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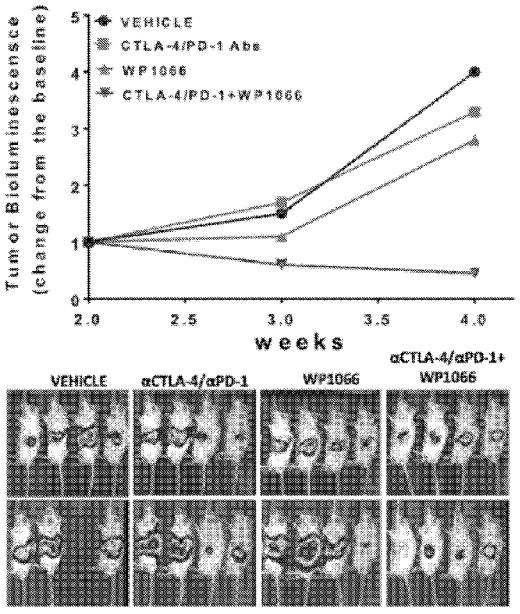

FIG. 1

(57) Abstract: Provided herein are methods of treating patients with a combination of a transcription inhibitor (e.g., a STAT3 inhibitor) and an immune checkpoint blockade therapy (e.g., anti- PD-1 therapy, anti-PD-L1 therapy, anti-CTLA-4 therapy). The patient may have a proliferative disease, such as cancer or psoriasis. The patient may have a pathogenic infection.

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DESCRIPTION

COMBINATIONS OF TRANSCRIPTION INHIBITORS AND IMMUNE CHECKPOINT INHIBITORS FOR TREATMENT OF DISEASE

REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the priority benefit of United States provisional application number 62/828,175, filed April 2, 2019, the entire contents of which is incorporated herein by reference.

BACKGROUND

1. Field

[0002] The present invention relates generally to the fields of medicine and oncology. More particularly, it concerns combination therapy comprising a STAT3 inhibitor and one or more immune checkpoint inhibitor for treating proliferative diseases.

2. Description of Related Art

[0003] Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies with limited treatment options besides surgery, the only curative modality. The surrounding tumor microenvironment is very complex and constituted mostly by a dense fibro-inflammatory stroma infiltrated by immunosuppressive cells, which have been implicated in tumorigenesis and the lack of responses to most therapies. Several immunotherapy approaches have emerged in the past decade, but single agent immune checkpoint inhibitors have not proven efficacious in treating pancreatic cancer. Thus, there is an urgent need to develop efficient strategies to favor anti-tumoral immunity.

SUMMARY

[0004] As such, provided herein are methods of increasing the efficacy of immunotherapies by inhibiting STAT3, thereby promoting an anti-tumor immune response by reducing the tumor induced immune suppression. These methods, which are useful for treating proliferative diseases, such as cancer, comprise treating patients with a combination of two therapeutic agents: 1) a STAT3 inhibitor (e.g., WP1066, WP1732, etc.) and 2) one or more immune checkpoint inhibitor.

[0005] In one embodiment, provided herein are methods of treating a disease in a patient, the method comprising administering to the patient a combined effective amount of a transcription inhibitor and an immune checkpoint inhibitor. In some aspects, the transcription inhibitor is a STAT3 inhibitor. In certain aspects, the transcription inhibitor is a α,β-unsaturated cyanocarboxamide. In certain aspects, the transcription inhibitor is WP1066 or WP1732. In some aspects, the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4 therapy. In certain aspects, the anti-PD1 therapy comprises nivolumab, pembrolizumab, pidilizumab, cemiplimab, tislelizumab, spartalizumab, PF-06801591, AK105, BCD-100, BI-754091, HLX10, JS001, LZM009, MEDI 0680, MGA012, Sym021, TSR-042, MGD013, AK104, and/or XmAb20717. In certain aspects, the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, FS118, BCD-135, BGB-A333, CBT502, CK-301, CS1001, FAZ053, KN035, MDX-1105, MSB2311, SHR-1316, M7824, LY3415244, CA-170, and CX-072. In certain aspects, the anti-CTLA-4 therapy comprises ipilimumab, tremelimumab, BMS-986218, AK104, and/or XmAb20717.

[0006] In some aspects, the disease is a proliferative disease. In certain aspects, the proliferative disease is cancer. In certain aspects, the methods inhibit the survival or proliferation of cancer cells. In certain aspects, the patient has previously failed to respond to the administration of an immune checkpoint inhibitor. In certain aspects, the methods are methods of overcoming resistance to immune checkpoint inhibitor therapy. In certain aspects, the methods further comprise administering a further anti-cancer therapy to the patient. In certain aspects, the further anti-cancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy, or cytokine therapy. In some aspects, the further anti-cancer therapy comprises gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, or docetaxel.

[0007] In some aspects, the cancer is a glioma, a pancreatic cancer, a breast cancer, a melanoma, a lymphoma, or a leukemia. In certain aspects, the leukemia is AML. In some aspects, the patient has previously undergone at least one round of anti-cancer therapy. In some aspects, the proliferative disease is psoriasis. In some aspects, the disease is a pathogenic infection. In some aspects, the patient is a human.

[0008] As used herein, "essentially free," in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a

composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0009] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one.

[0010] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[0011] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, the variation that exists among the study subjects, or a value that is within 10% of a stated value.

[0012] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0014] FIG. 1. Combination therapy of WP1066 and anti-PD-1/CTLA-4 antibodies in syngeneic, orthotopic model of pancreatic cancer.

[0015] FIGS. 2A-B. Combination therapy of WP1732 and anti-PD-1/CTLA-4 antibodies in syngeneic, orthotopic model of pancreatic cancer. FIG. 2A provides Kaplan-Meier curves showing percent survival for each treatment group. FIG. 2B provides bioluminescence images for mice in each treatment group over time.

DETAILED DESCRIPTION

[0016] The present invention provides methods of treating a patient having a proliferative disorder with a combination of a transcription inhibitor and an immune checkpoint inhibitor. Such treatment may also be in combination with another therapeutic regime, such as chemotherapy.

I. Transcription Inhibitors

[0017] A transcription inhibitor may be a STAT3 inhibitor, such as an α, β-unsaturated carboxamide containing compound, for example, WP1066 or WP1732. These compounds are potent p-STAT3 inhibitors with drug-like properties. Compound WP1066 is currently being evaluated in a Phase I clinical trial (NCT01904123) as an orally administered agent. WP1732 is an analog of WP1066 that is suitable for IV administration. Signal Transducer and Activators of Transcription 3 (STAT3) plays a pivotal role in carcinogenesis, chemo- and radio-sensitivity, metastasis, and immune evasion in multiple malignances including Pancreatic Ductal Adenocarcinoma (PDAC). Its activation decreases T-cell recruitment to the tumor and the tumor microenvironment, and it increases activity and accumulation of immunosuppressive cells, such as Treg and MDSC, thereby contributing to the immunosuppressive tumor microenvironment.

[0018] WP1066, WP1732, and other α,β-unsaturated cyanocarboxamide-containing compounds have a potent anti-tumor effect. The anti-proliferative effects of WP1066, WP1732, and similar compounds have been shown *in vitro* and *in vivo* in a broad range of cancer types. It is established that inflammatory and proliferative conditions, such as cancer, rely on co-opting normal signaling pathways that lead to transcription of factors responsible for cell growth, survival, and cell differentiation. WP1066 and related compound's anticancer activity is mediated via suppression of a particular class of proteins referred to as Signal Transducers and Activators of Transcription (known as "STATs"), more specifically STAT3, and related key oncogenic transcriptional factors, namely c-Myc and HIF-1α.

[0019] WP1066's effects on the activated form of STAT3 (p-STAT3 – tyrosine 705 phosphorylated STAT3) are well documented. p-STAT3 plays an important role in regulating the process of disease cell survival and proliferation, angiogenesis, and immune system function and is persistently activated in a large number of human inflammatory processes and in hyper-proliferating diseases. Specifically, STAT3, can be activated by any one of many different upstream inducers, ultimately leading to p-STAT3, which then forms a dimer that enters the cell nucleus and triggers gene transcription. By inhibiting the presence of p-STAT3, WP1066 directly affects tumor cells by inhibiting gene transcription.

[0020] The family of α, β-unsaturated carboxamide-containing compounds contemplated for use in the present methods include those described in specifications of U.S. Patent Nos. 7,745,468; 8,119,827; 8,143,412; 8,450,337; 8,648,102; 8,779,151; 9,096,499; 8,809,377; and 9,868,736; U.S. Appln. Ser. No. 16/185,669; U.S. Patent Appln. Publn. Nos. 2016/0237082; 2005/0277680; 2011/0021805; 2011/0053992; 2010/0152143; 2014/0329901; and 2012/0214850; and International (PCT) Appln. Publn. Nos. WO2010/005,807 and WO2015/187,427, each of which is incorporated by reference herein in its entirety.

II. Immune Checkpoint Inhibitors

[0021] Immune checkpoints either turn up a signal (*e.g.*, co-stimulatory molecules) or turn down a signal. Inhibitory immune checkpoints that may be targeted by immune checkpoint blockade include adenosine A2A receptor (A2AR), B7-H3 (also known as CD276), B and T lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin (KIR), lymphocyte activation gene-3 (LAG3), programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), T-cell immunoglobulin domain and mucin domain 3 (TIM-3), and V-domain Ig suppressor of T cell activation (VISTA). In particular, the immune checkpoint inhibitors target the PD-1 axis and/or CTLA-4.

[0022] The immune checkpoint inhibitors may be drugs, such as small molecules, recombinant forms of ligand or receptors, or antibodies, such as human antibodies (*e.g.*, International Patent Publication WO2015/016718; Pardoll, *Nat Rev Cancer*, 12(4): 252-264, 2012; both incorporated herein by reference). Known inhibitors of the immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized, or human forms of antibodies may be used. As the skilled person will know, alternative and/or

equivalent names may be in use for certain antibodies mentioned in the present disclosure. Such alternative and/or equivalent names are interchangeable in the context of the present disclosure. For example, it is known that lambrolizumab is also known under the alternative and equivalent names MK-3475 and pembrolizumab.

[0023] In some embodiments, a PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or an oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. 8,735,553, 8,354,509, and 8,008,449, all of which are incorporated herein by reference. Other PD-1 axis antagonists for use in the methods provided herein are known in the art, such as described in U.S. Patent Application Publication Nos. 2014/0294898, 2014/022021, and 2011/0008369, all of which are incorporated herein by reference.

[0024] In some embodiments, a PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of Nivolumab (also known as MDX-1106-04, MDX-1106, MK-347, ONO-4538, BMS-936558, and OPDIVO®; described in WO2006/121168), Pembrolizumab (also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475; WO2009/114335), Pidilizumab (also known as CT-011, hBAT or hBAT-1; WO2009/101611), Cemiplimab (also known as LIBTAYO®, REGN-2810, REGN2810, SAR-439684, SAR439684), Tislelizumab (also known as BGB-A317, hu317-1/IgG4mt2; U.S. Patent No. 8,735,553), Spartalizumab (also known as PDR001, PDR-001, NPV-PDR001, NPVPDR001; U.S. Patent No. 9,683,048), PF-06801591, AK105, BCD-100, BI-754091, HLX10, JS001, LZM009, MEDI 0680, MGA012, Sym021, TSR-042, MGD013, AK104 (bispecific with anti-CTLA4), and XmAb20717 (bispecific with anti-CTLA4).

[0025] In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2

fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence)). For example, AMP-224 (also known as B7-DCIg) is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0026] In some embodiment, a PD-L1 binding antagonist is an anti-PD-L1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-L1 antibody is selected from the group consisting of Atezolizumab (also known as Tencentriq, MPDL3280A; described in U.S. Patent No. 8,217,149), Avelumab (also known as BAVENCIO®, MSB-0010718C, MSB0010718C), Durvalumab (also known as IMFINZI®, MEDI-4736, MEDI4736; described in WO2011/066389), FS118, BCD-135, BGB-A333, CBT502 (also known as TQB2450), CK-301, CS1001 (also known as WBP3155), FAZ053, KN035, MDX-1105, MSB2311, SHR-1316, M7824, LY3415244, CA-170, and CX-072.

[0027] Another immune checkpoint protein that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CTLA-4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA-4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA-4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[0028] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in US Patent No. 8,119,129; PCT Publn. Nos. WO 01/14424, WO 98/42752, WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab); U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc Natl Acad Sci USA*, 95(17): 10067-10071; Camacho *et*

al. (2004) *J Clin Oncology*, 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res*, 58:5301-5304 can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No. WO2001/014424, WO2000/037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[0029] An exemplary anti-CTLA-4 antibody is ipilimumab (also known as 10D1, MDX-010, MDX-101, MDX-CTLA4, and YERVOY®) or antigen binding fragments and variants thereof (see, *e.g.*, WO 01/14424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises the CDR1, CDR2, and CDR3 domains of the VH region of ipilimumab, and the CDR1, CDR2, and CDR3 domains of the VL region of ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on CTLA-4 as the above-mentioned antibodies. In another embodiment, the antibody has an at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95%, or 99% variable region identity with ipilimumab).

[0030] In some embodiment, a CTLA-4 binding antagonist is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-CTLA-4 antibody is selected from the group consisting of ipilimumab (also known as 10D1, MDX-010, MDX-101, MDX-CTLA4, and YERVOY®; described in WO 01/14424), Tremelimumab (also known as CP-675,206, CP-675, ticilimumab; described in WO 00/37504), BMS-986218, AK104 (bispecific with anti-PD-1), and XmAb20717 (bispecific with anti-PD-1).

[0031] Other molecules for modulating CTLA-4 include CTLA-4 ligands and receptors such as described in U.S. Patent Nos. 5844905, 5885796 and International Patent Application Nos. WO1995001994 and WO1998042752; all incorporated herein by reference, and immunoadhesins such as described in U.S. Patent No. 8329867, incorporated herein by reference.

[0032] Another immune checkpoint protein that can be targeted in the methods provided herein is lymphocyte-activation gene 3 (LAG-3), also known as CD223. The

complete protein sequence of human LAG-3 has the Genbank accession number NP-002277. LAG-3 is found on the surface of activated T cells, natural killer cells, B cells, and plasmacytoid dendritic cells. LAG-3 acts as an "off" switch when bound to MHC class II on the surface of antigen-presenting cells. Inhibition of LAG-3 both activates effector T cells and inhibitor regulatory T cells. In some embodiments, the immune checkpoint inhibitor is an anti-LAG-3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-LAG-3 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-LAG-3 antibodies can be used. An exemplary anti-LAG-3 antibody is relatlimab (also known as BMS-986016) or antigen binding fragments and variants thereof (see, *e.g.*, WO 2015/116539). Other exemplary anti-LAG-3 antibodies include TSR-033 (see, *e.g.*, WO 2018/201096), MK-4280, and REGN3767. MGD013 is an anti-LAG-3/PD-1 bispecific antibody described in WO 2017/019846. FS118 is an anti-LAG-3/PD-L1 bispecific antibody described in WO 2017/220569.

[0033] Another immune checkpoint protein that can be targeted in the methods provided herein is V-domain Ig suppressor of T cell activation (VISTA), also known as C10orf54. The complete protein sequence of human VISTA has the Genbank accession number NP_071436. VISTA is found on white blood cells and inhibits T cell effector function. In some embodiments, the immune checkpoint inhibitor is an anti-VISTA3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-VISTA antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-VISTA antibodies can be used. An exemplary anti-VISTA antibody is JNJ-61610588 (also known as onvatilimab) (see, *e.g.*, WO 2015/097536, WO 2016/207717, WO 2017/137830, WO 2017/175058). VISTA can also be inhibited with the small molecule CA-170, which selectively targets both PD-L1 and VISTA (see, *e.g.*, WO 2015/033299, WO 2015/033301).

[0034] Another immune checkpoint protein that can be targeted in the methods provided herein is CD38. The complete protein sequence of human CD38 has Genbank accession number NP_001766. In some embodiments, the immune checkpoint inhibitor is an

anti-CD38 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-CD38 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CD38 antibodies can be used. An exemplary anti-CD38 antibody is daratumumab (see, *e.g.*, U.S. Pat. No. 7,829,673).

[0035] Another immune checkpoint protein that can be targeted in the methods provided herein is T cell immunoreceptor with Ig and ITIM domains (TIGIT). The complete protein sequence of human TIGIT has Genbank accession number NP_776160. In some embodiments, the immune checkpoint inhibitor is an anti-TIGIT antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-TIGIT antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-TIGIT antibodies can be used. An exemplary anti-TIGIT antibody is MK-7684 (see, *e.g.*, WO 2017/030823, WO 2016/028656).

[0036] Co-stimulatory molecules are ligands that interact with receptors on the surface of the immune cells, e.g., CD28, 4-1BB, OX40 (also known as CD134), ICOS, and GITR. As an example, the complete protein sequence of human OX40 has Genbank accession number NP_003318. In some embodiments, the immunomodulatory agent is an anti-OX40 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-OX40 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-OX40 antibodies can be used. An exemplary anti-OX40 antibody is PF-04518600 (see, *e.g.*, WO 2017/130076). ATOR-1015 is a bispecific antibody targeting CTLA4 and OX40 (see, *e.g.*, WO 2017/182672, WO 2018/091740, WO 2018/202649, WO 2018/002339).

[0037] Another co-stimulatory molecule that can be targeted in the methods provided herein is ICOS, also known as CD278. The complete protein sequence of human ICOS has Genbank accession number NP_036224. In some embodiments, the immune checkpoint inhibitor is an anti-ICOS antibody (*e.g.*, a human antibody, a humanized antibody, or a

chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-ICOS antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-ICOS antibodies can be used. Exemplary anti-ICOS antibodies include JTX-2011 (see, *e.g.*, WO 2016/154177, WO 2018/187191) and GSK3359609 (see, *e.g.*, WO 2016/059602).

[0038] Yet another co-stimulatory molecule that can be targeted in the methods provided herein is glucocorticoid-induced tumour necrosis factor receptor-related protein (GITR), also known as TNFRSF18 and AITR. The complete protein sequence of human GITR has Genbank accession number NP_004186. In some embodiments, the immunomodulatory agent is an anti-GITR antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-GITR antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-GITR antibodies can be used. An exemplary anti-GITR antibody is TRX518 (see, *e.g.*, WO 2006/105021).

[0039] Other immune inhibitory molecules that can be targeted for immunomodulation include STAT3 and indoleamine 2,3-dioxygenase (IDO). By way of example, the complete protein sequence of human IDO has Genbank accession number NP_002155. In some embodiments, the immunomodulatory agent is a small molecule IDO inhibitor. Exemplary small molecules include BMS-986205, epacadostat (INCB24360), and navoximod (GDC-0919).

III. Methods of Treatment

[0040] The term “subject” or “patient” as used herein refers to any individual to which the subject methods are performed. Generally the patient is human, although as will be appreciated by those in the art, the patient may be an animal. Thus other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs, etc., and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of patient.

[0041] "Treatment" and "treating" refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For example, a treatment may include administration chemotherapy, immunotherapy, radiotherapy, performance of surgery, or any combination thereof.

[0042] The methods described herein are useful in inhibiting survival or proliferation of cells (e.g., tumor cells), treating proliferative disease (e.g., cancer, psoriasis), and treating pathogenic infection. Generally, the terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. More specifically, cancers that are treated in connection with the methods provided herein include, but are not limited to, solid tumors, metastatic cancers, or non-metastatic cancers. In certain embodiments, the cancer may originate in the lung, kidney, bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, duodenum, small intestine, large intestine, colon, rectum, anus, gum, head, liver, nasopharynx, neck, ovary, pancreas, prostate, skin, stomach, testis, tongue, or uterus.

[0043] The cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; non-small cell lung cancer; renal cancer; renal cell carcinoma; clear cell renal cell carcinoma; lymphoma; blastoma; sarcoma; carcinoma, undifferentiated; meningioma; brain cancer; oropharyngeal cancer; nasopharyngeal cancer; biliary cancer; pheochromocytoma; pancreatic islet cell cancer; Li-Fraumeni tumor; thyroid cancer; parathyroid cancer; pituitary tumor; adrenal gland tumor; osteogenic sarcoma tumor; neuroendocrine tumor; breast cancer; lung cancer; head and neck cancer; prostate cancer; esophageal cancer; tracheal cancer; liver cancer; bladder cancer; stomach cancer; pancreatic cancer; ovarian cancer; uterine cancer; cervical cancer; testicular cancer; colon cancer; rectal cancer; skin cancer; giant and spindle cell carcinoma; small cell carcinoma; small cell lung cancer; papillary carcinoma; oral cancer; oropharyngeal cancer; nasopharyngeal cancer; respiratory cancer; urogenital cancer; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrointestinal cancer; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial

polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma with squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; lentigo maligna melanoma; acral lentiginous melanoma; nodular melanoma; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi’s sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; an endocrine or neuroendocrine cancer or hematopoietic cancer; pinealoma, malignant; chordoma; central or peripheral nervous system tissue cancer; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory

neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; B-cell lymphoma; malignant lymphoma; Hodgkin's disease; Hodgkin's; low grade/follicular non-Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; mantle cell lymphoma; Waldenstrom's macroglobulinemia; other specified non-hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and hairy cell leukemia.

[0044] The term "therapeutic benefit" or "therapeutically effective" as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

[0045] Likewise, an effective response of a patient or a patient's "responsiveness" to treatment refers to the clinical or therapeutic benefit imparted to a patient at risk for, or suffering from, a disease or disorder. Such benefit may include cellular or biological responses, a complete response, a partial response, a stable disease (without progression or relapse), or a response with a later relapse. For example, an effective response can be reduced tumor size or progression-free survival in a patient diagnosed with cancer.

[0046] Regarding neoplastic condition treatment, depending on the stage of the neoplastic condition, neoplastic condition treatment involves one or a combination of the following therapies: surgery to remove the neoplastic tissue, radiation therapy, and chemotherapy. Other therapeutic regimens may be combined with the administration of the anticancer agents, e.g., therapeutic compositions and chemotherapeutic agents. For example, the patient to be treated with such anti-cancer agents may also receive radiation therapy and/or may undergo surgery.

[0047] For the treatment of disease, the appropriate dosage of a therapeutic composition will depend on the type of disease to be treated, as defined above, the severity and course of the disease, previous therapy, the patient's clinical history and response to the agent, and the discretion of the physician. The agent may be suitably administered to the patient at one time or over a series of treatments.

IV. Combination Treatments

[0048] The methods and compositions, including combination therapies, enhance the therapeutic or protective effect, and/or increase the therapeutic effect of another anti-cancer or anti-hyperproliferative therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect, such as the killing of a cancer cell and/or the inhibition of cellular hyperproliferation. A tissue, tumor, or cell can be contacted with one or more compositions or pharmacological formulation(s) comprising one or more of the agents or by contacting the tissue, tumor, and/or cell with two or more distinct compositions or formulations. Also, it is contemplated that such a combination therapy can be used in conjunction with radiotherapy, surgical therapy, or immunotherapy.

[0049] Administration in combination can include simultaneous administration of two or more agents in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, the subject therapeutic composition and another therapeutic agent can be formulated together in the same dosage form and administered simultaneously. Alternatively, subject therapeutic composition and another therapeutic agent can be simultaneously administered, wherein both the agents are present in separate formulations. In another alternative, the therapeutic agent can be administered just followed by the other therapeutic agent or vice versa. In the separate administration protocol, the subject therapeutic composition and another therapeutic agent may be administered a few minutes apart, or a few hours apart, or a few days apart.

[0050] An anti-cancer first treatment may be administered before, during, after, or in various combinations relative to a second anti-cancer treatment. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the first treatment is provided to a patient separately from the second treatment, one would generally ensure that a significant period of time did not expire between the time of

each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the first therapy and the second therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[0051] In certain embodiments, a course of treatment will last 1-90 days or more (this such range includes intervening days). It is contemplated that one agent may be given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof, and another agent is given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment, it is contemplated that there is a period of time at which no anti-cancer treatment is administered. This time period may last 1-7 days, and/or 1-5 weeks, and/or 1-12 months or more (this such range includes intervening days), depending on the condition of the patient, such as their prognosis, strength, health, *etc.* It is expected that the treatment cycles would be repeated as necessary.

[0052] Various combinations may be employed. For the example below a combination of a transcription inhibitor and an immune checkpoint inhibitor is "A" and another anti-cancer therapy is "B":

[0053] A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

[0054] B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A

[0055] B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[0056] Administration of any compound or therapy of the present invention to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

A. Chemotherapy

[0057] A wide variety of chemotherapeutic agents may be used in accordance with the present invention. The term "chemotherapy" refers to the use of drugs to treat cancer. A "chemotherapeutic agent" is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[0058] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclosphosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics, such as the enediyne antibiotics (*e.g.,* calicheamicin, especially calicheamicin gammaI and calicheamicin omegaI1); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins,

peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKpolysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DFMO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine,plicomycin, gemcitabien, navelbine, farnesyl-protein tansferase inhibitors, transplatinum, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

B. Radiotherapy

[0059] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly

and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

C. Immunotherapy

[0060] The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the invention. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (Rituxan®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, *etc.*) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

[0061] In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[0062] Examples of immunotherapies currently under investigation or in use are immune adjuvants, *e.g.*, Mycobacterium bovis, Plasmodium falciparum, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, Infection Immun., 66(11):5329-5336, 1998; Christodoulides et al., Microbiology, 144(Pt 11):3027-3037, 1998); cytokine therapy, *e.g.*, interferons α, β, and γ, IL-1, GM-CSF, and TNF (Bukowski et al., Clinical Cancer Res., 4(10):2337-2347, 1998; Davidson et al., J. Immunother., 21(5):389-398, 1998; Hellstrand et al., Acta Oncologica,

37(4):347-353, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin et al., Proc. Natl. Acad. Sci. USA, 95(24):14411-14416, 1998; Austin-Ward and Villaseca, Revista Medica de Chile, 126(7):838-845, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hanibuchi et al., Int. J. Cancer, 78(4):480-485, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

[0063] In some embodiment, the immune therapy could be adoptive immunotherapy, which involves the transfer of autologous antigen- specific T cells generated *ex vivo*. The T cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific T cells or redirection of T cells through genetic engineering. Isolation and transfer of tumor specific T cells has been shown to be successful in treating melanoma. Novel specificities in T cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single fusion molecule. In general, the binding moiety of a CAR consists of an antigen-binding domain of a single-chain antibody (scFv), comprising the light and variable fragments of a monoclonal antibody joined by a flexible linker. Binding moieties based on receptor or ligand domains have also been used successfully. The signaling domains for first generation CARs are derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. CARs have successfully allowed T cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors.

[0064] In one embodiment, the present application provides for a combination therapy for the treatment of cancer wherein the combination therapy comprises adoptive T cell therapy and a checkpoint inhibitor. In one aspect, the adoptive T cell therapy comprises autologous and/or allogenic T-cells. In another aspect, the autologous and/or allogenic T-cells are targeted against tumor antigens.

D. Surgery

[0065] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other

therapies, such as the treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[0066] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

E. Other Agents

[0067] It is contemplated that other agents may be used in combination with certain aspects of the present invention to improve the therapeutic efficacy of treatment. These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present invention to improve the treatment efficacy.

V. Kits

[0068] In various aspects of the invention, a kit is envisioned containing, diagnostic agents, therapeutic agents and/or delivery agents. In some embodiments, the present invention contemplates a kit for preparing and/or administering a therapy of the invention.

The kit may comprise reagents capable of use in administering an active or effective agent(s) of the invention. Reagents of the kit may include one or more anti-cancer component of a combination therapy, as well as reagents to prepare, formulate, and/or administer the components of the invention or perform one or more steps of the inventive methods. In some embodiments, the kit may also comprise a suitable container means, which is a container that will not react with components of the kit, such as an eppendorf tube, an assay plate, a syringe, a bottle, or a tube. The container may be made from sterilizable materials such as plastic or glass. The kit may further include an instruction sheet that outlines the procedural steps of the methods, and will follow substantially the same procedures as described herein or are known to those of ordinary skill.

VI. Examples

[0069] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Combination therapy of WP1066 and anti-PD-1/CTLA-4 antibodies in syngeneic, orthotopic model of pancreatic cancer

[0070] WP1066 and WP1732 are inhibitors of p-STAT3 with demonstrated in vitro and in vivo activity against PDAC tumor models. The chemical synthesis of WP1066 and WP1732 and their characterization was performed at U.T. MD Anderson Cancer Center. *In vitro* efficacy of both inhibitors was assessed using proliferation and apoptosis induction assays in a panel of patient-derived and commercially-available PDAC cell lines. Inhibition of p-STAT3 was investigated using western blot (WB) and immunofluorescence. Both WP1066 and WP1732 were shown to induce apoptosis and inhibit p-STAT3 and its nuclear localization in all tested PDAC cell lines. Observed IC_{50} values ranged from 0.5 to 2 μM.

[0071] Acute and multiple dose toxicity of WP1732 was tested in CD-1 mice. WP1732 was well tolerated by mice (LD_{50} 85 mg/kg given IV). Pharmacokinetic parameters

of WP1732 after intravenous administration was evaluated in naïve CD-1 mice using Mass Spectrometry LC/MS/MS or rats by liquid scintillation counting (LSC) using radio-labeled agent. Pharmacokinetic and biodistribution studies indicate high plasma levels of the drug and significant accumulation of WP1732 in the pancreas of mice and rats after a single bolus injection of the drug. Efficacy of both agents alone or in combination with immune checkpoints inhibitors was tested in PDAC tumor models.

[0072] Orally administered WP1066 40 mg/kg was tested in a syngeneic, orthotopic mouse model of pancreatic ductal adenocarcinoma using Panc02 cells stably expressing firefly luciferase (FIG. 1). One group of mice was administered vehicle, one group was administered anti CTLA-4/PD-1 antibodies at 100 and 250 μg/mouse through intraperitoneal (IP) injection, one group was administered WP1066 at 40 mg/kg orally, and one group was administered the combination. Drug administration was on a 7-day schedule for 3 weeks with drugs administered on day 1 and day 5 of each week. After 4 weeks, mice receiving vehicle and mice receiving single agent therapy showed tumor bioluminescence of 2-4 times baseline measured at 2 weeks. This means that tumors grew with each monotherapy. Mice receiving combination therapy on average showed less than baseline bioluminescence (FIG. 1). Thus, an additive/synergistic effect of the combination of WP1066 with anti-PD-1/CTLA-4 antibodies was observed in an orthotopic model of Panc02 tumors in immunocompetent mice.

Example 2 – WP1732 (suboptimal dose) activity in syngeneic, orthotopic MT04-Lyt2 model of pancreatic cancer as a single agent and in combination with immune checkpoint inhibitors

[0073] A similar experiment in a syngeneic, orthotopic mouse model of a different type of pancreatic cancer using MT04-Lyt2 cells stably expressing firefly luciferase and WP1732 20 mg/kg IP was also conducted. BL6 albino male mice were surgically implanted with 2.5 x 10^5 MT04-Lyt2 mouse pancreatic cancer cells expressing luciferase. The dosing schedule was begun at day 10 post-surgery. Drug administration was performed on a 7-day schedule, for a total of three weeks. Immune checkpoint antibody cocktail (anti-CTLA4, 100 μg/mouse and anti-PD-1, 250 μg/mouse) was administered i.p. on days 1 and 5 of the dosing schedule, and WP1732 was administered i.p. (20 mg/kg) on days 1-5 of the dosing schedule. Mice were imaged with luciferin weekly, starting at day 7, 14, 21, and 28 post-surgery using an IVIS Spectrum imager, and radiance (total counts/second) were analyzed. Survival data

were analyzed in GraphPad Prism using the Kaplan-Meier method and Gehan-Breslow-Wilcoxon statistical analysis. Median survival was 18 days for untreated mice, 21.5 days for WP1732 monotherapy mice, 26 days for checkpoint inhibitor mice, and 40 days for combination therapy WP1732 and checkpoint inhibitor (CTLA4 and PD-1 antibodies) mice (FIGS. 2A-B). The present *in vivo* testing demonstrated that the anti-tumor activity of immune checkpoint inhibitors was boosted in a very significant way by WP1066 and WP1732.

* * *

[0074] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating a disease in a patient, the method comprising administering to the patient a combined effective amount of a transcription inhibitor and an immune checkpoint inhibitor.

2. The method of claim 1, wherein the transcription inhibitor is a STAT3 inhibitor.

3. The method of claim 2, wherein the transcription inhibitor is a α,β-unsaturated cyanocarboxamide.

4. The method of claim 2, wherein the transcription inhibitor is WP1066 or WP1732.

5. The method of any one of claims 1-4, wherein the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4 therapy.

6. The method of claim 5, wherein the anti-PD1 therapy comprises nivolumab, pembrolizumab, pidilizumab, cemiplimab, tislelizumab, spartalizumab, PF-06801591, AK105, BCD-100, BI-754091, HLX10, JS001, LZM009, MEDI 0680, MGA012, Sym021, TSR-042, MGD013, AK104, and/or XmAb20717.

7. The method of claim 5, wherein the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, FS118, BCD-135, BGB-A333, CBT502, CK-301, CS1001, FAZ053, KN035, MDX-1105, MSB2311, SHR-1316, M7824, LY3415244, CA-170, and CX-072.

8. The method of claim 5, wherein the anti-CTLA-4 therapy comprises ipilimumab, tremelimumab, BMS-986218, AK104, and/or XmAb20717.

9. The method of any one of claims 1-8, wherein the disease is a proliferative disease.

10. The method of claim 9, wherein the proliferative disease is cancer.

11. The method of claim 9, wherein the method inhibits the survival or proliferation of cancer cells.

12. The method of claim 10 or 11, wherein the patient has previously failed to respond to the administration of an immune checkpoint inhibitor.

13. The method of claim 12, wherein the method is a method of overcoming resistance to immune checkpoint inhibitor therapy.

14. The method of any one of claims 9-13, further comprising administering a further anti-cancer therapy to the patient.

15. The method of claim 14, wherein the further anti-cancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy, or cytokine therapy.

16. The method of claim 14, wherein the further anti-cancer therapy comprises gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, or docetaxel.

17. The method of any one of claims 10-16, wherein the cancer is a glioma, a pancreatic cancer, a breast cancer, a melanoma, a lymphoma, or a leukemia.

18. The method of claim 17, wherein the leukemia is AML.

19. The method of any one of claims 10-18, wherein the patient has previously undergone at least one round of anti-cancer therapy.

20. The method of claim 9, wherein the proliferative disease is psoriasis.

21. The method of claim 1, wherein the disease is a pathogenic infection.

22. The method of any one of claims 1-21, wherein the patient is a human.

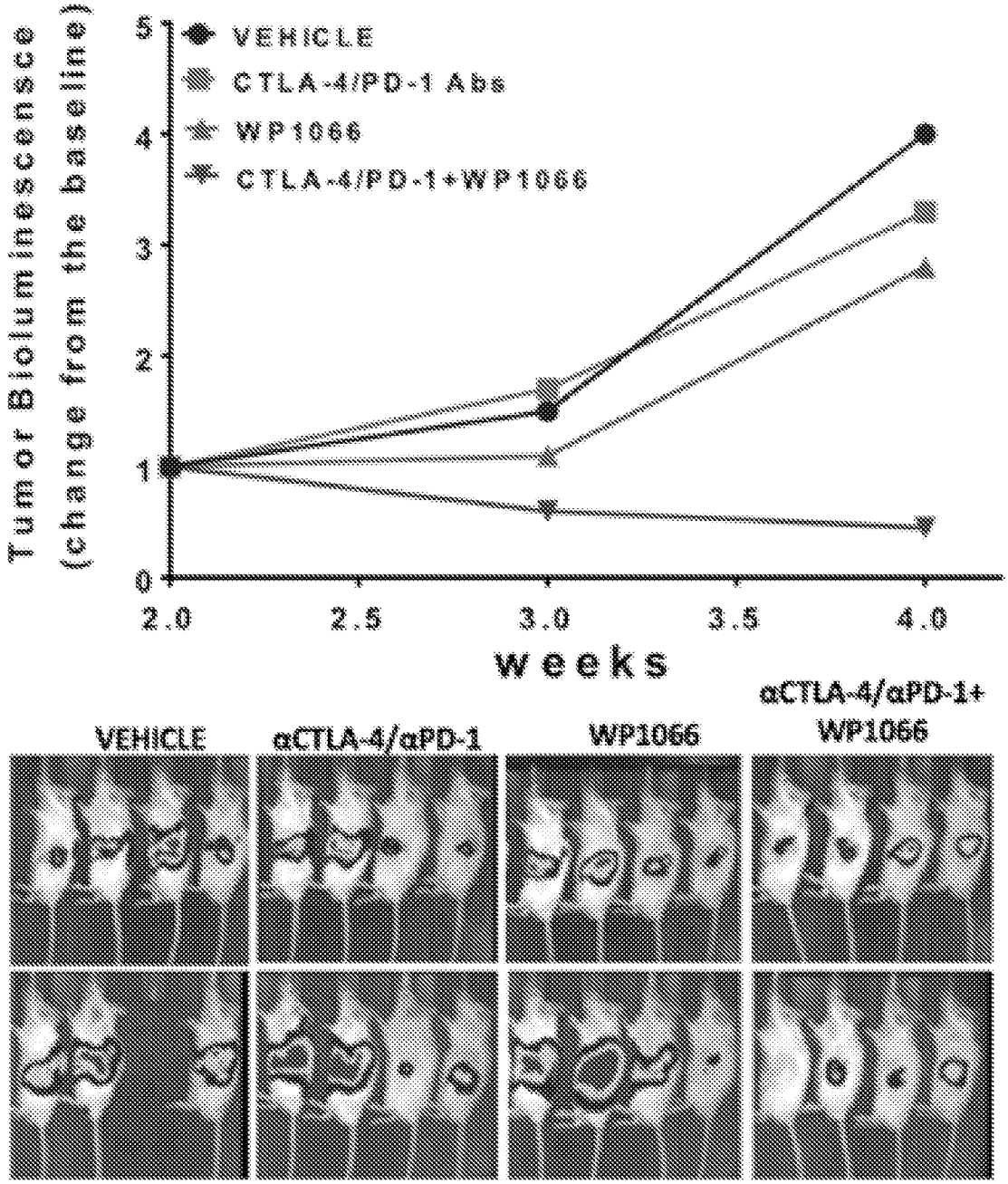
Tumor Bioluminescence
(change from the baseline)
VEHICLE
CTLA-4/PD-1 Abs
WP1066
CTLA-4/PD-1+WP1066
0
1
2
3
4
5
2.0
2.5
3.0
3.5
4.0
weeks
VEHICLE
αCTLA-4/αPD-1
WP1066
αCTLA-4/αPD-1+
WP1066

FIG. 1

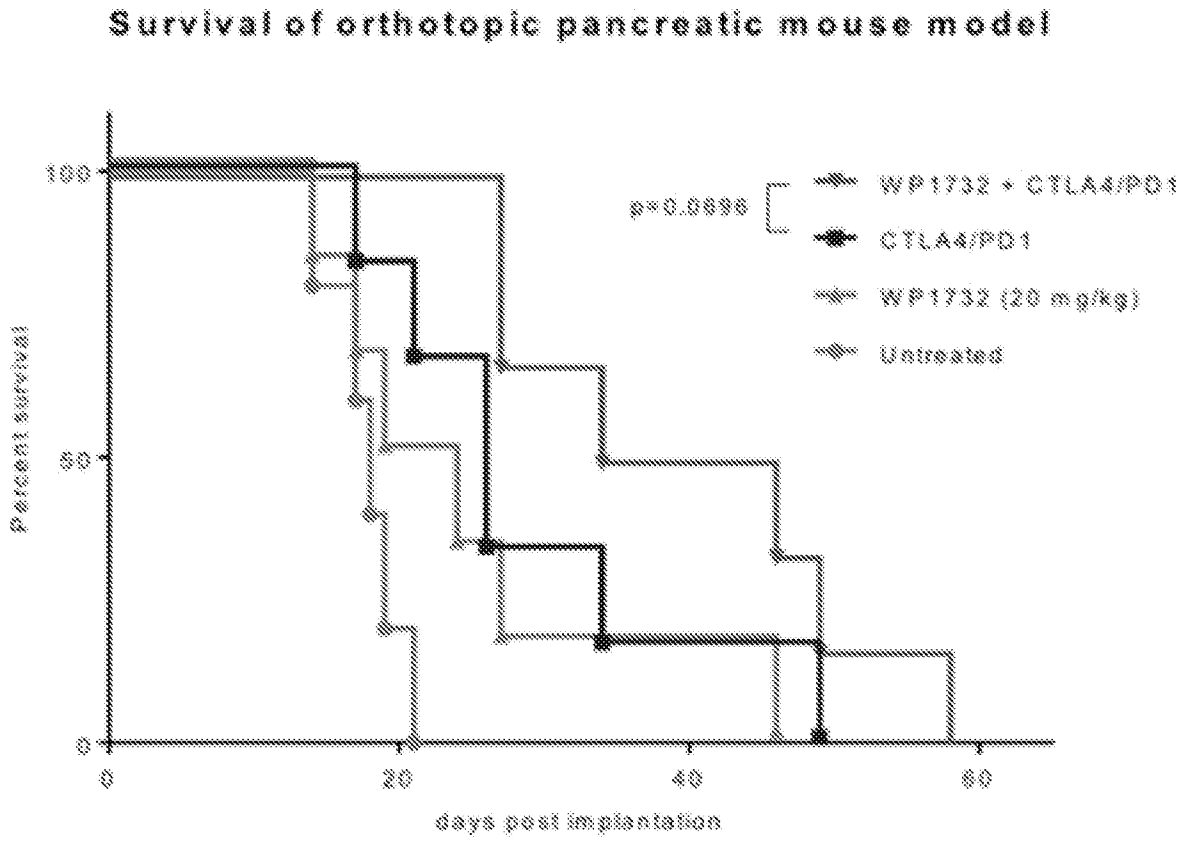
Survival of orthotopic pancreatic mouse model
Percent survival
100
50
0
0
20
40
60
days post implantation
p=0.0098
WP1732 + CTLA4/PD1
CTLA4/PD1
WP1732 (20 mg/kg)
Untreated

FIG. 2A

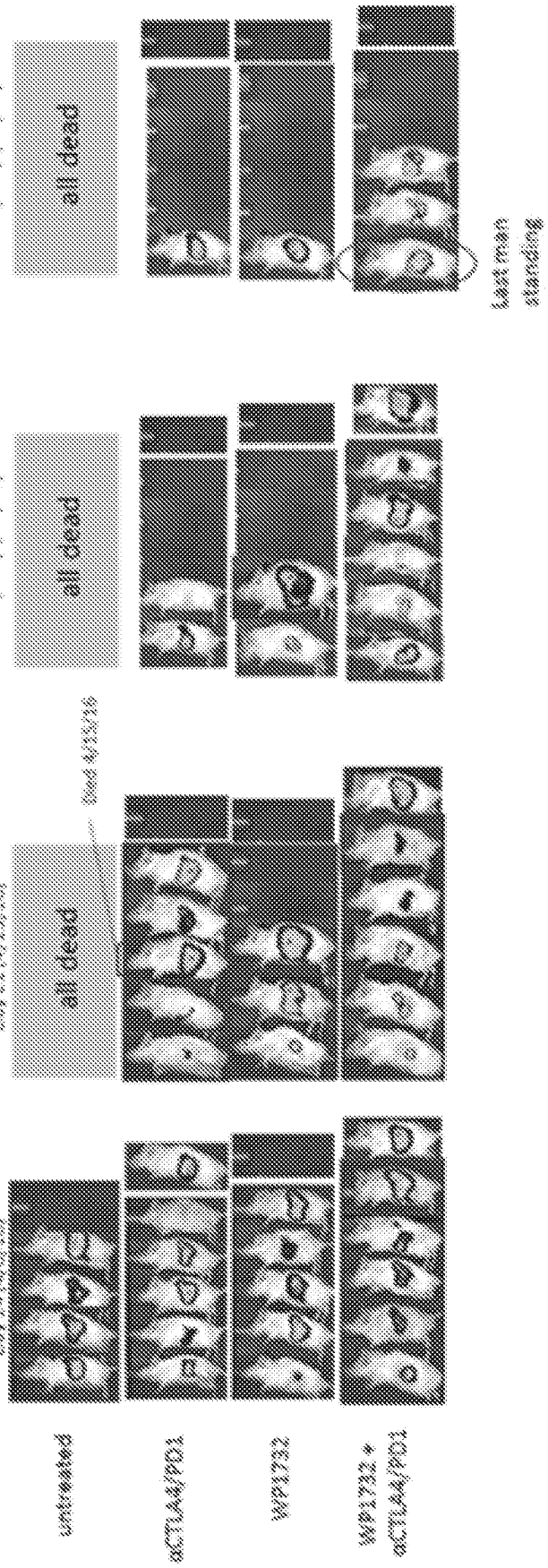
Day 14 (4/6/16)
Day 21 (4/13/16)
Day 28 (4/20/16)
Day 35 (4/27/16)
all dead
all dead
all dead
Died 4/13/16
Last man standing
untreated
αCTLA4/PD1
WP1732
WP1732 + αCTLA4/PD1

FIG. 2B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/026366

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/165; A61K 31/275; A61K 31/277; A61K 31/44; A61K 39/395; A61P 35/00 (2020.01)
CPC - A61K 31/275; A61K 31/33; A61K 2039/505; A61P 17/06; A61P 31/00; A61P 35/00; A61P 37/02; C07C 255/00 (2020.05)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/062722 A1 (ASTRAZENECA AB) 28 April 2016 (28.04.2016) entire document	1, 2, 5-8
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Y		3, 4
Y	US 2012/0114648 A1 (LANGERMANN et al) 10 May 2012 (10.05.2012) entire document	1, 21
Y	US 2014/0329901 A1 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 06 November 2014 (06.11.2014) entire document	1, 3, 4, 21
A	US 2018/0140572 A1 (BOSTON BIOMEDICAL, INC.) 24 May 2018 (24.05.2018) entire document	1-8, 21
A	WO 2019/023525 A1 (DANA-FARBER CANCER INSTITUTE, INC.) 31 January 2019 (31.01.2019) entire document	1-8, 21
A	WO 2016/071890 A1 (IMMUNID) 12 May 2016 (12.05.2016) entire document	1-8, 21
A	US 2016/0304873 A1 (MIRIMMUNE INC.) 20 October 2016 (20.10.2016) entire document	1-8, 21
A	US 2015/0094343 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 02 April 2015 (02.04.2015) entire document	1-8, 21

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2020	16 JUN 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/026366

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-20, 22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.