



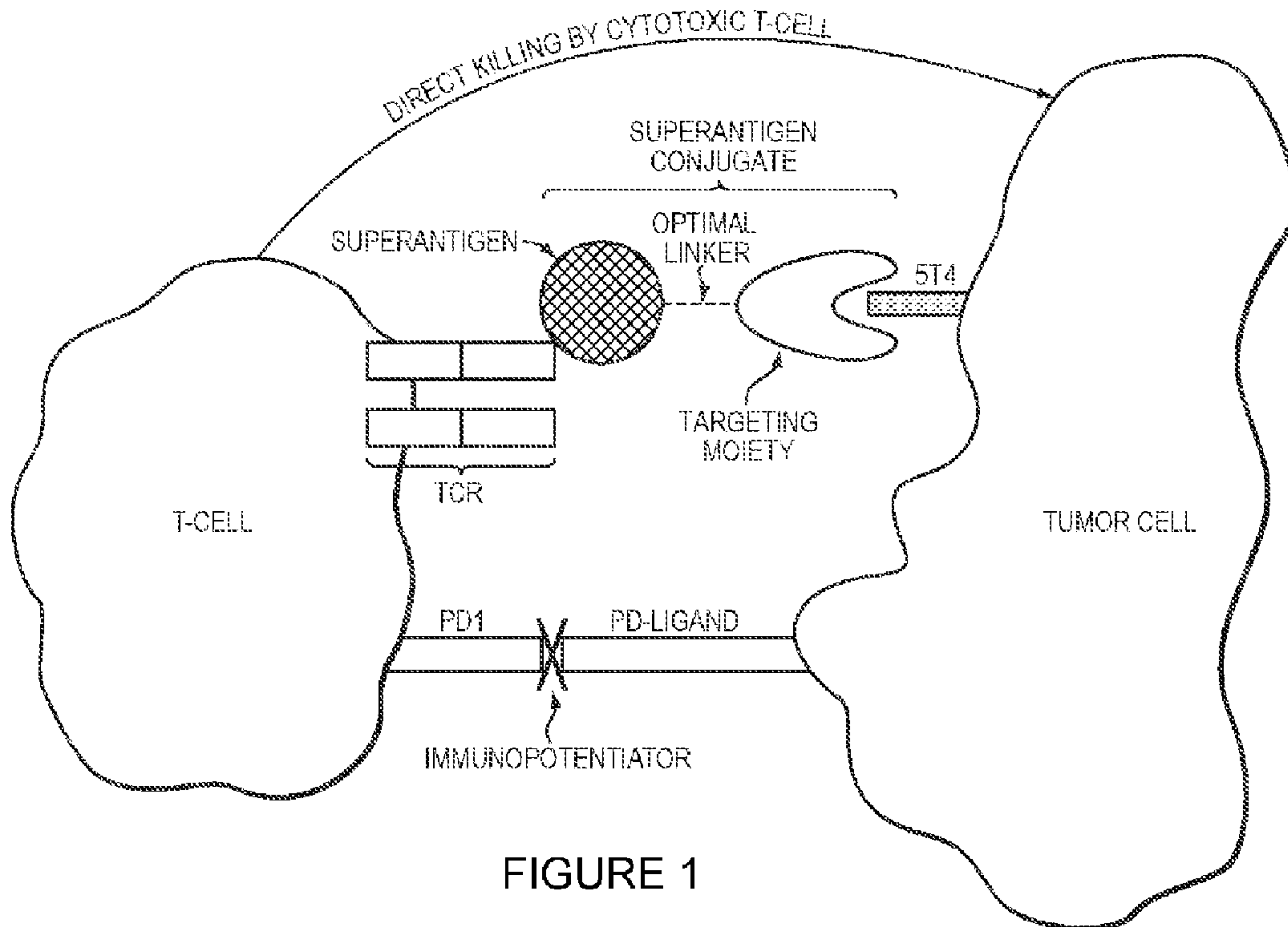
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(54) Titre : PROCÉDES ET COMPOSITIONS PERMETTANT D'AMÉLIORER LA PUISSANCE DE L'IMMUNOTHERAPIE ANTICANCÉREUSE MÉDIÉE PAR UN SUPERANTIGÈNE
(54) Title: METHODS AND COMPOSITIONS FOR ENHANCING THE POTENCY OF SUPERANTIGEN MEDIATED CANCER IMMUNOTHERAPY



(57) Abrégé/Abstract:

The invention provides methods or compositions for enhancing the potency of a targeted cancer immunotherapy in a subject by using a superantigen in combination with an immunopotentiator (for example, a PD-1 inhibitor).

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(54) Title: IMMUNOPOTENTIATOR ENHANCED SUPERANTIGEN MEDIATED CANCER IMMUNOTHERAPY

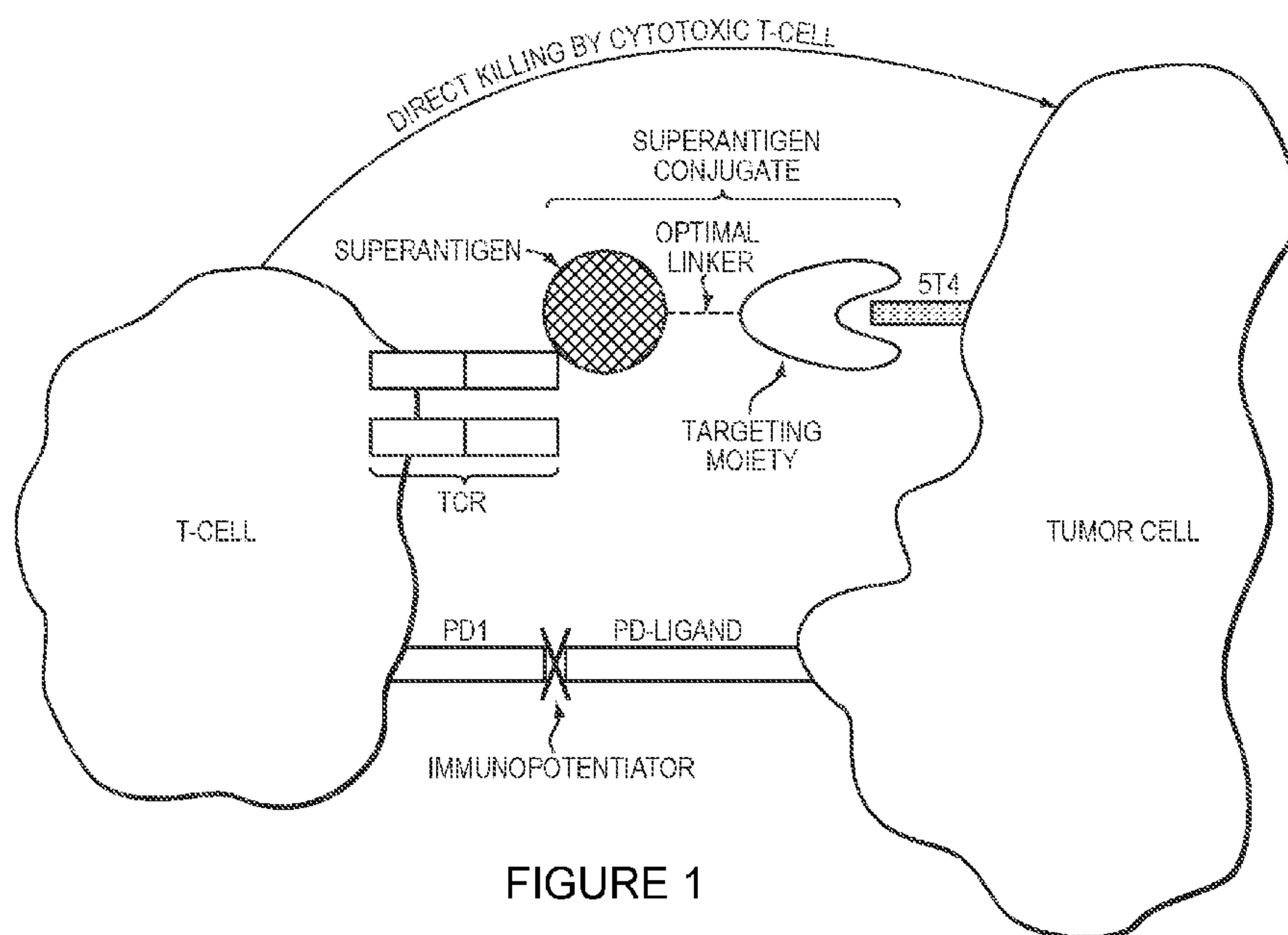


FIGURE 1

(57) Abstract: The invention provides methods or compositions for enhancing the potency of a targeted cancer immunotherapy in a subject by using a superantigen in combination with an immunopotentiator (for example, a PD-1 inhibitor).

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METHODS AND COMPOSITIONS FOR ENHANCING THE POTENCY OF SUPERANTIGEN MEDIATED CANCER IMMUNOTHERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to, U.S. Provisional Patent Application serial number 62/276,955, filed January 10, 2016, the entire contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The invention relates generally to methods and compositions for enhancing the
5 potency of superantigen mediated cancer immunotherapy, and more specifically relates to
superantigen mediated cancer immunotherapy with an immunopotentiator that prevents cancer
cells from evading the subject's immune system.

BACKGROUND

[0003] According to the American Cancer Society, more than one million people in the
United States are diagnosed with cancer each year. Cancer is a disease that results from
10 uncontrolled proliferation of cells that were once subject to natural control mechanisms but
have been transformed into cancerous cells that continue to proliferate in an uncontrolled
manner. In recent years, a number of immunotherapies have been developed that have
attempted to harness the subject's immune system to find and destroy cancer cells. Such
immunotherapies include, for example, those that are designed to boost the body's natural
15 defenses for fighting cancer using natural molecules made by the body, or alternatively,
through administration of recombinant molecules designed to improve, better target or restore
immune system function. Certain immunotherapies include the administration of compounds
known to be general immune system enhancers, such as cytokines, for example, IL-2 and
interferon. While various immunotherapies developed to date have shown efficacy, they can be
20 associated with side effects including, for example, off-target activities, allergic reactions to the
active agents administered including the potential for cytokine storms, a loss of potency caused

by the stimulation of antibodies that bind and neutralize the active agents, a decrease in blood cell number, and fatigue.

[0004] Other immunotherapies utilize molecules referred to as immune checkpoint inhibitors, which enhance immune responses to cancer. Such checkpoint inhibitors function to inhibit the ability of cancer cells to block immune inhibitory checkpoints thereby resulting in an enhancement of potency of an anti-cancer therapy. A first-generation immune checkpoint inhibitor ipilimumab (YERVOY[®]; Bristol-Myers Squibb) was approved by the U.S. Food and Drug Administration in 2011 and is an IgG1 monoclonal antibody that can cause ADCC-mediated regulatory T-cell (Treg) cytotoxicity. Over the years, immunochemotherapy, the combination of immunotherapy and chemotherapy, has become important in the treatment of certain cancers. For example, rituximab (RITUXAN[®]; Roche) is a CD-20-specific monoclonal antibody that depletes CD20-expressing cells and has become a standard component of the treatment of B-cell lymphomas, for example, non-Hodgkin's lymphoma using rituximab (R), cyclophosphamide (C), hydroxydaunorubicin (H), oncovin (O), and prednisone (P), known as R-CHOP.

[0005] Recently, PD-1 inhibitors have been approved such as Nivolumab and Pembrolizumab, which prevent the inhibitory signals between PD-1 and PD-L1. While these drugs have potentiated durable responses in some patients, the response rates of these drugs as monotherapy have been low and in the range of 21%, and the complete response rate has been about 1% in several studies.

[0006] Although there are ongoing efforts to combine various cancer therapies to improve patient outcomes and some combinations have shown benefits in efficacy, safety has become a major concern as combining drugs may potentiate serious side effects. For example, drug-related adverse events of grade 3 or 4 were reported in a significant number of patients who received anti-CTLA-4 and anti-PD1 antibodies in combination as compared to patients who received the anti-CTLA-4 antibody alone. Accordingly, despite the significant developments that have been made in the fields of immunotherapy and oncology, there is still a need for safe and effective immunotherapies for treating cancer.

SUMMARY OF THE INVENTION

[0007] In recent years, a number of immunotherapies have been developed that have attempted to harness the subject's immune system to find and destroy cancer cells. Although a

human immune system has the potential to eliminate cancer cells, certain cancer cells develop the ability to “turn off,” “down regulate,” or otherwise evade the host’s immune system allowing the cancerous cells to continue to grow and proliferate unchecked.

5 [0008] The invention is based, in part, upon the discovery that a targeted immune response against a cancer in a subject can be significantly enhanced by combining a superantigen-based therapy with an immunopotentiator that can (a) stimulate activating T-cell signaling, (b) repress T-cell inhibitory signalling between the cancerous cells and a T-cell, (c) repress inhibitory signalling that leads to T-cell expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated pathway, or (d) a combination of two or more of the foregoing.

10 [0009] In one aspect, the invention provides a method of treating a cancer in a subject in need thereof. The method comprises administering to the subject (i) an effective amount of a superantigen conjugate, the conjugate comprising a superantigen covalently linked to a targeting moiety that binds a first antigen preferentially expressed by cancerous cells within the subject, and (ii) an effective amount of an immunopotentiator (for example, a checkpoint
15 pathway inhibitor) effective to at least one or more of (a) stimulating activating T-cell signaling, (b) repressing T-cell inhibitory signalling between the cancerous cells and a T-cell, and/or (c) repressing inhibitory signalling that leads to T-cell expansion, activation and/or activity via human IgG4 immunoglobulin-mediated pathway (i.e., via a non-human IgG1-mediated immune response pathway), thereby to potentiate an immune response in the subject
20 against the cancerous cells to treat the cancer. Superantigen-based therapies may upregulate the secretion of interferon γ from T-cells, which in turn may upregulate PD-L1 expression. However, to date, it was not known if this negative effect could be mitigated by a PD-1 inhibitor that offsets the positive effects of increased antigenicity. It has now been discovered that superantigen conjugate-based therapies can potentially effect response rates of PD1
25 inhibitors or otherwise effect clinical outcome.

[0010] In certain embodiments, the superantigen conjugate may be administered to the subject before, at the same time as, or after the immunopotentiator. Furthermore, the superantigen conjugate and the immunopotentiator may be co-administered together or sequentially with one or more additional agents that enhance the potency and/or selectively of
30 the therapeutic effect. Such agents include, for example, corticosteroids, additional immune modulators, and those compounds designed to reduce the patient’s possible immunoreactivity

to the administered superantigen conjugate. For example, immunoreactivity to the administered superantigen may be reduced via co-administration with, for example, an anti-CD20 antibody and/or an anti-CD19 antibody, that reduces the production of anti-superantigen antibodies in the subject.

5 [0011] In certain embodiments, the superantigen present in the superantigen conjugate binds to a T-cell receptor on a surface of a T-cell, for example, a CD4⁺ and/or a CD8⁺ T-cell. The superantigen can comprise Staphylococcal enterotoxin A or B, an immunologically reactive variant thereof, or an immunologically reactive fragment of the Staphylococcal enterotoxin A or B or the immunologically variant thereof. The targeting moiety of the conjugate binds an
10 antigen preferably expressed on the cancer cell thereby to bind the superantigen to the cancer cell. The targeting moiety can be used to target the conjugate to the cancerous cells by preferentially binding one or more different antigens, for example, one or more cell surface antigens, expressed by the cancerous cells. In certain embodiments, the cell surface antigen is, for example, a 5T4 oncofetal cancer antigen, which is present on cancer cells as well as certain
15 cancer stem cells. Although a variety of targeting moieties can be used to target the superantigen to the cancerous cells expressing the first antigen, in certain embodiments, the targeting moiety is an antibody, for example, an anti-5T4 antibody. In certain embodiments, the targeting moiety is a Fab fragment that binds 5T4. In certain embodiments, the superantigen in the conjugate comprises amino acids 226-458 of SEQ ID NO: 7 (also residues
20 226-458 of SEQ ID NO: 8), or an immunologically reactive variant thereof, or an immunologically reactive fragment of each of the foregoing.

[0012] In certain embodiments, the immunopotentiator is a checkpoint pathway inhibitor. For example, in certain embodiments, the immunopotentiator reduces expression of, or activity of, a Programmed Cell Death-Ligand (PD-L), for example, PD-L1 or PD-L2, expressed on the
25 surface of the cancerous cells. PD-L is a ligand that binds to a Programmed Cell Death-1 receptor (PD-1), which is expressed on T-cells. Certain cancer cells express PD-L to reduce the activation or activity of T-cells via a PD-1 checkpoint pathway so as to evade the subject's immune system. In certain embodiments, the immunopotentiator is an anti-PD-1 antibody that prevents PD-L, for example, PD-L1 or PD-L2, from binding to PD-1 expressed on the surface
30 of the T-cell. In certain embodiments, the anti-PD-1 antibody has or is based on a human IgG4 immunoglobulin isotype that induces much lower antibody-dependent cell-mediated toxicity (ADCC) than a human IgG1 immunoglobulin isotype.

[0013] In certain embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab and pembrolizumab. Other PD-1 inhibitors include (i) anti-PD-1 antibodies, for example, MK-3475 (Merck & Co), pidlizumab (CureTech), AMP-224 (AstraZeneca/Medimmune) and AMP-514 (AstraZeneca/Medimmune), and (ii) anti-PD-L1 antibodies, for example, MPDL3280A (Genentech/Roche), MEDI-4736 (AstraZeneca/Medimmune) and MSB0010718C (EMD Serono/Merck KGA).

[0014] In certain embodiments, other potential immunopotentiators can be used such as a 4-1BB (CD137) agonist (*e.g.*, the fully human IgG4 anti-CD137 antibody Urelumab/BMS-663513), a LAG3 inhibitor (*e.g.*, the humanized IgG4 anti-LAG3 antibody LAG525, Novartis); an IDO inhibitor (*e.g.*, the small molecule INCB024360, Incyte Corporation), a TGF β inhibitor (*e.g.*, the small molecule Galunisertib, Eli Lilly) and other receptor or ligands that are found on T-cells and/or tumor cells and that are amenable to pharmaceutical intervention based on agonist/antagonist interactions but not through ADCC.

[0015] It is understood that the method can be used to treat a variety of cancers, for example, a cancer selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastric cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, and skin cancer.

[0016] In another aspect, the invention provides a pharmaceutical composition comprising (i) an effective amount of a superantigen conjugate, the conjugate comprising a superantigen covalently linked to a targeting moiety that binds to a first antigen expressed by cancerous cells in a subject, (ii) an effective amount of an immunopotentiator effective to at least one or more of the following (a) stimulating activating T-cell signaling, (b) repressing T-cell inhibitory signalling between the cancerous cells and a T-cell, and/or (c) repressing inhibitory signalling that leads to T-cell expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated pathway (*i.e.*, via a non-human IgG1-mediated immune response pathway), in the subject, and (iii) a pharmaceutically acceptable excipient.

[0017] In certain embodiments, the superantigen component of the conjugate binds to a T-cell receptor expressed on a cell surface of a T-cell. For example, in certain embodiments, the superantigen comprises Staphylococcal enterotoxin A or Staphylococcal enterotoxin B, an immunologically reactive variant thereof, or an immunologically reactive fragment of the Staphylococcal enterotoxin A or B or the variant thereof. It is understood that the targeting

moiety in the conjugate can target the conjugate to one or more antigens expressed in the cancerous cells. In certain embodiments, the first antigen targeted by the conjugate is a cell surface antigen, for example, a 5T4 oncofetal cancer antigen. Although a variety of targeting moieties can be used to target the conjugate to the antigen expressed in the cancerous cells, in one embodiment, the targeting moiety is an antibody, for example, an anti-5T4 antibody. In certain embodiments, the targeting moiety comprises a Fab fragment that binds the 5T4 antigen. In certain embodiments, the superantigen in the conjugate comprises amino acid residues 226-458 of sequence of SEQ ID NO: 7 (also residues 226-458 of SEQ ID NO: 8) or an immunologically reactive variant thereof, or an immunologically reactive fragment of each of the foregoing.

[0018] In certain embodiments, the immunopotentiator prevents PD-L (for example, PD-L1 or PD-L2) expressed in the cancerous cells from binding a PD-1 receptor expressed on the T-cell so as to reduce the activation and/or activity of the T-cell. For example, the immunopotentiator can be a PD-1 checkpoint pathway inhibitor, for example, an anti-PD-1 antibody. In certain embodiments, the anti-PD-1 antibody has or is based on a human immunoglobulin IgG4 isotype that preferably induces much lower ADCC than a human IgG1 immunoglobulin isotype. In certain embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab and pembrolizumab.

[0019] These and other aspects and features of the invention are described in the following detailed description, figures, and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The foregoing and other objects, features and advantages of the invention will become apparent from the following description of preferred embodiments, as illustrated in the accompanying drawings. Like referenced elements identify common features in the corresponding drawings. The drawings are not necessarily to scale, with emphasis instead being placed on illustrating the principles of the present invention, in which:

[0021] **FIGURE 1** is a schematic representation of an exemplary treatment method of the invention using a superantigen conjugate and an immunopotentiator;

[0022] **FIGURE 2** is a sequence alignment showing the homologous A-E regions in certain wild type and modified superantigens;

[0023] **FIGURE 3** is an amino acid sequence corresponding to an exemplary superantigen conjugate;

[0024] **FIGURE 4** is a bar chart illustrating the effect of ANYARA[®] and KEYTRUDA[®] alone or in combination on the viability of the non-small-cell lung (NSCLC) cell line, HCC827. The survival rates of HCC827 cells co-cultured with T-cells were measured after 48 hours of treatment with ANYARA[®] (0.1μg/ml) and/or KEYTRUDA[®] (0.2μg/ml) or medium alone (Control). n=3-6; mean ± SD * $p < 0.05$, ** $p < 0.02$; *** $p < 0.005$, as determined by two-tailed Student's t test;

[0025] **FIGURE 5** is a bar chart illustrating the effect of ANYARA[®] and KEYTRUDA[®] alone or in combination on the viability of the NSCLC cell line, HCC827. The survival rates of HCC827 cells co-cultured with T-cells were measured after 48 hours of treatment with ANYARA[®] (10μg/ml) and/or KEYTRUDA[®] (0.2μg/ml) or medium alone (Control). n=3-6; mean ± SD. * $p = 0.005$, ** $p < 0.005$; *** $p < 0.0005$, as determined by two-tailed Student's t test; and

[0026] **FIGURE 6** is a bar chart illustrating the effect of C215Fab-SEA and anti-PD-1 mAb alone or in combination on lung tumor burden in the B16-EpCAM mice melanoma model. Mice were inoculated with B16-EpCAM melanoma cells intravenously and were treated with C215Fab-SEA (0.5μg/mouse) and/or anti-PD-1 mAb (200μg/mouse). Control group was injected with PBS. On day 21, mice were sacrificed, lungs were removed and the number of tumors was counted. n=7-8 mice/group; mean ± SEM. * $p = 0.05$, ** $p < 0.001$, as determined by ANOVA.

DETAILED DESCRIPTION

[0027] The present invention relates to methods and compositions for treating cancer in a subject. In particular, the invention is based, in part, upon the discovery that a targeted immune response against a cancer in a subject can be significantly enhanced by combining a superantigen-based therapy with an immunopotentiator that can (a) stimulate activating T-cell signaling, (b) repress T-cell inhibitory signalling between the cancerous cells and a T-cell, (c) repress inhibitory signalling that leads to T-cell expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated pathway, or (d) a combination of two or more of the foregoing. It has been discovered that the administration of a tumor-targeted superantigen (TTS; a form of immunotherapy), together with an immunopotentiator (e.g., a PD-1 inhibitor), can result in enhanced anti-cancer effect for both the superantigen and the immunopotentiator when combined together (*i.e.*, the agents act synergistically) to produce an effect that is greater than the additive effect of each agent when administered alone.

[0028] In one aspect, the invention provides a method of treating a cancer in a subject in need thereof. The method comprises administering to the subject (i) an effective amount of a superantigen conjugate, the conjugate comprising a superantigen covalently linked to a targeting moiety that binds a first antigen preferentially expressed by cancerous cells within the subject, and (ii) an effective amount of an immunopotentiator (for example, a checkpoint pathway inhibitor) effective to at least one or more of (a) stimulating activating T-cell signaling, (b) repressing T-cell inhibitory signalling between the cancerous cells and a T-cell, and/or (c) repressing inhibitory signalling that leads to T-cell expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated pathway (*i.e.*, via a non-human IgG1-mediated immune response pathway), thereby to potentiate an immune response in the subject against the cancerous cells to treat the cancer.

[0029] In another aspect, the invention provides a pharmaceutical composition comprising (i) an effective amount of a superantigen conjugate, the conjugate comprising a superantigen covalently linked to a targeting moiety that binds to a first antigen expressed by cancerous cells in a subject, (ii) an effective amount of an immunopotentiator effective to at least one or more of (a) stimulating activating T-cell signaling, (b) repressing T-cell inhibitory signalling between the cancerous cells and a T-cell, and/or (c) repressing inhibitory signalling that leads to T-cell expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated pathway (*i.e.*, via a non-human IgG1-mediated immune response pathway), in the subject, and (iii) a pharmaceutically acceptable excipient.

I. Definitions

[0030] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. For purposes of the present invention, the following terms are defined below.

[0031] As used herein, the terms “a” or “an” may mean one or more. For example, a statement such as “treatment with a superantigen and an immunopotentiator,” can mean treatment: with one superantigen and one immunopotentiator; with more than one superantigen and one immunopotentiator; with one superantigen and more than one immunopotentiator; or with more than one superantigen and more than one immunopotentiator.

[0032] As used herein, unless otherwise indicated, the term “antibody” is understood to mean an intact antibody (*e.g.*, an intact monoclonal antibody) or antigen-binding fragment of an

antibody, including an intact antibody or antigen-binding fragment of an antibody (*e.g.*, a phage display antibody including a fully human antibody, a semisynthetic antibody or a fully synthetic antibody) that has been optimized, engineered or chemically conjugated. Examples of antibodies that have been optimized are affinity-matured antibodies. Examples of antibodies
5 that have been engineered are Fc optimized antibodies, antibodies engineered to reduce immunogenicity, and multi-specific antibodies (*e.g.*, bispecific antibodies). Examples of antigen-binding fragments include Fab, Fab', F(ab')₂, Fv, single chain antibodies (*e.g.*, scFv), minibodies and diabodies. An antibody conjugated to a toxin moiety is an example of a chemically conjugated antibody.

10 **[0033]** As used herein, the terms “cancer” and “cancerous” are understood to mean the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, melanoma, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of cancers include squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer
15 including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, bone cancer, brain cancer, retinoblastoma, endometrial cancer
20 or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, as well as head and neck cancer, gum or tongue cancer. The cancer comprises cancer or cancerous cells, for example, the cancer may comprise a plurality of individual cancer or cancerous cells, for example, a leukemia, or a tumor comprising a plurality of associated cancer
25 or cancerous cells.

[0034] As used herein, the term “immunogen” is a molecule that provokes (evokes, induces, or causes) an immune response. This immune response may involve antibody production, the activation of certain cells, such as, for example, specific immunologically-competent cells, or both. An immunogen may be derived from many types of substances, such as, but not limited
30 to, molecules from organisms, such as, for example, proteins, subunits of proteins, killed or inactivated whole cells or lysates, synthetic molecules, and a wide variety of other agents both biological and nonbiological. It is understood that essentially any macromolecule (including

naturally occurring macromolecules or macromolecules produced via recombinant DNA approaches), including virtually all proteins, can serve as immunogens.

[0035] As used herein, the term “immunogenicity” relates to the ability of an immunogen to provoke (evoke, induce, or cause) an immune response. Different molecules may have differing degrees of immunogenicity, and a molecule having an immunogenicity that is greater compared to another molecule is known, for example, to be capable of provoking (evoking, inducing, or causing) a greater immune response than would an agent having a lower immunogenicity.

[0036] As used herein, the term “antigen” as used herein refers to a molecule that is recognized by antibodies, specific immunologically-competent cells, or both. An antigen may be derived from many types of substances, such as, but not limited to, molecules from organisms, such as, for example, proteins, subunits of proteins, nucleic acids, lipids, killed or inactivated whole cells or lysates, synthetic molecules, and a wide variety of other agents both biological and non-biological.

[0037] As used herein, the term “antigenicity” relates to the ability of an antigen to be recognized by antibodies, specific immunologically-competent cells, or both.

[0038] As used herein, the term “major histocompatibility complex,” or “MHC,” refers to a specific cluster of genes, many of which encode evolutionarily related cell surface proteins involved in antigen presentation, that are important determinants of histocompatibility. Class I MHC, or MHC-I, function mainly in antigen presentation to CD8⁺ T lymphocytes (CD8⁺ T-Cells). Class II MHC, or MHC-II, function mainly in antigen presentation to CD4⁺ T lymphocytes (CD4⁺ T-Cells).

[0039] As used herein, the term “derived,” for example “derived from,” includes, but is not limited to, for example, wild-type molecules derived from biological hosts such as bacteria, viruses and eukaryotic cells and organisms, and modified molecules, for example, modified by chemical means or produced in recombinant expression systems.

[0040] As used herein, the terms “seroreactive,” “seroreaction” or “seroreactivity” are understood to mean the ability of an agent, such as a molecule, to react with antibodies in the serum of a mammal, such as, but not limited to, a human. This includes reactions with all types of antibodies, including, for example, antibodies specific for the molecule and nonspecific

antibodies that bind to the molecule, regardless of whether the antibodies inactivate or neutralize the agent. As is known in the art, different agents may have different seroreactivity relative to one another, wherein an agent having a seroreactivity lower than another would, for example, react with fewer antibodies and/or have a lower affinity and/or avidity to antibodies than would an agent having a higher seroreactivity. This may also include the ability of the agent to elicit an antibody immune response in an animal, such as a mammal, such as a human.

[0041] As used herein, the terms “soluble T-cell receptor,” or “soluble TCR,” are understood to mean a “soluble” T-cell receptor comprising the chains of a full-length (*e.g.*, membrane bound) receptor, except that the transmembrane region of the receptor chains are deleted or mutated so that the receptor, when expressed by a cell, will not insert into, traverse or otherwise associate with the membrane. A soluble T-cell receptor may comprise only the extracellular domains or extracellular fragments of the domains of the wild-type receptor (*e.g.*, lacks the transmembrane and cytoplasmic domains).

[0042] As used herein, the term “superantigen” is understood to mean a class of molecules that stimulate a subset of T-cells by binding to MHC class II molecules and V β domains of T-cell receptors, thereby activating T-cells expressing particular V β gene segments. The term includes wild-type, naturally occurring superantigens, for example, those isolated from certain bacteria or expressed from unmodified genes from same, as well as modified superantigens, wherein, for example, the DNA sequence encoding a superantigen has been modified, for example, by genetic engineering, to, for example, produce a fusion protein with a targeting moiety, and/or alter certain properties of the superantigen, such as, but not limited to, its MHC class II binding (for example, to reduce affinity) and/or its seroreactivity, and/or its immunogenicity, and/or antigenicity (for example, to reduce its seroreactivity). The definition includes wild-type and modified superantigens and any immunologically reactive variants and/or fragments thereof described herein or in the following U.S. patents and patent applications: U.S. Patent Nos. 5,858,363, 6,197,299, 6,514,498, 6,713,284, 6,692,746, 6,632,640, 6,632,441, 6,447,777, 6,399,332, 6,340,461, 6,338,845, 6,251,385, 6,221,351, 6,180,097, 6,126,945, 6,042,837, 6,713,284, 6,632,640, 6,632,441, 5,859,207, 5,728,388, 5,545,716, 5,519,114, 6,926,694, 7,125,554, 7,226,595, 7,226,601, 7,094,603, 7,087,235, 6,835,818, 7,198,398, 6,774,218, 6,913,755, 6,969,616, and 6,713,284, U.S. Patent Application Nos. 2003/0157113, 2003/0124142, 2002/0177551, 2002/0141981, 2002/0115190, 2002/0051765, and 2001/0046501, and PCT International Publication Number WO/03/094846.

[0043] As used herein, the term “targeting moiety” refers to any structure, molecule or moiety that is able to bind to a cellular molecule, for example, a cell surface molecule, preferably a disease specific molecule such as an antigen expressed preferentially on a cancer (or cancerous) cell. Exemplary targeting moieties include, but are not limited to, antibodies
5 (including antigen binding fragments thereof) and the like, soluble T-cell receptors, interleukins, hormones, and growth factors.

[0044] As used herein, the terms “tumor-targeted superantigen” or “TTS” or “cancer-targeted superantigen” are understood to mean a molecule comprising one or more superantigens covalently linked (either directly or indirectly) with one or more targeting
10 moieties.

[0045] As used herein, the term “T-cell receptor” is understood to mean a receptor that is specific to T-cells, and includes the understanding of the term as known in the art. The term also includes, for example, a receptor that comprises a disulfide-linked heterodimer of the highly variable α or β chains expressed at the cell membrane as a complex with the invariant
15 CD3 chains, and a receptor made up of variable γ and δ chains expressed at the cell membrane as a complex with CD3 on a subset of T-cells.

[0046] As used herein, the terms “therapeutically effective amount” and “effective amount,” are understood to mean an amount of an active agent, for example, a pharmaceutically active agent or a pharmaceutical composition that produces at least some effect in treating a disease or
20 a condition. The effective amount of pharmaceutically active agent(s) used to practice the present invention for a therapeutic treatment varies depending upon the manner of administration, the age, body weight, and general health of the subject. These terms include, but are not limited to synergistic situations such as those described in the instant invention wherein a single agent alone, such as a superantigen conjugate or an immunopotentiator (for
25 example, an anti-PD-1 antibody), may act weakly or not at all, but when combined with each other, for example, but not limited to, via sequential dosage, the two or more agents act to produce a synergistic result.

[0047] As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are
30 not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[0048] As used herein, the terms “treat,” “treating” and “treatment” are understood to mean the treatment of a disease in a mammal, *e.g.*, in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its development; and (b) relieving the disease, *i.e.*, causing regression of the disease state; and (c) curing the disease. As used in the context of a therapeutic treatment, the terms “prevent” or “block” are understood to completely prevent or block, or not completely prevent or block (*e.g.*, partially prevent or block) a given act, action, activity, or event.

[0049] As used herein, the term “inhibits the growth of a cancer” is understood to mean a measurably slowing, stopping, or reversing the growth rate of the cancer or cancerous cells *in vitro* or *in vivo*. Desirably, the growth rate is slowed by 20%, 30%, 50%, or 70% or more, as determined using a suitable assay for determination of cell growth rates. Typically, a reversal of growth rate is accomplished by initiating or accelerating necrotic or apoptotic mechanisms of cell death in neoplastic cells, resulting in a shrinkage of a neoplasm.

[0050] As used herein, the terms “variant,” “variants,” “modified,” “altered,” “mutated,” and the like, are understood to mean proteins or peptides and/or other agents and/or compounds that differ from a reference protein, peptide or other compound. Variants in this sense are described below and elsewhere in greater detail. For example, changes in a nucleic acid sequence of the variant may be silent, *e.g.*, they may not alter the amino acids encoded by the nucleic acid sequence. Where alterations are limited to silent changes of this type a variant will encode a peptide with the same amino acid sequence as the reference peptide. Changes in the nucleic acid sequence of the variant may alter the amino acid sequence of a peptide encoded by the reference nucleic acid sequence. Such nucleic acid changes may result in amino acid substitutions, additions, deletions, fusions and/or truncations in the protein or peptide encoded by the reference sequence, as discussed below. Generally, differences in amino acid sequences are limited so that the sequences of the reference and the variant are similar overall and, in many regions, identical. A variant and reference protein or peptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and/or truncations, which may be present in any combination. A variant may also be a fragment of a protein or peptide of the invention that differs from a reference protein or peptide sequence by being shorter than the reference sequence, such as by a terminal or internal deletion. Another variant of a protein or peptide of the invention also includes a protein or peptide which retains essentially the same function or activity as the reference protein or peptide. A variant may also be: (i) one in which

one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature protein or peptide is fused with another
5 compound, such as a compound to increase the half-life of the protein or peptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature protein or peptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature protein or peptide. Variants may be made by mutagenesis techniques, and/or altering mechanisms such as chemical alterations, fusions, adjuncts and the
10 like, including those applied to nucleic acids, amino acids, cells or organisms, and/or may be made by recombinant means.

[0051] As used herein, the term “sequential dosage” and related terminology refers to the administration of at least one superantigen, with at least one immunopotentiator, and includes staggered doses of these agents (*i.e.*, time-staggered) and variations in dosage amounts. This
15 includes one agent being administered before, overlapping with (partially or totally), or after administration of another agent. This term generally considers the best administration scheme to achieve a synergistic combination of at least one superantigen and at least one immunopotentiator. By such a dosing strategy (*e.g.*, a sequential dosage), one may be able to achieve synergistic effects of combined superantigen and immunopotentiator administration.
20 In addition, the term “sequential dosage” and related terminology also includes the administration of at least one superantigen, one immunopotentiator and more or more optional additional compounds such as, for example, a corticosteroid, an immune modulator, and another agent designed to reduce potential immunoreactivity to the superantigen conjugate administered to the subject.

[0052] As used herein, the terms “systemic” and “systemically” in the context of administration are understood to mean administration of an agent such that the agent is exposed to at least one system associated with the whole body, such as but not limited to the circulatory system, immune system, and lymphatic system, rather than only to a localized part of the body, such as but not limited to within a tumor. Thus, for example, a systemic therapy or an agent
30 administered systematically is a therapy or an agent in which at least one system associated with the entire body is exposed to the therapy or agent, as opposed to, rather than just a target tissue.

[0053] As used herein, the term “parenteral administration” includes any form of administration in which the compound is absorbed into the subject without involving absorption via the intestines. Exemplary parenteral administrations that are used in the present invention include, but are not limited to intramuscular, intravenous, intraperitoneal, or
5 intraarticular administration.

[0054] Where the use of the term “about” is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

10 [0055] At various places in the present specification, values are disclosed in groups or in ranges. It is specifically intended that the description include each and every individual subcombination of the members of such groups and ranges. For example, an integer in the range of 0 to 40 is specifically intended to individually disclose 0, 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,
15 37, 38, 39, and 40, and an integer in the range of 1 to 20 is specifically intended to individually disclose 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

[0056] Throughout the description, where compositions and kits are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are
20 compositions and kits of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing and method steps.

[0057] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit
25 and scope of the present invention, whether explicit or implicit herein. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and

depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

II. Superantigen Conjugate

A. Superantigens

[0058] Superantigens are bacterial proteins, viral proteins, and human-engineered proteins, capable of activating T lymphocytes, for example, at picomolar concentrations. Superantigens can also activate large subsets of T lymphocytes (T-cells). Superantigens can bind to the major histocompatibility complex I (MHCI) without being processed and, in particular, can bind to conserved regions outside the antigen-binding groove on MHC class II molecules, avoiding most of the polymorphism in the conventional peptide-binding site. Superantigens can also bind to the V β chain of the T-cell receptor (TCR) rather than binding to the hypervariable loops of the T-cell receptor. Examples of bacterial superantigens include, but are not limited to, Staphylococcal enterotoxin (SE), Streptococcus pyogenes exotoxin (SPE), *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), Streptococcal mitogenic exotoxin (SME), Streptococcal superantigen (SSA), Staphylococcal enterotoxin A (SEA), Staphylococcal enterotoxin A (SEB), and Staphylococcal enterotoxin E (SEE).

[0059] The polynucleotide sequences encoding many superantigens have been isolated and cloned and superantigens expressed from these or modified (reengineered) polynucleotide sequences have been used in anti-cancer therapy (see, ANYARA[®], discussed below). Superantigens expressed by these polynucleotide sequences may be wild-type superantigens, modified superantigens, or wild-type or modified superantigens conjugated or fused with targeting moieties. The superantigens may be administered to a mammal, such as a human, directly, for example by injection, or may be delivered, for example, by exposure of blood of a patient to the superantigen outside the body, or, for example, via placing a gene encoding a superantigen inside a mammal to be treated (*e.g.*, via known gene therapy methods and vectors such as, for example, via cells containing, and capable of expressing, the gene) and expressing the gene within the mammal.

[0060] Examples of superantigens and their administration to mammals are described in the following U.S. patents and patent applications: U.S. Patent Nos. 5,858,363, 6,197,299, 6,514,498, 6,713,284, 6,692,746, 6,632,640, 6,632,441, 6,447,777, 6,399,332, 6,340,461, 6,338,845, 6,251,385, 6,221,351, 6,180,097, 6,126,945, 6,042,837, 6,713,284, 6,632,640,

6,632,441, 5,859,207, 5,728,388, 5,545,716, 5,519,114, 6,926,694, 7,125,554, 7,226,595,
7,226,601, 7,094,603, 7,087,235, 6,835,818, 7,198,398, 6,774,218, 6,913,755, 6,969,616, and
6,713,284, U.S. Patent Application Nos. 2003/0157113, 2003/0124142, 2002/0177551,
2002/0141981, 2002/0115190, and 2002/0051765, and PCT International Publication Number
5 WO/03/094846.

B. Modified Superantigens

[0061] Within the scope of this invention, superantigens may be engineered in a variety of
ways, including modifications that retain or enhance the ability of a superantigen to stimulate T
lymphocytes, and may, for example, alter other aspects of the superantigen, such as, for
example, its seroreactivity or immunogenicity. Modified superantigens include synthetic
10 molecules that have superantigen activity (*i.e.*, the ability to activate subsets of T lymphocytes).

[0062] It is contemplated that various changes may be made to the polynucleotide sequences
encoding a superantigen without appreciable loss of its biological utility or activity, namely the
induction of the T-cell response to result in cytotoxicity of the tumor cells. Furthermore, the
affinity of the superantigen for the MHC class II molecule can be decreased with minimal
15 effects on the cytotoxicity of the superantigen. This, for example, can help to reduce toxicity
that may otherwise occur if a superantigen retains its wild-type ability to bind MHC class II
antigens (as in such a case, class II expressing cells, such as immune system cells, could also be
affected by the response to the superantigen).

[0063] Techniques for modifying superantigens (*e.g.*, polynucleotides and polypeptides),
20 including for making synthetic superantigens, are well known in the art and include, for
example PCR mutagenesis, alanine scanning mutagenesis, and site-specific mutagenesis (see,
U.S. Patent Nos. 5,220,007; 5,284,760; 5,354,670; 5,366,878; 5,389,514; 5,635,377; and
5,789,166).

[0064] In some embodiments, a superantigen may be modified such that its seroreactivity is
25 reduced compared to a reference wild-type superantigen, but its ability to activate T-cells is
retained or enhanced relative to wild-type. One technique for making such modified
superantigens includes substituting certain amino acids in certain regions from one
superantigen to another. This is possible because many superantigens, including but not
limited to, SEA, SEE, and SED, share sequence homology in certain areas that have been
30 linked to certain functions (Marrack and Kappler (1990) SCIENCE 248(4959): 1066; see also

FIGURE 2, which shows region of homology between different wild type and engineered superantigens). For example, in certain embodiments of the present invention, a superantigen that has a desired T-cell activation-inducing response, but a non-desired high seroreactivity, is modified such that the resulting superantigen retains its T-cell activation ability but has reduced seroreactivity.

[0065] It is known and understood by those of skill in the art that the sera of humans normally contain various titers of antibodies against superantigens. For the staphylococcal superantigens, for instance, the relative titers are TSST-1>SEB>SEC-1>SE3>SEC2>SEA>SED>SEE. As a result, the seroreactivity of, for example, SEE (Staphylococcal enterotoxin E) is lower than that of, for example, SEA (Staphylococcal enterotoxin A). Based on this data, one skilled in the art may prefer to administer a low titer superantigen, such as, for example SEE, instead of a high titer superantigen, such as, for example, SEB (Staphylococcal enterotoxin B). However, as has also been discovered, different superantigens have differing T-cell activation properties relative to one another, and for wild-type superantigens, the best T-cell activating superantigens often also have undesirably high seroreactivity.

[0066] These relative titers sometimes correspond to potential problems with seroreactivity, such as problems with neutralizing antibodies. Thus, the use of a low titer superantigen, such as SEA or SEE may be helpful in reducing or avoiding seroreactivity of parenterally administered superantigens. A low titer superantigen has a low seroreactivity as measured, for example, by typical anti-superantigen antibodies in a general population. In some instances it may also have a low immunogenicity. Such low titer superantigens may be modified to retain its low titer as described herein.

[0067] Approaches for modifying superantigens can be used to create superantigens that have both the desired T-cell activation properties and reduced seroreactivity, and in some instances also reduced immunogenicity. Given that certain regions of homology between superantigens relate to seroreactivity, it is possible to engineer a recombinant superantigen that has a desired T-cell activation and a desired seroreactivity and/or immunogenicity.

Furthermore, the protein sequences and immunological cross-reactivity of the superantigens or staphylococcal enterotoxins are divided into two related groups. One group consists of SEA, SEE and SED. The second group is SPEA, SEC and SEB. Thus, it is possible to select low

titer superantigens to decrease or eliminate the cross-reactivity with high titer or endogenous antibodies directed against staphylococcal enterotoxins.

[0068] Regions in the superantigens that are believed to play a role in seroreactivity include, for example, Region A, which comprises amino acid residues 20, 21, 22, 23, 24, 25, 26, and 27; 5 Region B, which comprises amino acid residues 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and 49; Region C, which comprises amino acid residues 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, and 84; Region D, which comprises amino acid residues 187, 188, 189 and 190; and Region E, which comprise the amino acid residues, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, and 227 (see, U.S. Patent No. 7,125,554, and **FIGURE 2** herein). Thus, it is 10 contemplated that these regions can be mutated using, for example amino acid substitution, to produce a superantigen having altered seroreactivity.

[0069] Polypeptide or amino acid sequences for the above listed superantigens can be obtained from any sequence data bank, for example Protein Data Bank and/or GenBank. Exemplary GenBank accession numbers include, but are not limited to, SEE is P12993; SEA is 15 P013163; SEB is P01552; SEC1 is P01553; SED is P20723; and SEH is AAA19777.

[0070] In certain embodiments of the present invention, the wild-type SEE sequence (SEQ ID NO: 1) or the wild type SEA sequence (SEQ ID NO: 2) can be modified such that amino acids in any of the identified regions A-E (see, **FIGURE 2**) are substituted with other amino acids. Such substitutions include for example, K79, K81, K83 and D227 or K79, K81, K83, 20 K84 and D227, or, for example, K79E, K81E, K83S and D227S or K79E, K81E, K83S, K84S and D227A. In certain embodiments, the superantigen is SEA/E-120 (SEQ ID NO: 3; see also U.S. Patent No. 7,125,554) or SEA_{D227A} (SEQ ID NO: 4; see also U.S. Patent No. 7,226,601).

1. Modified Polynucleotides and Polypeptides

[0071] A biological functional equivalent of a polynucleotide encoding a naturally occurring or a reference superantigen may comprise a polynucleotide that has been engineered to contain 25 distinct sequences while at the same time retaining the capacity to encode the naturally occurring or reference superantigen. This can be accomplished due to the degeneracy of the genetic code, *i.e.*, the presence of multiple codons, which encode for the same amino acids. In one example, it is possible to introduce a restriction enzyme recognition sequence into a polynucleotide while not disturbing the ability of that polynucleotide to encode a protein. 30 Other polynucleotide sequences may encode superantigens that are different but functionally

substantially equivalent in at least one biological property or activity (for example, at least 50%, 60%, 70%, 80%, 90%, 95%, 98% of the biological property or activity, for example, without limitation, the ability to induce a T-cell response that results in cytotoxicity of the tumor cells) to a reference superantigen.

5 **[0072]** In another example, a polynucleotide may be (and encode) a superantigen functionally equivalent to a reference superantigen even though it may contain more significant changes. Certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies, binding sites on substrate molecules, receptors, and such
10 like. Furthermore, conservative amino acid replacements may not disrupt the biological activity of the protein, as the resultant structural change often is not one that impacts the ability of the protein to carry out its designed function. It is thus contemplated that various changes may be made in the sequence of genes and proteins disclosed herein, while still fulfilling the goals of the present invention.

15 **[0073]** Amino acid substitutions may be designed to take advantage of the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and/or the like. An analysis of the size, shape and/or type of the amino acid side-chain substituents reveals that arginine, lysine and/or histidine are all positively charged residues; that alanine, glycine and/or serine are all a similar size; and/or that phenylalanine,
20 tryptophan and/or tyrosine all have a generally similar shape. Therefore, based upon these considerations, arginine, lysine and/or histidine; alanine, glycine and/or serine; and/or phenylalanine, tryptophan and/or tyrosine; are defined herein as biologically functional equivalents. In addition, it may be possible to introduce non-naturally occurring amino acids. Approaches for making amino acid substitutions with other naturally occurring and non-
25 naturally occurring amino acid are described in U.S. Patent No. 7,763,253.

[0074] In terms of functional equivalents, it is understood that, implicit in the definition of a “biologically functional equivalent” protein and/or polynucleotide, is the concept that there is a limited number of changes that may be made within a defined portion of the molecule while retaining a molecule with an acceptable level of equivalent biological activity. Biologically
30 functional equivalents are thus considered to be those proteins (and polynucleotides) where selected amino acids (or codons) may be substituted without substantially affecting biological

function. Functional activity includes the induction of the T-cell response to result in cytotoxicity of the tumor cells.

[0075] In addition, it is contemplated that a modified superantigen can be created by substituting homologous regions of various proteins via “domain swapping,” which involves the generation of chimeric molecules using different but, in this case, related polypeptides. By comparing various superantigen proteins to identify functionally related regions of these molecules (see, *e.g.*, **FIGURE 2**), it is possible to swap related domains of these molecules so as to determine the criticality of these regions to superantigen function. These molecules may have additional value in that these “chimeras” can be distinguished from natural molecules, while possibly providing the same function.

[0076] In certain embodiments, the superantigen comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the sequence of a reference superantigen selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, wherein the superantigen optionally retains at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% of a biological activity or property of the reference superantigen.

[0077] In certain embodiments, the superantigen comprises an amino acid sequence that is encoded by a nucleic acid that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to a nucleic acid encoding the superantigen selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, wherein the superantigen optionally retains at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% of a biological activity or property of the reference superantigen.

[0078] Sequence identity may be determined in various ways that are within the skill in the art, *e.g.*, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul, (1993) J. MOL. EVOL. 36, 290-300; Altschul *et al.*, (1997) NUCLEIC ACIDS RES. 25:3389-3402, incorporated by reference) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases see Altschul *et al.*, (1994) NATURE GENETICS 6:119-129, which is fully incorporated by reference. Those skilled in the art can determine appropriate parameters for

measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default

5 scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919, fully incorporated by reference). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The

10 equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (*e.g.*: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match

15 [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and -Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may

20 also be used (default parameters may include, *e.g.*, Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

C. Targeted Superantigens

25 [0079] In order to increase specificity, the superantigen preferably is conjugated to a targeting moiety to create a targeted superantigen conjugate that binds an antigen preferentially expressed by a cancer cell, for example, a cell surface antigen such as 5T4. The targeting moiety is a vehicle that can be used to bind superantigen to the cancerous cells, for example, the surface of the cancerous cells. The targeted superantigen conjugate should retain the ability

30 to activate large numbers of T lymphocytes. For example, the targeted superantigen conjugate should activate large numbers of T-cells and direct them to tissues containing the tumor-

associated antigen bound to the targeting moiety. In such situations, specific target cells are preferentially killed, leaving the rest of the body relatively unharmed. This type of therapy is desirable, as non-specific anti-cancer agents, such as cytostatic chemotherapeutic drugs, are nonspecific and kill large numbers of cells not associated with tumors to be treated. For
 5 example, studies with targeted superantigen conjugates have shown that inflammation with infiltration by cytotoxic T lymphocytes (CTLs) into tumor tissue increases rapidly in response to the first injection of a targeted superantigen (Dohlsten *et al.* (1995) PROC. NATL. ACAD. SCI. USA 92:9791-9795). This inflammation with infiltration of CTLs into the tumor is one of the major effectors of the anti-tumor therapeutic of targeted superantigens.

10 **[0080]** Tumor-targeted superantigens represent an immunotherapy against cancer and are therapeutic fusion proteins containing a targeting moiety conjugated to a superantigen (Dohlsten *et al.* (1991) PROC. NATL. ACAD. SCI. USA 88:9287-9291; Dohlsten *et al.* (1994) PROC. NATL. ACAD. SCI. USA 91:8945-8949).

[0081] The targeting moiety can in principle be any structure that is able to bind to a cellular
 15 molecule, for example, a cell surface molecule and preferably is a disease specific molecule. The targeted molecule (*e.g.*, antigen) against which the targeting moiety is directed is usually different from (a) the V β chain epitope to which superantigen binds, and (b) the MHC class II epitopes to which superantigens bind. The targeting moiety can be selected from antibodies, including antigen binding fragments thereof, soluble T-cell receptors, growth factors,
 20 interleukins (*e.g.*, interleukin-2), hormones, *etc.*

[0082] In certain preferred embodiments, the targeting moiety is an antibody (*e.g.*, Fab, F(ab)₂, Fv, single chain antibody, *etc.*). Antibodies are extremely versatile and useful cell-specific targeting moieties because they typically can be generated against any cell surface antigen of interest. Monoclonal antibodies have been generated against cell surface receptors,
 25 tumor-associated antigens, and leukocyte lineage-specific markers such as CD antigens. Antibody variable region genes can be readily isolated from hybridoma cells by methods well known in the art. Exemplary tumor-associated antigens that can be used to produce a targeting moiety can include, but are not limited to gp100, Melan-A/MART, MAGE-A, MAGE (melanoma antigen E), MAGE-3, MAGE-4, MAGEA3, tyrosinase, TRP2, NY-ESO-1, CEA
 30 (carcinoembryonic antigen), PSA, p53, Mammaglobin-A, Survivin, Muc1 (mucin1)/DF3, metalloproteinase-1 (MPS-1), Cytochrome P450 isoform 1B1, 90K/Mac-2 binding protein,

Ep-CAM (MK-1), HSP-70, hTERT (TRT), LEA, LAGE-1/CAMEL, TAGE-1, GAGE, 5T4, gp70, SCP-1, c-myc, cyclin B1, MDM2, p62, Koc, IMP1, RCAS1, TA90, OA1, CT-7, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-TES-14/SCP-1, HOM-TES-85, HDAC5, MBD2, TRIP4, NY--CO-45, KNSL6, HIP1R, Seb4D, KIAA1416, IMP1, 90K/Mac-2 binding protein, MDM2, NY/ESO, and LMNA.

[0083] Exemplary cancer-targeting antibodies can include, but are not limited to, anti-CD19 antibodies, anti-CD20 antibodies, anti-5T4 antibodies, anti-Ep-CAM antibodies, anti-Her-2/neu antibodies, anti-EGFR antibodies, anti-CEA antibodies, anti-prostate specific membrane antigen (PSMA) antibodies, and anti-IGF-1R antibodies. It is understood that the superantigen can be conjugated to an immunologically reactive antibody fragment such as C215Fab, 5T4Fab (see, WO8907947) or C242Fab (see, WO9301303).

[0084] Examples of tumor targeted superantigens that can be used in the present invention include C215Fab-SEA (SEQ ID NO: 5), 5T4Fab-SEA_{D227A} (SEQ ID NO: 6) and 5T4Fab-SEA/E-120 (SEQ ID NO: 7, see **FIGURE 3**).

[0085] In a preferred embodiment, a preferred conjugate is a superantigen conjugate known as Naptumomab estafenatox/ANYARA[®], which is the fusion protein of the Fab fragment of an anti-5T4 antibody and the SEA/E-120 superantigen. ANYARA[®] comprises two protein chains that cumulatively include an engineered Staphylococcal enterotoxin superantigen (SEA/E-120) and a targeting 5T4 Fab comprising modified 5T4 variable region sequences fused to the constant region sequences of the murine IgG1/κ antibody C242. The first protein chain comprises residues 1 to 458 of SEQ ID NO: 7 (see also, SEQ ID NO: 8), and includes a chimeric 5T4 Fab heavy chain, corresponding to residues 1 to 222 of SEQ ID NO: 7, and the SEA/E-120 superantigen, corresponding to residues 226 to 458 of SEQ ID NO: 7, covalently linked via a GGP tripeptide linker, corresponding to residues 223-225 of SEQ ID NO: 7. The second chain comprises residues 459 to 672 of SEQ ID NO: 7 (see also, SEQ ID NO: 9) and includes a chimeric 5T4 Fab light chain. The two protein chains are held together by non-covalent interactions between the Fab heavy and light chains. Residues 1-458 of SEQ ID NO: 7 correspond to residues 1-458 of SEQ ID NO: 8, and residues 459-672 of SEQ ID NO: 7 correspond to residues 1-214 of SEQ ID NO: 9. ANYARA[®] comprises the proteins of SEQ ID NOS: 8 and 9 held together by non-covalent interactions between the Fab heavy and Fab light chains. ANYARA[®] induces T-cell mediated killing of cancer cells at concentrations around 10

pM and the superantigen component of the conjugate has been engineered to have low binding to human antibodies and MHC Class II.

[0086] It is contemplated that other antibody based targeting moieties can be designed, modified, expressed, and purified using techniques known in the art and discussed in more
5 detail below.

[0087] Another type of targeting moiety includes a soluble T-cell receptor (TCR). Some forms of soluble TCR may contain either only extracellular domains or extracellular and cytoplasmic domains. Other modifications of the TCR may also be envisioned to produce a soluble TCR in which the transmembrane domains have been deleted and/or altered such that
10 the TCR is not membrane bound as described in U.S. Publication Application Nos. U.S. 2002/119149, U.S. 2002/0142389, U.S. 2003/0144474, and U.S. 2003/0175212, and International Publication Nos. WO2003020763; WO9960120 and WO9960119.

[0088] The targeting moiety can be conjugated to the superantigen by using either recombinant techniques or chemically linking of the targeting moiety to the superantigen.

1. Recombinant Linker (Fusion Protein)

15 [0089] It is contemplated that a gene encoding a superantigen linked directly or indirectly (for example, via an amino acid containing linker) to a targeting moiety can be created and expressed using conventional recombinant DNA technologies. For example, the amino terminal of a modified superantigen can be linked to the carboxy terminal of a targeting moiety or vice versa. For antibodies, or antibody fragments that may serve as targeting moieties, either
20 the light or the heavy chain may be utilized for creating a fusion protein. For example, for a Fab fragment, the amino terminus of the modified superantigen can be linked to the first constant domain of the heavy antibody chain (CH₁). In some instances, the modified superantigen can be linked to a Fab fragment by linking the VH and VL domain to the superantigen. Alternatively, a peptide linker can be used to join the superantigen and targeting
25 moiety together. When a linker is employed, the linker preferably contains hydrophilic amino acid residues, such as Gln, Ser, Gly, Glu, Pro, His and Arg. Preferred linkers are peptide bridges consisting of 1-10 amino acid residues, more particularly, 3-7 amino acid residues. An exemplary linker is the tripeptide - GlyGlyPro -. These approaches have been used successfully in the design and manufacture of the ANYARA[®] superantigen conjugate.

2. Chemical Linkage

[0090] It is also contemplated that the superantigen may be linked to the targeting moiety via a chemical linkage. Chemical linkage of the superantigen to the targeting moiety may require a linker, for example, a peptide linker. The peptide linker preferably is hydrophilic and exhibits one or more reactive moieties selected from amides, thioethers, disulfides *etc.* (See, 5 U.S. Patent Nos. 5,858,363, 6,197,299, and 6,514,498). It is also contemplated that the chemical linkage can use homo- or heterobifunctional crosslinking reagents. Chemical linking of a superantigen to a targeting moiety often utilizes functional groups (*e.g.*, primary amino groups or carboxy groups) that are present in many positions in the compounds.

D. Expression of Superantigens and Superantigen Conjugates

[0091] When recombinant DNA technologies are employed, the superantigen or the 10 superantigen-targeting moiety conjugate may be expressed using standard expression vectors and expression systems. The expression vectors, which have been genetically engineered to contain the nucleic acid sequence encoding the superantigen, are introduced (*e.g.*, transfected) into host cells to produce the superantigen (see, *e.g.* Dohlsten *et al.* (1994), Forsberg *et al.* (1997) J. BIOL. CHEM. 272:12430-12436, Erlandsson *et al.* (2003) J. MOL. BIOL. 333:893-905 15 and WO2003002143).

[0092] Host cells can be genetically engineered, for example, by transformation or transfection technologies, to incorporate nucleic acid sequences and express the superantigen. Introduction of nucleic acid sequences into the host cell can be affected by calcium phosphate transfection, DEAE-dextran mediated transfection, microinjection, cationic lipid-mediated 20 transfection, electroporation, transduction, scrape loading, ballistic introduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as, Davis *et al.* (1986) BASIC METHODS IN MOLECULAR BIOLOGY and Sambrook, *et al.* (1989) MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

25 [0093] Representative examples of appropriate host cells include bacterial cells, such as streptococci, staphylococci, *E. coli*, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and aspergillus cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells.

[0094] Examples of production systems for superantigens are found, for example, in U.S. Patent No. 6,962,694.

E. Protein Purification

[0095] The superantigen and/or the superantigen-targeting moiety conjugates preferably are purified prior to use, which can be accomplished using a variety of purification protocols.

5 Having separated the superantigen or the superantigen-targeting moiety conjugate from other proteins, the protein of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, size exclusion chromatography; affinity chromatography; 10 polyacrylamide gel electrophoresis; isoelectric focusing. The term “purified” as used herein, is intended to refer to a composition, isolatable from other components, wherein the macromolecule (*e.g.*, protein) of interest is purified to any degree relative to its original state. Generally, the terms “purified” refer to a macromolecule that has been subjected to fractionation to remove various other components, and which substantially retains its biological 15 activity. The term “substantially purified” refers to a composition in which the macromolecule of interest forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the content of the composition.

[0096] Various methods for quantifying the degree of purification of the protein are known 20 to those of skill in the art, including, for example, determining the specific activity of an active fraction, and assessing the amount of a given protein within a fraction by SDS-PAGE analysis, High Performance Liquid Chromatography (HPLC), or any other fractionation technique. Various techniques suitable for use in protein purification include, for example, precipitation with ammonium sulfate, PEG, antibodies and the like or by heat denaturation, followed by 25 centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxyapatite, affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. It is contemplated that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

III. Immunopotentiator

[0097] It is contemplated that the efficacy of the immunopotentiator can be enhanced by administering the immunopotentiator to the subject to be treated together with a superantigen conjugate comprising the superantigen and the targeting moiety. Exemplary immunopotentiators can, for example: (a) stimulate activating T-cell signaling, (b) repress T-cell inhibitory signalling between the cancerous cells and a T-cell, (c) repress inhibitory signalling that leads to T-cell expansion, activation and/or activity via a non-human IgG1-mediated immune response pathway, for example, a human IgG4 immunoglobulin-mediated pathway, (d) a combination of (a) and (b), (e) combination of (a) and (c), (f) a combination of (b) and (c), and (g) a combination of (a), (b), and (c).

10 [0098] In certain embodiments the immunopotentiator is a checkpoint pathway inhibitor. A number of T-cell checkpoint inhibitor pathways have been identified to date, for example, the PD-1 immune checkpoint pathway and Cytotoxic T-lymphocyte antigen-4 (CTLA-4) immune checkpoint pathway.

[0099] Sundstedt *et al.* (Sundstedt *et al.* (2012) J. IMMUNOTHER. 35:344–35), showed that a combination of a tumor targeted superantigen (TTS) with an anti-CTLA4 IgG1 antibody in a mouse B16 melanoma model was more active than the individual components. In that study, although the superantigen caused infiltration of both CD4+ and CD8+ T-cells, a large number of regulatory T-cells (Tregs) accumulated in the tumor microenvironment as well. The upregulation of these suppressive regulatory cells is believed to be a direct consequence of TTS therapy, which may limit its effectiveness. The authors noted that it was likely that the extreme upregulation of CTLA-4 by the TTS led to the effect of the anti-CTLA-4 antibody mainly being on the Treg population. Without wishing to be bound by theory, it is contemplated that the CTLA-4 IgG1 antibody is cytotoxic to the Treg population, which is central to its anti-cancer activity when used in combination with a TTS. In contrast, certain embodiments of the present invention use antibodies not known to deplete Tregs, *e.g.*, IgG4 antibodies directed at non-CTLA-4 checkpoints (for example, anti-PD-1 IgG4 inhibitors), and therefore represent a novel combination whose synergistic effects are mediated by other mechanisms of action.

[00100] PD-1 is a receptor present on the surface of T-cells that serves as an immune system checkpoint that inhibits or otherwise modulates T-cell activity at the appropriate time to prevent an overactive immune response. Cancer cells, however, can take advantage of this checkpoint by expressing ligands, for example, PD-L1, PD-L2, *etc.*, that interact with PD-1 on

the surface of T-cells to shut down or modulate T-cell activity. Using this approach, cancer can evade the T-cell mediated immune response.

[00101] In the CTLA-4 pathway, the interaction of CTLA-4 on the T-cell with its ligands (*e.g.*, CD80, also known as B7-1, and CD86) on the surface of an antigen presenting cells (rather than the cancer cells) leads to T-cell inhibition. As a result, the ligand that inhibits T-cell activation or activity (*e.g.*, CD80 or CD86) is provided by an antigen presenting cell (a key cell type in the immune system) rather than the cancer cell. Although CTLA-4 and PD-1 binding both have similar negative effects on T-cells the timing of downregulation, the responsible signaling mechanisms, and the anatomic locations of immune inhibition by these two immune checkpoints differ (American Journal of Clinical Oncology. Volume 39, Number 1, February 2016). Unlike CTLA-4, which is confined to the early priming phase of T-cell activation, PD-1 functions much later during the effector phase, (Keir *et al.* (2008) ANNU. REV IMMUNOL., 26:677–704). Consequently, T-cell inhibition mediated via the PD-1 checkpoint pathway is very different from T-cell inhibition mediated via the CTLA-4 checkpoint pathway.

[00102] In certain embodiments, the immunopotentiator prevents (completely or partially) an antigen expressed by the cancerous cell from repressing T-cell inhibitory signaling between the cancerous cell and the T-cell. In one embodiment, such an immunopotentiator is a checkpoint inhibitor, for example, a PD-1 inhibitor. Examples of such immunopotentiators include, for example, anti-PD-1 antibodies, anti-PD-L1 antibodies, and anti-PD-L2 antibodies.

Accordingly, in one embodiment the superantigen conjugate is administered with a PD-1-based immunopotentiator, which can include (1) a molecule (for example, an antibody or small molecule) that binds to a PD-1 ligand (for example, PD-L1 or PD-L2) to prevent the PD-1 ligand from binding to its cognate PD-1 on the T-cell, and/or (2) a molecule (for example, an antibody or small molecule) that binds to PD-1 on a T-cell to prevent the binding of a PD-ligand expressed by the cancer cell of interest.

[00103] Furthermore, in certain embodiments, the immunopotentiator prevents (completely or partially) an antigen expressed by the cancerous cell from repressing T-cell expansion, activation and/or activity via a human IgG4 (a non-human IgG1) mediated immune response pathway, for example, not via an ADCC pathway. It is contemplated that, although the immune response potentiated by the superantigen conjugate and the immunopotentiator may include some ADCC activity, the principal component(s) of the immune response do not

involve ADCC activity. In contrast, some of the antibodies currently being used in immunotherapy, such as ipilimumab (an anti-CTLA-4 IgG1 monoclonal antibody), can kill targeted cells via ADCC through signaling via their Fc domain through Fc receptors on effector cells. Ipilimumab, like many other therapeutic antibodies, was designed as a human IgG1 immunoglobulin, and although ipilimumab blocks interactions between CTLA-4 and CD80 or CD86, its mechanism of action is believed to include ADCC depletion of tumor-infiltrating regulatory T-cells that express high levels of cell surface CTLA-4. (Mahoney *et al.* (2015) NATURE REVIEWS, DRUG DISCOVERY 14: 561-584.) Given that CTLA-4 is highly expressed on a subset of T-cells (regulatory T-cells) that act to negatively control T-cells activation, when an anti-CTLA-4 IgG1 antibody is administered, the number of regulatory T-cells is reduced via ADCC.

[00104] In certain embodiments, it is desirable to use immunopotentiators whose mode of action is primarily to block the inhibitory signals between the cancer cells and the T-cells without significantly depleting the T-cell populations (for example, permitting the T-cell populations to expand). To achieve this, it is desirable to use an antibody, for example, an anti-PD-1 antibody, an anti-PD-L1 antibody or an anti-PD-L2 antibody, that has or is based on a human IgG4 isotype. Human IgG4 isotype is preferred under certain circumstances because this antibody isotype invokes little or no ADCC activity compared to the human IgG1 isotype (Mahoney *et al.* (2015) *supra*). Accordingly, in certain embodiments, the anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 has or is based on a human IgG4 isotype. As a result, it is not possible to extrapolate potential therapeutic activity of an anti-PD-1 antibody, anti-PD-L1 antibody, or anti-PD-L2 antibody having human IgG4 isotype based upon activity of an antibody having a different isotype, for example, IgG1 isotype, especially if that antibody is directed to a different antigen, for example, CTLA-4, such as an anti-CTLA-4 human IgG1 immunoglobulin. Furthermore, CTLA-4 and PD-1 represent two T-cell-inhibitory receptors with independent, non-redundant mechanisms of action. Despite their shared ability to block T-cell activation, the two proteins have unique structures and impact very different immune responses to cancer cells.

[00105] Exemplary PD-1/PD-L1 based immunopotentiators are described in U.S. Patent Nos. 8,728,474, 8,952,136, and 9,073,994, and EP Patent No. 1537878B1, and include anti-PD-1 antibodies. Exemplary anti-PD-1 antibodies include nivolumab (Bristol-Myers Squibb Co.),

pembrolizumab (KEYTRUDA[®], Merck & Co.) and Atezolizumab (formerly MPDL3280A), MEDI4736, Avelumab, and PDR001.

[00106] The protein based immunopotentiators may be designed, expressed, and purified using techniques known to those skilled in the art, for example, as described hereinabove. The anti-PD-1 antibodies may be designed, expressed, purified, formulated and administered as described in U.S. Patent Nos. 8,728,474, 8,952,136, and 9,073,994.

[00107] Other immunopotentiators (for example, antibodies, and various small molecules) may target signaling pathways involving one or more of the following ligands: B7-H3 (found on prostate, renal cell, non-small cell lung, pancreatic, gastric, ovarian, colorectal cells, among others); B7-H4 (found on breast, renal cell, ovarian, pancreatic, melanoma cells, among others); HHLA2 (found on breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney cells, among others); galectins (found on non-small cell lung, colorectal, and gastric cells, among others); CD30 (found on Hodgkin lymphoma, large cell lymphoma cells, among others); CD70 (found on non-Hodgkin's lymphoma, renal cells, among others); ICOSL (found on glioblastoma, melanoma cells, among others); and CD155 (found on kidney, prostate, pancreatic glioblastoma cells, among others). Similarly, other potential immunopotentiators that can be used include, for example, a 4-1BB (CD137) agonist (*e.g.*, the fully human IgG4 anti-CD137 antibody Urelumab/BMS-663513), a LAG3 inhibitor (*e.g.*, the humanized IgG4 anti-LAG3 antibody LAG525, Novartis); an IDO inhibitor (*e.g.*, the small molecule INCB024360, Incyte Corporation), a TGF β inhibitor (*e.g.*, the small molecule Galunisertib, Eli Lilly) and other receptor or ligands that are found on T-cells and/or tumor cells and that are amenable to pharmaceutical intervention based on agonist/antagonist interactions but not through ADCC.

A. Antibody Production

[00108] Methods for producing antibodies are known in the art. For example, DNA molecules encoding light chain variable regions and heavy chain variable regions can be chemically synthesized using the sequences of the CDRs and variable regions of the antibodies of interest, for example, the antibody sequences provided in U.S. Patent No. 8,952,136 and the hybridoma deposits described in U.S. Patent No. 9,073,994. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, *e.g.*, constant region coding sequences, and expression control sequences, to produce conventional gene expression

constructs encoding the desired antibodies. Production of defined gene constructs is within routine skill in the art. Alternatively, the sequences provided herein can be cloned out of hybridomas by conventional hybridization techniques or polymerase chain reaction (PCR) techniques, using synthetic nucleic acid probes whose sequences are based on sequence
5 information provided herein, or prior art sequence information regarding genes encoding the heavy and light chains of murine antibodies in hybridoma cells.

[00109] Nucleic acids encoding the antibodies disclosed herein can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese
10 hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells that do not otherwise produce IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions.

[00110] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. The expressed
15 secreted protein accumulates in refractile or inclusion bodies, and can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art.

[00111] If a DNA construct encoding an antibody disclosed herein is to be expressed in eukaryotic host cells, *e.g.*, CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, IgG enhancers, and various introns. This
25 expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy and/or light chain to be expressed. In some embodiments, a single expression vector contains both heavy and light chain variable regions to be expressed.

[00112] The gene construct can be introduced into eukaryotic host cells using conventional
30 techniques. The host cells express V_L or V_H fragments, V_L - V_H heterodimers, V_H - V_L or V_L - V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof,

each of which may be attached to a moiety having another function (*e.g.*, cytotoxicity). In some embodiments, a host cell is transfected with a single vector expressing a polypeptide expressing an entire, or part of, a heavy chain (*e.g.*, a heavy chain variable region) or a light chain (*e.g.*, a light chain variable region). In other embodiments, a host cell is transfected with
5 a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain. In still other embodiments, a host cell is co-transfected with more than one expression vector (*e.g.*, one expression vector expressing a polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, and
10 another expression vector expressing a polypeptide comprising an entire, or part of, a light chain or light chain variable region).

[00113] A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or a polypeptide comprising an immunoglobulin light chain variable region may comprise growing (culturing) a host cell transfected with an expression vector under conditions
15 that permits expression of the polypeptide comprising the immunoglobulin heavy chain variable region or the polypeptide comprising the immunoglobulin light chain variable region. The polypeptide comprising a heavy chain variable region or the polypeptide comprising the light chain variable region then may be purified using techniques well known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) and histidine tags.

20 [00114] A method of producing a monoclonal antibody that binds a target protein, for example, PD-1, PD-L1, or PD-L2, or an antigen-binding fragment of the antibody, may comprise growing a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression vector that encodes a complete or partial immunoglobulin light chain; or (b) a single expression vector that encodes
25 both chains (*e.g.*, complete or partial chains), under conditions that permit expression of both chains. The intact antibody (or antigen-binding fragment) can be harvested and purified using techniques well known in the art, *e.g.*, Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) and histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression
30 vectors.

B. Antibody Modifications

[00115] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When the antibodies are to be administered to a human, the antibodies preferably are “humanized” to reduce or eliminate antigenicity in humans. Preferably, a humanized antibody has the same or substantially the same affinity for the antigen
5 as the non-humanized mouse antibody from which it was derived.

[00116] In one humanization approach, chimeric proteins are created in which mouse immunoglobulin constant regions are replaced with human immunoglobulin constant regions. See, *e.g.*, Morrison *et al.* (1984) PROC. NAT. ACAD. SCI. 81:6851-6855, Neuberger *et al.* (1984) NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and
10 4,816,567 (Cabilly).

[00117] In an approach known as CDR grafting, the CDRs of the light and heavy chain variable regions are grafted into frameworks from another species. For example, murine CDRs can be grafted into human FRs. In some embodiments, the CDRs of the light and heavy chain variable regions of an anti-ErbB3 antibody are grafted onto human FRs or consensus human
15 FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described in U.S. Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986) NATURE 321: 522-
20 525; Riechmann *et al.* (1988) NATURE 332: 323-327; Verhoeyen *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[00118] In an approach called “SUPERHUMANIZATION™,” human CDR sequences are chosen from human germline genes, based on the structural similarity of the human CDRs to those of the mouse antibody to be humanized. See, *e.g.*, U.S. Patent No. 6,881,557 (Foote);
25 and Tan *et al.* (2002) J. IMMUNOL. 169:1119-1125.

[00119] Other methods to reduce immunogenicity include “reshaping,” “hyperchimerization,” and “veneering/resurfacing.” See, *e.g.*, Vaswami *et al.* (1998) ANNALS OF ALLERGY, ASTHMA, & IMMUNOL. 81:105; Roguska *et al.* (1996) PROT. ENGINEER 9:895-904; and U.S. Patent No. 6,072,035 (Hardman). In the veneering/resurfacing approach, the surface
30 accessible amino acid residues in the murine antibody are replaced by amino acid residues

more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, *e.g.*, in U.S. Patent No. 5,639,641 (Pedersen).

[00120] Another approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMABTM technology (Vaccinex, Inc., Rochester, NY), which
5 involves a vaccinia virus-based vector to express antibodies in mammalian cells. High levels of combinatorial diversity of IgG heavy and light chains are said to be produced. See, *e.g.*, U.S. Patent Nos. 6,706,477 (Zauderer); 6,800,442 (Zauderer); and 6,872,518 (Zauderer).

[00121] Another approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto,
10 CA). This technology involves the use of a proprietary human “acceptor” library to produce an “epitope focused” library for antibody selection.

[00122] Another approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERINGTM technology, which is practiced commercially by XOMA (US) LLC. See, *e.g.*, PCT Publication No. WO 93/11794 and U.S. Patent Nos.
15 5,766,886; 5,770,196; 5,821,123; and 5,869,619.

[00123] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody including the binding moiety component of the superantigen conjugate disclosed herein.

[00124] Methods of making multispecific antibodies are known in the art. Multi-specific
20 antibodies include bispecific antibodies. Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies bind to two different epitopes of the antigen of interest. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*, F(ab')₂ bispecific antibodies and diabodies) as described, for example, in Milstein *et al.*, NATURE 305:537-539 (1983), WO 93/08829,
25 Traunecker *et al.*, EMBO J., 10:3655-3659 (1991), WO 94/04690, Suresh *et al.* (1986) METHODS IN ENZYMOLOGY 121:210, WO96/27011, Brennan *et al.* (1985) SCIENCE 229: 81, Shalaby *et al.* (1992) J. EXP. MED. 175: 217-225, Kostelny *et al.* (1992) J. IMMUNOL. 148(5):1547-1553, Hollinger *et al.* (1993) PNAS, 90:6444-6448, Gruber *et al.* (1994) J. IMMUNOL. 152:5368, Wu *et al.* (2007) NAT. BIOTECHNOL. 25(11): 1290-1297, U.S. Patent
30 Publication No. 2007/0071675, and Bostrom *et al.*, SCIENCE 323:1640-1644 (2009).

IV. Formulations and Pharmaceutical Compositions

[00125] The superantigen conjugate and immunopotentiator can be administered together, sequentially, or intermittently to the subject so as to treat the cancer, for example, to slow the growth rate of cancer cells, reduce the incidence or number of metastases, reduce tumor size, inhibit tumor growth, reduce the blood supply to a tumor or cancer cells, promote an immune
5 response against cancer cells or a tumor, prevent or inhibit the progression of cancer, for example, by at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100%.

Alternatively, the superantigen conjugate and immunopotentiator can be administered together, sequentially, or intermittently to the subject so as to treat the cancer, for example, to increase the lifespan of a subject with cancer, for example, by 3 months, 6 months, 9 months, 12
10 months, 1 year, 5 years, or 10 years.

[00126] The superantigen conjugate and the immunopotentiator may be formulated separately or together using techniques known to those skilled in the art. For example, for therapeutic use, the superantigen conjugate and/or the immunopotentiator is combined with a pharmaceutically acceptable carrier. As used herein, “pharmaceutically acceptable carrier”
15 means buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be “acceptable” in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers include buffers, solvents,
20 dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[00127] Pharmaceutical compositions containing the superantigen and/or the immunopotentiator disclosed herein can be provided in a single dosage form or different
25 dosage forms. The pharmaceutical composition or compositions should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intramuscular, intradermal, inhalation, transdermal, topical, transmucosal, and rectal administration. Alternatively, the agents may be administered locally rather than systemically, for example, via injection of the agent or agents directly into the site of action,
30 often in a depot or sustained release formulation.

[00128] Useful formulations can be prepared by methods well known in the pharmaceutical art. For example, see Remington's Pharmaceutical Sciences, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[00129] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof.

[00130] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[00131] The combined superantigen conjugate and immunopotentiator of the present invention may be employed alone or in conjunction with other compounds, such as carriers or other therapeutic compounds. Pharmaceutical compositions of the present invention comprise an effective amount of one or more superantigen conjugates and one or more immunopotentiators, for example an anti-PD-1 antibody, and may also contain additional agents, dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases “pharmaceutical” or “pharmacologically acceptable” refer to substances, *e.g.*, compositions, that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, such as, for example, a human. The preparation of a pharmaceutical composition that contains at least one superantigen conjugate and an immunopotentiator will be known to those of skill in the art in light of the present disclosure, and as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for human administration, it will be understood that preparations should

meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[00132] In a specific embodiment of the invention, the compositions of the invention comprise tumor-targeted superantigen in combination with an immunopotentiator. Such combinations include, for example, any tumor-targeted superantigen and/or immunopotentiator as described herein.

[00133] In a specific embodiment of the invention, the tumor-targeted super antigen comprises a bacterial superantigen including, but are not limited to, Staphylococcal enterotoxin (SE), Streptococcus pyogenes exotoxin (SPE), *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), Streptococcal mitogenic exotoxin (SME), Streptococcal superantigen (SSA), Staphylococcal enterotoxin A (SEA), Staphylococcal enterotoxin B (SEB), and Staphylococcal enterotoxin E (SEE) conjugated to a targeting moiety. In another embodiment of the invention, the compositions comprise tumor-targeted superantigens comprising superantigens with the following Protein Data Bank and/or GenBank accession numbers include, but are not limited to, SEE is P12993; SEA is P013163; SEB is P01552; SEC1 is P01553; SED is P20723; and SEH is AAA19777, as well as variants thereof, conjugated to a targeting moiety.

[00134] In certain embodiments, the superantigen conjugate comprises a wild type or engineered superantigen sequence such as, the wild-type SEE sequence (SEQ ID NO: 1) or the wild type SEA sequence (SEQ ID NO: 2), either of which can be modified such that amino acids in any of the identified regions A-E (see, **FIGURE 2**) are substituted with other amino acids. In certain embodiments, the superantigen incorporated in the conjugate is SEA/E-120 (SEQ ID NO: 3) or SEA_{D227A} (SEQ ID NO: 4).

[00135] Specific examples of targeting moieties to be conjugated to the superantigens include, for example, any molecule that is able to bind to a cellular molecule and preferably a disease specific molecule such as a cancer cell specific molecule. The targeting moiety can be selected from antibodies, including antigen binding fragments, soluble T-cell receptors, growth factors, interleukins, hormones, *etc.* Exemplary cancer targeting antibodies can include, but are not limited to, anti-CD19, anti-CD20 antibodies, anti-5T4 antibodies, anti-Ep-CAM antibodies, anti-Her-2/neu antibodies, anti-EGFR antibodies, anti-CEA antibodies, anti-prostate specific membrane antigen (PSMA) antibodies, and anti-IGF-1R antibodies. In one embodiment, the

superantigen can be conjugated to an immunologically reactive antibody fragment such as C215Fab, 5T4Fab (see, WO8907947) or C242Fab (see, WO9301303).

[00136] Examples of such tumor-targeted superantigens include C215Fab-SEA (SEQ ID NO: 5), 5T4Fab-SEA_{D227A} (SEQ ID NO: 6) and 5T4Fab-SEA/E-120 (SEQ ID NO: 7). In a preferred embodiment, the superantigen conjugate is 5T4 Fab-SEA/E-120, known in the art as Naptumomab estafenatox/ANYARA[®], which comprises two polypeptide sequences that together define an Fab fragment of an anti-5T4 antibody, where one of the polypeptide sequences further comprises the SEA/E-120 superantigen namely SEQ ID NO: 8 (chimeric V_H chain of 5T4 Fab coupled by three amino acid linker to SEA/E-120) and SEQ ID NO: 9 (chimeric V_L chain of 5T4 Fab).

[00137] In a preferred embodiment, the compositions of the invention comprise the tumor-targeted superantigen 5T4Fab-SEA/E-120, known in the art as naptumomab estafenatox/ANYARA[®] in combination with a PD-1 inhibitor, such as an anti-PD-1 antibody, for example, nivolumab (Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA[®], Merck & Co.), MK-3475 (Merck & Co), pidlizumab (CureTech), AMP-224 (AstraZeneca/Medimmune) and AMP-514 (AstraZeneca/Medimmune) or an anti-PD-L1 antibody such as MPDL3280A (Genentech/Roche), MEDI-4736 (AstraZeneca/Medimmune) and MSB0010718C (EMD Serono/Merck KGaA).

[00138] In a specific embodiment of the invention, the compositions comprise the targeted super antigen naptumomab estafenatox (ANYARA[®]) in combination with one or more anti-PD-1 antibodies including nivolumab (Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA[®], Merck & Co.), Atezolizumab (formerly MPDL3280A), MEDI4736, Avelumab, and PDR001.

[00139] Formulations or dosage form containing the superantigen conjugate and immunopotentiator may comprise different types of carriers depending on whether they are to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection.

[00140] Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers and the like, or combinations thereof. The composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by

preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[00141] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable. Such determinations are known and used by those of skill in the art.

[00142] The active agents are administered in an amount or amounts effective to decrease, reduce, inhibit or otherwise abrogate the growth or proliferation of cancer cells, induce apoptosis, inhibit angiogenesis of a cancer or tumor, inhibit metastasis, or induce cytotoxicity in cells. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of cancer varies depending upon the manner of administration, the age, body weight, and general health of the subject. These terms include synergistic situations such as those presented and described in the instant invention wherein a single agent alone, such as a superantigen conjugate or an immunopotentiator such as an anti-PD-1 antibody, may act weakly or not at all, but when combined with each other, for example, but not limited to, via sequential dosage, the two or more agents act to produce a synergistic result.

[00143] In certain non-limiting examples, a dose of the superantigen conjugate may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 15 microgram/kg/body weight, about 20 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-

limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, about 1 microgram/kg/body weight to about 100 milligram/kg/body weight. Other exemplary dosage ranges, range from about 1

5 microgram/kg/body weight to about 1000 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 100 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 75 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 50 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 40 microgram/kg/body weight, from about 1

10 microgram/kg/body weight to about 30 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 20 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 15 microgram/kg/body weight, from about 1 microgram/kg/body weight to about 10 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 1000 microgram/kg/body weight, from about 5

15 microgram/kg/body weight to about 100 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 75 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 50 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 40 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 30 microgram/kg/body weight, from about 5

20 microgram/kg/body weight to about 20 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 15 microgram/kg/body weight, from about 5 microgram/kg/body weight to about 10 microgram/kg/body weight, from about 10 microgram/kg/body weight to about 1000 microgram/kg/body weight, from about 10 microgram/kg/body weight to about 100 microgram/kg/body weight, from about 10

25 microgram/kg/body weight to about 75 microgram/kg/body weight, from about 10 microgram/kg/body weight to about 50 microgram/kg/body weight, from about 10 microgram/kg/body weight to about 40 microgram/kg/body weight, from about 10 microgram/kg/body weight to about 30 microgram/kg/body weight, from about 10 microgram/kg/body weight to about 20 microgram/kg/body weight, from about 10

30 microgram/kg/body weight to about 15 microgram/kg/body weight, from about 15 microgram/kg/body weight to about 1000 microgram/kg/body weight, from about 15 microgram/kg/body weight to about 100 microgram/kg/body weight, from about 15

microgram/kg/body weight to about 75 microgram/kg/body weight, from about 15
microgram/kg/body weight to about 50 microgram/kg/body weight, from about 15
microgram/kg/body weight to about 40 microgram/kg/body weight, from about 15
microgram/kg/body weight to about 30 microgram/kg/body weight, from about 15
5 microgram/kg/body weight to about 20 microgram/kg/body weight, from about 20
microgram/kg/body weight to about 1000 microgram/kg/body weight, from about 20
microgram/kg/body weight to about 100 microgram/kg/body weight, from about 20
microgram/kg/body weight to about 75 microgram/kg/body weight, from about 20
microgram/kg/body weight to about 50 microgram/kg/body weight, from about 20
10 microgram/kg/body weight to about 40 microgram/kg/body weight, from about 20
microgram/kg/body weight to about 30 microgram/kg/body weight, *etc.*, can be administered,
based on the numbers described above.

[00144] In certain embodiments, for example, but not limited to, administration of the
superantigen conjugate, the effective amount or dose of the superantigen conjugate that is
15 administered is an amount in the range of 0.01 to 500 $\mu\text{g/kg}$ body weight of the subject, for
example, 0.1-500 $\mu\text{g/kg}$ body weight of the subject, and, for example, 1-100 $\mu\text{g/kg}$ body weight
of the subject.

[00145] It is envisioned that the effective amount or dose of the immunopotentiator that is
administered in combination with the superantigen conjugate is a dose that results in an at least
20 an additive but preferably a synergistic anti-tumor effect and does not interfere or inhibit the
enhancement of the immune system or T-cell activation. If the immunopotentiator is
administered simultaneously with the superantigen conjugate, then the immunopotentiator may
be administered in a low dose such that it does not interfere with the mechanism of action of
the superantigen conjugate.

25 [00146] Generally, a therapeutically effective amount of immunopotentiator, for example, the
anti-PD-1 antibody, is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1
mg/kg to 10 mg/kg. For example, pembrolizumab (KEYTRUDA[®]) can be administered
periodically at 2 mg/kg as an intravenous infusion. The amount of immunopotentiator
administered will depend on variables such as the type and extent of disease or indication to be
30 treated, the overall health of the patient, the *in vivo* potency of the superantigen conjugate and
the immunopotentiator, the pharmaceutical formulation, and the route of administration.

V. Treatment Regimens and Indications

[00147] Treatment regimens may vary as well, and often depend on tumor type, tumor location, disease progression, and health and age of the patient. Certain types of tumor may require more aggressive treatment protocols, but at the same time, the patients may be unable to tolerate more aggressive treatment regimens. The clinician may often be best suited to make
5 such decisions based on his or her skill in the art and the known efficacy and toxicity (if any) of the therapeutic formulations.

[00148] In a specific embodiment of the invention, the treatment methods of the invention comprise administration of a tumor-targeted superantigen in combination with an immunopotentiator to a patient in need thereof, *i.e.*, a cancer patient. Such combination
10 treatments include, for example, administration of any tumor-targeted superantigen and/or immunopotentiator as described herein. In a specific embodiment of the invention, the tumor-targeted super antigen comprises a bacterial superantigen including, but are not limited to, Staphylococcal enterotoxin (SE), Streptococcus pyogenes exotoxin (SPE), *Staphylococcus aureus* toxic shock-syndrome toxin (TSST-1), Streptococcal mitogenic exotoxin (SME),
15 Streptococcal superantigen (SSA), Staphylococcal enterotoxin A (SEA), Staphylococcal enterotoxin B (SEB), and Staphylococcal enterotoxin E (SEE) conjugated to a targeting moiety.

[00149] In certain embodiments, the superantigen conjugate comprises a wild type or engineered superantigen sequence such as, the wild-type SEE sequence (SEQ ID NO: 1) or the wild type SEA sequence (SEQ ID NO: 2), either of which can be modified such that amino
20 acids in any of the identified regions A-E (see, **FIGURE 2**) are substituted with other amino acids. In certain embodiments, the superantigen incorporated in the conjugate is SEA/E-120 (SEQ ID NO: 3) or SEA_{D227A} (SEQ ID NO: 4).

[00150] Specific examples of targeting moieties to be conjugated to the superantigens include, for example, any molecule that is able to bind to a cellular molecule and preferably a
25 disease specific molecule such as a cancer cell specific molecule. The targeting moiety can be selected from antibodies, including antigen binding fragments, soluble T-cell receptors, growth factors, interleukins, hormones, *etc.* Exemplary cancer targeting antibodies can include, but are not limited to, anti-CD19, anti-CD20 antibodies, anti-5T4 antibodies, anti-Ep-CAM antibodies, anti-Her-2/neu antibodies, anti-EGFR antibodies, anti-CEA antibodies, anti-prostate specific
30 membrane antigen (PSMA) antibodies, and anti-IGF-1R antibodies. In one embodiment, the

superantigen can be conjugated to an immunologically reactive antibody fragment such as C215Fab, 5T4Fab (see, WO8907947) or C242Fab (see, WO9301303).

[00151] Examples of such tumor-targeted superantigens include C215Fab-SEA (SEQ ID NO: 5), 5T4Fab-SEA_{D227A} (SEQ ID NO: 6) and 5T4Fab-SEA/E-120 (SEQ ID NO: 7). In a preferred embodiment, the superantigen conjugate is 5T4 Fab-SEA/E-120 known in the art as Naptumomab estafenatox/ANYARA[®], which comprises two polypeptide sequences that together define an Fab fragment of an anti-5T4 antibody, where one of the polypeptide sequences further comprises the SEA/E-120 superantigen namely SEQ ID. NO: 8 (chimeric V_H chain of 5T4 Fab coupled by three amino acid linker to SEA/E-120) and SEQ ID. NO: 9 (chimeric V_L chain of 5T4 Fab).

[00152] In a preferred embodiment, the compositions of the invention comprise the tumor-targeted superantigen 5T4Fab-SEA/E-120, known in the art as naptumomab estafenatox/ANYARA[®] in combination with a PD-1 inhibitor, such as an anti-PD-1 antibody, for example, nivolumab (Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA[®], Merck & Co.), MK-3475 (Merck & Co), pidilizumab (CureTech), AMP-224 (AstraZeneca/Medimmune) and AMP-514 (AstraZeneca/Medimmune) or an anti-PD-L1 antibody such as MPDL3280A (Genentech/Roche), MEDI-4736 (AstraZeneca/Medimmune) and MSB0010718C (EMD Serono/Merck KGA).

[00153] In a specific embodiment of the invention, the compositions comprise the targeted superantigen naptumomab estafenatox (ANYARA[®]) in combination with one or more anti-PD-1 antibodies including nivolumab (Bristol-Myers Squibb Co.), pembrolizumab (KEYTRUDA[®], Merck & Co.), Atezolizumab (formerly MPDL3280A), MEDI4736, Avelumab, and PDR001.

[00154] Furthermore, the superantigen conjugate and immunopotentiator may be co-administered together or sequentially with one or more additional agents that enhance the potency and/or selectively of the therapeutic effect. Such agents include, for example, corticosteroids, additional immune modulators, and those compounds designed to reduce the patient's possible immunoreactivity to the administered superantigen conjugate. For example, immunoreactivity to the administered superantigen may be reduced via co-administration with, for example, an anti-CD20 antibody and/or an anti-CD19 antibody, that reduces the production of anti-superantigen antibodies in the subject.

[00155] Preferably, patients to be treated will have adequate bone marrow function (defined as a peripheral absolute granulocyte count of $>2,000/\text{mm}^3$ and a platelet count of $100,000/\text{mm}^3$), adequate liver function (bilirubin $<1.5 \text{ mg/dl}$) and adequate renal function (creatinine $<1.5 \text{ mg/dl}$).

5 **[00156]** In certain embodiments, the treatment regimen of the present invention may involve contacting the neoplasm or tumor cells with the superantigen conjugate and the immunopotentiator at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations, at the same time, wherein one composition
10 includes the superantigen conjugate and the other includes the immunopotentiator.

[00157] Alternatively, the superantigen conjugate may precede or follow the immunopotentiator by intervals ranging from minutes, days to weeks. In embodiments where the other immunopotentiator and the superantigen conjugate are applied separately to the cell, one should ensure that a significant period of time does not expire between the time of each
15 delivery, such that the superantigen conjugate and immunopotentiator would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one may contact the cell with both modalities within about 12-72 hours of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the
20 respective administrations.

[00158] Various combinations may be employed, the superantigen conjugate being “A” and the immunopotentiator being “B”: 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, B/B/B/A, B/B/A/B, A/A/B/B, A/B/A/B, A/B/B/A, B/B/A/A, B/A/B/A, B/A/A/B, A/A/A/B, B/A/A/A, A/B/A/A, and A/A/B/A.

25 **[00159]** It is further envisioned that the present invention can be used in combination with surgical intervention. In the case of surgical intervention, the present invention may be used preoperatively, *e.g.*, to render an inoperable tumor subject to resection. Alternatively, the present invention may be used at the time of surgery, and/or thereafter, to treat residual or metastatic disease. For example, a resected tumor bed may be injected or perfused with a
30 formulation comprising the tumor-targeted superantigen and/or the immunopotentiator. The perfusion may be continued post-resection, for example, by leaving a catheter implanted at the

site of the surgery. Periodic post-surgical treatment also is envisioned. Any combination of the invention therapy with surgery is within the scope of the invention.

[00160] Continuous administration also may be applied where appropriate, for example, where a tumor is excised and the tumor bed is treated to eliminate residual, microscopic
5 disease. Delivery via syringe or cauterization is preferred. Such continuous perfusion may take place for a period from about 1-2 hours, to about 2-6 hours, to about 6-12 hours, to about 12-24 hours, to about 1-2 days, to about 1-2 weeks or longer following the initiation of treatment. Generally, the dose of the therapeutic composition via continuous perfusion will be equivalent to that given by a single or multiple injections, adjusted over a period of time during which the
10 perfusion occurs. It is further contemplated that limb perfusion may be used to administer therapeutic compositions of the present invention, particularly in the treatment of melanomas and sarcomas.

[00161] A typical course of treatment, for a primary tumor or a post-excision tumor bed, may involve multiple doses. Typical primary tumor treatment may involve a 6 dose application
15 over a two-week period. The two-week regimen may be repeated one, two, three, four, five, six or more times. During a course of treatment, the need to complete the planned dosings may be re-evaluated.

[00162] Immunotherapy with the superantigen conjugate often results in rapid (within hours) and powerful polyclonal activation of T lymphocytes. A superantigen conjugate treatment
20 cycle may include 4 to 5 daily intravenous superantigen conjugate drug injections. Such treatment cycles can be given in *e.g.*, 4 to 6 week intervals. The inflammation with infiltration of CTLs into the tumor is one of the major effectors of the anti-tumor therapeutic superantigens. After a short period of massive activation and differentiation of CTLs, the T-cell response declines rapidly (within 4-5 days) back to base line levels. Thus, the period of
25 lymphocyte proliferation, during which cytostatic drugs may interfere with superantigen treatment is short and well-defined. Only with the superantigen/immunopotentiator therapy of the instant invention is such a distinct time frame for activity plausible, thereby allowing the novel integrated high dose cytostatic agent/immunotherapy treatment.

[00163] It is contemplated that a number of cancers may be treated using the methods and
30 compositions described herein, including but not limited to primary or metastatic melanoma, adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma, thymoma,

lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin's lymphoma, Hodgkin's lymphoma, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, colon cancer, multiple myeloma, neuroblastoma, NPC, bladder cancer, cervical cancer and the like.

- 5 **[00164]** Moreover, the cancer that may be treated using the methods and compositions described herein may be based upon the body location and/or system to be treated, for example, but not limited to bone (*e.g.*, Ewing's Family of tumors, osteosarcoma); brain (*e.g.*, adult brain tumor, (*e.g.*, adult brain tumor, brain stem glioma (childhood), cerebellar astrocytoma (childhood), cerebral astrocytoma/malignant glioma (childhood), ependymoma (childhood).
- 10 medulloblastoma (childhood), supratentorial primitive neuroectodermal tumors and pineoblastoma (childhood), visual pathway and hypothalamic glioma (childhood) and childhood brain tumor (other)); breast (*e.g.*, female or male breast cancer); digestive/gastrointestinal (*e.g.*, anal cancer, bile duct cancer (extrahepatic), carcinoid tumor (gastrointestinal), colon cancer, esophageal cancer, gallbladder cancer, liver cancer (adult
- 15 primary), liver cancer (childhood), pancreatic cancer, small intestine cancer, stomach (gastric cancer); endocrine (*e.g.*, adrenocortical carcinoma, carcinoid tumor (gastrointestinal), islet cell carcinoma (endocrine pancreas), parathyroid cancer, pheochromocytoma, pituitary tumor, thyroid cancer); eye (*e.g.*, melanoma (intraocular), retinoblastoma); genitourinary (*e.g.*, bladder cancer, kidney (renal cell) cancer, penile cancer, prostate cancer, renal pelvis and ureter cancer
- 20 (transitional cell), testicular cancer, urethral cancer, Wilms' Tumor and other childhood kidney tumors); germ cell (*e.g.*, extracranial germ cell tumor (childhood), extragonadal germ cell tumor, ovarian germ cell tumor, testicular cancer); gynecologic (*e.g.*, cervical cancer, endometrial cancer, gestational trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, uterine sarcoma, vaginal cancer, vulvar
- 25 cancer); head and neck (*e.g.*, hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer, metastatic squamous neck cancer with occult primary, nasopharyngeal cancer, oropharyngeal cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, salivary gland cancer); lung (*e.g.*, non-small cell lung cancer, small cell lung cancer); lymphoma (*e.g.*, AIDS-Related Lymphoma, cutaneous T-cell lymphoma, Hodgkin's Lymphoma (adult),
- 30 Hodgkin's Lymphoma (childhood), Hodgkin's Lymphoma during pregnancy, mycosis fungoides, Non-Hodgkin's Lymphoma (adult), Non-Hodgkin's Lymphoma (childhood), Non-Hodgkin's Lymphoma during pregnancy, primary central nervous system lymphoma, Sezary

Syndrome, T-cell lymphoma (cutaneous), Waldenstrom's Macroglobulinemia); musculoskeletal (*e.g.*, Ewing's Family of tumors, osteosarcoma/malignant fibrous histiocyoma of bone, rhabdomyosarcoma (childhood), soft tissue sarcoma (adult), soft tissue sarcoma (childhood), uterine sarcoma); neurologic (*e.g.*, adult brain tumor, childhood brain tumor (*e.g.*,
5 brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors and pineoblastoma, visual pathway and hypothalamic glioma, other brain tumors), neuroblastoma, pituitary tumor primary central nervous system lymphoma); respiratory/thoracic (*e.g.*, non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, thymoma and thymic
10 carcinoma); and skin (*e.g.*, cutaneous T-cell lymphoma, Kaposi's sarcoma, melanoma, and skin cancer).

[00165] It is understood that the method can be used to treat a variety of cancers, for example, a cancer selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastric cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer,
15 prostate cancer, renal cell cancer, and skin cancer.

[00166] Yet further, the cancer may include a tumor comprised of tumor cells. For example, tumor cells may include, but are not limited to melanoma cell, a bladder cancer cell, a breast cancer cell, a lung cancer cell, a colon cancer cell, a prostate cancer cell, a liver cancer cell, a pancreatic cancer cell, a stomach cancer cell, a testicular cancer cell, a renal cancer cell, an
20 ovarian cancer cell, a lymphatic cancer cell, a skin cancer cell, a brain cancer cell, a bone cancer cell, or a soft tissue cancer cell. Examples of solid tumors that can be treated according to the invention include sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma,
25 mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma,
30 choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma,

hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

VI. Kits

[00167] In addition, the invention provides kits comprising, for example, a first container containing a superantigen conjugate and a second container containing an immunopotentiator
5 such as an anti-PD-1 antibody. Such a kit may also contain additional agents such as, for example, corticosteroid or another lipid modulator. The container means may itself be a syringe, pipette, and/or other such like apparatus, from which the formulation may be applied to a specific area of the body, injected into an animal, and/or applied and/or mixed with the other components of the kit.

10 [00168] The kits may comprise a suitably aliquoted superantigen conjugate and/or immunopotentiator, and optionally, lipid and/or additional agent compositions of the present invention. The components of the kits may be packaged either in aqueous media or in lyophilized form. When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is a sterile aqueous solution.

15 [00169] Practice of the invention will be more fully understood from the foregoing examples, which are presented herein for illustrative purposes only, and should not be construed as limiting the invention in any way.

EXAMPLES

Example 1: Combination Therapy Of ANYARA[®] And An Anti-PD-1 Inhibitor Against The NSCLC Tumor Cell Line

[00170] This example describes an *in-vitro* study testing the anti-cancer effect of the combination of a tumor targeted superantigen, ANYARA[®], and an anti-PD-1 antibody,
20 KEYTRUDA[®], against the HCC827 non-small-cell lung (NSCLC) tumor cell line.

[00171] Peripheral blood mononuclear cells (PBMCs) from healthy donors were incubated for 4 days with 10 ng/ml of Staphylococcal enterotoxin A (SEA). T-cells were then isolated and incubated with IL-2 for 1 additional day. 1×10^4 HCC827 cells per well were incubated in 96-well plates for 1 hour with T-cells and KEYTRUDA[®] at a concentration of 0.2 μ g/ml.
25 Effector:Target ratio (T-cells:HCC827 cells) was 8:1. After 1 hour of incubation with or without KEYTRUDA[®], ANYARA[®] in different concentrations (0, 0.1 μ g/ml, and 10 μ g/ml)

was added to the wells and the plates were incubated for an additional 48 hours. At the end of the treatment the culture supernatant was removed, including suspended T-cells and tumors cells, and the attached tumor cells were washed one time with culture medium. The viability of residual HCC827 was tested with a CCK8 kit (Cell Counting Kit-8, Sigma Aldrich) according to the manufacturer's protocol. The viability of control group was normalized to 100%.

Viability of the cancer cells (%) = (OD value of treatment group/OD value of control group) x 100.

[00172] As shown in **FIGURE 4** and **FIGURE 5**, the combination of ANYARA[®] with KEYTRUDA[®] had the strongest effect on HCC827 cell viability. Although ANYARA[®] alone at both concentrations decreased the viability of HCC827 cells, it was much less effective in comparison to the combination of ANYARA[®] with KEYTRUDA[®]. KEYTRUDA[®] at the tested concentration of 0.2 µg/ml had no effect on the viability of the cancer cells. ANYARA[®] at the lower concentration of 0.1 µg/ml decreased the viability of HCC827 cells to 60 ± 9.4% (p < 0.05 vs. control) whereas the combination of ANYARA[®] with KEYTRUDA[®] reduced the viability of the cells to 33 ± 4.9% (p < 0.005 vs. control; p < 0.05 vs. ANYARA[®]) (**FIGURE 4**). ANYARA[®] at the higher concentration (10 µg/ml) had a stronger effect than at the lower concentration, reducing the viability of the cancer cells to 40 ± 6.6% (p < 0.005 vs. control), however, also at this concentration the combination of ANYARA[®] with KEYTRUDA[®] was significantly more effective and reduced the viability of the cells to 14 ± 4.2% (p < 0.0005 vs. control; p = 0.005 vs. ANYARA[®]) (**FIGURE 5**).

[00173] Taken together, these results demonstrate the synergistic effect of immunopotentiator anti-PD-1 (KEYTRUDA[®]) with tumor targeted superantigen (ANYARA[®]) and support the concept that administration of a cancer targeted superantigen, together with an immunopotentiator, can result in an enhanced anti-cancer effect that is greater than the additive effect of each agent when administered alone.

Example 2: Combination Therapy Of Tumor Targeted Superantigen And A Murine Anti-PD-1 Inhibitor In A B16 Melanoma Mouse Model

[00174] This example describes a study testing the effect of a tumor targeted superantigen, C215Fab-SEA, and a murine anti-PD-1 antibody against the murine B16-EpCAM melanoma model *in vivo*. The combination therapy of tumor targeted superantigen and anti-PD-1 was

tested in a syngeneic tumor model using low immunogenic B16 melanoma transfected with the human colon carcinoma antigen EpCAM, which is recognized by the C215 antibody. The tumor targeted superantigen C215Fab-SEA is a fusion protein which includes a tumor-reactive mAb (C215Fab) and the bacterial superantigen *staphylococcal enterotoxin A* (SEA). C215Fab-SEA was used instead of ANYARA[®] to facilitate *in vivo* murine experiments.

[00175] For the study, C57Bl/6 mice were inoculated intravenously (IV) with 1.75×10^5 of B16-EpCAM melanoma cells into the tail vein to induce lung tumors. Mice were treated on days 5 to 8 with daily IV injections of C215Fab-SEA (0.5 μ g/mouse) and/or intraperitoneal (IP) injections of anti-PD-1 mAb (200 μ g/mouse) twice a week. The control group was treated with PBS at the same mode of administration and regiment as the combination therapy group. On day 21 the mice were killed and the lungs were removed. After fixation in Bouin's solution for at least 24 hours, the number of lung tumors were counted.

[00176] The results of the study are presented in **FIGURE 6**. As was expected, because B16 melanoma is a low immunogenic tumor, anti-PD-1 mAb monotherapy had no effect on the number of lung tumors. C215Fab-SEA alone reduced the number of B16 lung tumors by approximately 50%, from 217.1 ± 15.9 (mean \pm SEM) tumors in the control treated mice to 98.8 ± 16.4 at day 21. C215Fab-SEA and the anti-PD-1 mAb in combination reduced the number of tumors by approximately 80%, a significant reduction ($p < 0.05$) compared to the monotherapy.

[00177] These results further demonstrate the potential of combining tumor targeted superantigen together with an immunopotentiator for the treatment of low immunogenic tumors.

INCORPORATION BY REFERENCE

[00178] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00179] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the

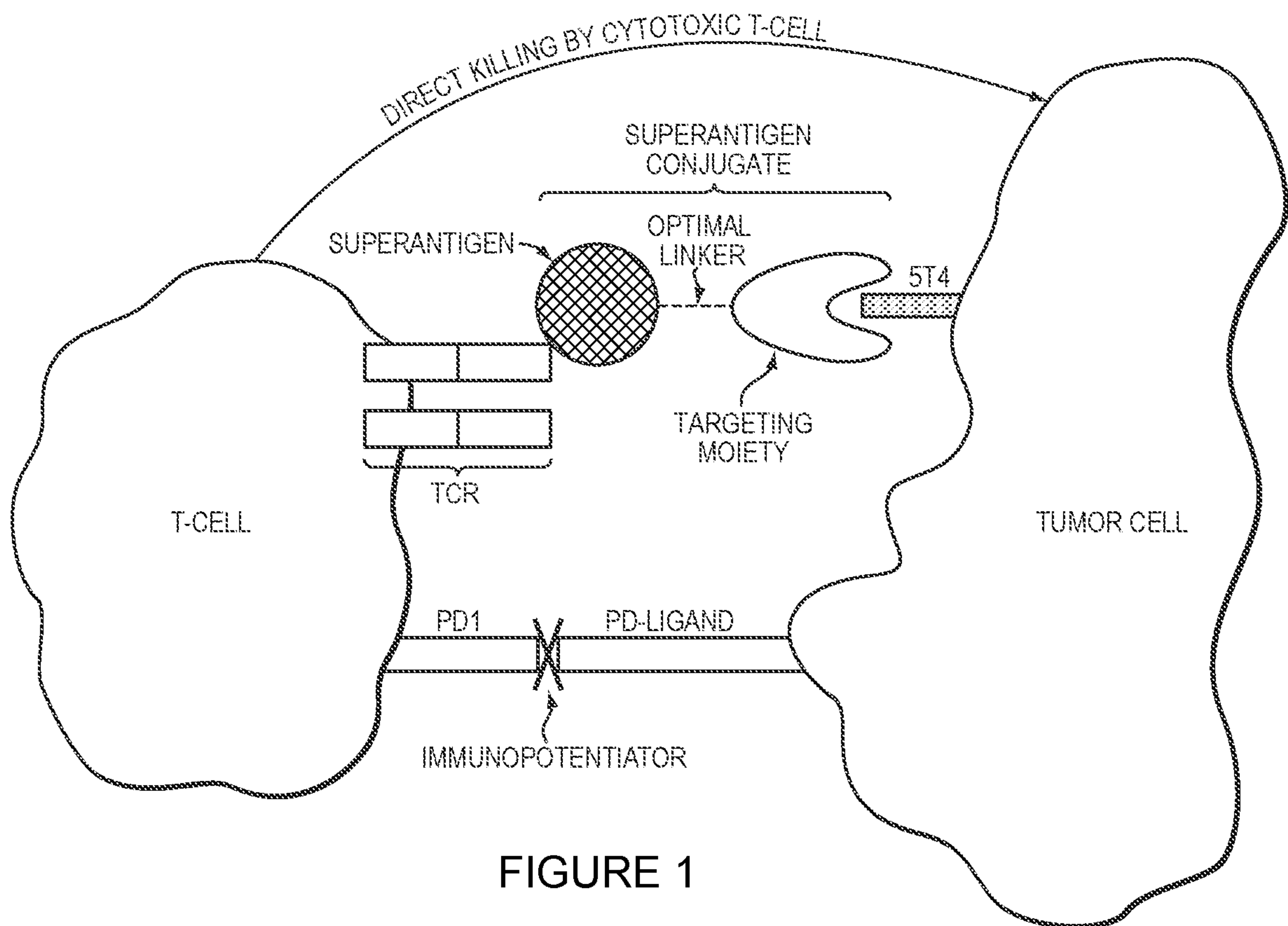
claims are intended to be embraced therein.

What is claimed is:

- 1 1. A method of treating a cancer in a subject in need thereof, the method comprising:
 2 administering to the subject (i) an effective amount of a superantigen conjugate
 3 comprising a superantigen covalently linked to a targeting moiety that binds a first
 4 antigen expressed by cancerous cells within the subject and (ii) an effective amount of an
 5 immunopotentiator capable of one or more of the following activities (a) stimulating
 6 activating T-cell signaling, (b) repressing T-cell inhibitory signalling between the
 7 cancerous cells and a T-cell, and/or (c) repressing inhibitory signalling that leads to T-cell
 8 expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated
 9 pathway, thereby to potentiate an immune response in the subject against the cancerous
 10 cells to treat the cancer.
- 1 2. The method of claim 1, wherein the superantigen is administered to the subject before, at
 2 the same time as, or after the immunopotentiator.
- 1 3. The method of claim 1 or 2, wherein the superantigen binds to a T-cell receptor on a
 2 surface of a T-cell.
- 1 4. The method of any one of claims 1-3, wherein the superantigen comprises Staphylococcal
 2 enterotoxin A or an immunologically variant and/or fragment thereof.
- 1 5. The method of claim 1, wherein the first antigen is a cell surface antigen.
- 1 6. The method of claim 5, wherein the cell surface antigen is a 5T4 cancer antigen.
- 1 7. The method of any one of claims 1-6, wherein the targeting moiety is an antibody.
- 1 8. The method of claim 7, wherein the antibody is an anti-5T4 antibody.
- 1 9. The method of claim 8, wherein the anti-5T4 antibody comprises a Fab fragment that
 2 binds a 5T4 cancer antigen.
- 1 10. The method of any one of claims 1-9, wherein the superantigen comprises amino acid
 2 residues 226 to 458 of SEQ ID NO: 7 or an immunologically reactive variant and/or
 3 fragment thereof.
- 1 11. The method of any one of claims 1-10, wherein a Programmed Cell Death-Ligand (PD-L)
 2 is expressed on the surface of the cancerous cells that binds to Programmed Cell Death-1
 3 protein (PD-1) expressed by the T-cell.

- 1 12. The method of claim 11, wherein the immunopotentiator is an anti-PD-1 antibody that
2 prevents PD-L from binding PD-1 expressed on the surface of the T-cell.
- 1 13. The method of claim 12, wherein the anti-PD-1 antibody has or is based on a human IgG4
2 isotype.
- 1 14. The method of claim 12 or 13, wherein the anti-PD-1 antibody is selected from the group
2 consisting of nivolumab and pembrolizumab.
- 1 15. The method of any one of claims 1-14, wherein the cancer is selected from the group
2 consisting of breast cancer, cervical cancer, colorectal cancer, gastric cancer, non-small
3 cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, and
4 skin cancer.
- 1 16. A pharmaceutical composition comprising an effective amount of a superantigen
2 conjugate comprising a superantigen covalently linked to a targeting moiety that binds to
3 a first antigen expressed by cancerous cells in a subject, an effective amount of an
4 immunopotentiator that is capable of or more of the following activities (a) stimulating
5 activating T-cell signaling, (b) repressing T-cell inhibitory signalling between the
6 cancerous cells and a T-cell, and/or (c) repressing inhibitory signalling that leads to T-cell
7 expansion, activation and/or activity via a human IgG4 immunoglobulin-mediated
8 pathway in the subject, and a pharmaceutically acceptable excipient.
- 1 17. The composition of claim 16, wherein the superantigen binds to a T-cell receptor
2 expressed on a cell surface of a T-cell.
- 1 18. The composition of claim 16 or 17, wherein the superantigen comprises Staphylococcal
2 enterotoxin A or an immunologically variant and/or fragment thereof.
- 1 19. The composition of any one of claims 16-18, wherein the first antigen is a cell surface
2 antigen.
- 1 20. The composition of any one of claims 16-19, wherein the antigen is a 5T4 cancer antigen.
- 1 21. The composition of any one of claims 16-20, wherein the targeting moiety is an antibody.
- 1 22. The composition of claim 20, wherein the antibody is an anti-5T4 antibody.
- 1 23. The composition of claim 22, wherein the antibody comprises a Fab fragment that binds
2 to a 5T4 antigen.

- 1 24. The composition of any one of claims 16-23, wherein the superantigen comprises amino
2 acid residues 226 to 458 of SEQ ID NO: 7 or an immunologically reactive variant and/or
3 fragment thereof.
- 1 25. The composition of any one of claims 16-24, wherein the immunopotentiator prevents
2 PD-L expressed in the cancerous cells from binding PD-1 expressed by the T-cell.
- 1 26. The composition of claim 25, wherein the immunopotentiator is an anti-PD-1 antibody.
- 1 27. The composition of claim 26, wherein the antibody has or is based on a human IgG4
2 isotype.
- 1 28. The composition of 26 or 27, wherein the anti-PD-1 antibody is selected from the group
2 consisting of nivolumab, and pembrolizumab.



| | | | | |
|---------------|-----------|--|--------------------------------------|-----|
| | | <u>A</u> | <u>B</u> | |
| SEQ ID NO: 3 | SEA/E-120 | SEKSEEINEKDLRKKSELQGTALGNLKQIYYYN | SKAITSSEKSADQFLTNTLLFKGFFTG | 60 |
| SEQ ID NO: 10 | SEA/E-18 | SEKSEEINEKDLRKKSELQGTALGNLKQIYYYN | NEKAITENKESDDQFLENTLLFKGFFTG | 60 |
| SEQ ID NO: 1 | SEE | SEKSEEINEKDLRKKSELQRNALSNLRQIYYYN | NEKAITENKESDDQFLENTLLFKGFFTG | 60 |
| SEQ ID NO: 2 | SEA | SEKSEEINEKDLRKKSELQGTALGNLKQIYYYN | NEKAKTENKESHQFLQHTILFKGFFTD | 60 |
| | | ***** | ..**.*:*****.* *..:.* **** :*:*****. | |
| | | <u>C</u> | | |
| | SEA/E-120 | HPWYNDLLVDLGSTAATSEYEGSSVDLYGAYYGYQCAGGTPNK | TACMYGGVTLHDNNRLT | 120 |
| | SEA/E-18 | HPWYNDLLVDLGSKDATNKYKGKKVDLYGAYYGYQCAGGTPNK | TACMYGGVTLHDNNRLT | 120 |
| | SEE | HPWYNDLLVDLGSKDATNKYKGKKVDLYGAYYGYQCAGGTPNK | TACMYGGVTLHDNNRLT | 120 |
| | SEA | HSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNK | TACMYGGVTLHDNNRLT | 120 |
| | | *.*****:.*. ..:.*:..***** | | |
| | SEA/E-120 | EEKKVPINLWIDGKQTTVPIDKVKTSKKEVTVQELDLQARHYLHGK | FGLYNSDSFGGKVQ | 180 |
| | SEA/E-18 | EEKKVPINLWIDGKQTTVPIDKVKTSKKEVTVQELDLQARHYLHGK | FGLYNSDSFGGKVQ | 180 |
| | SEE | EEKKVPINLWIDGKQTTVPIDKVKTSKKEVTVQELDLQARHYLHGK | FGLYNSDSFGGKVQ | 180 |
| | SEA | EEKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEKYNLYNSDVFDGKVQ | 180 | |
| | | *****:****.**:..***.**:*****:***: *:.***** *.**** | | |
| | | <u>D</u> | <u>E</u> | |
| | SEA/E-120 | RGLIVFHSSEGSTVSYDLFDAQGGQYPDTLLRIYRDNTTISSTSL | SISLYLYTT | 233 |
| | SEA/E-18 | RGLIVFHSSEGSTVSYDLFDAQGGQYPDTLLRIYRDNKTINSEN | LHIALYLYTT | 233 |
| | SEE | RGLIVFHSSEGSTVSYDLFDAQGGQYPDTLLRIYRDNKTINSEN | LHIDL YLYTT | 233 |
| | SEA | RGLIVFHTSTEPSVNYDLFGAQQQYSNTLLRIYRDNKTINSEN | MHIDIYLYTS | 233 |
| | | *****:* ..:*,****,*****.:*****.*.* * ..: * :*****: | | |

FIGURE 2

| | | |
|--------------|---------|---|
| SEQ ID NO: 7 | 1-50 | 5T4 Variable Heavy chain |
| | | EVQLQ QSGPD LVKPG ASVKI SCKAS GYSET GYYMH WVKQS PGKGL EWIGR |
| | 51-100 | INPNN GVTLY NQKEK DKATL TVDKS STTAY MELRS LTSED SAVYY CARST |
| | | C242 Constant Heavy chain |
| | 101-150 | MITNY VMDYW GQGTS VTVSS AKTTP PSVYP LAFGS AAQTN SMVTL GCLVK |
| | 151-200 | GYFPE PVTVT WNSGS LSSGV HTEPA VLQSD LYTLS SSVTV PSSTW PSETV |
| | | SEA/E-120 |
| | 201-250 | TCNVA HPASS TKVDK KIVPR DSGGP SEKSE EINEK DLRKK SELQG TALGN |
| | 251-300 | LKQIY YYNSK AITSS EKSAD QFLTN TLLFK GFFTG HPWYN DLLVD LGSTA |
| | 301-350 | ATSEY EGSSV DLYGA YGYQ CAGGT PNKTA CMYGG VTLHD NNRLT EEKKV |
| | 351-400 | PINLW IDGKQ TTVPI DKVKT SKKEV TVQEL DLQAR HYLHG KFGLY NSDSF |
| | 401-450 | GGKVQ RGLIV FHSSE GSTVS YDLED AQQQY PPTLL RIYRD NTTIS STSLS |
| | | 5T4 Variable Light chain |
| | 451-500 | ISLYL YTTSI VMTQT PTSLL VSACD RVTIT CKASQ SVSND VAWYQ QKPGQ |
| | 501-550 | SPKLL ISYTS SRYAG VPDRF SGSGY GTDET LTISS VQAEV AAVYF CQQDY |
| | | C242 Constant Light chain |
| | 551-600 | NSPPT FGGGT KLEIK RADAA PTVSI FPPSS EQLTS GGASV VCFLN NFYPK |
| | 601-650 | DINVK WKIDG SERQN GVLNS WTDQD SKDST YSMSS TLTLT KDEYE RHNSY |
| | 651-672 | TCEAT HKTST SPIVK SFNRN ES |

FIGURE 3

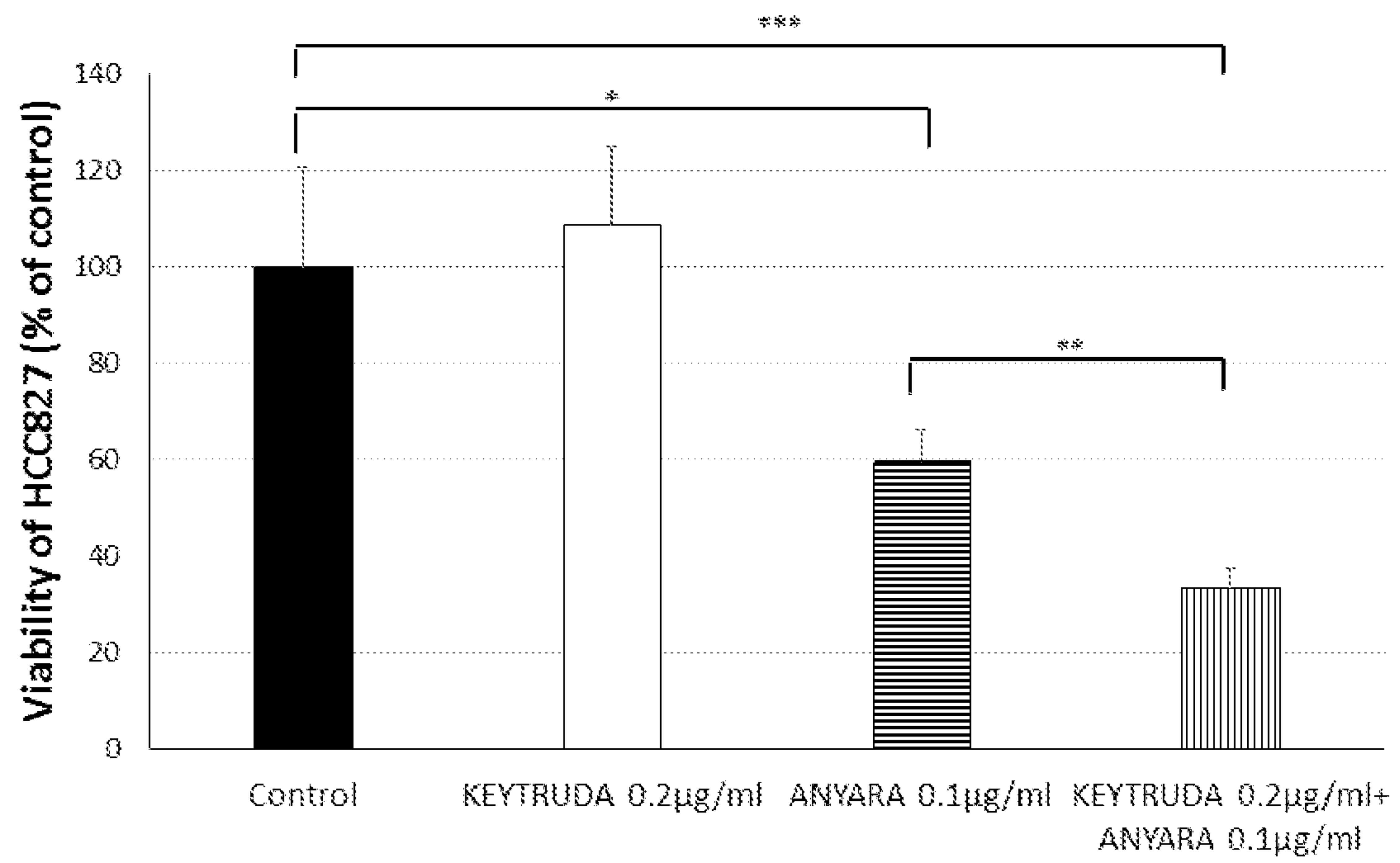


FIGURE 4

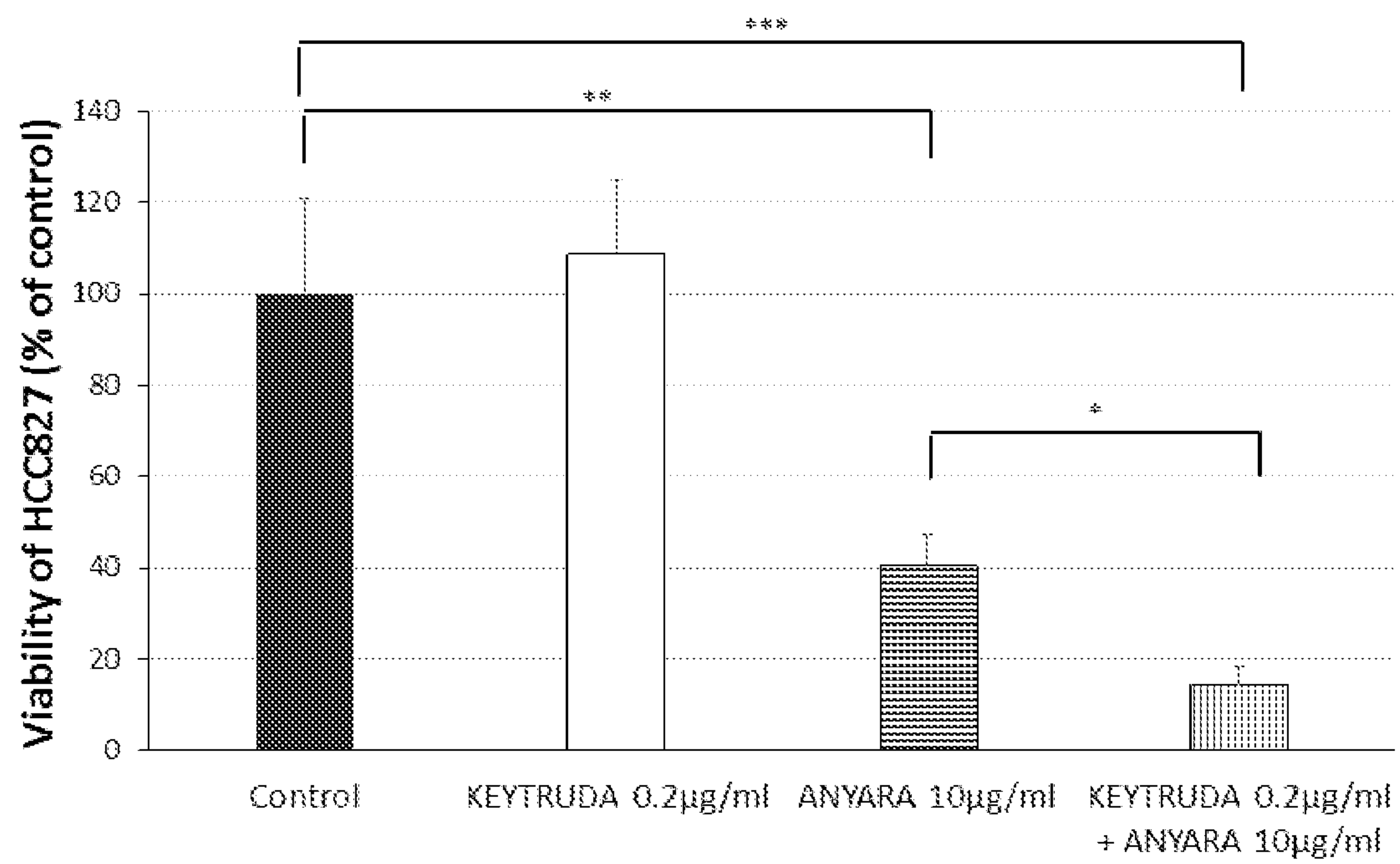


FIGURE 5

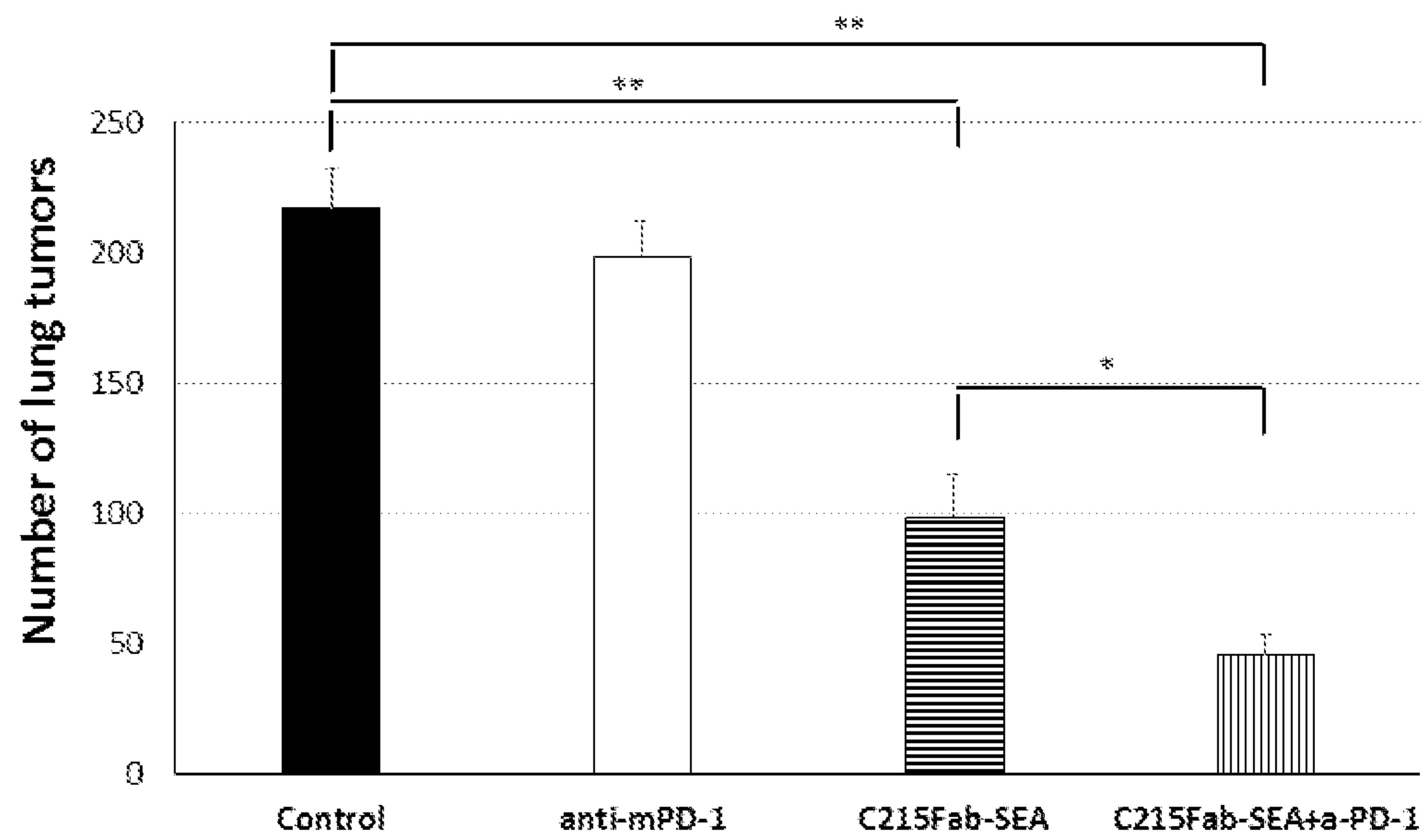


FIGURE 6

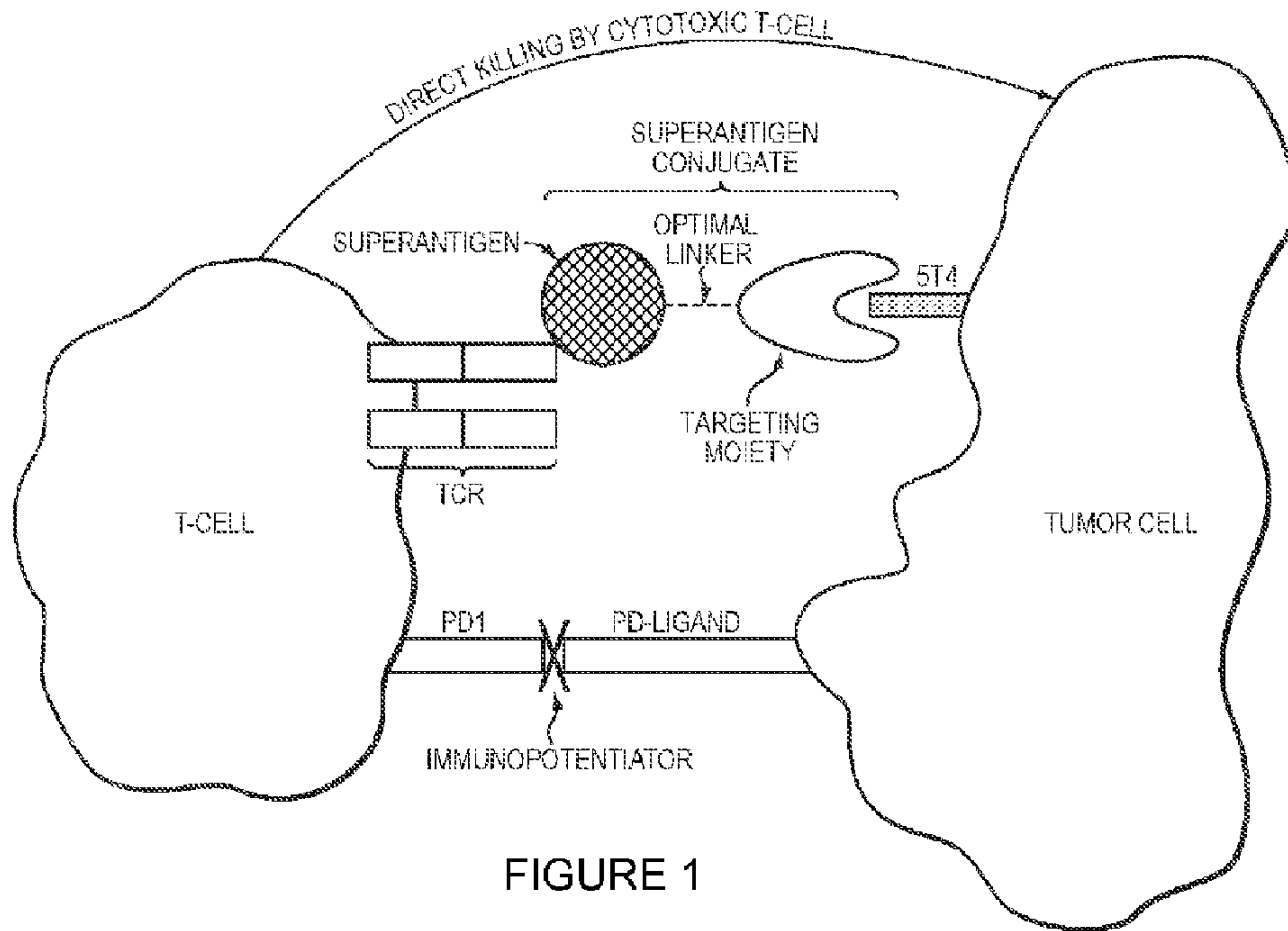


FIGURE 1