2 shows an estimate of plasma exposures of Conjugate **1** and niraparib, and tumor xenograft exposures of Conjugate **1** to maximize efficacy and/or minimize toxicity based on a 7-Day schedule. Conjugate **1** data were from mouse plasma and mouse tumor xenograft. PARP inhibitor plasma concentrations were estimated/extrapolated from published niraparib human data.

[0125] In some embodiments, Component I is Conjugate **1** and is administered to a subject in need thereof at a dose of at least 150 mg/m^2 on Days 1, 8, and 15 of a 28-day cycle; Component II is a PARP inhibitor and is administered to the subject on Days 2, 3, 4, 5 and 6 of a 7-day cycle. The subject may have SCLC, have no more than 1 prior line of therapy, performance status (PS) 0/1, be irinotecan naïve and/or have PARP wildtype.

II. Methods of Using Conjugate 1 and/or the Combination Therapy

[0126] One aspect of the present disclosure provides methods of treating of a variety of different disease conditions with Conjugate **1**. The disease conditions include cellular proliferative diseases, such as neoplastic diseases, autoimmune diseases, central nervous system or neurodegenerative diseases, cardiovascular diseases, hormonal abnormality diseases, infectious diseases, and the like.

[0127] By 'treatment' is meant at least an amelioration of the symptoms associated with the disease condition afflicting the host, where amelioration is used in a broad sense to refer

to at least a reduction in the magnitude of a parameter, *e.g.*, symptom, associated with the pathological condition being treated, such as inflammation and pain associated therewith. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.*, prevented from happening, or stopped, *e.g.*, terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

[0128] A variety of hosts are treatable according to the present disclosure. Generally such hosts are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class Mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice, guinea pigs, and rats), and primates (*e.g.*, humans, chimpanzees, and monkeys). In many embodiments, the hosts will be humans.

[0129] The invention also provides methods for treatment of a disease or disorder in which the subject to be treated is selected for treatment based on the presence of, or the overexpression of, a particular protein. For example, subjects may be selected for treatment of cancer based on the presence of greater the normal levels of Hsp90. The HSP90 may be extracellular HSP90 (eHSP90) on the tumor cells or the circulating HSP90 in the body fluids (such as plasma or serum). The method includes obtaining a sample from the patient and measuring the expression of human extracellular Hsp90 or circulating HSP90 in the sample.

[0130] The expression of eHSP90 can be measured using the method known in the art, such as ELISA, RIA, EIA, sandwich assay, Western Blot analysis, immunostaining, flow cytometry and immunohistological staining.

[0131] Circulating HSP90 can be measured in blood serum samples of patients. In some embodiments, the serum samples of patients can be collected and frozen at -20°C until analysis. HSP90 ELISA uses a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of human HSP90 in samples.

[0132] In some embodiments, the circulating HSP90 is HSP90α. According to US8580519 (Univ. of Maryland), the contents of which are incorporated by reference in their entirety, the amount or concentration of HSP90α in the plasma or serum of a patient can be used to determine cancer progression. The Hsp90α polypeptide may be detected using an agent that interacts with Hsp90α polypeptide. For example, the agent may be an antibody that binds Hsp90α or Hsp90α fragments. The agent may comprise a label used for detection, such as a chemiluminescent tag, colorimetric tag, fluorescent tag, or radioactive label.

[0133] In some embodiments, radiolabeled isoxazole derivatives disclosed in WO2007096194 (Novartis), the contents of which are incorporated herein by reference in their entirety, are used to probe HSP90 using molecular imaging modalities. In some embodiments, aryl substituted adenine-based HSP90 inhibitors with radiolabels such as 124I, 131I or 123I, disclosed in US7834181 (Sloan-Kettering Institute), the contents of which are incorporated herein by reference in their entirety, are used to conduct radio imaging of tumor tissues and determine HSP90 levels.

[0134] In some embodiments, nuclear magnetic resonance imaging of small molecule inhibitors of HSP90 as contrast agents is conducted to measure the expression of HSP90. The steps include (a) providing a contrast enhancement agent comprising a small molecule that binds to HSP90 in the target tissue; (b) introducing the contrast enhancement agent into the target tissue; and (c) scanning the target tissue using magnetic resonance imaging, whereby a visible image of the target tissue is non-invasively generated. The HSP90-binding contrast enhancement small molecule disclosed in US2009/0088578 (Haystead et al.), the contents of which are incorporated herein by reference in their entirety, may be used.

[0135] In some embodiment, a method of treating cancer in a patient is provided, wherein the method comprises the steps of:

1). measuring the HSP90 levels in the patient, and

2). administering a therapeutically effective amount of Conjugate **1** to the patient.

[0136] As non-limiting examples, the carcinoma which may be treated may be Acute granulocytic leukemia, Acute lymphocytic leukemia, Acute myelogenous leukemia, Adenocarcinoma, Adenosarcoma, Adrenal cancer, Adrenocortical carcinoma, Anal cancer, Anaplastic astrocytoma, Angiosarcoma, Appendix cancer, Astrocytoma, Basal cell carcinoma, B-Cell lymphoma), Bile duct cancer, Bladder cancer, Bone cancer, Bowel cancer, Brain cancer, Brain stem glioma, Brain tumor, Breast cancer, Carcinoid tumors, Cervical cancer, Cholangiocarcinoma, Chondrosarcoma, Chronic lymphocytic leukemia, Chronic myelogenous leukemia, Colon cancer, Colorectal cancer, Craniopharyngioma, Cutaneous lymphoma, Cutaneous melanoma, Diffuse astrocytoma, Ductal carcinoma in situ, Endometrial cancer, Ependymoma, Epithelioid sarcoma, Esophageal cancer, Ewing sarcoma, Extrahepatic bile duct cancer, Eye cancer, Fallopian tube cancer, Fibrosarcoma, Gallbladder cancer, Gastric cancer, Gastrointestinal cancer, Gastrointestinal carcinoid cancer, Gastrointestinal stromal tumors, General, Germ cell tumor, Glioblastoma multiforme,

Glioma, Hairy cell leukemia, Head and neck cancer, Hemangioendothelioma, Hodgkin lymphoma, Hodgkin's disease, Hodgkin's lymphoma, Hypopharyngeal cancer, Infiltrating ductal carcinoma, Infiltrating lobular carcinoma, Inflammatory breast cancer, Intestinal Cancer, Intrahepatic bile duct cancer, Invasive / infiltrating breast cancer, Islet cell cancer, Jaw cancer, Kaposi sarcoma, Kidney cancer, Laryngeal cancer, Leiomyosarcoma, Leptomeningeal metastases, Leukemia, Lip cancer, Liposarcoma, Liver cancer, Lobular carcinoma in situ, Low-grade astrocytoma, Lung cancer, Lymph node cancer, Lymphoma, Male breast cancer, Medullary carcinoma, Medulloblastoma, Melanoma, Meningioma, Merkel cell carcinoma, Mesenchymal chondrosarcoma, Mesenchymous, Mesothelioma, Metastatic breast cancer, Metastatic melanoma, Metastatic squamous neck cancer, Mixed gliomas, Mouth cancer, Mucinous carcinoma, Mucosal melanoma, Multiple myeloma, Nasal cavity cancer, Nasopharyngeal cancer, Neck cancer, Neuroblastoma, Neuroendocrine tumors, Non-Hodgkin lymphoma, Non-Hodgkin's lymphoma, Non-small cell lung cancer, Oat cell cancer, Ocular cancer, Ocular melanoma, Oligodendroglioma, Oral cancer, Oral cavity cancer, Oropharyngeal cancer, Osteogenic sarcoma, Osteosarcoma, Ovarian cancer, Ovarian epithelial cancer, Ovarian germ cell tumor, Ovarian primary peritoneal carcinoma, Ovarian sex cord stromal tumor, Paget's disease, Pancreatic cancer, Papillary carcinoma, Paranasal sinus cancer, Parathyroid cancer, Pelvic cancer, Penile cancer, Peripheral nerve cancer, Peritoneal cancer, Pharyngeal cancer, Pheochromocytoma, Pilocytic astrocytoma, Pineal region tumor, Pineoblastoma, Pituitary gland cancer, Primary central nervous system lymphoma, Prostate cancer, Rectal cancer, Renal cell cancer, Renal pelvis cancer, Rhabdomyosarcoma, Salivary gland cancer, Sarcoma, Sarcoma, bone, Sarcoma, soft tissue, Sarcoma, uterine, Sinus cancer, Skin cancer, Small cell lung cancer, Small intestine cancer, Soft tissue sarcoma, Spinal cancer, Spinal column cancer, Spinal cord cancer, Spinal tumor, Squamous cell carcinoma, Stomach cancer, Synovial sarcoma, T-cell lymphoma), Testicular cancer, Throat cancer, Thymoma/ thymic carcinoma, Thyroid cancer, Tongue cancer, Tonsil cancer, Transitional cell cancer, Transitional cell cancer, Transitional cell cancer, Triple-negative breast cancer, Tubal cancer, Tubular carcinoma, Ureteral cancer, Ureteral cancer, Urethral cancer, Uterine adenocarcinoma, Uterine cancer, Uterine sarcoma, Vaginal cancer, and Vulvar cancer.

[0137] In some embodiments, Conjugate **1** is administered to a subject having cancer, wherein the cancer is selected from small cell lung cancer (SCLC), gastric or

gastroesophageal junction (GEJ) cancer, pancreatic ductal adenocarcinoma (PDAC), ovarian cancer, endometrial cancer, small cell cancer (SCC) or squamous cell cancer of anus, cervix, vulva, or penis, Ewings Sarcoma, and Rhabdomyocarcoma.

[0138] In some embodiments, Conjugate **1** is administered to a subject having tumor, wherein the tumor is selected from tumor in anus, breast, cholangiocarcioma, colon, duodental, esophageal, hepatocellular, lung (small cell), neuroendocrine, ovary, pancreas, prostate, or sarcoma.

[0139] In some embodiments, Conjugate **1** is administered to a subject having cancer, wherein the cancer is selected from squamous carcinoma, pancreatic adenocarcinoma, acinar cell cancer of the pancreas, and liposarcoma.

[0140] In some embodiments, Conjugate **1** is administered to a subject having cancer, wherein the subject has received at least one previous anticancer therapy. Non-limiting examples of anticancer therapies include:

Tumor Type	**Previous Anticancer Therapies**
LIPOSARCOMA	Ifosfamide/Epirubicin Doxorubicin/Olaratumab
RHABDOMYOSARCOMA	Cycophosphamide/Dactinomycin/Doxorubicin/Etoposide/Ifosfamide/Irinotecan/Vincristine Irinotecan/Temodar/Vincristine
EWINGS SARCOMA	Cyclophosphamide/Doxorubicin/Etoposide/Ifosfamide/Vincristine Irinotecan/Temodar TK215 Irinotecan/Niraparib Cyclophosphamide
NEUROENDOCRINE TUMOR OF UNKNOWN ORIGIN	Carboplatin/Etoposide AZD1775/Olaparib Docetaxel/RGX104
SQUAMOUS CELL CARCINOMA OF THE ANUS	Mitomycin C/Capecitabine 5FU/Cisplatin/Pembrolizumzb
OVARIAN CANCER	Carboplatin/Taxol/Avastin Cisplatin/Taxol Doxil Demcizumab/Pembrolizumab DMUC antibody drug conjugate

	Taxol/VEGF monoclonal antibody
SCLC	Carboplatin/Irinotecan Sunitinib/Carboplatin/Irinotecan Carboplatin/Etoposide LCL161/RM228/Taxol IAP inhibitor/RM206 RM327 LUN259 Ipilimumab/Nivolumab PARP inhibitor Nivolumab Temodar LAG-3
ACINAR CELL CARCINOMA OF THE PANCREAS	5FU/Mitomycin C FF-10502
ADENOCARCINOMA OF THE PANCREAS	Abraxane/Gemzar 5FU Leucovorin/5FU/Irinotecan/Oxaliplatin Capecitabine Taxol/LMB100 Leucovorin/5FU/Irinotecan Gemcitabine Ipilimumab/Nivolumab Abraxane
CHOLANGIOCARCINOMA	Cisplatin/Gemzar Regorafenib Capecitabine Leucovorin/5FU/Oxaliplatin PI3K inhibitor Sulfatinib Nucleoside Analog
ADENOCARCINOMA OF THE COLON	Leucovorin/5FU/Oxaliplatin/Avastin Irinotecan/Panitumumab/Cetuximab Irinotecan/Panitumumab RM505 LAG-3/PD-1 WNT pathway inhibitor

	Avastin/Leucovorin/5FU/Irinotecan
	Avastin/Leucovorin/5FU
	Tipiracil
	Regorafenib
	Amygdalin
	Pembrolizumab
	Leucovorin/5FU/Irinotecan/Ramucirumab
	Gemzar
	Oxaliplatin/Leucovorin/5FU
	Avastin/Capecitabine
	Avastin/5FU
	Tumor Vaccine
	Avastin/Oxaliplatin/Capecitabine
	Avastin/Binimetnib/Pembrolizumab

[0141] In one example, a pharmaceutical composition comprising an effective amount of Conjugate **1** Sodium, a tautomer thereof, or a pharmaceutically acceptable salt thereof, and 5% Mannitol is provided. The pharmaceutical composition has a pH in the range of about 9.4 to about 10.3. The concentration of Conjugate **1** Sodium, a tautomer thereof, or a pharmaceutically acceptable salt thereof is in the range of around 1 mg/mL to around 20 mg/mL, such as about 3 mg/mL, 6 mg/mL, or 12 mg/mL.

[0142] Another aspect of the present disclosure provides methods for treating a subject having a hyperproliferative disorder such as cancer, wherein the method comprises a combination therapy of at least two distinct therapeutic agents. In some embodiments, the method comprises administering to the patient: (A) a first component which comprises, as an active agent, Compound I, or a prodrug, derivative, or pharmaceutically-acceptable salt thereof; and (B) a second component which comprises, as an active agent, Compound II, or a prodrug, derivative, or a pharmaceutically-acceptable salt thereof.

[0143] According to the present disclosure, cancer may be characterized by tumors, e.g., solid tumors or any neoplasm. In some embodiments, the cancer is a solid tumor. Large drug molecules have limited penetration in solid tumors. The penetration of large drug molecules is slow. On the other hand, small molecules such as small molecule conjugates may penetrate solid tumors rapidly and more deeply. Regarding penetration depth of the drugs, larger molecules penetrate less, despite having more durable pharmacokinetics.

[0144] In some embodiments, the combination therapy inhibits cancer and/or tumor growth. The combination therapy may also reduce, including cell proliferation, invasiveness, and/or metastasis, thereby rendering them useful for the treatment of a cancer.

[0145] In some embodiments, the combination therapy may be used to prevent the growth of a tumor or cancer, and/or to prevent the metastasis of a tumor or cancer. In some embodiments, the combination therapy may be used to shrink or destroy a cancer.

[0146] In some embodiments, the combination therapy is useful for inhibiting proliferation of a cancer cell. In some embodiments, the combination therapy is useful for inhibiting cellular proliferation, e.g., inhibiting the rate of cellular proliferation, preventing cellular proliferation, and/or inducing cell death. In general, the combination therapy can inhibit cellular proliferation of a cancer cell or both inhibiting proliferation and/or inducing cell death of a cancer cell. In some embodiments, cell proliferation is reduced by at least about 25%, about 50%, about 75%, or about 90% after treatment with the combination therapy of the present disclosure compared with cells with no treatment. In some embodiments, cell cycle arrest marker phospho histone H3 (PH3 or PHH3) is increased by at least about 50%, about 75%, about 100%, about 200%, about 400% or about 600% after treatment with combination therapy compared with cells with no treatment. In some embodiments, cell apoptosis marker cleaved caspase-3 (CC3) is increased by at least 50%, about 75%, about 100%, about 200%, about 400% or about 600% after treatment with combination therapy compared with cells with no treatment.

[0147] Furthermore, in some embodiments, combination therapy is effective for inhibiting tumor growth, whether measured as a net value of size (weight, surface area or volume) or as a rate over time, in multiple types of tumors.

[0148] In some embodiments, the size of a tumor is reduced by about 60 % or more after treatment with the combination therapy. In some embodiments, the size of a tumor is reduced by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 100%, by a measure of weight, and/or area and/or volume.

[0149] In some embodiments, tumor growth inhibition (TGI) of a subject receiving the combination therapy may be at least about 80%, 85%, 90%, 95%, or 99%.

[0150] The cancers treatable by combination therapy of the present disclosure generally occur in mammals. Mammals include, for example, humans, non-human primates, dogs, cats, rats, mice, rabbits, ferrets, guinea pigs, horses, pigs, sheep, goats, and cattle. In various embodiments, the cancer is lung cancer, breast cancer, e.g., mutant BRCA1 and/or mutant BRCA2 breast cancer, non-BRCA-associated breast cancer, colorectal cancer, ovarian cancer, pancreatic cancer, colorectal cancer, bladder cancer, prostate cancer, cervical cancer, renal cancer, leukemia, central nervous system cancers, myeloma, and melanoma.

[0151] In some embodiments, the cancer is a neuroendocrine cancer such as but not limited to small cell lung cancer (SCLC), adrenal medullary tumors (e.g., pheochromocytoma, neuroblastoma, ganglioneuroma, or paraganglioma), gastroenteropancreatic neuroendocrine tumors (e.g., carcinoids, gastrinoma, glucagonoma, vasoactive intestinal polypeptide-secreting tumor, pancreatic polypeptide-secreting tumor, or nonfunctioning gastroenteropancreatic tumors), meduallary thyroid cancer, Merkel cell tumor of the skin, pituitary adenoma, and pancreatic cancer. In some embodiments, the neuroendocrine cancer is a primary neuroendocrine cancer. In some embodiments, the neuroendocrine cancer is a neuroendocrine metastasis. Neuroendocrine metastatic may be in liver, lung, bone, or brain of a subject. In certain embodiments, the cancer is brain cancer, human lung carcinoma, ovarian cancer, pancreatic cancer or colorectal cancer.

[0152] In one embodiment, the combination therapy of the present disclosure is used to treat small cell lung cancer. About 12%-15% of patients having lung cancer have small cell lung cancer. Survival in metastatic small cell lung cancer is poor. Survival rate is below 5% five years after diagnosis. US incidence of small cell lung cancer is about 26K-30K. Among these patients, about 40%-80% are SSTR2 positive.

[0153] In one embodiment, the combination therapy of the present disclosure is used to treat patients having a histologically proven locally advanced or metastatic high grade neuroendocrine carcinoma (NEC). In some embodiments, the patients may have small cell and large cell neuroendocrine carcinoma of unknown primary or any extrapulmonary site. In some embodiments, the patients may have well differentiated G3 neuroendocrine neoplasms if Ki-67>30%. In some embodiments, the patients may have neuroendocrine prostate cancer (de novo or treatment-emergent) of prostate if small cell or large cell histology. In some embodiments, the patients may have mixed tumors, e.g. mixed adenoneuroendocrine carcinoma (MANEC) or mixed squamous or acinar cell NEC if the high grade (small or large

cell) NEC component comprises >50% of the original sample or subsequent biopsy. In some embodiments, the patients may have castrate resistant prostate cancer (CRPC).

[0154] A feature of the components of the combination therapy is relatively low toxicity to an organism while maintaining efficacy at inhibiting, e.g. slowing or stopping tumor growth. As used herein, "toxicity" refers to the capacity of a substance or composition to be harmful or poisonous to a cell, tissue organism or cellular environment. Low toxicity refers to a reduced capacity of a substance or composition to be harmful or poisonous to a cell, tissue organism or cellular environment. Such reduced or low toxicity may be relative to a standard measure, relative to a treatment or relative to the absence of a treatment. For example, Conjugate **1**, which comprises SN-38 as an active agent, has a toxicity lower than SN-38 administered alone.

[0155] Toxicity may further be measured relative to a subject's weight loss where weight loss over 15%, over 20% or over 30% of the body weight is indicative of toxicity. Other metrics of toxicity may also be measured such as patient presentation metrics including lethargy and general malaiase. Neutropenia, thrombopenia, white blood cell (WBC) count, complete blood cell (CBC) count may also be metrics of toxicity. Pharmacologic indicators of toxicity include elevated aminotransferases (AST/ALT) levels, neurotoxicity, kidney damage, GI damage and the like. In one embodiment, the combination therapy of the present disclosure do not cause a significant change of a subject's body weight. The body weight loss of a subject is less about 30%, about 20%, about 15%, about 10%, or about 5% after treatment with the combination therapy of the present disclosure. In another embodiment, the combination therapy of the present disclosure does not cause a significant increase of a subject's AST/ALT levels. The AST or ALT level of a subject is increased by less than about 30%, about 20%, about 15%, about 10%, or about 5% after treatment with the combination therapy of the present disclosure. In yet another embodiment, the combination therapy of the present disclosure does not cause a significant change of a subject's CBC or WBC count after treatment with the combination therapy of the present disclosure. The CBC or WBC level of a subject is decreased by less than about 30%, about 20%, about 15%, about 10%, or about 5% after treatment with the combination therapy of the present disclosure.

III. Kits and Devices

[0156] One aspect of the present disclosure provides a variety of kits and devices for conveniently and/or effectively carrying out methods of the present disclosure. Typically kits

will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[0157] In one embodiment, the present disclosure provides kits for inhibiting tumor cell growth *in vitro* or *in vivo*, comprising at least two distinct therapeutic agents. In some embodiments, the kit for inhibiting tumor cell growth comprises: (A) a first component which comprises, as an active agent, Compound I, or a prodrug, derivative, or pharmaceutically-acceptable salt thereof; and (B) a second component which comprises, as an active agent, Compound II, or a prodrug, derivative, or a pharmaceutically-acceptable salt thereof.

[0158] The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, or any delivery agent disclosed herein. The amount of each agent may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The agents may also be varied in order to increase the stability of the components of the combination therapy over a period of time and/or under a variety of conditions.

[0159] The present disclosure provides devices which may incorporate components of the combination therapy. These devices contain in a stable formulation available to be immediately delivered to a subject in need thereof, such as a human patient. In some embodiments, the subject has cancer.

[0160] Non-limiting examples of the devices include a pump, a catheter, a needle, a transdermal patch, a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices. The devices may be employed to deliver components of the combination therapy according to single, multi- or split-dosing regiments. The devices may be employed to deliver components of the combination therapy across biological tissue, intradermal, subcutaneously, or intramuscularly.

IV. Definitions

[0161] The term "compound", as used herein, is meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted. In the present application, compound is used interechangably with conjugate. Therefore, conjugate, as used herein, is also meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted.

[0162] The compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless

otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[0163] Compounds of the present disclosure also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Examples prototropic tautomers include ketone – enol pairs, amide – imidic acid pairs, lactam – lactim pairs, amide – imidic acid pairs, enamine – imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

[0164] Compounds of the present disclosure also include all of the isotopes of the atoms occurring in the intermediate or final compounds. “Isotopes” refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium.

[0165] The compounds and salts of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[0166] The terms "subject" or "patient", as used herein, refer to any organism to which the combination therapy may be administered, e.g., for experimental, therapeutic, diagnostic, and/or prophylactic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, guinea pigs, cattle, pigs, sheep, horses, dogs, cats, hamsters, lamas, non-human primates, and humans).

[0167] The terms "treating" or “preventing”, as used herein, can include preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having the disease, disorder or condition; inhibiting the disease, disorder or condition, e.g., impeding its

progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain.

[0168] A "target", as used herein, shall mean a site to which targeted constructs bind. A target may be either *in vivo* or *in vitro*. In certain embodiments, a target may be cancer cells found in leukemias or tumors (e.g., tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast and colon as well as other carcinomas and sarcomas). In still other embodiments, a target may refer to a molecular structure to which a targeting moiety or ligand binds, such as a hapten, epitope, receptor, dsDNA fragment, carbohydrate or enzyme. A target may be a type of tissue, e.g., neuronal tissue, intestinal tissue, pancreatic tissue, liver, kidney, prostate, ovary, lung, bone marrow, or breast tissue.

[0169] The "target cells" that may serve as the target for the combination therapy, are generally animal cells, e.g., mammalian cells. The present method may be used to modify cellular function of living cells *in vitro*, i.e., in cell culture, or *in vivo*, in which the cells form part of or otherwise exist in animal tissue. Thus, the target cells may include, for example, the blood, lymph tissue, cells lining the alimentary canal, such as the oral and pharyngeal mucosa, cells forming the villi of the small intestine, cells lining the large intestine, cells lining the respiratory system (nasal passages/lungs) of an animal (which may be contacted by inhalation of the subject disclosure), dermal/epidermal cells, cells of the vagina and rectum, cells of internal organs including cells of the placenta and the so-called blood/brain barrier, etc. In general, a target cell expresses at least one type of SSTR. In some embodiments, a target cell can be a cell that expresses an SSTR and is targeted by a conjugate described herein, and is near a cell that is affected by release of the active agent of the conjugate. For example, a blood vessel expressing an SSTR that is in proximity to a tumor may be the target, while the active agent released at the site will affect the tumor.

[0170] The term "therapeutic effect" is art-recognized and refers to a local or systemic effect in animals, particularly mammals, and more particularly humans caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease, disorder or condition in the

enhancement of desirable physical or mental development and conditions in an animal, e.g., a human.

[0171] The term "modulation" is art-recognized and refers to up regulation (i.e., activation or stimulation), down regulation (i.e., inhibition or suppression) of a response, or the two in combination or apart. The modulation is generally compared to a baseline or reference that can be internal or external to the treated entity.

[0172] The terms "sufficient" and "effective", as used interchangeably herein, refer to an amount (e.g., mass, volume, dosage, concentration, and/or time period) needed to achieve one or more desired result(s). A "therapeutically effective amount" is at least the minimum concentration required to affect a measurable improvement or prevention of at least one symptom or a particular condition or disorder, to effect a measurable enhancement of life expectancy, or to generally improve patient quality of life. The therapeutically effective amount is thus dependent upon the specific biologically active molecule and the specific condition or disorder to be treated. Therapeutically effective amounts of many active agents, such as antibodies, are known in the art. The therapeutically effective amounts of compounds and compositions described herein, e.g., for treating specific disorders may be determined by techniques that are well within the craft of a skilled artisan, such as a physician.

[0173] The terms "bioactive agent" and "active agent", as used interchangeably herein, include, without limitation, physiologically or pharmacologically active substances that act locally or systemically in the body. A bioactive agent is a substance used for the treatment (e.g., therapeutic agent), prevention (e.g., prophylactic agent), diagnosis (e.g., diagnostic agent), cure or mitigation of disease or illness, a substance which affects the structure or function of the body, or pro-drugs, which become biologically active or more active after they have been placed in a predetermined physiological environment.

[0174] The term "prodrug" refers to an agent, including a small organic molecule, peptide, nucleic acid or protein, that is converted into a biologically active form *in vitro* and/or *in vivo*. Prodrugs can be useful because, in some situations, they may be easier to administer than the parent compound (the active compound). For example, a prodrug may be bioavailable by oral administration whereas the parent compound is not. The prodrug may also have improved solubility in pharmaceutical compositions compared to the parent drug. A prodrug may also be less toxic than the parent. A prodrug may be converted into the parent drug by various mechanisms, including enzymatic processes and metabolic hydrolysis. Harper, N.J. (1962)

Drug Latentiation in Jucker, ed. *Progress in Drug Research*, 4:221-294; Morozowich et al. (1977) Application of Physical Organic Principles to Prodrug Design in E. B. Roche ed. *Design of Biopharmaceutical Properties through Prodrugs and Analogs*, APhA; Acad. Pharm. Sci.; E. B. Roche, ed. (1977) *Bioreversible Carriers in Drug in Drug Design, Theory and Application*, APhA; H. Bundgaard, ed. (1985) *Design of Prodrugs*, Elsevier; Wang et al. (1999) Prodrug approaches to the improved delivery of peptide drug, Curr. Pharm. Design. 5(4):265-287; Pauletti et al. (1997) Improvement in peptide bioavailability: Peptidomimetics and Prodrug Strategies, Adv. Drug. Delivery Rev. 27:235-256; Mizen et al. (1998). The Use of Esters as Prodrugs for Oral Delivery of β-Lactam antibiotics, Pharm. Biotech. 11:345-365; Gaignault et al. (1996) Designing Prodrugs and Bioprecursors I. Carrier Prodrugs, Pract. Med. Chem. 671-696; M. Asgharnejad (2000). Improving Oral Drug Transport Via Prodrugs, in G. L. Amidon, P. I. Lee and E. M. Topp, Eds., *Transport Processes in Pharmaceutical Systems*, Marcell Dekker, p. 185-218; Balant et al. (1990) Prodrugs for the improvement of drug absorption via different routes of administration, *Eur. J. Drug Metab. Pharmacokinet.*, 15(2): 143-53; Balimane and Sinko (1999). Involvement of multiple transporters in the oral absorption of nucleoside analogues, *Adv. Drug Delivery Rev.*, 39(1-3):183-209; Browne (1997). Fosphenytoin (Cerebyx), *Clin. Neuropharmacol.* 20(1): 1-12; Bundgaard (1979). Bioreversible derivatization of drugs--principle and applicability to improve the therapeutic effects of drugs, *Arch. Pharm. Chemi.* 86(1): 1-39; H. Bundgaard, ed. (1985) *Design of Prodrugs,* New York: Elsevier; Fleisher et al. (1996) Improved oral drug delivery: solubility limitations overcome by the use of prodrugs, *Adv. Drug Delivery Rev.* 19(2): 115-130; Fleisher et al. (1985) Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting, *Methods Enzymol.* 112: 360-81; Farquhar D, et al. (1983) Biologically Reversible Phosphate-Protective Groups, *J. Pharm. Sci.*, 72(3): 324-325; Han, H.K. et al. (2000) Targeted prodrug design to optimize drug delivery, *AAPS PharmSci.*, 2(1): E6; Sadzuka Y. (2000) Effective prodrug liposome and conversion to active metabolite, *Curr. Drug Metab.*, 1(1):31-48; D.M. Lambert (2000) Rationale and applications of lipids as prodrug carriers, *Eur. J. Pharm. Sci.*, 11 Suppl. 2:S15-27; Wang, W. et al. (1999) Prodrug approaches to the improved delivery of peptide drugs. *Curr. Pharm. Des.,* 5(4):265-87.

[0175] The term "biocompatible", as used herein, refers to a material that along with any metabolites or degradation products thereof that are generally non-toxic to the recipient and do not cause any significant adverse effects to the recipient. Generally speaking,

biocompatible materials are materials which do not elicit a significant inflammatory or immune response when administered to a patient.

[0176] The term "biodegradable" as used herein, generally refers to a material that will degrade or erode under physiologic conditions to smaller units or chemical species that are capable of being metabolized, eliminated, or excreted by the subject. The degradation time is a function of composition and morphology. Degradation times can be from hours to weeks.

[0177] The term "pharmaceutically acceptable", as used herein, refers to compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio, in accordance with the guidelines of agencies such as the U.S. Food and Drug Administration. A "pharmaceutically acceptable carrier", as used herein, refers to all components of a pharmaceutical formulation that facilitate the delivery of the composition *in vivo*. Pharmaceutically acceptable carriers include, but are not limited to, diluents, preservatives, binders, lubricants, disintegrators, swelling agents, fillers, stabilizers, and combinations thereof.

[0178] The term "molecular weight", as used herein, generally refers to the mass or average mass of a material. If a polymer or oligomer, the molecular weight can refer to the relative average chain length or relative chain mass of the bulk polymer. In practice, the molecular weight of polymers and oligomers can be estimated or characterized in various ways including gel permeation chromatography (GPC) or capillary viscometry. GPC molecular weights are reported as the weight-average molecular weight (M_w) as opposed to the number-average molecular weight (M_n). Capillary viscometry provides estimates of molecular weight as the inherent viscosity determined from a dilute polymer solution using a particular set of concentration, temperature, and solvent conditions.

[0179] The term "small molecule", as used herein, generally refers to an organic molecule that is less than 2000 g/mol in molecular weight, less than 1500 g/mol, less than 1000 g/mol, less than 800 g/mol, or less than 500 g/mol. Small molecules are non-polymeric and/or non-oligomeric.

[0180] The terms "polypeptide," "peptide" and "protein" generally refer to a polymer of amino acid residues. As used herein, the term also applies to amino acid polymers in which one or more amino acids are chemical analogs or modified derivatives of corresponding

naturally-occurring amino acids or are unnatural amino acids. The term "protein", as generally used herein, refers to a polymer of amino acids linked to each other by peptide bonds to form a polypeptide for which the chain length is sufficient to produce tertiary and/or quaternary structure. The term “protein” excludes small peptides by definition, the small peptides lacking the requisite higher-order structure necessary to be considered a protein.

[0181] The terms "nucleic acid," "polynucleotide," and "oligonucleotide" are used interchangeably to refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. These terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general and unless otherwise specified, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. The term “nucleic acid” is a term of art that refers to a string of at least two base-sugar-phosphate monomeric units. Nucleotides are the monomeric units of nucleic acid polymers. The term includes deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the form of a messenger RNA, antisense, plasmid DNA, parts of a plasmid DNA or genetic material derived from a virus. An antisense nucleic acid is a polynucleotide that interferes with the expression of a DNA and/or RNA sequence. The term nucleic acids refers to a string of at least two base-sugar-phosphate combinations. Natural nucleic acids have a phosphate backbone. Artificial nucleic acids may contain other types of backbones, but contain the same bases as natural nucleic acids. The term also includes PNAs (peptide nucleic acids), phosphorothioates, and other variants of the phosphate backbone of native nucleic acids.

[0182] As used herein, the term “linker” refers to a carbon chain that can contain heteroatoms (e.g., nitrogen, oxygen, sulfur, etc.) and which may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 atoms long. Linkers may be substituted with various substituents including, but not limited to, hydrogen atoms, alkyl, alkenyl, alkynl, amino, alkylamino, dialkylamino, trialkylamino, hydroxyl, alkoxy, halogen, aryl, heterocyclic, aromatic heterocyclic, cyano, amide, carbamoyl, carboxylic acid, ester, thioether, alkylthioether, thiol, and ureido groups. Those of skill in the art will recognize that each of these groups may in turn be substituted. Examples of linkers include, but are not

limited to, pH-sensitive linkers, protease cleavable peptide linkers, nuclease sensitive nucleic acid linkers, lipase sensitive lipid linkers, glycosidase sensitive carbohydrate linkers, hypoxia sensitive linkers, photo-cleavable linkers, heat-labile linkers, enzyme cleavable linkers (e.g., esterase cleavable linker), ultrasound-sensitive linkers, and x-ray cleavable linkers.

[0183] The term "pharmaceutically acceptable salt(s)" refers to salts of acidic or basic groups that may be present in compounds used in the present compositions. Compounds included in the present compositions that are basic in nature are capable of forming a variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, including but not limited to sulfate, citrate, malate, acetate, oxalate, chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds included in the present compositions that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds included in the present compositions, that are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium, lithium, zinc, potassium, and iron salts.

[0184] If the compounds described herein are obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds. Those skilled in the art will recognize various synthetic methodologies that may be used to prepare non-toxic pharmaceutically acceptable addition salts.

[0185] A pharmaceutically acceptable salt can be derived from an acid selected from 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-

oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isethionic, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, pantothenic, phosphoric acid, proprionic acid, pyroglutamic acid, salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tartaric acid, thiocyanic acid, toluenesulfonic acid, trifluoroacetic, and undecylenic acid.

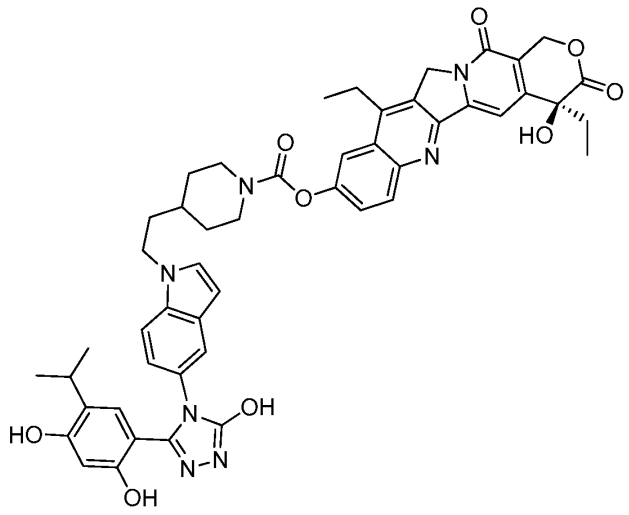
[0186] The term "bioavailable" is art-recognized and refers to a form of the subject disclosure that allows for it, or a portion of the amount administered, to be absorbed by, incorporated to, or otherwise physiologically available to a subject or patient to whom it is administered.

[0187] It will be appreciated that the following examples are intended to illustrate but not to limit the present disclosure. Various other examples and modifications of the foregoing description and examples will be apparent to a person skilled in the art after reading the disclosure without departing from the spirit and scope of the disclosure, and it is intended that all such examples or modifications be included within the scope of the appended claims. All publications and patents referenced herein are hereby incorporated by reference in their entirety.

EXAMPLES

Example 1: Synthesis of Conjugate 1

Conjugate **1** (SDC-TRAP-0063)

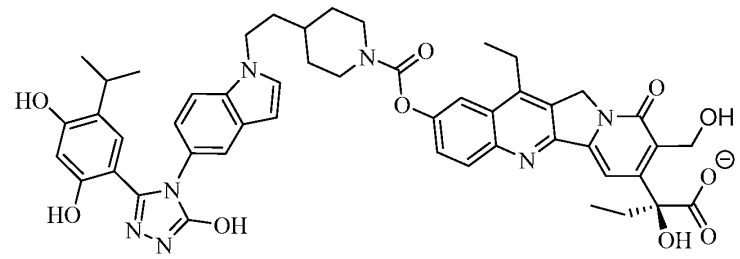


((*S*)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl 4-(2-(5-(3-(2,4-dihydroxy-5-isopropylphenyl)-5-hydroxy-4H-1,2,4-triazol-4-yl)-1H-indol-1-yl)ethyl)piperidine-1-carboxylate) or its tautomer.

[0188] A synthesis scheme of the synthesis of SDC-TRAP-0063 is provided in Example 6 of PCT Application No. PCT/US2013/036783. The person of ordinary skill in the art would be able, without undue experimentation, to adapt this synthetic scheme for making other targeting molecule conjugates within the scope of the invention.

Example 2: Salt form and formulation of HSP90 binding drug conjugate

[0189] In solution, Conjugate **1** contains a lactone ring at pH-dependent equilibrium with the corresponding open chain carboxylic acid form. At high pH (above pH of 9.3, pKa value) the equilibrium shifts toward an open ring carboxylic acid form and at low pH it shifts toward the closed ring lactone form shown below:

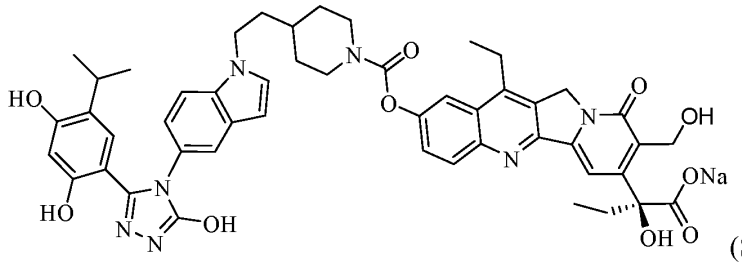


[0190] The open ring carboxylic acid form may form a salt with cationic ions include, but not limited to, lithium, aluminum, calcium, magnesium, potassium, sodium, zinc, barium,

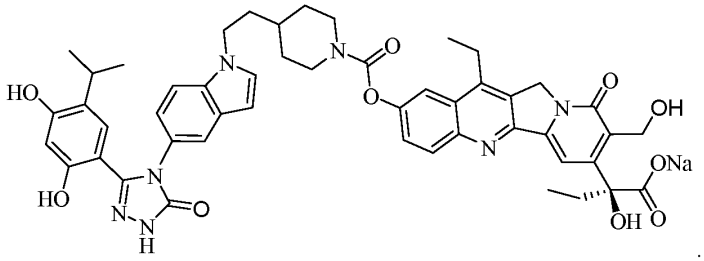
bismuth, benethamine, diethylamine, tromethamine, benzathid, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, or procaine.

Sodium salt derivative of Conjugate **1** (SDC-TRAP-0063)

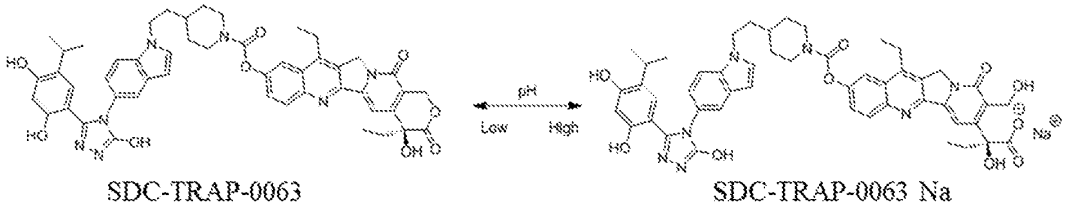
[0191] The sodium salt (SDC-TRAP-0063 Sodium or SDC-TRAP-0063 Na) of the carboxylic acid derivative has a structure of



(Sodium (*S*)-2-(2-((4-(2-(5-(3-(2,4-dihydroxy-5-isopropylphenyl)-5-hydroxy-4*H*-1,2,4-triazol-4-yl)-1*H*-indol-1-yl)ethyl)piperidine-1-carbonyl)oxy)-12-ethyl-8-(hydroxymethyl)-9-oxo-9,11-dihydroindolizino[1,2-b]quinolin-7-yl)-2-hydroxybutanoate) or its tautomer:



[0192] Structure of SDC-TRAP-0063 in both lactone and sodium salt form:



SDC-TRAP-0063 SDC-TRAP-0063 Na

[0193] SDC-TRAP-0063 drug substance is isolated and stored in the lactone form and SDC-TRAP-0063 Sodium drug product is converted and stored in the carboxylic acid sodium salt form.

[0194] SDC-TRAP-0063 can be prepared with the following process: a portion of tert-butanol was melted at 28 – 32°C and dispensed into an 8-liter glass mixing vessel jacketed at 28 – 32°C. SDC-TRAP-0063 powder was added slowly into the stirring tert-butanol and mixed for at least 20 minutes. The quantity of SDC-TRAP-0063 added was determined

gravimetrically and the target drug product batch size was calculated. A second portion of tert-butanol was then added by weight quantity sufficient (Q.S. or QS) and mixed for at least 15 minutes with a ~6" magnetic stir bar to adequately wet and suspend it. 0.3 normal aqueous sodium hydroxide solution was then slowly added and allowed to mix for at least an additional 1 hour. Complete dissolution of SDC-TRAP-0063 powder was confirmed by visual observation both while mixing and while the mixer was stopped. Water for Injection (WFI) was then dispensed up to ~95% of the target total batch volume and mixed for 20 minutes. A sample was taken and measured to ensure pH was greater than or equal to 9.8, with option to adjust by incremental addition of 5-gram aliquots of 0.3 normal aqueous sodium hydroxide solution with at least 15 minutes of mixing if necessary. Water for Injection was again added to weight QS and mixed for 15 minutes to complete compounding of the bulk drug solution. The 8 liter glass mixing vessel jacket temperature was then reduced to within 20 – 25°C. Product sterilization was achieved by filtration through one at least two Millipore Opticap XL3 0.2 µm filters in series, and samples were taken immediately pre-filter for microbial enumeration testing. Depyrogenated 10 milliliter nominal size borosilicate glass vials were then aseptically filled with 1.1 milliliters of bulk drug solution per vial. Vials were stoppered into the lyophilization position and loaded into a lyophilizer. Vials were lyophilized per the recipe in Table 1, and fully stoppered. Vials were aseptically removed from the lyophilizer and caps were crimped to seal the vials. Exterior vial washing and visual inspection were conducted to complete production of the drug product in its primary enclosure.

Table 1: Lyophilization steps and conditions

Step #	Step Description	Temperature	Pressure	Duration
1	Loading	5°C	Atmospheric	Not applicable
2	Freezing	5°C	Atmospheric	120 minutes
3	Freezing ramp	5°C to -50°C	Atmospheric	120 minutes
4	Freezing	-50°C	Atmospheric	210 minutes
5	Freezing ramp	-50°C to -40°C	Atmospheric	15 minutes
6	Evacuation	-40°C	80 µbar	Not applicable
7	Primary drying ramp	-40°C to -15°C	80 µbar	50 minutes
8	Primary drying	-15°C	80 µbar	2,520 minutes
9	Secondary drying ramp	-15°C to 25°C	80 µbar	156 minutes
10	Secondary drying	25°C	80 µbar	960 minutes
11	Pre-aeration with nitrogen	25°C	800 µbar	Not applicable
12	Stoppering	25°C	800 µbar	Not applicable
13	Aeration with nitrogen	25°C	Atmospheric	Not applicable
14	Unloading*	25°C	Atmospheric	Not applicable

* If unloading is not immediate, maintain the shelves at 5°C; before starting unloading, move the shelf temperature to the unloading temperature.

[0195] During the manufacturing process, SDC-TRAP-0063 was converted to SDC-TRAP-0063 Sodium, which is the dominant form at pH above 9.3. SDC-TRAP-0063 Sodium drug product was aseptically manufactured as a sterile-filtered solution that was lyophilized. The composition of the lyophilized drug product is shown below:

Ingredient	Role	Amount (mg/vial)
SDC-TRAP-0063 sodium	Active	105

[0196] This solution is filled to deliver 105 mg/vial into a container closure system consisting of a USP Type 1 clear glass vial, stopper, and overseal. The drug product is stored at 2°C to 8°C, away from light. Prior to administration the lyophilized powder is reconstituted with Water for Injection and then is further diluted in 5% Mannitol, USP to the target concentration prior to use. SDC-TRAP-0063 Sodium may have a concentration of between about 20 to about 25 mg/mL, about 25 to about 50 mg/mL, between about 50 to about 100 mg/mL, between about 100 to about 150 mg/mL, or between about 150 to 200 mg/mL. The drug product is intended for intravenous administration by infusion.

[0197] The reconstituted solution of SDC-TRAP-0063 Sodium has a pH of about 10.0. This solution is diluted to the target dose in 5% Mannitol, USP. The pH of the infusion solution depends on the concentration of SDC-TRAP-0063 Sodium in the diluted infusion solution. Across the dose ranges employed in the clinical study protocol, the volume of the diluted infusion solution administered will range from 50 to 500 mL, and the pH will range from 8.1 to 9.6. In order to reduce the potential risks of injection site pain and/or damage to the venous endothelium during IV administration, a central venous access line is used for administration of the diluted SDC-TRAP-0063 Sodium.

Example 3: First-in-Human Phase 1/2a Study of Conjugate 1 for Patients with Advanced Solid Tumors: Phase 1 Results

[0198] In a Phase 1/2a study of Conjugate **1**, patients received weekly Conjugate **1** treatment for 3 of 4 weeks in a 28-day cycle. The patients have histologically or cytologically confirmed advanced solid tumors. Their tumor types included anus, breast, cholangiocarcinoma, colon, Ewing sarcoma, liver, lung, neuroendocrine (unknown primary), ovary, pancreas, salivary gland, and sarcoma. They have received one or more prior lines of anticancer therapy. The patients received flat dosings or dosings based on body surface area (BSA). The doses included 30mg, 60mg, 120mg, 240mg, 360mg, 175 mg/m^2, and 200

mg/m^2. The maximum tolerated dose (MTD) for Conjugate **1** monotherapy was determined to be 175 mg/m^2. BSA-based dosing will be used for future studies.

[0199] To study pharmacokinetics (PK) profile, multiple plasma samples were collected to measure Conjugate **1** and SN-38 concentrations. As shown in Table 2, Conjugate **1** exhibits non-linear PK at lower doses, but greater proportionality was observed at higher doses. Elimination half-life was ~10 hours across all dose ranges. Free SN-38 levels were generally low. AUC is <2% of the conjugate (Conjugate **1**) AUC. Inter-patient PK variability was observed with flat doses which were generally correlated with BSA. PK analyses show the linker is stable in circulation and systemic exposure to free SN-28 is low.

Table 2. Pharmacokinetic Parameters (Cycle 1 Day 1)

Dose range (mg/m2)	n	Mean t1/2 (h)	Mean Cmax (μg/mL)	Mean AUCinf (h·μg/mL)
16-18	2	8.2	0.69	0.80
30-40	2	7.1	1.68	1.89
60-64	3	6.0	6.04	6.83
120-140	3	6.7	25.8	35.0
172-184	5	8.3	40.4	72.2
197-228	6	18	53.7	87.5

[0200] Of the 17 patients who received Conjugate **1** treatment, one patient achieved partial response (PR; a reduction of tumor size by 30% or more) and 5 had stable disease (SD; the tumor has responded and did not grow in size). 120-360mg flat dose and 175 mg/m2 BSA dose were associated with stable disease that was maintained for ≥12 weeks.

[0201] Overall, Conjugate **1** is safe and has an expected adverse (AE) event profile. AEs are well-managed with dose modification. The most common AEs were nausea, fatigue, diarrhea, vomiting and alopecia.

[0202] Conjugate **1** will be evaluated in 3 Phase 2a expansion disease cohorts: previously treated pancreatic adenocarcinoma; endometrial adenocarcinoma; and squamous cell carcinoma of the anus, cervix, or head and neck.

Example 4: Levels of Conjugate 1 and SN-38 in Tumor and Plasma

[0203] A pancreatic cancer patient received Conjugate **1** at a dose of 150 mg/m^2 and underwent a tumor biopsy to determine Conjugate **1** and SN-38 levels in tumor and plasma. Samples were taken 24 h after the second dose of 150 mg/m^2 Conjugate **1** from a liver metastasis.

[0204] As shown in Table 3, significant levels of Conjugate **1** and SN-38 were observed in tumor, while a fraction of the concentration was observed in the plasma (compared to 5 other patients dosed at 175 mg/m^2) at hour 24. Conjugate **1** and SN-38 levels in tumor are much higher than the levels in plasma. These data demonstrate focused tumor uptake, retention of the conjugate (Conjugate **1**), and intratumoral release of the payload (SN-38) over time from ongoing slow linker cleavage. These data are also consistent with preclinical data which show retained Conjugate **1** slowly releases SN-38 payload in cancer cells over multiple days.

Table 3. Levels of Conjugate **1** and SN-38 in patient biopsy at 24 h

	Amount in Plasma (nM)	Amount in Tumor (nM)	Ratio of tumor level / plasma level
Conjugate **1**	52.3*	318	About 6.1
SN-38	25.5*	86.6	About 3.4

*Average of 5 patients dosed at 175 mg/m^2

[0205] The SN-38 exposure at 24 h of the pancreatic patient was also compared with the SN-38 exposure at 72 h of patients who received Onivyde (irinotecan) treatments (13 patients), data shown in Table 4. The patient who received Conjugate **1** treatment had 86.6nM of SN-38 in tumor, about 3.5 fold of the SN-38 tumor level (24.5nM) of patients who received Onivyde treatment.

Table 4. Levels of Irinotecan and SN-38 in patient biopsies after Onivyde treatment at 72 h

	Amount in Plasma (nM)	Amount in Tumor (nM)	Ratio of tumor level / plasma level
Irinotecan	7921	4043	About 0.5
SN-38	5.9	24.5	About 4.2

[0206] For patients who received Onivyde treatment, low SN-38 levels in tumor and high irinotecan levels over time in circulation are consistent with the fact that the non-targeted liposome formulations of irinotecan spend a long time in circulation.

[0207] For patients who received Onivyde treatment, tumor levels of irinotecan were only about ½ of plasma levels, illustrating the lack of tumor targeting, which contrasts with the patients who received treatment of the targeted conjugate Conjugate **1** and had higher levels of the conjugate and payload in tumor relative to plasma.

[0208] Onivyde tumor irinotecan levels inflated due to very high plasma levels and levels in vasculature of encapsulated irinotecan confounding actual tumor intracellular levels. Onyvide's untargeted approach results in low levels of SN-38 in the tumor.

Example 5: Efficacious Tumor Exposure in Mouse Xenograft Model

[0209] Conjugate **1** dosed in an H1975 (NSCLC) mouse xenograft model showed 24h tumor exposures of Conjugate **1** and SN-38 that are similar to levels measured in patient biopsy in Example 4. Efficacies in the H1975 model at Conjugate **1** weekly doses of 72, 100 and 150 mg/kg are shown in Fig. 1A and Fig. 1B.

[0210] Exposures from doses of 50 mg/kg and 100 mg/kg in tumor PK (while variable), are similar to patient biopsy exposures and weekly doses of 72 mg/kg and 100 mg/kg result in significant efficacy in the xenograft model. Dose of 150 mg/kg that results in tumor regression, provides 24 h tumor exposure that is significantly higher for Conjugate **1** but only marginally higher for SN-38 relative to the patient biopsy data.

Table 5. Levels of PEN-866 and SN-38 in H1975 Model at 24 h

	Conjugate **1** in tumor at 24 h (nM)	SN-38 in tumor at 24 h (nM)
H1975 (50 mg/kg)	163	98
H1975 (100 mg/kg)	149	18.4
H1975 (150 mg/kg)	4990	221
Patient Biopsy (Example 4)	318	86.6

Example 6: Antitumor efficacy of Conjugate 1 in combination with Talazoparib

[0211] Mice bearing SKOV3 (ovarian cancer) tumors were treated with the following:

1). Vehicle controls;

2). 75 mg/kg of Conjugate **1** once per week (day 1) via intravenous administration (IV) for 6 weeks;

3). 0.33 mg/kg Talazoparib daily for 4 days on/3 days off (days 2-5 of the week) via oral administration (PO) for 6 weeks;

4). 75 mg/kg Conjugate **1** once per week via IV, and 0.33mpk Talazoparib daily for 4 days on/3 days off starting 24hrs post Conjugate **1** dose for 6 weeks.

[0212] Tumor volumes were measured after treatment. As shown in Fig. 3A, a statistically significant improvement in efficacy was observed with the combination treatment when compared to Conjugate **1** treatment alone and talazoparib treatment alone.

[0213] In a similar study, Conjugate **1** and talazoparib combo treatment is studied in mice bearing NCI-H460 (non-small cell lung cancer (NSCLC)) model. Mice bearing SKOV3 (ovarian cancer) tumors were treated with the following:

1). Vehicle controls;

2). 100 mg/kg of Conjugate **1** once per week (day 1) via intravenous administration (IV) for 2 weeks;

3). 0.33 mg/kg Talazoparib daily for 4 days on/3 days off (days 2-5 of the week) via oral administration (PO) for 2 weeks;

4). 100 mg/kg Conjugate **1** once per week via IV, and 0.33mpk Talazoparib daily for 4 days on/3 days off starting 24hrs post Conjugate **1** dose for 2 weeks.

[0214] Tumor volumes were measured after treatment. As shown in Fig. 3B, a statistically significant improvement in efficacy was observed with the combination treatment when compared to Conjugate **1** treatment alone and talazoparib treatment alone. The pH2AX quantity data in Fig. 3C demonstrated that treatment with Conjugate **1** in combination with talazoparib produced sustained and synergistic DNA damage 7 days after dosing.

[0215] In another similar study, Conjugate **1** and veliparib combo treatment is evaluated in mice models.

[0216] The scope of the present disclosure is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[0217] In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[0218] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

[0219] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0220] In addition, it is to be understood that any particular embodiment of the present disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the disclosure can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0221] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[0222] Section and table headings are not intended to be limiting.