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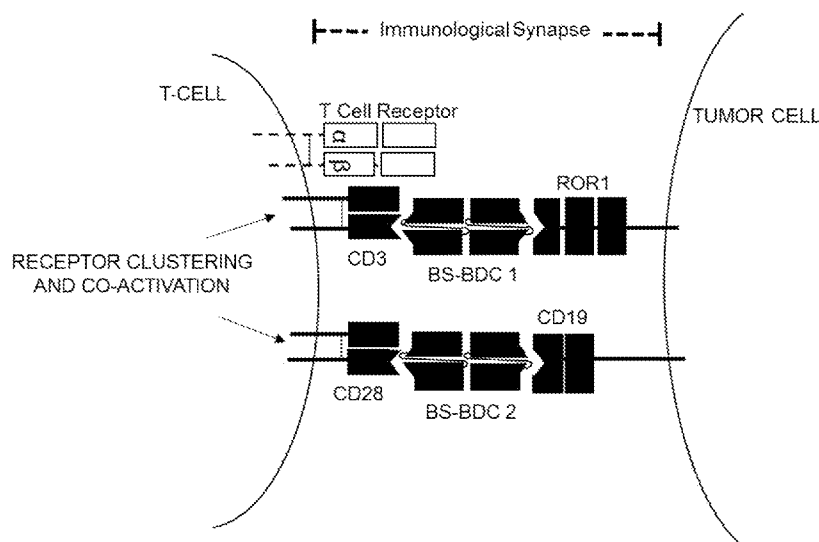
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(54) Title: METHODS TO ENHANCE THE SELECTIVITY AND EFFECTIVENESS OF CANCER TREATMENTS

FIG. 1A



(57) Abstract: Methods to enhance the selectivity and effectiveness of cancer treatments are described. The methods utilize strategies based on selecting and administering groups of molecules that have little to no effect at a therapeutic dose individually but are synergistically active in combination at a cancer site that co-expresses two different cancer antigen epitopes that are not significantly co-expressed in non-cancerous tissues.

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METHODS TO ENHANCE THE SELECTIVITY AND EFFECTIVENESS OF CANCER TREATMENTS

REFERENCE TO A SEQUENCE LISTING

[0001] This application incorporates by reference the Sequence Listing submitted in Computer Readable Form as file 19-053-WO-PCT_Sequence.txt, created on January 13, 2020 and containing 214, 271 bytes.

FIELD OF THE DISCLOSURE

[0002] The current disclosure provides methods to enhance the selectivity and effectiveness of cancer treatments. The methods utilize strategies based on selecting and administering groups of molecules that have little to no individual effects but are synergistically active in combination at a cancer site that co-expresses two different cancer antigen epitopes that are not significantly co-expressed in non-cancerous tissues.

BACKGROUND OF THE DISCLOSURE

[0003] Despite advances in cancer treatments, mortality associated with the disease remains too high. For example, despite improvements in outcome for many pediatric patients, cancer remains the leading cause of death past infancy among children in the United States. Thus, the need for effective new therapies for cancers, including childhood cancers is unquestioned.

[0004] Targeting cancer cells with antibodies raised high expectations as a potent means of eliminating tumor cells with limited non-specific toxicities. For many patients, however, use of single antibodies has not been effective.

[0005] Bispecific T cell engaging antibodies bind both a cancer antigen on cancer cells and a T cell activating epitope, with the goal of bringing T cells to cancer cells to destroy the cancer cells. See, for example, US 2008/0145362. Most current bispecific T cell engaging antibody therapeutics include paired monospecific, antibody-derived binding domains. One member of the pair targets a cancer antigen epitope and the other member of the pair targets a T cell activating epitope. Some have explored use of such antibodies in combinations that target two different T cell activating epitopes (e.g., CD3 and CD28).

[0006] PCT/US2017/042264 describes multiple bispecific binding domain constructs (BS-BDC) to treat cancer. Each BS-BDC within a group binds a cancer antigen epitope and an immune cell activating epitope that is different from the cancer antigen epitope and immune cell activating epitope bound by another BS-BDC within the group. This advance provided several benefits. First, because BS-BDC within a group bind different cancer antigen epitopes, there can be less competition for

binding and reduced steric hindrance. Second, by binding different immune cell activating epitopes, immune cell co-stimulation signals are achieved. Because the binding domains recognizing the immune cell activating epitopes (e.g., a T cell receptor and a co-stimulatory receptor), are located on different BS-BDC within a group, the groups can be designed to induce strong T cell activation only in the presence of cancer cells. This approach provided a versatile platform that can be utilized to target a large variety of cancers.

[0007] In the context of such combination therapies that target cancer cells based on the particular antigens that they express, such approaches used to date have focused on (i) choosing members for inclusion in a combination therapy based on their independently effective therapeutic effects; and (ii) identifying cancer antigens that are significantly differentially expressed between cancerous and healthy tissue. That is, cancer antigens are selected for targeting when they are highly expressed by cancerous tissue with significantly less expression in healthy tissue. This current requirement for cancer antigen selection severely restricts antigens available for targeting by combination therapies.

[0008] Moreover, while previously used approaches to select combination therapies have provided important advances, side-effects and on-target/off-cancer toxicities of group members remain a significant concern. For example, Cetuximab, an anti-EGFR cancer antigen antibody is associated with severe skin rashes thought to be due to EGFR expression in the skin. Another example is Herceptin (trastuzumab), an anti-HER2 (ERBB2) antibody used in the treatment of breast cancer. Herceptin is associated with cardiotoxicity due to Her2 expression in the heart. Moreover, targeting Her2 with CAR-T cells was lethal in at least one patient due to on-target, off-cancer expression in the lung. Molecular Therapy, Vol. 18 No. 4, 843–851 (April 2010). Thus, there remains a need for significant improvements in the selectivity and effectiveness of cancer treatments.

SUMMARY OF THE DISCLOSURE

[0009] The current disclosure provides methods to select individual components of anti-cancer combination therapies. The new methods contravene many widely-accepted principles of combination drug development leading to enhanced selectivity and effectiveness of cancer treatments.

[00010] In particular embodiments, the methods provide that members of a combination therapy have little to no effect at a therapeutic dose when not in the presence of other members of the combination therapy. In particular embodiments, the methods provide that members of a combination therapy target at least two different cancer antigen epitopes that are co-expressed by a cancer cell or tumor but that are not significantly co-expressed in non-cancerous tissue. Further, particular embodiments require that the combination therapy exhibits a synergistic anti-cancer effect in an environment where the targeted cancer antigen epitopes are co-expressed.

[0011] By requiring that (i) individual members of a combination therapy have little to no effect at a

therapeutic dose when not in the presence of other group members; and (ii) targeted cancer antigen epitopes be co-expressed at a cancer site but not significantly co-expressed on healthy tissue, several important advances are achieved. First, individual targeted cancer antigen epitopes can be expressed, and even significantly expressed, by healthy tissues, so long as they are not significantly co-expressed on the non-cancerous tissues with the other targeted cancer antigen epitopes. Permitting expression on healthy tissues significantly expands the number of cancer antigens that can be targeted.

[0012] Because an individual member of the combination therapy has little to no effect at a therapeutic dose when not in the presence of its combination therapy group, off-site toxicities are significantly reduced, if not eliminated, even if the individual group member binds to its targeted antigen within non-cancerous tissue.

[0013] In addition to the stringent selection criteria described above, the current disclosure also provides methods of prioritizing potential combination therapy group members for assessment. Aspects of these prioritization schemes similarly contravene most commonly followed methods of combination therapy development. For example, in particular embodiments, a potential group member can be prioritized for assessment based on having passed a regulatory safety test but having failed a regulatory efficacy test. In particular embodiments, a potential group member can be prioritized for assessment based on binding a cancer antigen epitope that is significantly expressed in non-cancerous tissue. In particular embodiments, a potential group member can be prioritized for assessment based on low individual effector function. In particular embodiments, a potential group member can be prioritized for assessment based on eliciting cytokine expression or cancer cell killing, but de-prioritized if it elicits cytokine expression and cancer cell killing.

[0014] Particular embodiments prioritize multiple bispecific binding domain construct (BS-BDC) groups as described in PCT/US2017/042264 for assessment. Importantly, there is currently no mechanism to predict groups of BS-BDC that will meet the selection criteria described herein. Accordingly, the current disclosure provides groups of potential BS-BDC group members, curated according to likelihood of cancer antigen epitope co-expression in cancerous tissue. The current disclosure also provides detailed testing and selection criteria to allow selection of groups that meet the criteria.

[0015] In particular embodiments, BS-BDC can be altered to meet selection criteria disclosed herein. For example, a BS-BDC with high individual anti-cancer activity can be altered to reduce its physiological activity at a given dose.

[0016] The current disclosure also provides additional methods to enhance the effectiveness and selectivity of combination therapies based on the selection criteria disclosed herein. These additional

methods refine, for example, characteristics of the BS-BDC to achieve additional treatment goals and administration protocols regarding the timing, site, and mode of administration.

[0017] As examples of the additional methods and refinements, the size of BS-BDC within a combination therapy can be tailored to modulate in vivo half-life and/or tumor penetration.

[0018] Administration protocols can be timed to alter the tumor microenvironment. For example, a BS-BDC that may upregulate a targeted cancer antigen epitope and/or an immune stimulating epitope can be administered. In coordination, a BS-BDC that binds the upregulated target may be administered to take advantage of the altered microenvironment. Other compounds may also be used to modulate the tumor microenvironment. For example, interferon gamma (IFN γ) can be administered to upregulate targeted cancer antigen epitopes, such as PD-L1. In coordination, a BS-BDC that binds the upregulated cancer antigen epitope may be administered to take advantage of the altered microenvironment.

[0019] Particular embodiments also include linked forms of BS-BDC within a group. In particular embodiments, the BS-BDC remain linked following administration. In other embodiments, BS-BDC can be linked as prodrugs that are cleaved into individual elements following administration. In additional embodiments, BS-BDC can be linked during manufacturing but cleaved prior to administration.

[0020] In various embodiments, any of the features or components of embodiments discussed above or herein may be combined, and such combinations are encompassed within the scope of the present disclosure. Any specific value discussed above or herein may be combined with another related value discussed above or herein to recite a range with the values representing the upper and lower ends of the range, and such ranges are encompassed within the scope of the present disclosure.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0021] Many of the drawings submitted herein are better understood in color. Applicant considers the color versions of the drawings as part of the original submission and reserve the right to present color images of the drawings in later proceedings.

[0022] FIGs. 1A-1C provide strategies behind BS-BDC group selection. (1A) depicts silence and synergy gating. Silence is provided by selecting BS-BDC with little to no individual anti-cancer activity at a therapeutic dose. Due to this feature, healthy tissue expressing a targeted cancer antigen epitope is not affected. Synergy is observed when at least two immune stimulating epitopes are engaged so that a T cell receives both stimulation and co-stimulation. This synergy can result in a powerful signal to kill the tumor cell and/or to secrete cytokines that alter the tumor microenvironment. (1B) the selected silence and synergy gate allows targeting of cancer antigens that are expressed in healthy tissues, so long as both targeted cancer antigens are not significantly co-expressed in healthy tissues.

(1C) depiction of exemplary protocols and testing equipment to evaluate potential silence and synergy BS-BDC groups.

[0023] FIG. 2 provides examples of individual BS-BDC, each targeting a cancer antigen epitope and an immune cell stimulating epitope.

[0024] FIGs. 3A-3G provide curated examples of potential combinations of BS-BDC for prioritized testing based on likelihood of cancer antigen co-expression in particular types of cancer, as follows: (3A) ovarian cancer; (3B) breast cancer; (3C) lung cancer; (3D) colorectal cancer; (3E) leukemia; (3F) acute myeloid leukemia (AML); and (3G) embryonal carcinoma.

[0025] FIGs. 4A-4C show exemplary data evaluating silence and synergy criteria as described herein: (4A) cell death (note that in certain tests, Blincyto has been found to be too potent for inclusion with a silence and synergy BS-BDC group; (4B) % cytotoxicity; (4C) cytokine release (e.g., IL-2, IFN γ , TNF α). Here, low levels of a costimulatory BS-BDC were sufficient for optimized cytokine production.

[0026] FIGs. 5A and 5B. Optional beneficial features of selected binding domains and associated considerations. (5A) binding domains that ignore shed cancer antigen epitopes. (5B) crystal structure of OKT3 bound to CD3E (PDB: 1SY6).

[0027] FIGs. 6A-6H. Exemplary BS-BDC formats, strategies, and associated data. (6A) schematic of a ScFv-based BS-BDC with signal peptide (SP), variable light chain (V_L), Gly-Ser linker (G₄S)₃, variable heavy chain (V_H), Gly-Ser linker (G₄S), variable heavy chain (V_H), Gly-Ser linker (G₄S)₃, variable light chain (V_L) and Histag (His₆); (6B) schematic of a ScFv-based BS-BDC bound to a cancer cell and a T cell; (6C) a canonical bispecific T cell engager (e.g., adopting the format of a BiTE® (Amgen, Thousand Oaks, CA)) has a serum half-life of several hours requiring continuous intravenous (iv) infusion whereas a BS-BDC including IgG has a serum half-life of 21 days requiring only intermittent iv administration; (6D) depiction of a canonical bispecific T cell engager and an IgG-containing BS-BDC bound to the surface of a T cell through CD3; (6E) an IgG-containing BS-BDC shows equivalent cytotoxicity to a canonical bispecific T cell engager; (6F) IgG-containing BS-BDC show half-lives very similar to that of an antibody; (6G) schematics of 3 BS-BDC formats, each containing Fc (left panel: DS1; middle panel: DS2; right panel: DS3 knob/hole); these designs can improve half-life and manufacturability; (6H) scFvs can be stabilized with a disulfide bond. According to Kabat numbering, disulfide bond positioning can include VH44-VL100 (Reiter et al., (1994) Biochemistry, 33, 5451–5459); VH105-VL43 (Jung, et al., (1994) Proteins, Struct. Func. Genet., 19, 35–47); VH100b-VL49 (Glockshuber et al., (1990) Biochemistry, 29, 1362– 1367); VH100-VL50 (Glockshuber et al., (1990) Biochemistry, 29, 1362– 1367); and/or VH101-VL46 (Zhu, et al., (1997) Prot. Sci., 6, 781– 788) (see also Eve et al., Protein Engineering, Design & Selection vol. 25 no. 7 pp. 321– 329, 2012 for a more recent review).

[0028] FIG. 7 provides representative sequences supporting the disclosure (SEQ ID NOs: 1-18).

DETAILED DESCRIPTION

[0029] Despite advances in cancer treatments, mortality associated with the disease remains too high. For example, despite improvements in outcome for many pediatric patients, cancer remains the leading cause of death past infancy among children in the United States. Thus, the need for effective new therapies for cancers, including childhood cancers is unquestioned.

[0030] Targeting cancer cells with antibodies raised high expectations as a potent means of eliminating tumor cells with limited non-specific toxicities. For many patients, however, use of single antibodies has not been effective.

[0031] Bispecific T cell engaging antibodies bind both a cancer antigen on cancer cells and a T cell activating epitope, with the goal of bringing T cells to cancer cells to destroy the cancer cells. See, for example, US 2008/0145362. Most current bispecific T cell engaging antibody therapeutics include paired monospecific, antibody-derived binding domains. One member of the pair targets a cancer antigen epitope and the other member of the pair targets a T cell activating epitope. Some have explored use of such antibodies in combinations that target two different T cell activating epitopes (e.g., CD3 and CD28).

[0032] PCT/US2017/042264 describes multiple bispecific binding domain constructs (BS-BDC) to treat cancer. Each BS-BDC within a group binds a cancer antigen epitope and an immune cell activating epitope that is different from the cancer antigen epitope and immune cell activating epitope bound by another BS-BDC within the group. The different cancer antigen epitopes can be on the same or different cancer antigens. This advance provided several benefits. First, because BS-BDC within a group bind different cancer antigen epitopes, there can be less competition for binding and reduced steric hindrance. Second, by binding different immune cell activating epitopes, immune cell co-stimulation signals are achieved. Because the binding domains recognizing the immune cell activating epitopes (e.g., a T cell receptor and a co-stimulatory receptor), are located on different BS-BDC within a group, the groups can be designed to induce strong T cell activation only in the presence of cancer cells. This approach provided a versatile platform that can be utilized to target a large variety of cancers.

[0033] In the context of such combination therapies that target cancer cells based on the particular antigens that they express, such approaches used to date have focused on (i) choosing members for inclusion in a combination therapy based on their independently effective therapeutic effects; and (ii) identifying cancer antigens that are significantly differentially expressed between cancerous and healthy tissue. That is, cancer antigens are selected for targeting when they are highly expressed by cancerous tissue with less expression in healthy tissue. This current requirement for cancer antigen

selection severely restricts antigens available for targeting by combination therapies.

[0034] Moreover, while previously used approaches to select combination therapies have provided important advances, side-effects and on-target/off-cancer toxicities remain a significant concern. For example, Cetuximab, an anti-EGFR cancer antigen antibody is associated with severe skin rashes thought to be due to EGFR expression in the skin. Another example is Herceptin (trastuzumab), an anti-HER2 (ERBB2) antibody used in the treatment of breast cancer. Herceptin is associated with cardiotoxicity due to Her2 expression in the heart. Moreover, targeting Her2 with CAR-T cells was lethal in at least one patient due to on-target, off-cancer expression in the lung. Thus, there remains a need for significant improvements in the selectivity and effectiveness of cancer treatments.

[0035] The current disclosure provides methods to select individual components of anti-cancer combination therapies. The new methods contravene many widely-accepted principles of combination drug development leading to enhanced selectivity and effectiveness of cancer treatments.

[0036] In particular embodiments, the methods provide that members of a combination therapy have little to no effect when not in the presence of other members of the combination therapy. In particular embodiments, the methods provide that members of a combination therapy target two different cancer antigen epitopes that are co-expressed by a cancer cell or tumor but that are not significantly co-expressed in healthy tissue. Further, particular embodiments require that the combination therapy exhibits a synergistic anti-cancer effect in an environment where the targeted cancer antigen epitopes are co-expressed. "Co-expressed in cancerous tissue", as used herein, means that the first and second cancer antigen epitopes are co-expressed, respectively, at a density of at least 100 copies per cell in the cancerous tissue. In some cases, the density is at least 250 or 500 copies per cell. In some cases, the density is at least 1000 copies per cell. In some cases, the density is at least 2500 or 5000 copies per cell. In some cases, the density is at least 10,000 copies per cell. Unless otherwise noted, "not significantly co-expressed in healthy tissue" refers to an expression level for the first and second cancer antigens that is less than the expression level, respectively, in cancerous tissue. For example, if the first and second cancer antigens are co-expressed, respectively, at ≥ 1000 copies per cell in cancerous tissue, references to not significantly co-expressed in healthy tissue means that the cancer antigens are expressed, respectively, at less than 1000 copies per cell in healthy tissue.

[0037] By requiring that (i) individual members of a combination therapy have little to no effect when not in the presence of other group members; and (ii) targeted cancer antigen epitopes be co-expressed at a cancer site but not significantly co-expressed on healthy tissue, several important advances are achieved. First, individual targeted cancer antigen epitopes can be expressed, and even significantly expressed, by healthy tissues, so long as they are not significantly co-expressed on

the healthy tissues with the other targeted cancer antigen epitopes. Permitting expression on healthy tissues significantly expands the number of cancer antigens that can be targeted.

[0038] Because an individual member of the combination therapy has little to no effect when not in the presence of its combination therapy group at its therapeutic dose, off-site toxicities are significantly reduced, if not eliminated, even if the individual member binds to its targeted antigen within healthy tissue.

[0039] In addition to the stringent selection criteria described above, the current disclosure also provides methods of prioritizing potential combination therapy group members for assessment. Aspects of these prioritization schemes similarly contravene most commonly followed methods of combination therapy development. For example, in particular embodiments, a potential group member can be prioritized for assessment based on having passed a regulatory safety test but having failed a regulatory efficacy test. If the potential member that passed a regulatory safety test but failed a regulatory efficacy test is a bispecific antibody that binds a cancer antigen epitope and an immune cell stimulating epitope and/or an antibody drug conjugate the potential group member can remain prioritized. If the potential member that passed a regulatory safety test but failed a regulatory efficacy test is a bispecific antibody that binds a cancer antigen epitope and an immune cell stimulating epitope and/or an antibody drug conjugate and the binding domain is based on an antibody that passed a regulatory safety test and a regulatory efficacy test, the potential group member can remain prioritized. If, however, the potential group member is an antibody that passed a regulatory safety test but failed a regulatory efficacy test, binding domains based on that antibody can be de-prioritized.

[0040] In particular embodiments, a potential group member can be prioritized for assessment based on binding a cancer antigen epitope that is significantly expressed in non-cancerous tissue. In particular embodiments, a potential group member can be prioritized for assessment based on low individual effector function. In particular embodiments, a potential group member can be prioritized for assessment based on eliciting cytokine expression or cancer cell killing, but de-prioritized if it elicits cytokine expression and cancer cell killing.

[0041] Particular embodiments prioritize multiple bispecific binding domain constructs (BS-BDC) groups as described in PCT/US2017/042264 for assessment. Importantly, there is currently no mechanism to predict groups of BS-BDC that will meet the selection criteria described herein. Accordingly, the current disclosure provides groups of potential BS-BDC group members, curated according to likelihood of cancer antigen epitope co-expression. The current disclosure also provides detailed testing and selection criteria to allow selection of groups that meet the criteria.

[0042] In particular embodiments, BS-BDC can be altered to meet selection criteria disclosed herein. For example, a BS-BDC with high individual anti-cancer activity can be altered to reduce its

physiological activity at a given dose.

[0043] The current disclosure also provides additional methods to enhance the effectiveness and selectivity of combination therapies based on the selection criteria disclosed herein. These additional methods refine, for example, characteristics of the BS-BDC to achieve additional treatment goals and administration protocols regarding the timing, site, and mode of administration.

[0044] As examples of the additional methods and refinements, the size of BS-BDC within a combination therapy can be tailored to modulate in vivo half-life and/or tumor penetration.

[0045] Administration protocols can be timed to alter the tumor microenvironment. For example, a BS-BDC that may upregulate a targeted cancer antigen epitope and/or an immune stimulating epitope can be administered. In coordination, a BS-BDC that binds the upregulated target may be administered to take advantage of the altered microenvironment. Other compounds may also be used to modulate the tumor microenvironment. For example, interferon gamma (IFN γ) can be administered to upregulate targeted cancer antigen epitopes, such as PD-L1. In coordination, a BS-BDC that binds the upregulated cancer antigen epitope may be administered to take advantage of the altered microenvironment.

[0046] Particular embodiments also include linked forms of BS-BDC within a group. In particular embodiments, the BS-BDC remain linked following administration. In other embodiments, BS-BDC can be linked as prodrugs that are cleaved into individual elements following administration. In additional embodiments, BS-BDC can be linked during manufacturing but cleaved prior to administration.

[0047] Anti-cancer effects can be assessed based on one or more of: cytotoxicity assays, cytokine expression or release assays, activation of CD4 $^{+}$ and CD8 $^{+}$ T cell assays, lymphocyte proliferation assays, in vivo cancer models, and clinical trials.

[0048] Cytotoxicity Assays. In particular embodiments, cytotoxicity assays can include flow cytometry-based cell cytotoxicity assays. For example, CD3 $^{+}$ T cell enriched human peripheral blood mononuclear cells (PBMCs) can be isolated from healthy donors using methods including Ficoll density gradient centrifugation. Target cells, e.g., cells expressing a cancer antigen epitope, can be labeled with a fluorescent membrane dye such as DiOC18(3) (3,3'-dioctadecyloxacarbocyanine perchlorate or DiO) or CellVue™ dyes (Thermo Fisher Scientific, Waltham, MA). Increasing concentrations of a BS-BDC can be incubated with fluorescent labeled target cells as well as effector cells at PBMC effector-to-target (E:T) cell ratios. E:T ratios can include 10:1, 5:1, 2.5:1, 1.25:1, 1:1, 0.625:1, and 0.3125:1. Cell lysis can be assessed by flow cytometry as loss of target-cell membrane integrity, which can be indicated by nuclear uptake of a nucleic acid staining agent such as propidium iodide (PI) or (DAPI). Target cell lysis can then be calculated as the percentage of fluorescent label

positive cells staining positive by the nucleic acid staining agent. A dose response curve can also be generated and effective concentration for 50% cell lysis (EC50 values) can be determined. In particular embodiments, PBMCs can be from *Cynomolgus* macaque. Controls can include absence of T cells and use of an inactive analogue molecule that solely binds to the immune stimulating epitope.

[0049] Cell viability can be measured through metabolic indications such as adenosine triphosphate (ATP) levels measured via luminescence detection, for example, with CellTiter-Glo Luminescent Cell Viability Assay System (Promega, Madison, WI). Cell membrane integrity can also be measured via a fluorometric lactate dehydrogenase (LDH) release assay. Apoptosis is a more defined stage of cell death that can be measured via caspase enzyme activation. Morphological changes in dying cells can be observed and measured using vital dyes and can be measured via cellular imaging and flow cytometry. Flow cytometry and high-content imaging methods allow simultaneous quantification of both live and dead cell populations with multiple fluorescent reagents (e.g., esterase substrates, nucleic acid stains, reagents that measure oxidation or reduction, potentiometric dyes, acidotropic stains, fluorescent glucose analogs, and fluorescent antibiotics).

[0050] Another fluorescence-based assay is DELFIA® (dissociation-enhanced lanthanide fluorescence immunoassay (Wallac Oy Corp., Turku, Finland). DELFIA® is a time-resolved fluorescence (TRF) intensity technology. These assays detect the presence of a compound or biomolecule using lanthanide chelate labeled reagents, separating unbound reagent using wash steps.

[0051] Cell fates including decreased cell viability, apoptosis, and necrosis, also can be quantified by various other biochemical and cell-based assay methods. For example, cytotoxicity assays can be performed using chromium (⁵¹Cr) and Calcein release assays, in which target tumor cells are pre-loaded with a radioisotope or fluorescent dye, respectively, and cell death is quantified by measuring release of the entrapped labels into the supernatant during T cell or natural killer cell mediated cytotoxicity. The amount of label in the media can be measured to determine the level of cytotoxicity the effector cells have induced.

[0052] In particular embodiments, the cytotoxicity assay is a T cell dependent cellular cytotoxicity assay (TDCC).

[0053] In particular embodiments, a BS-BDC has little or no anti-cancer effect when the percentage of target cell death includes less than 20%, less than 15%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1% when administered within a silence dose range. In particular embodiments, an anti-cancer effect can be assessed in relation to a positive control, such as relative to the percentage of target cell death in a cytotoxicity

assay with blinatumomab. In particular embodiments, the BS-BDC does not mediate lysis of cells from normal tissues or normal cells from tissues of a healthy subject or control at the selected dose.

[0054] Cytokine Release Assays. To quantify BS-BDC-induced cytokine release, a number of assay formats can be used. Cytokine release assays (CRAs) can be solution and/or solid phase based; use dilutions of whole blood, PBMCs, or peripheral blood leukocytes (PBLs) as responder cells; and can capture antibodies on plates or beads via Fc using protein A or antibodies to Fc. In particular embodiments, PBMCs can be incubated with a BS-BDC either wet or dry coated onto tissue culture plates for solid phase presentation. In particular embodiments, whole blood can be incubated with a BS-BDC bound to protein A capture beads for solid phase presentation. In particular embodiments, high density pre-culture of PBMCs can be followed by incubation with an aqueous phase BS-BDC. In particular embodiments, whole blood or diluted whole blood can be incubated with an aqueous phase BS-BDC. In particular embodiments, PBMCs can be co-cultured with an aqueous phase BS-BDC over human umbilical vein endothelial cells. In particular embodiments, positive CRA controls can include anti-CD3 reagents, anti-CD28 superagonist monoclonal antibodies (mAbs) (such as TGN 1412 homologs), other marketed mAbs, or lipopolysaccharide (LPS). In particular embodiments, negative controls include phosphate buffered saline, tissue culture medium, isotype mAb controls or marketed mAbs that do not cause cytokine release.

[0055] In particular embodiments, cytokine concentrations can be measured using the human Th1/Th2 or non-human primate Th1/Th2 cytometric bead arrays (CBA-kit, BD Bioscience, Heidelberg, Germany) in accordance with the manufacturer's protocol. Cytokines such as IL-2, IL-4, IL-6, IL-8, IL-10, IFN- γ , and TNF- α can be measured. Particular embodiments assess TNF α , IFN- γ , and IL-2 expression.

[0056] In some embodiments, measurements of anti-cancer activity utilize an assay to measure: increased T cell proliferation; increased T cell survival; delayed T cell dysfunction; deletion of inhibitory immune cells such as regulatory T cells; improved anti-tumor activity of other infiltrating immune cells such as macrophages, dendritic cells or natural killer cells; or selective activation of CD4+ versus CD8+ T cells.

[0057] In particular embodiments, a BS-BDC has little or no anti-cancer effect when the amount of a given cytokine released includes less than a 20% increase, less than 15% increase, less than 10% increase, less than 9% increase, less than 8% increase, less than 7% increase, less than 6% increase, less than 5% increase, less than 4% increase, less than 3% increase, less than 2% increase, less than 1% increase, less than 0.5% increase, less than 0.4% increase, less than 0.3% increase, less than 0.2% increase, or less than 0.1% increase when administered within a silence dose range (also referred to herein as a therapeutic dose. In particular embodiments, an anti-cancer

effect can be assessed in relation to a positive control, such as relative to the percentage of cytokine expression or release caused by blinatumomab. In particular embodiments, the BS-BDC does not mediate cytokine expression or release at the silence dose. In particular embodiments, baseline expression depends on particular assay conditions, but little to no anti-cancer effect is evidenced by remaining below the lower limit of detection within the silence dose range.

[0058] Activation of CD4⁺ and CD8⁺ T cells. Activation of T cells by a BS-BDC can be determined by measuring expression of a T cell activation marker such as CD25 or CD69 by flow cytometry. For example, an allophycocyanine (APC)-conjugated anti-CD25 antibody can be used in combination with either an phycoerythrin (PE)-labeled anti-CD4 or an APC-CyTM7-labeled anti-CD8 antibody (BD Biosciences, Heidelberg, Germany). In particular embodiments, a BS-BDC has little or no anti-cancer effect when the percentage of T cell activation includes less than 20%, less than 15%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, or less when administered within a silence dose range and relative to the percentage of T cell activation in a T cell activation assay with a positive control BS-BDC such as blinatumomab. In particular embodiments, the BS-BDC does not mediate T cell activation within the silence dose range.

[0059] Lymphocyte proliferation assays. Lymphocyte proliferation assays typically measure the ability of lymphocytes placed in tissue culture to undergo a clonal proliferation when stimulated *in vitro* by a foreign molecule, antigen, mitogen, or BS-BDC. In particular embodiments, the proliferation of lymphocytes can be measured by [³H] thymidine or 5-bromodeoxyuridine incorporation into DNA, which is measured upon harvesting cell cultures after stimulation by a BS-BDC. In particular embodiments, the proliferation of lymphocytes can be indirectly measured by an MTT assay. The yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate an intracellular purple formazan that can be solubilized and quantified by spectrophotometric means or detected by ELISA. In particular embodiments, lymphocyte proliferation can be measured by a dye dilution method where intracellular molecules in e.g., PBMCs, can be labeled with a fluorescent dye such as carboxyfluorescein by use of a carboxyfluorescein succinimidyl ester (CFSE) reagent. Cell division can be assessed by measuring a decrease in cell fluorescence via flow cytometry, as progeny of CFSE-labeled cells are endowed with half the number of carboxyfluorescein-tagged molecules at each cell division. In particular embodiments, lymphocyte proliferation can be measured by flow-cytometric assay for specific cell-mediated immune-response in activated whole blood (FASCI^A), which measures blast formation of proliferating lymphocytes. During division a lymphocyte increases in size and can be detected as an increase in forward scatter with flow cytometry. In

FASCIA, whole blood can be diluted into a FASCIA medium and the mixture can be stimulated with a BS-BDC. Addition of fluorophore-conjugated monoclonal antibodies directed to T- and B-cell lineage markers in combination with known reference beads can allow quantification of proliferating T-and B-cells.

[0060] In particular embodiments, a BS-BDC has little or no anti-cancer effect when the percentage of proliferating lymphocytes includes less than 20%, less than 15%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, or less when administered within a silence dose range relative to the percentage of proliferating lymphocytes measured in a lymphocyte proliferation assay by a positive control BS-BDC such as blinatumomab. In particular embodiments, the BS-BDC does not stimulate lymphocyte proliferation within the silence dose range.

[0061] In Vivo Models. The BS-BDCs of the present disclosure can be tested in suitable animal model systems prior to testing in humans, including in rats, mice, chicken, cows, monkeys, rabbits, and hamsters.

[0062] In particular embodiments, suitable animal models for the study of cancer include the severe combined immunodeficiency (SCID) mouse model or transgenic mice where a mouse cancer antigen is replaced with the corresponding human cancer antigen, nude mice with human xenografts, or any animal model (including hamsters, rabbits, etc.) known in the art and described in Relevance of Tumor Models for Anticancer Drug Development (1999, eds. Fiebig and Burger); Contributions to Oncology (1999, Karger); The Nude Mouse in Oncology Research (1991, eds. Boven and Winograd); and Anticancer Drug Development Guide (1997 ed. Teicher).

[0063] In particular embodiments, the cytotoxic effects of the BS-BDCs of the disclosure can be tested *in vivo* with a mouse model such as the non-diabetic/severe combined immunodeficiency (NOD/SCID) mouse model. This mouse carries a double mutation that results in a lack of T and B cells as well as impaired natural killer cell function. The mouse model can use a tumor xenograft such as a human cancer cell line that expresses one or more cancer antigens of interest. Briefly, animals can be injected with a mixture of target tumor cells and effector (e.g., human CD3+ T cells from healthy donors) or target cells alone without effector cells. The T cells can be activated and expanded before injection using a T cell activation/expansion kit (Miltenyi Biotech, Bergisch Gladbach, Germany). The BS-BDC, vehicle control, and positive control BS-BDC (e.g. blinatumomab) can be administered at various time intervals after tumor cell injection. Tumor growth kinetics may be measured between treatment groups. The percentage of mice alive over time and at a given dose of the BS-BDC can be measured. Tumor volume or size in the mice over time and at a given dose of the

BS-BDC can be measured.

[0064] In particular embodiments, little to no anti-cancer effect includes less than 20%, less than 15%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, or less decrease in tumor burden when administered within a silence dose range.

[0065] An anti-cancer effect of a BS-BDC can be selected for or screened by assessing outcomes for the BS-BDC from a clinical trial. Endpoints of clinical trials that can be considered include overall survival (OS); progression-free survival (PFS); time to progression (TTP); time to treatment failure (TTF); time to next treatment (TTNT); event-free survival (EFS); overall response rate (ORR); duration of response (DoR); quality of life (QOL) symptoms reported by patients; toxicity; response rate (RR); stable disease (SD) or no change (NC); and disease control rate (DCR) or clinical benefit rate (CBR). OS can include time from randomization (or time from study enrollment) until death from any cause. PFS can include time from randomization (or time from study enrollment) until disease progression or death. TTP and TTF can include time from randomization (or time from study enrollment) until objective disease progression, and does not include deaths. TTNT can include time from end of primary treatment to institution of next therapy. EFS can include time from randomization (or time from study enrollment) to disease progression, death, or discontinuation of treatment for any reason (e.g. toxicity, patient preference, or initiation, of a new treatment without documented progression). ORR can include a proportion of patients with reduction in disease burden of a predefined amount. DoR can include time from documentation of disease response to disease progression. QOL symptoms can include outcome self-reported by patients using wellness scales, presence of adverse effects and toxicity therapeutic. Toxicity can include rate of adverse effects. RR can measure disease size, usually using a scan or X-ray. SD or NC can include between a 30% reduction or <25% increase in the size of all detectable disease. DCR or CBR can include percentage of patients whose disease shrinks or remains stable over a certain time period. DCR includes the sum of the complete, partial and stable disease rates. In particular embodiments, a BS-BDC has little or no anti-cancer effect when values for OS, PFS, TTP, TTF, TTNT, EFS, ORR, DoR, RR, SD or NC, or DCR or CBR obtained in a clinical trial for a BS-BDC are 20% less, 25% less, 30% less, 35% less, 40% less, 45% less, 50% less, 55% less, 60% less, 65% less, 70% less, 75% less, 80% less, 85% less, 90% less, 95% less, or more when administered within a silence dose range relative to the corresponding values obtained in a clinical trial for an effective BS-BDC such as blinatumomab. In particular embodiments, a BS-BDC has little or no anti-cancer effect when QOL symptoms reported in a clinical trial for a BS-BDC are worse relative to the corresponding QOL symptoms reported in a clinical trial

for an effective BS-BDC such as blinatumomab.

[0066] In particular embodiments, stringent selection criteria require that while each individual BS-BDC in a group have little to no anti-cancer effect when administered within a silence dose range, when administered in a group, the BS-BDC group exhibit a synergistic anti-cancer combination effect at a site expressing at least two selected cancer antigen epitopes, resulting in a silence and synergy dose range.

[0067] A combination effect can result from a treatment with at least two structurally different therapeutic agents, such as two structurally different BS-BDCs. Combination effects can be additive, synergistic, or antagonistic. In particular embodiments, an additive effect means that the sum of the effects resulting from the individual BS-BDC equals the effect resulting from the BS-BDCS applied in combination. In particular embodiments, a synergistic effect means that the combination effect is greater than the sum of the effects resulting from the individual BS-BDC. In particular embodiments, an antagonistic effect means that the effect of the combination treatment is smaller than the sum of the effects resulting from the individual BS-BDC. In some cases, additive, synergistic, or antagonistic effects can be readily detected by manual inspection of individual and combination treatment effects.

[0068] In particular embodiments, synergy means that individual members of a BS-BDC group have little or no activity within a concentration range, but when administered together result in significant T cell killing and/or cytokine release in an vitro assay or in vivo cancer model.

[0069] One widely accepted method to identify different types of combination effects includes determining the "combination index" (CI) as described by Chou and Talalay (Chou, Pharmacol Rev, 58(3):621-681, 2006; Chou, Cancer Res, 70(2):440-446, 2010; and US 2009/0324744). Drugs (e.g., BS-BDC) with a $CI < 1$ are thought to act synergistically, with a $CI = 1$ additively, and with a $CI > 1$ antagonistically. CI values for two mutually exclusive (similar mechanisms/modes of action) drugs are described by the classic isobologram formula $CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2$ (Formula 1), wherein $(Dx)_1$ means the concentration of Drug 1 without Drug 2 that results in a particular effect, for instance, 50% cell killing, and $(Dx)_2$ refers to the concentration of Drug 2 without Drug 1 that leads to the same effect. $(D)_1$ and $(D)_2$ are the individual concentrations of Drug 1 and Drug 2 in Drug 1-Drug 2 combinations.

[0070] Classic isobolograms are graphical representations of the concentrations of two drugs which in combination induce a particular effect, e.g., 50% cell killing, under the assumption the effects of the two drugs are additive ($CI = 1$). If Drug 1 concentrations are shown on the X-axis, and Drug 2 concentrations on the Y-axis, such isobolograms may be generated by drawing a straight line from the position on the X-axis representing the concentration of Drug 1 by which the particular effect is achieved without Drug 2 to the position on the Y-axis representing the concentration of Drug 2 by

which the same effect is achieved without Drug 1. Actual data points (with combinations of drugs at concentrations resulting in the particular effect) outside the triangle given by the X-axis, Y-axis, and the aforementioned "straight line" refer to drugs acting (at the tested concentrations) antagonistically ($CI > 1$), on the "straight line" additively ($CI = 1$), and inside the triangle synergistically ($CI < 1$). To assess whether experimental data supports synergistic, additive, or antagonistic relationships between two therapeutic agents, CI values may be calculated via software such as CompuSyn (by ComboSyn, Inc., available at combosyn.com).

[0071] In particular embodiments, synergy is found when a group shows an effect that is more than additive over the individual members of the group within each member's silent dose range in a T cell killing assay. In particular embodiments, synergy is found when a group shows an effect that is more than additive over the individual members of the group within each member's silent dose range in eliciting cytokine release. In particular embodiments, synergy is found when a group shows an effect that is more than additive over the individual members of the group within each member's silent dose range in a T cell killing assay and in eliciting cytokine release. In particular embodiments, synergy is found when a group shows an effect that is more than additive in eliciting cell killing or cytokine release in an isogenic cell line that expresses targeted co-expressed cancer antigen epitopes over cell lines that express only one or a subset of targeted co-expressed cancer antigen epitopes. In particular embodiments, synergy is found when a group shows an effect that is more than additive over the individual members of the group in an in vivo cancer model.

[0072] In particular embodiments, synergy is found when a group is 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10, 5 times, or more than 2 times more effective over the individual members of the group in a T cell killing assay. In particular embodiments, synergy is found when a group is 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10, 5 times, or more than 2 times more effective over the individual members of the group in eliciting cytokine release. In particular embodiments, synergy is found when a group is 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10, 5 times, or more than 2 times more effective over the individual members of the group in a T cell killing assay and in eliciting cytokine release. In particular embodiments, synergy is found when a group is 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10, 5 times, or more than 2 times more effective in eliciting cell killing or cytokine release in an isogenic cell line that expresses targeted co-expressed cancer antigen epitopes over cell lines that express only one or a subset of targeted co-expressed cancer antigen epitopes. In particular embodiments, synergy is found when a group is 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10, 5 times, or more than 2 times more effective over the individual members of the group in an in vivo cancer model.

[0073] In particular embodiments, synergy is found when a group is 1, 2, or 3 standard deviations above the effect of the individual members of the group in a T cell killing assay. In particular embodiments, synergy is found when a group is 1, 2, or 3 standard deviations above the effect of the individual members of the group in eliciting cytokine release. In particular embodiments, synergy is found when a group is 1, 2, or 3 standard deviations above the effect of the individual members of the group in a T cell killing assay and in eliciting cytokine release. In particular embodiments, synergy is found when a group is 1, 2, or 3 standard deviations above the effect of individual members of the group in eliciting cell killing or cytokine release in an isogenic cell line that expresses targeted co-expressed cancer antigen epitopes over cell lines that express only one or a subset of targeted co-expressed cancer antigen epitopes. In particular embodiments, synergy is found when a group is 1, 2, or 3 standard deviations above the effect of the individual members of the group in an in vivo cancer model.

[0074] In particular embodiments, synergy can be based on the Highest single agent model (HSA) or Gaddum's non-interaction model. Berenbaum *Pharmacological Reviews* June 1989 41(20) 93-141. In particular embodiments, synergy is based on the Lowe additivity model. Lowe *Arzneimittelforschung* 1939; 3:285-90. In particular embodiments, synergy is based on the Bliss Model. Ann Appl Biol. 1939; 26:585-615. Particular embodiments may also utilize Isobole analysis or zero interaction potency to determine synergy. Jia et al., (2009) *Nature Reviews Drug Discovery* 8(2) 111-128 provides a review of synergy in medicine.

[0075] At least two potential cancer antigen epitope targets that are co-expressed by a cancer cell or tumor are selected. In particular embodiments, the selected cancer antigen epitope must not be co-expressed by healthy, normal, or non-cancerous tissue. Candidate targets with high expression in cancer cells can be prioritized over other candidate targets with quantitatively or qualitatively lower expression levels. While not necessary, potential cancer antigen epitopes can be prioritized if they are highly expressed in cancers, difficult for a cell to down-regulate, and/or slow to internalize.

[0076] While particular embodiments require a lack of targeted cancer antigen epitope co-expression, in other embodiments, co-expression may be permitted in certain circumstances, so long as there is no significant co-expression (or not significantly co-expressed). For example, in particular embodiments, no significant co-expression means that co-expression only occurs in non-critical tissues. Non-critical tissues can include non-vital organs, or tissue or cells that are temporarily or permanently expendable or replaceable (e.g., white blood cells, red blood cells, platelets, non-critical cells within organs). In particular embodiments, no significant co-expression in non-cancerous tissue can be acceptable so long as there is significantly more expression by cancer cells or in tumors. In particular embodiments, it can be acceptable for the first targeted cancer antigen epitope and the

second targeted cancer antigen epitope to be co-expressed in a non-cancerous tissue so long as they are expressed by different cell populations within the tissue. In particular embodiments, the first targeted cancer antigen epitope and the second targeted cancer antigen epitope can advance as potential targeted cancer antigen epitopes when both are expressed on the same cells in non-cancerous tissue if the expression level of at least one target is significantly higher in cancer cells than in normal cells or if the potential toxicity to cells expressing both antigens by T cells is expected to be medically manageable (e.g., analogous to loss of normal B cells in response to CD19 directed CAR-T cells or bispecific antibodies). Further, in some instances healthy tissue may express a targeted cancer antigen in such a way that it is not exposed to the blood stream, whereas cancers do expose the targeted cancer antigen to the bloodstream. For example, Mesothelin and MUC16 can be normally expressed at high levels on luminal or intraperitoneal surfaces. When cancer expresses these targets, however, tissue architecture is not retained and the antigens become exposed to the blood. Thus, despite the potential for co-expression by healthy or non-cancerous tissues, cancer antigens can still be selected within these circumstances.

[0077] In particular embodiments, cancer antigen epitopes that are both highly expressed in cancer (e.g., as determined by immunohistochemistry (IHC)) and in which each is expressed in at least 75% of cancer cells by IHC are prioritized. IHC is qualitative, so some cancer cells may express targeted cancer antigen epitopes but not at a given threshold. Also, tumors contain a mixture of cancer cells and stromal cells which are often difficult to distinguish, so it can be important not to set a cut-off too high. While 75% is provided as an example, ranges or categories can also be used (e.g., >75%, 50-75%, 25-50% and 0-25%) to prioritize cancer antigen epitope candidates.

[0078] Particular embodiments can utilize commercially-available antibodies for this cancer antigen epitope prioritization and selection step. In particular embodiments, more than one antibody can be used for robustness. When possible, public databases are used to inform decisions regarding cancer antigen epitope prioritization and selection.

[0079] Once at least two co-expressed cancer antigen epitopes are selected, potential cancer antigen epitope binding domains from numerous antibodies or other binding molecules can be considered. Upon selection, the cancer antigen epitope binding domain can be formatted into a BS-BDC wherein the BS-BDC additionally binds an immune stimulating epitope. Generally, one BS-BDC in a group will bind a primary immune stimulating epitope such as CD3, while another BS-BDC in the group will bind a co-stimulatory epitope, such as CD28 or 4-1BB.

[0080] Each BS-BDC can be tested for anti-cancer activity across a range of doses from pg/ml to µg/ml to mg/ml doses. If a BS-BDC shows little to no anti-cancer effect within a dose range, it can continue to be assessed for inclusion in a combination therapy. As indicated previously, the dose

range at which a particular BS-BDC shows little to no anti-cancer effect can be referred to herein as its “silence dose range”.

[0081] Following identification of a BS-BDC with a silent dose range, its potential for synergism within the silence dose range of another BS-BDC can be assessed.

[0082] Tests for synergism can be based on the tests for anti-cancer activity described above or elsewhere herein. In particular embodiments, these assays are performed on cells that have high levels of the first targeted cancer antigen epitope and the second cancer antigen epitope (high/high); on cells that have high levels of the first targeted cancer antigen epitope and low levels of the second cancer antigen epitope (high/low); on cells that have low levels of the first targeted cancer antigen epitope and high levels of the second cancer antigen epitope (low/high); and/or on cells that have low levels of the first targeted cancer antigen epitope and low levels of the second cancer antigen epitope (low/low). Tests for synergism can also be conducted on cells that lack the first targeted cancer antigen epitope or the second cancer antigen epitope as a surrogate to determine whether the pair has potential to damage non-cancerous tissue that expresses the first targeted cancer antigen epitope or the second cancer antigen epitope.

[0083] Generally, one BS-BDC in a tested group will bind a primary immune stimulating epitope such as CD3, while another BS-BDC in the group will bind a co-stimulatory epitope, such as CD28 or 4-1BB.

[0084] If the tested pair shows synergistic anti-cancer activity within each member’s silent dose range, then the synergistic dose range within each member’s silent dose range is noted as the group’s “silence and synergy dose range”. Importantly, this synergism should only be observed when both targeted cancer antigen epitopes are present. In particular embodiments, BS-BDC groups that show a synergistic anti-cancer effect in the presence of cells that express high levels of both first targeted cancer antigen epitope and second targeted cancer antigen epitope (high/high) as well as cells that express low levels of one or both first targeted cancer antigen epitope and/or second targeted cancer antigen epitope (high/low or low/high) can be prioritized for further development. In particular embodiments, if the group shows synergistic anti-cancer activity in the absence of a targeted cancer antigen epitope, the group does not qualify as a BS-BDC group.

[0085] Aspects of the disclosure are now described in additional detail as follows: (I) Targeted Cancer Antigen Epitopes; (II) Immune Cell Activating Epitopes; (III) BS-BDC Formats and Modifications; (IV) Cancer Antigen Epitope and Immune Stimulating Epitope Binding Domains; (V) Compositions for Administration; (VI) Methods of Use; (VII) Expression Methods; and (VIII) Additional Exemplary Experimental Protocols.

[0086] (I) Targeted Cancer Antigen Epitopes. Cancer cell antigens are expressed by cancer cells or

tumors. One of the significant features of the current disclosure is that the targeted cancer antigen need not be preferentially expressed by cancer cells or tumors. This is because meaningful BS-BDC-induced immune cell activation occurs only in the presence of the cancer cells or tumors that co-express the targeted cancer antigen epitopes. As one example, PD-L1 is expressed by cancer cells and non-cancer cells.

[0087] In particular embodiments, cancer cell antigen epitopes are preferentially expressed by cancer cells. “Preferentially expressed” means that a cancer cell antigen is found at higher levels on cancer cells as compared to other cell types. In some instances, a cancer antigen epitope is only expressed by the targeted cancer cell type. In other instances, the cancer antigen is expressed on the targeted cancer cell type at least 25%, 35%, 45%, 55%, 65%, 75%, 85%, 95%, 96%, 97%, 98%, 99%, or 100% more than on non-targeted cells.

[0088] In particular embodiments, cancer cell antigens are significantly expressed on cancerous and healthy tissue. In particular embodiments, significantly expressed means that the use of a bispecific antibody was stopped during development based on on-target/off-cancer toxicities. In particular embodiments, significantly expressed means the use of a bispecific antibody requires warnings regarding potential negative side effects based on on-target/off-cancer toxicities. As one example, Cetuximab is anti-EGFR antibody associated with a severe skin rashes thought to be due to EGFR expression in the skin. Another example is Herceptin (trastuzumab), which is an anti-HER2 (ERBB2) antibody. Herceptin is associated with cardiotoxicity due to target expression in the heart. Moreover, targeting Her2 with a CAR-T cell was lethal in a patient due to on-target, off-cancer expression in the lung.

[0089] The following provides examples of cancer antigens that are more likely to be co-expressed in particular cancers: CD19, ROR1, PD-L1, EFGR; PD-L1, EFGR; HER2, ERBB2, ROR1, PD-L1, EFGR, MUC16, folate receptor (FOLR); L1-CAM, MUC16, FOLR, Lewis Y, ROR1, mesothelin, PD-L1, EFGR; mesothelin, PD-L1, EFGR; PD-L1, EFGR; GD2, PD-L1, EFGR; mesothelin, ROR1, PD-L1, EFGR, MUC16; ROR1, PD-L1, EFGR, mesothelin, MUC16, FOLR; mesothelin, PD-L1, EFGR; MUC16, PD-L1, EFGR; and ROR1, glypican-2, disialoganglioside, PD-L1, EFGR.

[0090] The following table provides examples of cancer antigens that are more likely to be co-expressed in particular cancer types:

Cancer Antigens Likely to be Co-Expressed	Cancer Type
CD19, ROR1, PD-L1, EFGR	Leukemia/Lymphoma
PD-L1, EFGR	Multiple Myeloma
PD-L1, EFGR	Prostate Cancer
HER2, ROR1, PD-L1, EFGR, MUC16, folate receptor (FOLR)	Breast Cancer
PD-L1, EFGR	Stem Cell Cancer

MUC16, FOLR, ROR1, mesothelin, PD-L1, EFGR	Ovarian Cancer
Mesothelin, PD-L1, EFGR	Mesothelioma
PD-L1, EFGR	Renal Cell Carcinoma
GD2, PD-L1, EFGR	Melanoma
Mesothelin, ROR1, PD-L1, EFGR, MUC16	Pancreatic Cancer
ROR1, PD-L1, EFGR, mesothelin, MUC16, FOLR	Lung Cancer
Mesothelin, PD-L1, EFGR	Cholangiocarcinoma
MUC16, PD-L1, EFGR,	Bladder Cancer
ROR1, glypican-2, disialoganglioside, PD-L1, EFGR	Neuroblastoma
PD-L1, EFGR	Colorectal Cancer
PD-L1, EFGR	Merkel Cell Carcinoma

[0091] In more particular examples, cancer cell antigens include: Mesothelin, MUC16, FOLR, PD-L1, ROR1, glypican-2 (GPC2), disialoganglioside (GD2), HER2, CD19, and EGFR. Representative sequences of cancer antigens are provided in FIG. 7. As will be understood by one of ordinary skill in the art, targeted antigens can lack signal peptides.

[0092] Disialoganglioside GalAc β 1-4(NeuAc α 2-8NeuAc α 2-3)Gal β 1-4Glc β 1-1Cer (GD2) is expressed on various tumors, including neuroblastoma. The disialoganglioside antigen GD2 includes a backbone of oligosaccharides flanked by sialic acid and lipid residues. See, e.g., Cheresch, 1987, *Surv. Synth. Pathol. Res.* 4:97 and US 5653977.

[0093] In particular embodiments, BS-BDC targeting PD-L1 can be used to combat checkpoint inhibition.

[0094] Targeted cancer antigen epitopes that are co-expressed in cancerous tissues but not in non-cancerous tissues are different from one another. In particular embodiments, “different from” means that the targeted epitopes are distinct from one another in sequence and/or structure. In particular embodiments, in addition to being different, targeted epitopes are also non-overlapping. “Non-overlapping” means that the binding of one BS-BDC in a group to an epitope is not decreased to a statistically-significant degree in a competitive binding assay by the presence of at least one other BS-BDC in the group. Non-overlapping epitopes may be epitopes on different molecules (e.g., ROR1 and CD19; CD3 and CD28) or may be non-overlapping epitopes located on the same molecule (e.g., non-overlapping ROR1 epitopes; non-overlapping CD3 epitopes). Non-repetitive different epitopes on the same antigen exclude epitopes that are physically distinct in space from one another yet repetitive in sequence to each other. For example, MUC1 has a repetitive sequence, and the repeats within the sequence are not non-repetitive and different, as defined herein.

[0095] In particular embodiments, a targeted cancer antigen epitope can have high expression by a targeted cancer cell or tumor or low expression by a targeted cancer cell or tumor. In particular embodiments, high and low expression can be determined using flow cytometry or fluorescence-activated cell-sorting (FACs). As is understood by one of ordinary skill in the art of flow cytometry, “hi”,

“lo”, “+” and “-” refer to the intensity of a signal relative to negative or other populations. In particular embodiments, positive expression (+) means that the marker is detectable on a cell using flow cytometry. In particular embodiments, negative expression (-) means that the marker is not detectable using flow cytometry. In particular embodiments, “hi” means that the positive expression of a marker of interest is brighter as measured by fluorescence (using for example FACS) than other cells also positive for expression. In these embodiments, those of ordinary skill in the art recognize that brightness is based on a threshold of detection. Generally, one of skill in the art will analyze a negative control tube first, and set a gate (bitmap) around the population of interest by FSC and SSC and adjust the photomultiplier tube voltages and gains for fluorescence in the desired emission wavelengths, such that 97% of the cells appear unstained for the fluorescence marker with the negative control. Once these parameters are established, stained cells are analyzed and fluorescence recorded as relative to the unstained fluorescent cell population. In particular embodiments, and representative of a typical FACS plot, hi implies to the farthest right (x line) or highest top line (upper right or left) while lo implies within the left lower quadrant or in the middle between the right and left quadrant (but shifted relative to the negative population). In particular embodiments, “hi” refers to greater than 20-fold of +, greater than 30-fold of +, greater than 40-fold of +, greater than 50-fold of +, greater than 60-fold of +, greater than 70-fold of +, greater than 80-fold of +, greater than 90-fold of +, greater than 100-fold of +, or more of an increase in detectable fluorescence relative to + cells. Conversely, “lo” can refer to a reciprocal population of those defined as “hi”.

[0096] (II) Immune Cell Activating Epitopes. Immune cells that can be targeted for localized activation by BS-BDC groups of the current disclosure include, for example, T cells, natural killer (NK) cells, and macrophages.

[0097] T cell activation can be mediated by two distinct signals: those that initiate antigen-dependent primary activation and provide a T cell receptor like signal (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). BS-BDC groups disclosed herein can target any combination of T cell activating epitopes that upon binding induce T cell activation. Examples of such T cell activating epitopes are on T cell markers including CD3, CD28, and 4-1BB.

[0098] CD3 is a primary signal transduction element of T cell receptors. CD3 is composed of a group of invariant proteins called gamma (γ), delta (Δ), epsilon (Σ), zeta (Z) and eta (H) chains. The γ , Δ , and Σ chains are structurally-related, each containing an Ig-like extracellular constant domain followed by a transmembrane region and a cytoplasmic domain of more than 40 amino acids. The Z and H chains have a distinctly different structure: both have a very short extracellular region of only 9

amino acids, a transmembrane region and a long cytoplasmic tail including 113 and 115 amino acids in the Z and H chains, respectively. The invariant protein chains in the CD3 complex associate to form noncovalent heterodimers of the Σ chain with a γ chain ($\Sigma\gamma$) or with a Δ chain ($\Sigma\Delta$) or of the Z and H chain (ZH), or a disulfide-linked homodimer of two Z chains (ZZ). 90% of the CD3 complex incorporate the ZZ homodimer.

[0099] The cytoplasmic regions of the CD3 chains include a motif designated the immunoreceptor tyrosine-based activation motif (ITAM). This motif is found in a number of other receptors including the Ig- α /Ig- β heterodimer of the B-cell receptor complex and Fc receptors for IgE and IgG. The ITAM sites associate with cytoplasmic tyrosine kinases and participate in signal transduction following TCR-mediated triggering. In CD3, the γ , Δ and Σ chains each contain a single copy of ITAM, whereas the Z and H chains harbor three ITAMs in their long cytoplasmic regions. Indeed, the Z and H chains have been ascribed a major role in T cell activation signal transduction pathways. CD3 is expressed on all mature T cells.

[00100] CD28 is a member of a subfamily of costimulatory molecules characterized by an extracellular variable immunoglobulin-like domain. CD28 is the receptor for CD80 (B7-1) and CD86 (B7-2) proteins.

[00101] CD28 is one of the proteins expressed on T cells that provide co-stimulatory signals required for T cell activation and survival. CD28 is expressed on 80% of human CD4⁺ T cells and 50% of CD8⁺ T cells. CD28 expression increases after T cell activation. CD28 interacts with molecules of the B7 family present mainly at the surface of antigen presenting cells (APCs), as well as on activated T and B cells. After engagement of the TCR with a class II (or I) MHC molecule on the APC, IL-2 production and IL-2 receptor expression are initiated. The second signal provided by the CD28/CD80 or CD28/CD86 interaction stabilizes IL-2 mRNA and increases IL-2 secretion, resulting in T cell proliferation and clonal expansion, thereby promoting immune response. T cell stimulation through CD28 in addition to the T cell receptor (TCR) also provides a potent signal for the production of various other interleukins such as IL-6 and IL-13.

[00102] 4-1BB (also referred to as CD137 and TNFRSF9) is a transmembrane protein of the Tumor Necrosis Factor receptor superfamily. In particular embodiments, 4-1BB is a 255 amino acid protein as described in Accession No. NM_001561 or NP_001552. In particular embodiments, 4-1BB includes a signal sequence (amino acid residues 1-17), followed by an extracellular domain (169 amino acids), a transmembrane region (27 amino acids), and an intracellular domain (42 amino acids) (Cheuk et al. 2004 Cancer Gene Therapy 11: 215-226).

[00103] 4-1BB expression is generally activation dependent. It is present in a broad subset of immune cells including activated T cells, regulatory T cells, dendritic cells (DC), activated NK and NKT cells,

stimulated mast cells, differentiating myeloid cells, monocytes, neutrophils, and eosinophils (Wang, 2009, Immunological Reviews 229: 192-215). 4-1BB expression has also been demonstrated on tumor vasculature (Broll, 2001, Amer. J Clin. Pathol. 115(4):543-549; Seaman, 2007, Cancer Cell 11: 539-554). The ligand that stimulates 4-1BB, i.e., 4-1BB Ligand (4-1BBL), is expressed on activated antigen-presenting cells (APCs), myeloid progenitor cells, and hematopoietic stem cells.

[0104] 4-1BB agonists can increase costimulatory molecule expression and markedly enhance cytolytic T lymphocyte responses, resulting in anti-tumor efficacy in various models. Further, 4-1BB monotherapy and combination therapy tumor models have established durable anti-tumor protective T cell memory responses (Lynch, 2008, Immunol Rev. 22: 277-286). 4-1BB agonists also have been shown to inhibit autoimmune reactions in a variety of art-recognized autoimmunity models (Vinay, 2006, J Mol Med 84:726-736). This dual activity of 4-1BB offers the potential to provide anti-tumor activity while dampening autoimmune side effects that can be associated with immunotherapy approaches that break immune tolerance.

[0105] In particular embodiments, BS-BDC targeting 4-1BB can be used for co-stimulation when T cells become exhausted. In particular embodiments, T cell exhaustion can be determined by measuring the expression level of CD28. In particular embodiments, T cell exhaustion can be determined by measuring the expression level of K (lysine) acetyltransferase 2B gene (KAT2B); calcium/calmodulin-dependent serine protein kinase 3 gene (CASK); ATP-binding cassette sub-family D member 2 gene (ABCD2); disks large homolog 1 gene (DLG1); synovial sarcoma translocation, chromosome 18 gene (SS18); retinoblastoma-like protein 2 gene (RBL2); RAS oncogene family-like 1 gene (RAB7L1); methylenetetrahydrofolate dehydrogenase 1 gene (MTHFD1); keratoc gene (KERA); B cell-specific Moloney murine leukemia virus integration site 1 gene (BMI1); conserved oligomeric Golgi complex subunit 5 gene (COG5); cAMP-specific 3',5'-cyclic phosphodiesterase 4D gene (PDE4D); and variable charge, Y-linked gene (VCY). In particular embodiments, a subject has an exhausted CD8⁺ T cell or lack of CD4⁺ T cell costimulation phenotype characterized by upregulated expression of genes KAT2B, CASK, ABCD2, DLG1, SS18, RBL2, RAB7L1, MTHFD1, BMI1, COG5, and PDE4D, and downregulated expression of genes KERA and VCY, relative to the level of expression of these genes in a subject who does not have said phenotype.

[0106] The level of expression of genes KAT2B, CASK, ABCD2, DLG1, SS18, RBL2, RAB7L1, MTHFD1, BMI1, COG5, PDE4D, KERA, and VCY may be determined by any convenient means and many suitable techniques are known in the art. For example, suitable techniques include: reverse-transcription quantitative PCR (RT-qPCR), microarray analysis, enzyme-linked immunosorbent assays (ELISA), protein chips, flow cytometry (such as Flow-FISH for RNA, also referred to as FlowRNA, described in Porichis et al., Nature Comm (2014) 5:5641), mass spectrometry, Western

blotting, and northern blotting.

[0107] T cells are classified into helper cells (CD4⁺ T cells) and cytotoxic T cells (CTLs, CD8⁺ T cells), which include cytolytic T cells. T helper cells assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and activation of cytotoxic T cells and macrophages, among other functions. These cells are also known as CD4⁺ T cells because they express the CD4 protein on their surface. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of APCs. Once activated, they divide rapidly and secrete cytokines that regulate or assist in the active immune response.

[0108] Particular embodiments can include activating CD4 T cells by binding CD3 or CD28. This targeted T cell type activation could be achieved with a first cancer associated antigen (CAA1)-CD3 and a second cancer associated antigen (CAA2)-CD28 BS-BDC group. This approach can be beneficial because CD4⁺ T cells are not inherently cytotoxic and can provide an enhanced safety profile. CD4⁺ T cells also secrete cytokines that modulate the solid tumor microenvironment.

[0109] Cytotoxic T cells destroy tumor cells. These cells are also known as CD8⁺ T cells because they express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body. Particular embodiments can include activating CD8 T cells by binding CD3, CD28, or 4-1BB.

[0110] (III) BS-BDC Formats and Modifications. BS-BDC formats include a protein with a first binding domain that binds a cancer antigen epitope and a second binding domain that binds an immune cell activating epitope. FIG. 2 provides examples of combinations of cancer antigen epitope and immune stimulating epitope pairs that can be bound by BS-BDC. FIGs. 3A-3G provide examples of combinations for prioritized testing based on likelihood of cancer antigen co-expression in particular types of cancer. Exemplary bispecific antibody formats are described in, e.g., WO2009/080251, WO2009/080252, WO2009/080253, WO2009/080254, WO2010/112193, WO2010/115589, WO2010/136172, WO2010/145792, WO2010/145793, and Brinkmann & Kontermann, mAbs, 2017. 9:2, 182-212, DOI: 10.1080/19420862.2016.1268307.

[0111] Different binding domains can be derived from multiple sources such as antibodies, fibronectin, affibodies, natural ligands (e.g., CD80 and CD86 for CD28 or folate for the folate receptor), etc. In particular embodiments, binding domains can be derived from whole antibodies or binding fragments of an antibody, e.g., Fv, Fab, Fab', F(ab')₂, Fc, and single chain Fv fragments (scFvs) or any biologically effective fragments of an immunoglobulin that bind specifically to a cancer antigen epitope or immune cell activating epitope (e.g., T cell receptor). Antibodies or antigen binding fragments include all or a portion of polyclonal antibodies, monoclonal antibodies, human antibodies,

humanized antibodies, synthetic antibodies, chimeric antibodies, bispecific antibodies, mini bodies, and linear antibodies.

[0112] BS-BDC including binding domains from human origin or humanized antibodies have lowered immunogenicity in humans and have a lower number of non-immunogenic epitopes compared to non-human antibodies. Binding domains will generally be selected to have reduced antigenicity in human subjects. Binding domains can particularly include any peptide that specifically binds a selected cancer antigen epitope or immune cell activating epitope. Sources of binding domains include antibody variable regions from various species (which can be in the form of antibodies, sFvs, scFvs, Fabs, scFv-based grababody, or soluble VH domain or domain antibodies). These antibodies can form antigen-binding regions using only a heavy chain variable region, i.e., these functional antibodies are homodimers of heavy chains only (referred to as "heavy chain antibodies") (Jespers et al., *Nat. Biotechnol.* 22:1161, 2004; Cortez-Retamozo et al., *Cancer Res.* 64:2853, 2004; Baral et al., *Nature Med.* 12:580, 2006; and Barthelemy et al., *J. Biol. Chem.* 283:3639, 2008).

[0113] Phage display libraries of partially or fully synthetic antibodies are available and can be screened for an antibody or fragment thereof that can bind a selected epitope. For example, binding domains may be identified by screening a Fab phage library for Fab fragments that specifically bind to a target of interest (see Hoet et al., *Nat. Biotechnol.* 23:344, 2005). Phage display libraries of human antibodies are also available. Additionally, traditional strategies for hybridoma development using a target of interest as an immunogen in convenient systems (e.g., mice, HuMAb mouse®, TC mouse™, KM-mouse®, llamas, chicken, rats, hamsters, rabbits, etc.) can be used to develop binding domains. In particular embodiments, binding domains specifically bind to selected epitopes expressed by targeted cancer cells and/or T cells and do not cross react with nonspecific components or unrelated targets. Once identified, the amino acid sequence or polynucleotide sequence coding for the CDR within a binding domain can be isolated and/or determined.

[0114] An alternative source of binding domains includes sequences that encode random peptide libraries or sequences that encode an engineered diversity of amino acids in loop regions of alternative non-antibody scaffolds, such as scTCR (see, e.g., Lake et al., *Int. Immunol.* 11:745, 1999; Maynard et al., *J. Immunol. Methods* 306:51, 2005; U.S. Patent No. 8,361,794), mAb2 or Fcab™ (F-star Biotechnology Ltd., Cambridge, UK, see, e.g., PCT Patent Application Publication Nos. WO 2007/098934; WO 2006/072620), affibodies, avimers, fynomers, cytotoxic T-lymphocyte associated protein-4 (Weidle et al., *Cancer Gen. Proteo.* 10:155, 2013), and the like (Nord et al., *Protein Eng.* 8:601, 1995; Nord et al., *Nat. Biotechnol.* 15:772, 1997; Nord et al., *Euro. J. Biochem.* 268:4269, 2001; Binz et al., *Nat. Biotechnol.* 23:1257, 2005; Boersma and Plückthun, *Curr. Opin. Biotechnol.* 22:849, 2011).

[0115] In particular embodiments, an antibody fragment is used as one or more binding domains in a BS-BDC. An "antibody fragment" denotes a portion of a complete or full length antibody that retains the ability to bind to an epitope. Examples of antibody fragments include Fv, scFv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; and linear antibodies.

[0116] A single chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy and light chains of immunoglobulins connected with a short linker peptide. Fv fragments can include the VL and VH domains of a single arm of an antibody. Although the two domains of the Fv fragment, VL and VH, are coded by separate genes, they can be joined, using, for example, recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (single chain Fv (scFv)). For additional information regarding Fv and scFv, see e.g., Bird, et al., *Science* 242 (1988) 423-426; Huston, et al., *Proc. Natl. Acad. Sci. USA* 85 (1988) 5879-5883; Plueckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore (eds.), Springer-Verlag, New York), (1994) 269-315; WO1993/16185; US Patent 5,571,894; and US Patent 5,587,458.

[0117] A Fab fragment is a monovalent antibody fragment including VL, VH, CL and CH1 domains. A F(ab')₂ fragment is a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region. For discussion of Fab and F(ab')₂ fragments having increased in vivo half-life, see U.S. Patent 5,869,046. Diabodies include two epitope-binding sites that may be bivalent. See, for example, EP 0404097; WO1993/01161; and Holliger, et al., *Proc. Natl. Acad. Sci. USA* 90 (1993) 6444-6448. Dual affinity retargeting antibodies (DART® (MacroGenics, Inc., Rockville, MD); based on the diabody format but featuring a C-terminal disulfide bridge for additional stabilization (Moore et al., *Blood* 117, 4542-51 (2011))) can also be used. Antibody fragments can also include isolated CDRs. For a review of antibody fragments, see Hudson, et al., *Nat. Med.* 9 (2003) 129-134.

[0118] Antibody fragments can be made by various techniques, including proteolytic digestion of an intact antibody as well as production by recombinant host-cells (e.g. human suspension cell lines, *E. coli* or phage), as described herein. Antibody fragments can be screened for their binding properties in the same manner as intact antibodies.

[0119] In particular embodiments, binding domains of a BS-BDC may be joined through a linker. A linker is an amino acid sequence which can provide flexibility and room for conformational movement between the binding domains of a BS-BDC. Any appropriate linker may be used. Examples of linkers can be found in Chen et al., *Adv Drug Deliv Rev.* 2013 Oct 15; 65(10): 1357–1369. Linkers can be flexible, rigid, or semi-rigid, depending on the desired functional domain presentation to a target. Commonly used flexible linkers include Gly-Ser linkers such as GGSGGGSGGSG (SEQ ID NO: 19), GGSGGGSGGSG (SEQ ID NO: 20) and GGSGGGSG (SEQ ID NO: 21). Additional examples include:

GGGGSGGGGS (SEQ ID NO: 22); GGGSGGGGS (SEQ ID NO: 23); and GGS GGS (SEQ ID NO: 24). See also FIG. 6A depicting $(G_4S)_3$ (SEQ ID NO: 25) and G_4S (SEQ ID NO: 26). Linkers that include one or more antibody hinge regions and/or immunoglobulin heavy chain constant regions, such as CH3 alone or a CH2CH3 sequence can also be used.

[0120] In some situations, flexible linkers may be incapable of maintaining a distance or positioning of binding domains needed for a particular use. In these instances, rigid or semi-rigid linkers may be useful. Examples of rigid or semi-rigid linkers include proline-rich linkers. In particular embodiments, a proline-rich linker is a peptide sequence having more proline residues than would be expected based on chance alone. In particular embodiments, a proline-rich linker is one having at least 30%, at least 35%, at least 36%, at least 39%, at least 40%, at least 48%, at least 50%, or at least 51% proline residues. Particular examples of proline-rich linkers include fragments of proline-rich salivary proteins (PRPs).

[0121] Particular embodiments include monomeric agents on the end of a BS-BDC that engages an immune stimulating epitope, such that receptors do not become cross-linked until the BS-BDC has also bound its targeted cancer antigen epitope. Formats that provide these features include canonical bispecific antibodies as well as IgG-containing BS-BDC that are dimeric but sterically hindered on the immune stimulating epitope portion. A canonical bispecific antibody is one that has the simple architecture of blinatumomab: two single-chain antibodies fused together as one single chain.

[0122] In particular embodiments, BS-BDC can have an antibody-like architecture with the heavy and light chain portions that bind an immune stimulating epitope attached to an Fc. The heavy and light chain come together when the BS-BDC is assembled. This architecture may utilize knobs and holes Fc with a single chain employed on the N-terminus (e.g., a maxibody-like architecture).

[0123] In particular embodiments, modified BS-BDC include those wherein one or more amino acids have been replaced with a non-amino acid component, or where the amino acid has been conjugated to a functional group or a functional group has been otherwise associated with an amino acid. The modified amino acid may be, e.g., a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, or an amino acid conjugated to an organic derivatizing agent. Amino acid(s) can be modified, for example, co-translationally or post-translationally during recombinant production (e.g., N-linked glycosylation at N-X-S/T motifs during expression in mammalian cells) or modified by synthetic means. The modified amino acid can be within the sequence or at the terminal end of a sequence. Modifications also include nitrated constructs.

[0124] In particular embodiments, BS-BDC can be modified to produce an administration benefit, a regulatory benefit, and/or a stoichiometric benefit. Exemplary administration benefits can include, for

example, extended half-life, lowered immunogenicity, modified (e.g., reduced) effector function, and/or enhanced tumor penetration. An exemplary regulatory benefit is based on expressing a BS-BDC group as a single molecule. An exemplary stoichiometric benefit is based on providing tailored amounts of BS-BDC that target different cancer antigen epitopes and/or immune stimulating epitopes. As just one example, a BS-BDC group could include two BS-BDC that target the first targeted cancer antigen epitope for every one BS-BDC that targets a second targeted cancer antigen epitope.

[0125] Particular modifications include attachment of a half-life extender. Exemplary half-life extenders include a single chain Fc, albumin, transferrin, a hydrophobic tail such as palmitic acid, albumin-binding peptides, and polyethylene glycol (PEG).

[0126] Particular embodiments include a single chain antibody attached to the C-terminus of a light chain (see, e.g., *Oncoimmunology*. 2017; 6(3): e1267891). This format can be useful because the presence of the Fc region can help preserve the protein half-life. The presence of the Fc region can also be useful because Fc interacts with several receptors and can contribute to the immune response. Antibody-scFv fusions can also be useful because the antibody portion binds to its epitope in a dimeric fashion, which enhances avidity and the scFv portion binds its epitope in a monomeric fashion, which can be useful, for example, for binding T cell epitopes and only allowing multimerization in the presence of a target (e.g., cancer cell). These embodiments can be “tri-specific”.

[0127] PEGylation particularly is a process by which polyethylene glycol (PEG) polymer chains are covalently conjugated to other molecules such as proteins. Several methods of PEGylating proteins have been reported in the literature. For example, N-hydroxy succinimide (NHS)-PEG was used to PEGylate the free amine groups of lysine residues and N-terminus of proteins; PEGs bearing aldehyde groups have been used to PEGylate the amino-termini of proteins in the presence of a reducing reagent; PEGs with maleimide functional groups have been used for selectively PEGylating the free thiol groups of cysteine residues in proteins; and site-specific PEGylation of acetyl-phenylalanine residues can be performed.

[0128] Covalent attachment of proteins to PEG has proven to be a useful method to increase the half-lives of proteins in the body (Abuchowski, A. et al., *Cancer Biochem. Biophys.*, 1984, 7:175-186; Hershfield, M. S. et al., *N. Engl. J. Medicine*, 1987, 316:589-596; and Meyers, F. J. et al., *Clin. Pharmacol. Ther.*, 1991, 49:307-313). The attachment of PEG to proteins not only protects the molecules against enzymatic degradation, but also reduces their clearance rate from the body. The size of PEG attached to a protein has significant impact on the half-life of the protein. The ability of PEGylation to decrease clearance is generally not a function of how many PEG groups are attached to the protein, but the overall molecular weight of the altered protein. Usually the larger the

PEG is, the longer the *in vivo* half-life of the attached protein. In addition, PEGylation can also decrease protein aggregation (Suzuki et al., Biochem. Bioph. Acta vol. 788, pg. 248 (1984)), alter protein immunogenicity (Abuchowski et al.; J. Biol. Chem. vol. 252 pg. 3582 (1977)), and increase protein solubility as described, for example, in PCT Publication No. WO 92/16221).

[0129] Several sizes of PEGs are commercially available (Nektar Advanced PEGylation Catalog 2005-2006; and NOF DDS Catalogue Ver 7.1), which are suitable for producing proteins with targeted circulating half-lives. A variety of active PEGs have been used including mPEG succinimidyl succinate, mPEG succinimidyl carbonate, and PEG aldehydes, such as mPEG-propionaldehyde.

[0130] In particular embodiments, a BS-BDC is split around a half-life extender. For example, half of a BS-BDC can be at the N-terminus of a half-life extender and half of the BS-BDC can be at the C-terminus of the half-life extender.

[0131] In particular embodiments, BS-BDC can include Fc with modifications to decrease or increase effector function. For example, effector function can be reduced by eliminating glycosylation sites. In particular embodiments, the human IgG1 variant L234A/L235A can be used. In particular embodiments, the IgG4 variant F234A/L235A can be used. In particular embodiments, an IgG2 variant with point mutations from IgG4 (i.e., H268Q/V309L/A330S/P331S) can be used. In particular embodiments, a variant that contains the IgG2 to IgG4 cross-subclass mutations V309L/A330S/P331S combined with the non-germline mutations V234A/G237A/P238S/H268A, and the N297A, N297Q, and N297G mutations can be used. These modifications can reduce or eliminate glycosylation reducing effector function through modulation of FcγRs and C1q binding capability. In addition, some mutations such as M252Y/S254T/T256E and M428L/N434S can increase antibody half-life while reducing effector function.

[0132] In particular embodiments, mutations of amino acids such as S298A/E333A/K334A, S239D/I332E or S239D/I332E/A330L, the variant F243L/R292P/Y300L/V305I/P396L, the combination of L234Y/L235Q/G236W/S239M/H268D/D270E/S298A changes in one Fc domain and D270E/K326D/A330M/K334E changes in the other, and/or the elimination of the fucose molecule that is normally part of the carbohydrate located at asparagine 297 (while maintaining other forms of glycosylation at this site) can lead to antibodies with enhanced ADCC effector functions mediated by binding to their corresponding FcγRs (receptors). In addition, mutations such as G236A/S239D/I332E can lead to antibodies with enhanced ADCP effector functions and mutations such as K326W/E333S and S267E/H268F/S324T can lead to antibodies with enhanced CDC effector functions mediated by an increased binding affinity to C1q.

[0133] In particular embodiments, modifications to a BS-BDC binding domain are made to alter the binding affinity of the BS-BDC to its targeted epitope. Depending on the activity of the BS-BDC,

modifications can be chosen to increase or decrease binding affinity. For example, binding affinity can be enhanced through increasing avidity which arises from multimerization of binding domains.

Exemplary multimerization domains include C4b and ferritin.

[0134] Affinity of each binding domain of a BS-BDC for an epitope can be measured by an assay known to one of ordinary skill in the art such as (enzyme linked immunosorbent assays (ELISAs), gel-shift assays, immunoprecipitation assays, equilibrium dialysis, analytical ultracentrifugation, surface plasmon resonance (SPR), spectroscopic assays, and isothermal titration calorimetry (ITC).

[0135] SPR assays may be performed using the Sensor Chip CM5 (Biacore AB, Uppsala, Sweden) which contains a carboxymethyl (CM) dextran matrix and a Biacore® 3000 SPR biosensor (Biacore AB, Uppsala, Sweden). A target molecule including a cancer antigen epitope or an immune stimulating epitope can be covalently attached to the CM dextran matrix using amine coupling chemistry. A reference surface is created by omission of the recombinant molecule coupling step. Different concentrations of a BS-BDC can be prepared by serial dilution (e.g., 50 nM, 25 nM, 12.5 nM, 6.25 nM and 3.13 nM) and injected in a serial-flow manner across the cancer antigen-specific or immune stimulating molecule-specific surface and its corresponding reference surface. Dissociation of bound target molecule – BS-BDC can be monitored. Remaining bound material can be removed with appropriate buffers. A BS-BDC such as blinatumomab can be used as a positive reference control.

[0136] In particular embodiments, the disclosure provides a BS-BDC including a binding domain that specifically binds to a cancer antigen epitope or immune stimulating epitope with an association rate constant or k_{on} rate of, either before and/or after modification, (e.g., binding domain + epitope $\rightarrow$ binding domain-epitope) not more than $10^7 \text{ M}^{-1}\text{s}^{-1}$, less than $5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, less than $2.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, less than $2 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, less than $1.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, less than $10^6 \text{ M}^{-1}\text{s}^{-1}$, less than $5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, less than $2.5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, less than $2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, less than $1.5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, less than $10^5 \text{ M}^{-1}\text{s}^{-1}$, less than $5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, less than $2.5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, less than $2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, less than $1.5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, less than $10^4 \text{ M}^{-1}\text{s}^{-1}$, less than $10^3 \text{ M}^{-1}\text{s}^{-1}$, less than $10^2 \text{ M}^{-1}\text{s}^{-1}$, or in a range of $10^2 \text{ M}^{-1}\text{s}^{-1}$ to $10^7 \text{ M}^{-1}\text{s}^{-1}$, in a range of $10^3 \text{ M}^{-1}\text{s}^{-1}$ to $10^6 \text{ M}^{-1}\text{s}^{-1}$, in a range of $10^4 \text{ M}^{-1}\text{s}^{-1}$ to $10^5 \text{ M}^{-1}\text{s}^{-1}$, or in a range of $10^3 \text{ M}^{-1}\text{s}^{-1}$ to $10^7 \text{ M}^{-1}\text{s}^{-1}$.

[0137] In particular embodiments, the disclosure provides a BS-BDC including a binding domain that specifically binds to a cancer antigen epitope or immune stimulating epitope with a k_{off} rate (binding domain-epitope $\rightarrow$ binding domain + epitope) of, either before and/or after modification, not less than 0.5 s^{-1} , not less than 0.25 s^{-1} , not less than 0.2 s^{-1} , not less than 0.1 s^{-1} , not less than $5 \times 10^{-2} \text{ s}^{-1}$, not less than $2.5 \times 10^{-2} \text{ s}^{-1}$, not less than $2 \times 10^{-2} \text{ s}^{-1}$, not less than $1.5 \times 10^{-2} \text{ s}^{-1}$, not less than 10^{-2} s^{-1} , not less than $5 \times 10^{-3} \text{ s}^{-1}$, not less than $2.5 \times 10^{-3} \text{ s}^{-1}$, not less than $2 \times 10^{-3} \text{ s}^{-1}$, not less than $1.5 \times 10^{-3} \text{ s}^{-1}$, not less than 10^{-3} s^{-1} , not less than $5 \times 10^{-4} \text{ s}^{-1}$, not less than $2.5 \times 10^{-4} \text{ s}^{-1}$, not less than $2 \times 10^{-4} \text{ s}^{-1}$, not less than $1.5 \times 10^{-4} \text{ s}^{-1}$, not less than 10^{-4} s^{-1} , not less than $5 \times 10^{-5} \text{ s}^{-1}$, not less than $2.5 \times 10^{-5} \text{ s}^{-1}$,

not less than $2 \times 10^{-5} \text{ s}^{-1}$, not less than $1.5 \times 10^{-5} \text{ s}^{-1}$, not less than 10^{-5} s^{-1} , not less than $5 \times 10^{-6} \text{ s}^{-1}$, not less than $2.5 \times 10^{-6} \text{ s}^{-1}$, not less than $2 \times 10^{-6} \text{ s}^{-1}$, not less than $1.5 \times 10^{-6} \text{ s}^{-1}$, not less than 10^{-6} s^{-1} , or in a range of 0.5 to 10^{-6} s^{-1} , in a range of 10^{-2} s^{-1} to 10^{-5} s^{-1} , or in a range of 10^{-3} s^{-1} to 10^{-4} s^{-1} .

[0138] In particular embodiments, the disclosure provides a BS-BDC including a binding domain that specifically binds to a cancer antigen epitope or immune stimulating epitope with an affinity constant or K_a (k_{on}/k_{off}) of, either before and/or after modification, less than 10^6 M^{-1} , less than $5 \times 10^5 \text{ M}^{-1}$, less than $2.5 \times 10^5 \text{ M}^{-1}$, less than $2 \times 10^5 \text{ M}^{-1}$, less than $1.5 \times 10^5 \text{ M}^{-1}$, less than 10^5 M^{-1} , less than $5 \times 10^4 \text{ M}^{-1}$, less than $2.5 \times 10^4 \text{ M}^{-1}$, less than $2 \times 10^4 \text{ M}^{-1}$, less than $1.5 \times 10^4 \text{ M}^{-1}$, less than 10^4 M^{-1} , less than $5 \times 10^3 \text{ M}^{-1}$, less than $2.5 \times 10^3 \text{ M}^{-1}$, less than $2 \times 10^3 \text{ M}^{-1}$, less than $1.5 \times 10^3 \text{ M}^{-1}$, less than 10^3 M^{-1} , less than 500 M^{-1} , less than 250 M^{-1} , less than 200 M^{-1} , less than 150 M^{-1} , less than 100 M^{-1} , less than 50 M^{-1} , less than 25 M^{-1} , less than 20 M^{-1} , less than 15 M^{-1} , or less than 10 M^{-1} , or in a range of 10 M^{-1} to 10^6 M^{-1} , in a range of 10^2 M^{-1} to 10^5 M^{-1} , or in a range of 10^3 M^{-1} to $1 \times 10^4 \text{ M}^{-1}$.

[0139] In particular embodiments, the disclosure provides a BS-BDC including a binding domain that specifically binds to a cancer antigen epitope or immune stimulating epitope with a dissociation constant or K_d (k_{off}/k_{on}) of, either before and/or after modification, not less than $.05 \text{ M}$, not less than $.025 \text{ M}$, not less than $.02 \text{ M}$, not less than $.01 \text{ M}$, not less than $5 \times 10^{-3} \text{ M}$, not less than $2.5 \times 10^{-3} \text{ M}$, not less than $2 \times 10^{-3} \text{ M}$, not less than $1.5 \times 10^{-3} \text{ M}$, not less than 10^{-3} M , not less than $5 \times 10^{-4} \text{ M}$, not less than $2.5 \times 10^{-4} \text{ M}$, not less than $2 \times 10^{-4} \text{ M}$, not less than $1.5 \times 10^{-4} \text{ M}$, not less than 10^{-4} M , not less than $5 \times 10^{-5} \text{ M}$, not less than $2.5 \times 10^{-5} \text{ M}$, not less than $2 \times 10^{-5} \text{ M}$, not less than $1.5 \times 10^{-5} \text{ M}$, not less than 10^{-5} M , not less than $5 \times 10^{-6} \text{ M}$, not less than $2.5 \times 10^{-6} \text{ M}$, not less than $2 \times 10^{-6} \text{ M}$, not less than $1.5 \times 10^{-6} \text{ M}$, not less than 10^{-6} M , or not less than 10^{-7} M , or in a range of $.05 \text{ M}$ to 10^{-7} M , in a range of $5 \times 10^{-3} \text{ M}$ to 10^{-6} M , or in a range of 10^{-4} M to 10^{-7} M .

[0140] In particular embodiments, BS-BDC of different sizes are selected to achieve in vivo effects. For example, as described above, inclusion of IgG1 can extend serum half-life of BS-BDC. In some instances, small BS-BDC may better penetrate solid tumors. In particular embodiments, small BS-BDC can be 20 amino acids or less. In particular embodiments, small BS-BDC are under the limit of glomerular filtration (60kDa). Wittrup et al., Methods Enzymol. 2012; 503: 255-268 describe relationships between tumor uptake and size of the targeting agent.

[0141] Particular embodiments may include IgG4 instead of or in addition to IgG1. IgG4 is generally less active than IgG1.

[0142] In particular embodiments, BS-BDC can be created as linked groups. In particular embodiments, the linkage can be cleaved by an in vivo physiological factor or an ex vivo factor. For example, particular embodiments can utilize a disulfide-linked fusion protein. Particular embodiments can utilize an in vivo cleavable disulfide linker LEAGCKNFFPR↓SFTSCGSLE (SEQ ID NO: 27). This

linker is based on a dithiocyclopeptide containing an intramolecular disulfide bond formed between two cysteine (Cys) residues on the linker, as well as a thrombin-sensitive sequence (PRS) between the two Cys residues. Exposure to thrombin results in cleavage of the thrombin-sensitive sequence, while the reversible disulfide linkage between the two domains of a fusion protein can remain.

Resultant disulfide-linked proteins are designated as "G-S-S-T".

[0143] In particular embodiments, BS-BDC can be linked with a peptide linker that is cleaved *in vivo* by tumor proteases. Examples of such sequences include GPLGMLSQ (SEQ ID NO: 28), GPLGLWAQ (SEQ ID NO: 29) and GPLGIAGQ (SEQ ID NO: 30) that are substrates for tumor proteases MMP2 and MMP9 (Netzel-Arnett, JBC, 266 6747-6755 (1991); Netzel-Arnett, Biochemistry 32 6427-6432 (1993)) and GGGR (SEQ ID NO: 31) which is a uPA substrate (Chung & Kratz (2006) Bioorganic & Medicinal Chemistry Letters, 16(19), 5157–5163).

[0144] In particular embodiments, BS-BDC can be linked with a peptide linker, such as GGGSGGGSENLYFQSAAA (SEQ ID NO: 32) that can be conveniently cleaved by TEV protease during manufacturing in a mammalian system (J Struct Biol. 2016 Apr; 194(1): 1–7).

[0145] Other linkers that are not cleaved may also be used to link BS-BDC groups described herein. Gly-Ser linkers and many proline-rich linkers provide examples of such linkers.

[0146] BS-BDC can be linked, for example, as linker-to-linker (a butterfly design), from the C-terminus of one BS-BDC to the N-terminus of another BS-BDC (or vice versa), or at any other appropriate position along the length of a BS-BDC, given the particular BS-BDC grouping and associated formats within a group.

[0147] (IV) Cancer Antigen Epitope and Immune Stimulating Epitope Binding Domains. In particular embodiments, binding domains for cancer antigen epitopes or immune stimulating epitopes are based on binding domains of antibodies known within the public domain.

[0148] In particular embodiments, a binding domain VH region and/or VL region can be derived from or based on a VH or VL of a known monoclonal antibody and can include one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the VH or VL of a known monoclonal antibody. An insertion, deletion or substitution may be anywhere in the VH region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided a binding domain including the modified VH region can still specifically bind its target with an affinity similar to the wild type binding domain.

[0149] In particular embodiments, a binding domain includes or is a sequence that is at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to a known amino acid sequence of a light chain variable region (VL) or to a heavy chain variable region (VH), or both, wherein each CDR includes zero changes or at most one, two, or three changes, from a monoclonal antibody or fragment or derivative thereof that specifically binds to target of interest.

[0150] In particular embodiments, a binding domain is a single chain T cell receptor (scTCR) including $V\alpha/\beta$ and $C\alpha/\beta$ chains (e.g., $V\alpha$ - $C\alpha$, $V\beta$ - $C\beta$, $V\alpha$ - $V\beta$) or including $V\alpha$ - $C\alpha$, $V\beta$ - $C\beta$, $V\alpha$ - $V\beta$ pair specific for a target epitope of interest. In particular embodiments, T cell activating epitope binding domains can be derived from or based on a $V\alpha$, $V\beta$, $C\alpha$, or $C\beta$ of a known TCR (e.g., a high-affinity TCR).

[0151] In particular embodiments, T cell activating epitope binding domains include one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the $V\alpha$, $V\beta$, $C\alpha$, or $C\beta$ of a known TCR. An insertion, deletion or substitution may be anywhere in a $V\alpha$, $V\beta$, $C\alpha$, or $C\beta$ region, including at the amino- or carboxy-terminus or both ends of these regions, provided that each CDR includes zero changes or at most one, two, or three changes and provided a binding domain including a modified $V\alpha$, $V\beta$, $C\alpha$, or $C\beta$ region can still specifically bind its target with an affinity similar to wild type.

[0152] In particular embodiments, a binding domain includes or is a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to a known TCR or fragment or derivative thereof that specifically binds to target of interest.

[0153] In particular embodiments, TCR can be targeted with an MHC engager. For example, CD3 and/or CD28 binding domains can be replaced with single chain MHC binding ends.

[0154] In particular embodiments, BS-BDC can also include a natural receptor or ligand for an epitope as a binding domain. For example, if a target for binding includes PD-L1, the binding domains can include PD-1 (including, e.g., a PD-1/antiCD3 fusion). One example of a receptor fusion for binding is Enbrel® (Amgen). Natural receptors or ligands can also be modified to enhance binding. For example, betalacept is a modified version of abatacept. In particular embodiments, the BS-BDC can include a natural receptor or ligand that induces phagocytosis. Calreticulin (UniProt ID No. P27797) is a protein that is localized to the endoplasmic reticulum of healthy cells, but in dying cells it translocates to the cell surface and induces phagocytosis by immune cells such as macrophages. In particular embodiments, the binding domains can include calreticulin or a portion of calreticulin that is capable of inducing phagocytosis.

[0155] (V) Compositions for Administration. BS-BDC can be formulated alone or in combination into compositions for administration to subjects. In particular embodiments, compositions include at least two BS-BDC disclosed herein formulated with a pharmaceutically acceptable carrier. Salts and/or prodrugs of BS-BDC can also be used.

[0156] A pharmaceutically acceptable salt includes any salt that retains the activity of the BS-BDC and is acceptable for pharmaceutical use. A pharmaceutically acceptable salt also refers to any salt which may form in vivo as a result of administration of an acid, another salt, or a prodrug which is converted into an acid or salt.

[0157] Suitable pharmaceutically acceptable acid addition salts can be prepared from an inorganic acid or an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids can be selected from aliphatic, cycloaliphatic, aromatic, arylaliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids.

[0158] Suitable pharmaceutically acceptable base addition salts include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylene-diamine, chlorprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, lysine, arginine and procaine.

[0159] A prodrug includes an active ingredient which is converted to a therapeutically active compound after administration, such as by cleavage of a linked BS-BDC or by hydrolysis of a biologically labile group.

[0160] Exemplary generally used pharmaceutically acceptable carriers include any and all absorption delaying agents, antioxidants, binders, buffering agents, bulking agents or fillers, chelating agents, coatings, disintegration agents, dispersion media, gels, isotonic agents, lubricants, preservatives, salts, solvents or co-solvents, stabilizers, surfactants, and/or delivery vehicles.

[0161] Exemplary antioxidants include ascorbic acid, methionine, and vitamin E.

[0162] Exemplary buffering agents include citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0163] An exemplary chelating agent is EDTA.

[0164] Exemplary isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0165] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0166] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the BS-BDC or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran. Stabilizers are typically present in the range of from 0.1 to 10,000 parts by weight based on therapeutic weight.

[0167] For injection, compositions can be formulated as aqueous solutions, such as in buffers including Hanks' solution, Ringer's solution, or physiological saline. The aqueous solutions can include formulatory agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the formulation can be in lyophilized and/or powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0168] For oral administration, the compositions can be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For oral solid formulations such as powders, capsules and tablets, suitable excipients include binders (gum tragacanth, acacia, cornstarch, gelatin), fillers such as sugars, e.g. lactose, sucrose, mannitol and sorbitol; dicalcium phosphate, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxy- methylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; and binding agents. If desired, disintegrating agents can be added, such as corn starch, potato starch, alginic acid, cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. If desired, solid dosage forms can be sugar-coated or enteric-coated using standard techniques. Flavoring agents, such as peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc. can also be used.

[0169] Compositions can be formulated as an aerosol. In particular embodiments, the aerosol is provided as part of an anhydrous, liquid or dry powder inhaler. Aerosol sprays from pressurized packs or nebulizers can also be used with a suitable propellant, e.g., dichlorodifluoromethane,

trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, a dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may also be formulated including a powder mix of BS-BDC and a suitable powder base such as lactose or starch.

[0170] Compositions can also be formulated as depot preparations. Depot preparations can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salts.

[0171] Additionally, compositions can be formulated as sustained-release systems utilizing semipermeable matrices of solid polymers including at least one BS-BDC group. Various sustained-release materials have been established and are well known by those of ordinary skill in the art. Sustained-release systems may, depending on their chemical nature, release BS-BDC following administration for a few weeks up to over 100 days. Depot preparations can be administered by injection; parenteral injection; instillation; or implantation into soft tissues, a body cavity, or occasionally into a blood vessel with injection through fine needles.

[0172] Depot formulations can include a variety of bioerodible polymers including poly(lactide), poly(glycolide), poly(caprolactone) and poly(lactide)-co(glycolide) (PLG) of desirable lactide:glycolide ratios, average molecular weights, polydispersities, and terminal group chemistries. Blending different polymer types in different ratios using various grades can result in characteristics that borrow from each of the contributing polymers.

[0173] The use of different solvents (for example, dichloromethane, chloroform, ethyl acetate, triacetin, N-methyl pyrrolidone, tetrahydrofuran, phenol, or combinations thereof) can alter microparticle size and structure in order to modulate release characteristics. Other useful solvents include water, ethanol, dimethyl sulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP), acetone, methanol, isopropyl alcohol (IPA), ethyl benzoate, and benzyl benzoate.

[0174] Exemplary release modifiers can include surfactants, detergents, internal phase viscosity enhancers, complexing agents, surface active molecules, co-solvents, chelators, stabilizers, derivatives of cellulose, (hydroxypropyl)methyl cellulose (HPMC), HPMC acetate, cellulose acetate, pluronics (e.g., F68/F127), polysorbates, Span® (Croda Americas, Wilmington, Delaware), poly(vinyl alcohol) (PVA), Brij® (Croda Americas, Wilmington, Delaware), sucrose acetate isobutyrate (SAIB), salts, and buffers.

[0175] Excipients that partition into the external phase boundary of microparticles such as surfactants including polysorbates, dioctylsulfosuccinates, poloxamers, PVA, can also alter properties including particle stability and erosion rates, hydration and channel structure, interfacial transport, and kinetics

in a favorable manner.

[0176] Additional processing of the disclosed sustained release depot formulations can utilize stabilizing excipients including mannitol, sucrose, trehalose, and glycine with other components such as polysorbates, PVAs, and dioctylsulfosuccinates in buffers such as Tris, citrate, or histidine. A freeze-dry cycle can also be used to produce very low moisture powders that reconstitute to similar size and performance characteristics of the original suspension.

[0177] Particular embodiments include formulation of BS-BDC within hydrogels. Exemplary hydrogels include collagen hydrogels; type I collagen, fibrin, or a mixture thereof cross-linked, as the cross-linked state of these molecules in vivo; type I collagen hydrogels naturally cross-linked by lysyl oxidase-derived aldimine bonds (Sabeh et al., (2009) J Cell Biol 185:11-19); or other synthetic hydrogels as described in, for example, Rowe & Weiss (2008) Trends Cell Biol 18:560-574; Rowe & Weiss (2009) Annu Rev Cell Dev Biol 25:567-595; Egeblad et al., (2010) Curr Opin Cell Biol 22:697-706; Harunaga & Yamada (2011) Matrix Biol 30:363-368; Willis et al., (2013) J Microsc 251:250-260; and Gill et al. (2012) Cancer Res 72:6013-6023. In particular embodiments, a hydrogel refers to a network of polymer chains that are hydrophilic in which water or an aqueous medium is the dispersion medium. Particular embodiments may utilize a zwitterionic polymer as described in WO2016/040489.

[0178] Any composition disclosed herein can advantageously include any other pharmaceutically acceptable carriers which include those that do not produce significantly adverse, allergic, or other untoward reactions that outweigh the benefit of administration. Exemplary pharmaceutically acceptable carriers and formulations are disclosed in Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990. Moreover, formulations can be prepared to meet sterility, pyrogenicity, general safety, and purity standards as required by U.S. FDA Office of Biological Standards and/or other relevant foreign regulatory agencies.

[0179] In particular embodiments, BS-BDC may be formulated to remain inert at an administration site. In particular embodiments, this feature can be achieved with a proteolytically cleavable blocking peptide that is cleaved by proteases, such as (i) serum proteases, (ii) proteases secreted by cancers, and/or (iii) administered proteases.

[0180] Exemplary serum proteases include thrombin, Factor X, dipeptidyl peptidase IV, plasmin, and Hageman factor. Exemplary proteases secreted by cancers include ADAMTSs (A disintegrin and metalloprotease domains with thrombospondins motifs (ADAMTS-1, ADAMTS-4 and ADAMTS-5), metalloproteinases or matrix-degrading proteases (MMP3 and MMP7), urokinase-type plasminogen activator (uPA), the serine protease prostate-specific antigen (PSA), and cathepsins including *aspartic cathepsins* (cathepsins D and E) and *cysteine cathepsins* (cathepsins B, C, F, H, K, L, O, S, V, X and W).

[0181] In particular embodiments, BS-BDC may be formulated to remain inert at an administration site by altering the pH of the environment and/or by using a crystalline form of a BS-BDC, as previously applied to insulin.

[0182] In particular embodiments, the compositions include BS-BDC of at least 0.1% w/v or w/w of the composition; at least 1% w/v or w/w of composition; at least 10% w/v or w/w of composition; at least 20% w/v or w/w of composition; at least 30% w/v or w/w of composition; at least 40% w/v or w/w of composition; at least 50% w/v or w/w of composition; at least 60% w/v or w/w of composition; at least 70% w/v or w/w of composition; at least 80% w/v or w/w of composition; at least 90% w/v or w/w of composition; at least 95% w/v or w/w of composition; or at least 99% w/v or w/w of composition.

[0183] In particular embodiments, BS-BDC within a group are formulated into separate individual compositions. In particular embodiments, BS-BDC groups may be formulated into compositions together or in selected subsets of the grouping. When formulated together, the BS-BDC may be included in the same amounts or in different amounts or ratios. For example, if a BS-BDC group included 3 BS-BDC ratios of inclusion within a composition could include a 1:1:1 ratio, 2:1:1 ratio, 1:2:1 ratio, 1:1:2 ratio, 5:1:1 ratio, 1:5:1 ratio, 1:1:5 ratio, 10:1:1 ratio, 1:10:1 ratio, 1:1:10 ratio, 2:2:1 ratio, 1:2:2 ratio, 2:1:2 ratio, 5:5:1 ratio, 1:5:5 ratio, 5:1:5 ratio, 10:10:1 ratio, 1:10:10 ratio, 10:1:10 ratio, etc. If a BS-BDC group included 2 BS-BDC, the ratio can include any 2 number combination that can be created from the 3 number combinations provided above.

[0184] Compositions disclosed herein can be formulated for administration by, for example, injection, infusion, perfusion, or lavage. Protein-based compositions can further be formulated for administration by inhalation or ingestion. The compositions disclosed herein can further be formulated for intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral and/or subcutaneous administration and more particularly by intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, intrathecal, intratumoral, intramuscular, intravesicular, and/or subcutaneous injection.

[0185] (VI) Methods of Use. Methods disclosed herein include treating subjects (humans, veterinary animals (dogs, cats, reptiles, birds, etc.) livestock (horses, cattle, goats, pigs, chickens, etc.) and research animals (monkeys, rats, mice, fish, etc.) with compositions disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic treatments.

[0186] An "effective amount" is the amount of a composition necessary to result in a desired physiological change in the subject. For example, an effective amount can provide an immunogenic effect. Effective amounts are often administered for research purposes. Effective amounts disclosed

herein can cause a statistically-significant effect in an animal model or in vitro assay relevant to the assessment of a cancer's development or progression. An immunogenic composition can be provided in an effective amount, wherein the effective amount stimulates an immune response.

[0187] A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a cancer or displays only early signs or symptoms of a cancer such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the cancer further. Thus, a prophylactic treatment functions as a preventative treatment against a cancer. In particular embodiments, prophylactic treatments reduce, delay, or prevent metastasis from a primary a cancer tumor site from occurring.

[0188] A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a cancer and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the cancer. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the cancer and/or reduce control or eliminate side effects of the cancer.

[0189] Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

[0190] In particular embodiments, therapeutically effective amounts provide therapeutic anti-cancer effects. Therapeutic anti-cancer effects include a decrease in the number of cancer cells, decrease in the number of metastases, a decrease in tumor volume, an increase in life expectancy, induced chemo- or radiosensitivity in cancer cells, inhibited angiogenesis near cancer cells, inhibited cancer cell proliferation, inhibited tumor growth, prevented or reduced metastases, prolonged subject life, reduced cancer-associated pain, and/or reduced relapse or re-occurrence of cancer following treatment.

[0191] A tumor is one type of cancerous tissue. A tumor refers to a swelling or lesion formed by an abnormal growth of cells (called neoplastic cells or tumor cells). A "tumor cell" is an abnormal cell that grows by a rapid, uncontrolled cellular proliferation and continues to grow after the stimuli that initiated the new growth cease. Tumors show partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue, which may be benign, pre-malignant or malignant.

[0192] Non-cancerous tissue includes cells that exhibit what is considered normal cellular proliferation and structural organization for the particular tissue type.

[0193] For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from in vitro assays and/or animal model studies. Such information

can be used to more accurately determine useful doses in subjects of interest. The actual dose amount administered to a particular subject can be determined by a physician, veterinarian or researcher taking into account parameters such as physical and physiological factors including target, body weight, severity of condition, type of cancer, stage of cancer, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

[0194] Useful doses can range from 0.1 to 5 pg/kg or µg/kg or from 0.5 to 1 pg/kg µg/kg. In other examples, a dose can include 1 µg /kg, 15 µg /kg, 30 µg /kg, 50 µg/kg, 55 µg/kg, 70 µg/kg, 90 µg/kg, 150 µg/kg, 350 µg/kg, 500 µg/kg, 750 µg/kg, 1000 µg/kg, 0.1 to 5 mg/kg or from 0.5 to 1 mg/kg. In other non-limiting examples, a dose can include 1 mg/kg, 10 mg/kg, 30 mg/kg, 50 mg/kg, 70 mg/kg, 100 mg/kg, 300 mg/kg, 500 mg/kg, 700 mg/kg, 1000 mg/kg or more.

[0195] Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (e.g., daily, every other day, every 3 days, every 4 days, every 5 days, every 6 days, weekly, every 2 weeks, every 3 weeks, monthly, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every 8 months, every 9 months, every 10 months, every 11 months or yearly).

[0196] In particular embodiments, BS-BDC can be administered through a pump such as a programmable pump (e.g., an insulin pump). In particular embodiments, staged administration of different BS-BDC can be achieved using, for example, a programmed pump. In particular embodiments, BS-BDC can be slowly infused.

[0197] In particular embodiments, BS-BDC have a short half-life (e.g., short in vivo half-life) such that the BS-BDC are administered using continuous infusion with a pump. In particular embodiments, any BS-BDC with an in vivo half-life of less than 5 hours can be administered through continuous infusion. In contrast, antibodies can have in vivo half-lives of several weeks due to their larger size and Fc portion, and bispecific formats that contain an Fc portion can similarly have extended in vivo half-lives.

[0198] In particular embodiments, therapeutically effective amounts are administered at a time interval to reduce or eliminate cancer recurrence without causing autoimmune toxicity.

[0199] The pharmaceutical compositions described herein can be administered by injection, inhalation, infusion, perfusion, lavage or ingestion. Routes of administration can include intravenous, intradermal, intraarterial, intraparenteral, intranasal, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral, subcutaneous, and/or sublingual administration and more particularly by intravenous, intradermal, intraarterial, intraparenteral, intranasal, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral, subcutaneous, and/or sublingual injection.

[0200] In particular embodiments BS-BDC can be locally or regionally administered. For example, BS-BDC can be administered to the peritoneal cavity for abdominal cancers, such as ovarian cancer. BS-BDC can be administered in the surgical resection bed of solid tumors. BS-BDC can be administered directly into solid tumors, for example in a hydrogel, as described above.

[0201] In particular embodiments, BS-BDC are administered to different portions or areas of the body.

[0202] In particular embodiments, BS-BDC can be administered using a needle array assembly as developed by Presage Biosciences (Seattle, WA; see, for example, US Patent No. 8,475,412). In particular embodiments, BS-BDC can be administered using the CIVO® intratumoral microdosing delivery platform (Presage Biosciences).

[0203] As indicated, in particular embodiments, the administration of BS-BDC evolve over time during the course of a subject's treatment regimen. Groups of BS-BDC can combinatorically address many different types of cancer and be customized for individual subjects (e.g., A + B; A + C; A + F; B + F; etc). Likewise, there can be a very personalized aspect to the administration of BS-BDC groups in which subject samples (e.g., liquid biopsies, standard biopsies) are assessed using, for example, polymerase chain reaction (PCR), deep sequencing, flow cytometry, or immunohistochemistry (IHC) to identify emerging clones and to choose pairs of BS-BDC to specifically address an emerging clone. This "cassette" approach can involve monitoring the emergence of resistant clones and rapidly addressing them through new combinations of BS-BDC. "Emerging clone" can refer to a cancer cell or a clonal population of cancer cells with one or more alleles that are distinct from the dominant genotype of the population of cancer cells the clone was derived from. "Drug resistant clone" can refer to a cancer cell or a clonal population of cancer cells that have acquired a new allele that confers resistance to one or more cancer drugs. A patient's cancer can be monitored for the emergence of new cancer clones and/or treatment resistant clones, for example, by sequencing the DNA from a cancer sample derived from the patient.

[0204] As indicated, in particular embodiments, an in vitro analysis of optimal BS-BDC groupings can be assessed for personalized cancer cell killing. In these embodiments, a biopsy sample from a subject can be obtained along with a sample including the subject's peripheral blood mononuclear cells (PBMCs). A high throughput automated platform can be used to analyze optimal BS-BDC groupings and/or dose concentrations.

[0205] In particular embodiments, a patient can be monitored for immune suppression in the tumor microenvironment and/or T cell suppression. Immune suppression in the microenvironment and/or T cell suppression can be monitored, for example, by measuring cytokine levels and/or the number of T cells in a sample derived from the patient.

[0206] Particular embodiments include bringing a sample obtained from an individual subject into

contact with a reagent suitable for determining the expression level of CD28, KAT2B, CASK, ABCD2, DLG1, SS18, RBL2, RAB7L1, MTHFD1, BMI1, COG5, PDE4D, KERA and/or VCY, e.g., a reagent or reagents suitable for determining the expression level of one or more of said genes using RT-qPCR, microarray analysis, ELISA, protein chips, flow cytometry, mass spectrometry, or Western blotting to assess potential T cell exhaustion. For example, the reagent may be a pair or pairs of nucleic acid primers, suitable for determining the expression level of one or more of said genes using RT-qPCR. Alternatively, the reagent may be an antibody suitable for determining the expression level of said one or more genes using ELISA or Western blotting. In particular embodiments, the level of expression of said genes is determined using RT-qPCR or Flow-FISH. In particular embodiments, the level of expression of said genes is determined using RT- qPCR.

[0207] In particular embodiments, methods disclosed herein include activating immune cells in the tumor microenvironment. In particular embodiments, activating immune cells in the tumor microenvironment includes reducing or reversing T cell suppression in the tumor microenvironment. T cell suppression can refer to a block of or reduction in T cell activation, such as can be caused by regulatory T cells. Methods to measure T cell suppression can be found, for example in McMurchy & Levings, European Journal of Immunology 42(1): 27-34. Reducing or reversing T cell suppression in the tumor microenvironment can include replacing a CD28-binding BS-BDC with a BS-BDC that reduces the activity of an immune cell suppressor. This approach is beneficial when T cells in the tumor microenvironment reduce expression of CD28 following on-going activation.

[0208] In particular embodiments, one member of a BS-BDC group can be administered before others in a group. For example, injection of a subsequent BS-BDC in a group can occur after previously administered BS-BDC of the group have distributed through the body. This approach can be useful when appearance of a cancer antigen epitope or immune stimulating epitope may occur later in a timeline of events. For example, in some situations, co-stimulatory immune stimulating epitopes (e.g., CD28) will not be expressed until after a primary immune stimulating epitope is stimulated (e.g., CD3). In this scenario it can be beneficial to administer a BS-BDC that binds CD3 before administering a BS-BDC that binds CD28.

[0209] In particular embodiments, different amounts of BS-BDC within a group are administered. For example, if a BS-BDC group included 3 BS-BDC ratios of administration to a subject could include a 1:1:1 ratio, 2:1:1 ratio, 1:2:1 ratio, 1:1:2 ratio, 5:1:1 ratio, 1:5:1 ratio, 1:1:5 ratio, 10:1:1 ratio, 1:10:1 ratio, 1:1:10 ratio, 2:2:1 ratio, 1:2:2 ratio, 2:1:2 ratio, 5:5:1 ratio, 1:5:5 ratio, 5:1:5 ratio, 10:10:1 ratio, 1:10:10 ratio, 10:1:10 ratio, etc. If a BS-BDC group included 2 BS-BDC, the ratio can include any 2 number combination that can be created from the 3 number combinations provided above. In particular embodiments, a BS-BDC that binds a co-stimulatory immune stimulating epitope may be

administered at 1/10 the dose of a BS-BDC that binds a primary immune stimulating epitope.

[0210] In particular embodiments, BS-BDC with different half-lives are administered. For example, if a BS-BDC has no anti-cancer effect individually, this BS-BDC could be administered on a time and dose schedule to remain at or near the top of its therapeutic window as a long-lived architecture (e.g., a BS-BDC fused to a half-life extender). Subsequently, a BS-BDC with a shorter in vivo half-life could be administered. This approach would allow fast clearance of the shorter half-live BS-BDC in the event of an adverse reaction, such as a cytokine storm.

[0211] In particular embodiments, BS-BDC groups can be administered with other treatments, such as macrophage modulators, NK cell modulators and/or chemotherapeutic agents.

[0212] Exemplary macrophage modulators include bacterial cell wall products such as lipopolysaccharide (LPS) and muramyl dipeptide (MDP) or cytokines such as interferon-gamma (IFN- γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Additional examples of macrophage modulators include aminoalkyl glucosaminide phosphates (AGPs) and/or Mifamurtide, a fully synthetic derivative of MDP. Frampton, *Paediatr Drugs*. 2010 Jun; 12(3): 141-53. Additional examples of macrophage modulators include etherally monosubstituted monosaccharides including 3-O-3'-(N', N'-dimethylamino-n-propyl)-D-glucopyranose, 3-O-4'-(N-methyl piperidyl)-D-glucopyranose, 3-O-2'-(N', N'-dimethylaminoethyl)-D-glucopyranose, 3-O-3'-(2', N', N'-trimethylamino-n-propyl)-D-glucopyranose, α -N', N'-dimethylaminoisopropyl-D-glucoside, 6-O-3'-(N', N'-dimethylamino-n-propyl)-D-galactopyranose, 3-O-2'-(N', N'-dimethylaminopropyl)-D-galactopyranose, and 6-O-2'-(N', N'-dimethylaminopropyl)-D-galactopyranose. Emodin, a trihydroxy-anthraquinone which is found in several Chinese herbs including rhubarb (*Rheum palmatum*) and tuber fleece flower (*Polygonum multiflorum*, also commonly known as Chinese knotweed or he shou wu) may also be used.

[0213] Exemplary natural killer cells modulators include α -GalCer (also known as KRN7000), a simplified glycolipid analogue of agelasphin, a compound originally isolated from a marine sponge *Agelas mauritianu*, and its analogues, such as α -C-galactosylceramide (α -C-GalCer), a synthetic C-glycoside analogue, phenyl α -GalCer (C34), and OCH, an α -GalCer analogue with a shorter phytosphingosine chain, 7DW8-5, a phenyl glycolipid, and PBS-25, C6Ph, C8Ph, C8PhF, and C10Ph, analogues with Phenyl ring substitutions (Jung, *J Biomed Sci*. 2017; 24: 22). Additional compounds with natural killer cell modulator effects include cytokines such as interleukin (IL)-2, IL-12, IL-15, IL-18 and IL-21, and type 1 interferons (IFNs) (Zwirner, *NW Biofactors*. 2010 Jul-Aug; 36(4):274-88), Phorbol myristate acetate (PMA), ionomycin, an ionophore produced by the bacterium *Streptomyces conglobatus*, calcimycin (A23187), a mobile ion-carrier that forms stable complexes with divalent cations produced at fermentation of *Streptomyces chartreusensis* (Gasteiger

G, J Exp Med. 2013 Jun 3;210(6):1167-78), and bacterial lipopolysaccharide, or LPS (Kepell MP. J Immunol. 2015 Feb 15; 194(4): 1954–1962). Other examples include several groups of NK cell-activating natural compounds such as vitamins belonging to classes A, B, C, D, and E, polysaccharides, such as sulfated polysaccharides (SP) from the seaweed *Codium fragile*, lectins such as the mistletoe extract Iscador® (Verein für Krebsforschung Association, Arlesheim Switzerland), and a number of phytochemicals used in cancer research such as genistein, curcumin, ginseng extract, garlic extract, resveratrol, ashwagandha extract, ingenol mebutate, kumquat pericarp extract, and prostratin (Grudzien and Rapak, Journal of Immunology Research, Volume 2018, Article ID 4868417).

[0214] (VII). Expression Methods. Sequence information provided by public databases can be used to identify additional gene and protein sequences that can be used with the systems and methods disclosed herein.

[0215] In particular embodiments, BS-BDC disclosed herein are formed using the Daedalus expression system as described in Pechman et al., Am J Physiol 294: R1234-R1239, 2008. The Daedalus system utilizes inclusion of minimized ubiquitous chromatin opening elements in transduction vectors to reduce or prevent genomic silencing and to help maintain the stability of decigram levels of expression. This system can bypass tedious and time-consuming steps of other protein production methods by employing the secretion pathway of serum-free adapted human suspension cell lines, such as 293 Freestyle. Using optimized lentiviral vectors, yields of 20-100 mg/l of correctly folded and post-translationally modified, endotoxin-free protein of up to 70 kDa in size, can be achieved in conventional, small-scale (100 ml) culture. At these yields, most proteins can be purified using a single size-exclusion chromatography step, immediately appropriate for use in structural, biophysical or therapeutic applications. Bandaranayake et al., Nucleic Acids Res., 2011 (Nov); 39(21). In some instances, purification by chromatography may not be needed due to the purity of manufacture according the methods described herein.

[0216] Particular embodiments utilize DNA constructs (e.g., chimeric genes, expression cassettes, expression vectors, recombination vectors, etc.) including a nucleic acid sequence encoding the protein or proteins of interest operatively linked to appropriate regulatory sequences. Such DNA constructs are not naturally-occurring DNA molecules and are useful for introducing DNA into host-cells to express selected proteins of interest.

[0217] Operatively linked refers to the linking of DNA sequences (including the order of the sequences, the orientation of the sequences, and the relative spacing of the various sequences) in such a manner that the encoded protein is expressed. Methods of operatively linking expression control sequences to coding sequences are well known in the art. See, e.g., Maniatis et al., Molecular

Cloning: A Laboratory Manual, Cold Spring Harbor, N. Y., 1982; and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N. Y., 1989.

[0218] Expression control sequences are DNA sequences involved in any way in the control of transcription or translation. Suitable expression control sequences and methods of making and using them are well known in the art. Expression control sequences generally include a promoter. The promoter may be inducible or constitutive. It may be naturally-occurring, may be composed of portions of various naturally-occurring promoters, or may be partially or totally synthetic. Guidance for the design of promoters is provided by studies of promoter structure, such as that of Harley and Reynolds, *Nucleic Acids Res.*, 15, 2343-2361, 1987. Also, the location of the promoter relative to the transcription start may be optimized. See, e.g., Roberts et al., *Proc. Natl. Acad. Sci. USA*, 76:760-764, 1979.

[0219] The promoter may include, or be modified to include, one or more enhancer elements. In particular embodiments, the promoter will include a plurality of enhancer elements. Promoters including enhancer elements can provide for higher levels of transcription as compared to promoters that do not include them.

[0220] For efficient expression, the coding sequences can be operatively linked to a 3' untranslated sequence. In particular embodiments, the 3' untranslated sequence can include a transcription termination sequence and a polyadenylation sequence. The 3' untranslated region can be obtained, for example, from the flanking regions of genes.

[0221] In particular embodiments, a 5' untranslated leader sequence can also be employed. The 5' untranslated leader sequence is the portion of an mRNA that extends from the 5' CAP site to the translation initiation codon.

[0222] In particular embodiments, a "hisavi" tag can be added to the N-terminus or C-terminus of a gene by the addition of nucleotides coding for the Avitag amino acid sequence, "GLNDIFEAQKIEWHE" (SEQ ID NO: 33), as well as the 6xhistidine tag coding sequence "HHHHHH" (SEQ ID NO: 34)". The Avitag avidity tag can be biotinylated by a biotin ligase to allow for biotin-avidin or biotin-streptavidin based interactions for protein purification, as well as for immunobiology (such as immunoblotting or immunofluorescence) using anti-biotin antibodies. The 6xhistidine tag allows for protein purification using Ni-2+ affinity chromatography.

[0223] Nucleic acid sequences encoding proteins disclosed herein can be derived by those of ordinary skill in the art. Nucleic acid sequences can also include one or more of various sequence polymorphisms, mutations, and/or sequence variants. In particular embodiments, the sequence polymorphisms, mutations, and/or sequence variants do not affect the function of the encoded protein. The sequences can also include degenerate codons of a native sequence or sequences that

may be introduced to provide codon preference.

[0224] In some aspects, the DNA constructs can be introduced by transfection, a technique that involves introduction of foreign DNA into the nucleus of eukaryotic cells. In some aspects, the proteins can be synthesized by transient transfection (DNA does not integrate with the genome of the eukaryotic cells, but the genes are expressed for 24-96 hours). Various methods can be used to introduce the foreign DNA into the host-cells, and transfection can be achieved by chemical-based means including by the calcium phosphate, by dendrimers, by liposomes, and by the use of cationic polymers. Non-chemical methods of transfection include electroporation, sono-poration, optical transfection, protoplast fusion, impalefection, and hydrodynamic delivery. In some embodiments, transfection can be achieved by particle-based methods including gene gun where the DNA construct is coupled to a nanoparticle of an inert solid which is then "shot" directly into the target-cell's nucleus. Other particle-based transfection methods include magnet assisted transfection and impalefection.

[0225] As indicated previously in relation to the discussion of binding domain sequences and encoding gene sequences, variants of the sequences disclosed and referenced herein are also included. Variants of proteins can include those having one or more conservative amino acid substitutions or one or more non-conservative substitutions that do not adversely affect the function of the protein in a measure described in for example, FIGs. 4A-4C. In particular embodiments, variants are created to reduce the physiological effect of a protein at a given dose according to a measure as described in FIGs. 4A-4C.

[0226] A "conservative substitution" involves a substitution found in one of the following conservative substitutions groups: Group 1: Alanine (Ala), Glycine (Gly), Serine (Ser), Threonine (Thr); Group 2: Aspartic acid (Asp), Glutamic acid (Glu); Group 3: Asparagine (Asn), Glutamine (Gln); Group 4: Arginine (Arg), Lysine (Lys), Histidine (His); Group 5: Isoleucine (Ile), Leucine (Leu), Methionine (Met), Valine (Val); and Group 6: Phenylalanine (Phe), Tyrosine (Tyr), Tryptophan (Trp).

[0227] Additionally, amino acids can be grouped into conservative substitution groups by similar function or chemical structure or composition (e.g., acidic, basic, aliphatic, aromatic, sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other groups containing amino acids that are considered conservative substitutions for one another include: sulfur-containing: Met and Cysteine (Cys); acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. Additional information is found in Creighton (1984) *Proteins*, W.H. Freeman and Company.

[0228] As indicated elsewhere, variants of gene sequences can include codon optimized variants, sequence polymorphisms, splice variants, and/or mutations that do not affect the function of an encoded product to a statistically-significant degree.

[0229] Variants of the protein and nucleic acid sequences disclosed herein also include sequences with at least 70% sequence identity, 80% sequence identity, 85% sequence, 90% sequence identity, 95% sequence identity, 96% sequence identity, 97% sequence identity, 98% sequence identity, or 99% sequence identity to the protein and nucleic acid sequences described or disclosed herein.

[0230] "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between protein and nucleic acid sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily calculated by known methods, including (but not limited to) those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp CABIOS, 5, 151-153 (1989) with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul, et al., J. Mol. Biol. 215:403-410 (1990); DNASTAR (DNASTAR, Inc., Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.). Within the context of this disclosure it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program referenced. "Default values" will mean any set of values or parameters, which originally load with the software when first initialized.

[0231] (VIII) Additional Exemplary Experimental Protocols. (a) Healthy donor T cells: Unstimulated mononuclear cells will be collected from healthy adult volunteers via leukapheresis by the FHCRC Hematopoietic Cell Processing Core under IRB-approved research protocols as used in prior

bispecific antibody studies. T cells will be enriched through magnetic cell sorting, and then frozen in de-identified fashion in aliquots and stored in liquid nitrogen until use. Thawed cell aliquots will be labeled with CellVue Burgundy to allow separation from cancer cells.

[0232] Cytolytic properties of all bispecific T cell engaging antibodies will be determined in comparative in vitro assays that have been successfully employed to characterize other bispecific T cell engaging antibodies. ROR1+ primary tumor cells (JeKo) and transfected cells (K562/ROR1) are available for these studies, and ROR1 expression constructs that permit the generation of additional cell lines if necessary are also available. Appropriate ROR1- cells (e.g. parental K562 cells or MKN45 cells) will serve as negative controls. ROR1+ or ROR1- cell lines will be incubated in 96-well round bottom plates at 5-10,000 cells/well in 225 μ L of appropriate culture medium including various concentrations of individual bispecific T cell engaging antibodies in the presence or absence of healthy donor T cells added at different E:T cell ratios. After 48 hours, cell numbers and drug-induced cytotoxicity, using 4',6-diamidino-2-phenylindole (DAPI) to detect non-viable cells, will be determined using a LSRII cytometer. In experiments where healthy donor T cells are added, cancer cells will be identified by forward/side scatter properties and negativity for CellVue Burgundy dye.

[0233] Constructs of interest will be tested for their ability to mediate anti-tumor activity in NSG mice engrafted with human T- cells and firefly luciferase-expressing ROR1+ tumor cells (JeKo and MDA-MB231) that represent hematological (JeKo) and solid tumor (MDA-MB231) cell models. NSG mice will also be used for studies to determine serum half-lives of antibodies of interest. In these studies, blood will be collected by cardiac puncture at euthanasia and analyzed by mass spectrometry or scintillation counting. These studies will identify lead candidate humanized antibody bispecific T cell engaging molecule(s) that can be used for further testing.

[0234] Once lead candidate antibodies have been identified, larger-scale production of clinical-grade antibodies for preclinical safety and initial human clinical trials will be initiated. It is important to note that the Biologics Production Facility at FHCRC, as a current Good Manufacturing Processes (cGMP) laboratory, can generate validated biologics for Phase 1/2 studies. For example, the scFv to CD3, derived from the OKT3 antibody, is the same as the one utilized in blinatumomab, whereas the CD28 antibody that is being derived from scFv sequences has been demonstrated to activate T cells in vivo.

[0235] Statistical considerations: Predominantly, standard descriptive statistics for paired analyses will be used, which will be performed in consultation with a biostatistician.

[0236] (b) To quantify T cell activation, target antigen-negative and target antigen-positive cancer cells can be labeled with CellVue dye and incubated in 96-well plates together with unlabeled healthy-donor T cells at an effector:target (E:T) ratio of 1:1. Parallel cultures can be treated with a CD3 antibody (clone OKT3, low endotoxin/azide-free; BioLegend, San Diego, CA, USA) or a CD3 BiTE in

combination with either a CD28 antibody (clone CD28.2, low endotoxin/azide-free; BioLegend) or a CD28 BiTE. After 12–24 hours, T cell activation can be assessed by flow cytometry after staining of cells with fluorescently labeled antibodies recognizing CD3 (clone UCHT1, FITC-labeled; BD Biosciences, San Jose, CA, USA), CD4 (clone SK3, BV786-labeled; BD Biosciences), CD8 (clone RPA-T8, PE-Cy7-labeled; BD Biosciences), CD25 (clone M-A2451, APC-labeled; BD Biosciences), and CD69 (clone FN50, PE-labeled; BD Biosciences). Using 4',6-diamidino-2-phenylindole (DAPI) to separate non-viable cells, induction of CD25 and CD69 can be analyzed on CellVue dye-negative cells with FlowJo Software (Tree Star, Ashland, OR). To quantify antibody-induced cytotoxicity, cancer cells can be incubated in 96-well plates with various concentrations of monoclonal or bispecific antibodies as well as CellVue dye-labeled T cells at different E:T cell ratios. After 48 hours, cell numbers and drug-induced cytotoxicity, using DAPI to detect non-viable cells, can be determined by flow cytometry. AML cells can be identified by forward/side scatter properties and negativity for the CellVue dye. Repeated measures one-way or two-way ANOVA method with Tukey's multiple comparison testing can be used for statistical analysis with provision of two-sided *P*-values (Prism 7.0c; GraphPad [La Jolla, CA, USA]).

[0237] (c) Construction, expression, and purification of bispecific antibody constructs. BS-BDC can be constructed using the canonical architecture of blinatumomab (FIG. 6A) and variable domain sequences available from the literature. Protein sequences can be reverse-translated using human codons and cloned into a modified pCVL lentiviral vector as described in Bandaranayake et al., *Nucleic Acids Res* 2011; 39(21): e143. Lentivirus can be produced by transient co-transfection with psPAX2 and pMD2.G of 293T cells and used to transduce Freestyle™ 293-F cells. Cultures can be expanded to 2 liters and supernatants can be harvested by centrifugation. Secreted fusion protein can be extracted from the conditioned media using immobilized metal-affinity chromatography (Ni-NTA) and subsequently polished using size exclusion chromatography on an ÄKTA pure instrument (GE Healthcare Life Sciences, Pittsburgh, PA, USA) equipped with a Superdex 200 Increase 10/300GL running at 0.75 mL/min in PBS. Fractions corresponding to the monomeric proteins can be pooled, quantitated using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and further analyzed by SDS-PAGE under non-reducing and reducing conditions.

[0238] Preparation of healthy donor T cells. Unstimulated peripheral blood mononuclear cells can be collected from healthy adult volunteers via leukapheresis by the Fred Hutchinson Cancer Research Center (Fred Hutch) Hematopoietic Cell Processing Core under research protocols approved by the Fred Hutch Institutional Review Board (IRB) after written informed consent is obtained. T cells can be enriched via negative selection through magnetic cell sorting (Pan T Cell Isolation Kit; Miltenyi Biotec, Auburn, CA, USA), and then frozen in aliquots and stored in liquid nitrogen as described in Reusch et

al., Clin Cancer Res 2016; 22(23): 5829-5838. Thawed cell aliquots can either be used unlabeled or labeled with 3 μ M CellVue Burgundy or Jade (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. See, e.g., Reusch et al., Clin Cancer Res 2016; 22(23): 5829-5838; Laszlo et al., Blood 2014; 123(4): 554-561; Laszlo, et al., Blood Cancer J 2015; 5: e340; Harrington et al., PLoS One 2015; 10(8): e0135945.

[0239] Parental and engineered human cancer cell lines. Human myeloid K562 cells can be maintained in RPMI 1640 supplemented with 10% fetal bovine serum. Human lymphoid RCH-ACV and REH cells can be maintained as described in Laszlo et al., Oncotarget 2016; 7(28): 43281-43294. Sublines of cells overexpressing PD-L1 can be generated through transduction with a pRRLsin.cPPT.MSCV lentivirus containing a human PD-L1-IRES-Enhanced Green Fluorescent Protein (EGFP) cassette⁴ at a multiplicity of infection (MOI) of 0.25-25. See Laszlo et al., Blood 2014; 123(4): 554-561; Laszlo et al., Oncotarget 2016; 7(28): 43281-43294; Walter et al., Blood 2005; 105(3): 1295-1302; Laszlo, et al., Blood Cancer J 2015; 5: e340.

[0240] EGFP-positive cells can be isolated by flow cytometry and re-cultured for further analysis. Sublines of K562, RCH-ACV, and REH cells overexpressing ROR1 can be generated through transduction with a pMP71 retrovirus. ROR1-positive cells can be isolated by flow cytometry and re-cultured for further analysis.

[0241] Quantification of T cell activation. Target antigen-negative and target antigen-positive cancer cells can be labeled with 3 μ M CellVue Burgundy and incubated at 8×10^3 cells/well in 240 μ L culture medium in 96-well round bottom plates together with unlabeled healthy donor T cells at an effector:target (E:T) ratio of 1:1. Parallel cultures can be treated with a CD3 BS-BDC in combination or a CD28 BS-BDC. After 12-24 hours, T cell activation can be assessed using a LSRII flow cytometer (BD Biosciences) after staining of cells with fluorescently labeled antibodies recognizing CD3 (clone UCHT1, FITC-labeled; BD Biosciences, San Jose, CA, USA), CD4 (clone SK3, BV786-labeled; BD Biosciences), CD8 (clone RPA-T8, PE-Cy7-labeled; BD Biosciences), CD25 (clone M-A2451, APC-labeled; BD Biosciences), and CD69 (clone FN50, PE-labeled; BD Biosciences). Using 4',6-diamidino-2-phenylindole (DAPI) to separate non-viable cells, induction of CD25 and CD69 can be analyzed on CellVue dye-negative cells with FlowJo Software (Tree Star, Ashland, OR).

[0242] Quantification of drug-induced cytotoxicity. BS-BDC-induced cytotoxicity can be determined as described in Reusch et al., Clin Cancer Res 2016; 22(23): 5829-5838; Laszlo et al., Blood 2014; 123(4): 554-561; Laszlo, et al., Blood Cancer J 2015; 5: e340; Harrington et al., PLoS One 2015; 10(8): e0135945. Briefly, cancer cells can be incubated at 37°C (in 5% CO₂ and air) in 96-well round bottom plates (BD Falcon™) at 8×10^3 cells/well in 240 μ L culture medium containing various concentrations of monoclonal or bispecific antibodies as well as CellVue dye-labeled T cells at

different E:T cell ratios. After 48 hours, cell numbers and drug-induced cytotoxicity, using DAPI to detect non-viable cells, can be determined using a LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo Software. AML cells can be identified by forward/side scatter properties and negativity for the CellVue dye.

[0243] Statistical considerations. Repeated measures one-way or two-way ANOVA method with Tukey's multiple comparison testing can be used for statistical analysis. All *P*-values are two-sided. Statistical analyses were performed using Prism 7.0c (GraphPad; La Jolla, CA). Tests for silence and synergism as described herein can also be used.

[0244] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms "include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." The transition term "comprise" or "comprises" means includes, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase "consisting of" excludes any element, step, ingredient or component not specified. The transition phrase "consisting essentially of" limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause either: (i) a BS-BDC to show anti-cancer activity at a previously silent dose according to a measure shown in FIGs. 4A-4C or (ii) a group of BS-BDC to lose its synergistic effect at a previous silence and synergy dose according to a measure shown in FIGs. 4A-4C.

[0245] While the term "dose" is used throughout the specification, in particular embodiments, in vitro studies evaluate concentrations while in vivo studies evaluate doses. Thus, a dose includes a concentration in an vitro study and an administered amount in an in vivo study.

[0246] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term "about" has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of $\pm 20\%$ of the

stated value; $\pm 19\%$ of the stated value; $\pm 18\%$ of the stated value; $\pm 17\%$ of the stated value; $\pm 16\%$ of the stated value; $\pm 15\%$ of the stated value; $\pm 14\%$ of the stated value; $\pm 13\%$ of the stated value; $\pm 12\%$ of the stated value; $\pm 11\%$ of the stated value; $\pm 10\%$ of the stated value; $\pm 9\%$ of the stated value; $\pm 8\%$ of the stated value; $\pm 7\%$ of the stated value; $\pm 6\%$ of the stated value; $\pm 5\%$ of the stated value; $\pm 4\%$ of the stated value; $\pm 3\%$ of the stated value; $\pm 2\%$ of the stated value; or $\pm 1\%$ of the stated value.

[0247] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0248] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0249] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0250] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all

possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0251] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0252] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0253] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0254] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

What is claimed is:

1. A method of testing a bispecific binding domain construct (BS-BDC) that binds a first cancer antigen epitope and a first immune cell stimulating epitope for inclusion in a combination therapy comprising:

Obtaining the BS-BDC that binds the first cancer antigen epitope, wherein the first cancer antigen epitope is co-expressed in cancerous tissue with a second cancer antigen epitope wherein the first cancer antigen epitope and the second cancer antigen epitope are not significantly co-expressed in non-cancerous tissue;

Testing the BS-BDC for anti-cancer activity across a range of doses in the presence of (i) cancerous tissue expressing the first cancer antigen epitope; and (ii) T cells expressing the first immune cell stimulating epitope;

Identifying a dose where the BS-BDC shows little to no anti-cancer activity in the test thereby identifying a silence dose range for the BS-BDC;

Testing the BS-BDC for anti-cancer activity across the silence dose range within a silence dose range of a second BS-BDC that binds the second cancer antigen epitope a second immune cell stimulating epitope;

Identifying a silence and synergy dose range for the BS-BDC and the second-BS-BDC if the BS-BDC and second BS-BDC showed synergistic anti-cancer activity in the presence of (i) cancerous tissue expressing the first cancer antigen epitope; (ii) T cells expressing the first immune cell stimulating epitope; (iii) cancerous tissue expressing the second cancer antigen epitope; and (iv) T cells expressing the second immune cell stimulating epitope;

Thereby testing the BS-BDC for inclusion in a combination therapy wherein:

the BS-BDC can be included in the combination therapy with the second BS-BDC if a silence and synergy dose is identified;

the BS-BDC can be modified to reduce its anti-cancer activity if a silence and synergy dose is not identified; or

the BS-BDC can be discarded as a potential combination therapy group member if a silence and synergy dose is not identified.

2. The method of claim 1, wherein the testing for anti-cancer activity utilizes a T cell cytotoxicity assay.

3. The method of claim 1, wherein little to no anti-cancer effect defining a silence dose range is less than 20%, less than 15%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than

0.5%, less than 0.4%, less than 0.3%, less than 0.2%, or less than 0.1% T cell mediated cytotoxicity in the T cell cytotoxicity assay.

4. The method of claim 1, wherein the testing for anti-cancer activity is a cytokine release assay.

5. The method of claim 4, wherein the cytokine release assay measures IL-2, IL-4, IL-6, IL-8, IL-10, IFN- γ , and/or TNF- α .

6. The method of claim 1, wherein the testing for anti-cancer activity utilizes an assay to measure: increased T cell proliferation; increased T cell survival; delayed T cell dysfunction; deletion of inhibitory immune cells such as regulatory T cells; improved anti-tumor activity of other infiltrating immune cells such as macrophages, dendritic cells or natural killer cells; or selective activation of CD4+ versus CD8+ T cells.

7. The method of claim 4, wherein little to no anti-cancer effect defining a silence dose range is less than a 20% increase, less than 15% increase, less than 10% increase, less than 9% increase, less than 8% increase, less than 7% increase, less than 6% increase, less than 5% increase, less than 4% increase, less than 3% increase, less than 2% increase, less than 1% increase, less than 0.5% increase, less than 0.4% increase, less than 0.3% increase, less than 0.2% increase, or less than 0.1% increase in cytokine release.

8. The method of claim 4, wherein little to no anti-cancer effect defining a silence dose range is cytokine release remaining below the cytokine release assay's lower limit of detection.

9. The method of any one of claims 1-8, wherein synergistic anti-cancer activity defining a silence and synergy dose range is defined by a combination effect of the BS-BDC and the second BS-BDC that is greater than the sum of the effects resulting from the BS-BDC and the second BS-BDC individually in the T cell cytotoxicity assay or cytokine release assay.

10. The method of any one of claims 1-8, wherein synergistic anti-cancer activity defining a silence and synergy dose range is defined by a combination index (CI) < 1.

11. The method of claim 10, wherein $CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2$ wherein (D)₁ is the individual concentration of BS-BDC1 in BS-BDC1 and BS-BDC2 combinations; (D)₂ is the individual concentration of BS-BDC2 in BS-BDC1 and BS-BDC2 combinations; (Dx)₁ is the concentration of BS-BDC1 without BS-BDC2 that results in a particular effect;

and

(Dx)₂ is the concentration of BS-BDC₂ without BS-BDC₁ that results in the particular effect.

12. The method of any one of claims 1-8, wherein synergistic anti-cancer activity defining a silence and synergy dose range is defined by a combination effect of the BS-BDC and the second BS-BDC that is 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10, 5 times, or more than 2 times more than the effects resulting from the individual BS-BDC and second BS-BDC in the T cell cytotoxicity assay or cytokine release assay.

13. The method of any one of claims 1-8, wherein synergistic anti-cancer activity defining a silence and synergy dose range is defined by a combination effect of the BS-BDC and the second BS-BDC that is 1, 2, or 3 standard deviations above the effects resulting from the individual BS-BDC and second BS-BDC in the T cell cytotoxicity assay or cytokine release assay.

14. The method of any one of claims 1-13, wherein the anti-cancer effect is an in vitro anti-cancer effect.

15. The method of any one of claims 1-13, wherein the anti-cancer effect is an in vivo anti-cancer effect.

16. The method of any one of claims 1-15, wherein the first cancer antigen epitope is expressed in non-cancerous tissue.

17. The method of any one of claims 1-16, wherein the second cancer antigen epitope is expressed in non-cancerous tissue.

18. The method of any one of claims 1-17, wherein the first and second cancer antigen epitopes are expressed in non-cancerous tissue.

19. The method of any one of claims 1-18, wherein the first and second cancer antigen epitopes are not co-expressed in non-cancerous tissue.

20. The method of any one of claims 1-19, wherein the first and second cancer antigen epitopes are not expressed by the same cell type within a tissue.

21. The method of any one of claims 1-19, wherein the first and second cancer antigen epitopes are expressed by different cell types within a tissue.

22. The method of any one of claims 1-19, wherein the first and second cancer antigen epitopes are co-expressed by a cell type that is temporarily expendable.

23. The method of claim 22, wherein the cell type that is temporarily expendable comprises red blood cells, white blood cells, platelets, or non-critical cells within an organ.

24. The method of any one of claims 1-23, wherein the BS-BDC does not bind the first cancer antigen epitope in its shed form.

25. The method of any one of claims 1-18, wherein the first cancer antigen epitope and the second cancer antigen epitope are expressed in different non-cancerous tissues at different levels and wherein the BS-BDC binds a primary immune stimulating epitope and the cancer antigen epitope with the lower expression in non-cancerous tissue and the second BS-BDC binds a co-stimulatory immune stimulating epitope and the cancer antigen epitope with the higher expression in non-cancerous tissue.

26. The method of any one of claims 1-25, wherein the sequences contained within the BS-BDC obtained for testing are prioritized and obtained for testing based on having passed a regulatory safety test and failed a regulatory efficacy test.

27. The method of any one of claims 1-25, wherein the sequences contained within the BS-BDC obtained for testing are prioritized and obtained for testing based on showing on-target/off-cancer toxicities in previous experimental and/or regulatory testing.

28. The method of any one of claims 1-27, wherein the first cancer antigen epitope is selected from Mesothelin; MUC16; FOLR; PD-L1; ROR1; GPC2; GD2; HER2; EGFR; or CD19.

29. The method of any one of claims 1-28, wherein the first immune cell stimulating epitope is selected from CD3, CD28, CD2, CD27, OX40, or 4-1BB.

30. The method of any one of claims 1-28, wherein the first immune cell stimulating epitope is a primary immune cell stimulating epitope.

31. The method of claim 30, wherein the primary immune cell stimulating epitope is CD3.

32. The method of claim 31, wherein the primary immune cell stimulating epitope is CD3Δ.

33. The method of any one of claims 1-32, wherein the second immune cell stimulating epitope is a co-stimulatory immune cell stimulating epitope.
34. The method of claim 33, wherein the co-stimulatory immune cell stimulating epitope is CD28, CD2, CD27, OX40, or 4-1BB.
35. The method of any one of claims 1-34, wherein the BS-BDC comprises an scFv.
36. The method of any one of claims 1-34, wherein the BS-BDC comprises IgG.
37. The method of any one of claims 1-36, wherein at least one binding domain of the BS-BDC is mutated to decrease or increase binding affinity.
38. The method of any one of claims 1-37, wherein the BS-BDC and second BS-BDC are linked.
39. The method of claim 38, wherein the BS-BDC and second BS-BDC are linked through a flexible linker.
40. The method of claim 38, wherein the BS-BDC and second BS-BDC are linked through a cleavable linker.
41. The method of any one of claims 1-40, wherein the BS-BDC is modified to produce an administration benefit, a regulatory benefit and/or a stoichiometric benefit.
42. The method of claim 41, wherein the administration benefit is extended half-life, altered effector function, or enhanced tumor penetration.
43. The method of claim 42, wherein half-life is extended by associating the BS-BDC with a single chain Fc, albumin, transferrin, palmitic acid, an albumin binding domain, and/or polyethylene glycol (PEG).
44. The method of any one of claims 1-43, wherein the first and second cancer antigen epitopes are co-expressed, respectively, at a density of at least 100 copies per cell in the cancerous tissue.
45. The method of claim 44, wherein the density is at least 250 or 500 copies per cell.
46. The method of claim 44, wherein the density is at least 1000 copies per cell.

47. The method of claim 44, wherein the density is at least 2500 or 5000 copies per cell.

48. The method of claim 44, wherein the density is at least 10,000 copies per cell.

49. A method comprising testing bispecific binding domain constructs (BS-BDC) for silence and synergy combination therapy groupings comprising

Identifying cancer antigen epitopes that are co-expressed in cancerous tissue but not co-expressed in non-cancerous tissue;

Testing BS-BDC that bind one of the co-expressed cancer antigen epitopes and an immune cell stimulating epitope for anti-cancer activity in the presence of (i) cancerous tissue expressing the co-expressed cancer antigen epitope bound by the BS-BDC and (ii) T cells expressing the immune cell stimulating epitope bound by the BS-BDC;

Identifying a silence dose range wherein the BS-BDC shows little to no anti-cancer activity in the test;

Testing a second BS-BDC that binds a different co-expressed cancer antigen epitope and a different immune cell stimulating epitope for anti-cancer activity in the presence of (i) cancerous tissue expressing the different co-expressed cancer antigen epitope bound by the second BS-BDC and (ii) T cells expressing the second immune cell stimulating epitope bound by the second BS-BDC;

Identifying a silence dose range wherein the second BS-BDC shows little to no anti-cancer activity in the test;

Testing the BS-BDC and the second BS-BDC for anti-cancer activity together within the silence dose range of each in the presence of (i) cancerous tissue expressing the co-expressed cancer antigen epitopes; (ii) T cells expressing the immune cell stimulating epitopes bound by the BS-BDC and the second BS-BDC;

Identifying a silence and synergy dose range if the testing reveals a synergistic anti-cancer effect.

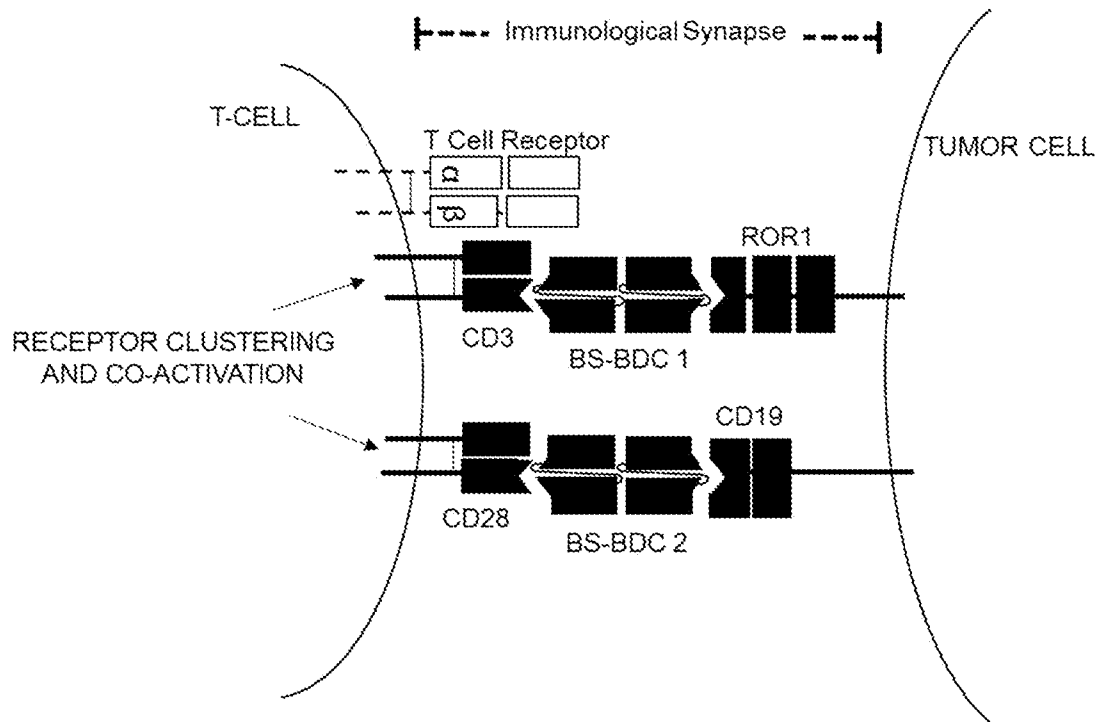
50. A method of providing an anti-cancer effect in a subject in need thereof comprising administering a combination therapy identified according to a method of any of claims 1-48 to the subject thereby providing an anti-cancer effect in the subject in need thereof.

51. The method of claim 50, comprising adjusting members of the combination therapy based on changed expression levels of targeted cancer antigen epitopes and/or immune stimulating epitopes within the subject at the subject's cancer site.

52. The method of claim 50, wherein a BS-BDC binding 4-1BB replaces a BS-BDC binding CD28 when CD28 expression is reduced in the subject at the subject's cancer site as compared to a previous level of CD28 expression in the subject at the subject's cancer site.
53. The method of claim 50, further comprising monitoring the subject for T cell exhaustion.
54. The method of claim 53, further comprising adjusting members of the combination therapy when T cell exhaustion is observed within the subject.
55. The method of claim 50, further comprising monitoring the subject's for upregulated PD-L1 expression.
56. The method of claim 55, further comprising administering a BS-BDC that binds PD-L1 to the subject when upregulated PD-L1 expression is observed within the subject as compared to a previous level of PD-L1 expression in the subject at the subject's cancer site.
57. The method of claim 50, further comprising administering a compound that alters the subject's cancer site and administering a BS-BDC based on the altered cancer site.
58. The method of claim 57, wherein the compound is interferon gamma (IFN γ).
59. The method of claim 58, wherein the BS-BDC binds PD-L1 upregulated at the subject's cancer site following administration of the IFN γ .

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FIG. 1A



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FIG. 1B

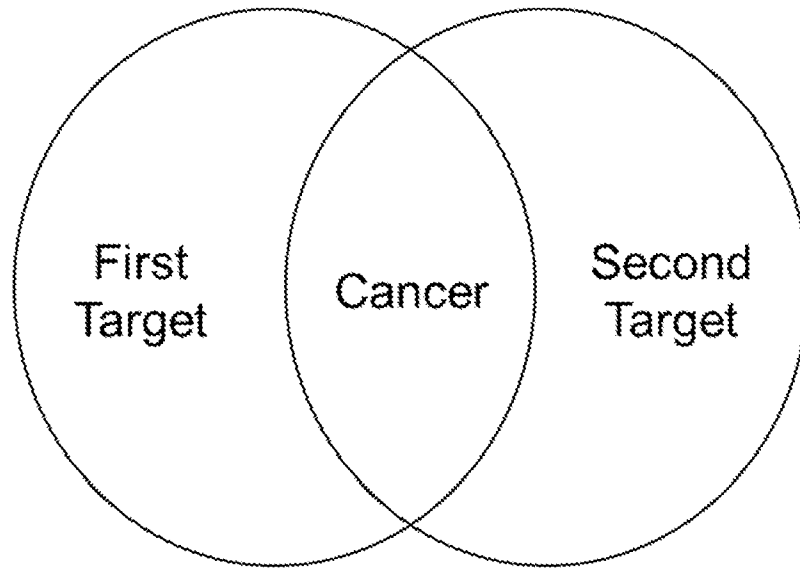
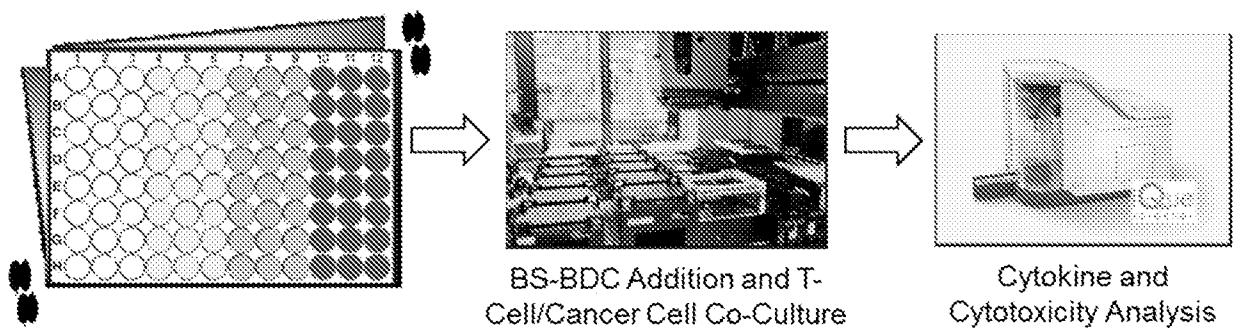


FIG. 1C



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FIG. 2

	CD3	CD28	41BB
Mesothelin	Mesothelin : CD3	Mesothelin : CD28	Mesothelin : 41BB
MUC16	MUC16 : CD3	MUC16 : CD28	MUC16 : 41BB
FOLR	FOLR : CD3	FOLR : CD28	FOLR : 41BB
PD-L1	PD-L1 : CD3	PD-L1 : CD28	PD-L1 : 41BB
ROR1	ROR1 : CD3	ROR1 : CD28	ROR1 : 41BB
GPC2	GPC2 : CD3	GPC2 : CD28	GPC2 : 41BB
GD2	GD2 : CD3	GD2 : CD28	GD2 : 41BB
HER2	HER2 : CD3	HER2 : CD28	HER2 : 41BB
EGFR	EGFR : CD3	EGFR : CD28	EGFR : 41BB
CD19	CD19 : CD3	CD19 : CD28	CD19 : 41BB

FIG. 3A

MUC16 : CD28 & Mesothelin : CD3	MUC16 : CD3 & Mesothelin : CD28	MUC16 : CD3 & Mesothelin : 41BB
MUC16 : 41BB & Mesothelin : CD3	MUC16 : 41BB & Mesothelin : CD28	MUC16 : CD28 & Mesothelin : 41BB
FOLR : CD28 & Mesothelin : CD3	FOLR : CD3 & Mesothelin : CD28	FOLR : CD3 & Mesothelin : 41BB
FOLR : 41BB & Mesothelin : CD3	FOLR : 41BB & Mesothelin : CD28	FOLR : CD28 & Mesothelin : 41BB
PD-L1 : CD28 & Mesothelin : CD3	PD-L1 : CD3 & Mesothelin : CD28	PD-L1 : CD3 & Mesothelin : 41BB
PD-L1 : 41BB & Mesothelin : CD3	PD-L1 : 41BB & Mesothelin : CD28	PD-L1 : CD28 & Mesothelin : 41BB
EGFR : CD28 & Mesothelin : CD3	EGFR : CD3 & Mesothelin : CD28	EGFR : CD3 & Mesothelin : 41BB
EGFR : 41BB & Mesothelin : CD3	EGFR : 41BB & Mesothelin : CD28	EGFR : CD28 & Mesothelin : 41BB
FOLR : CD28 & MUC16 : CD3	FOLR : CD3 & MUC16 : CD28	FOLR : CD3 & MUC16 : 41BB
FOLR : 41BB & MUC16 : CD3	FOLR : 41BB & MUC16 : CD28	FOLR : CD28 & MUC16 : 41BB
PD-L1 : CD28 & MUC16 : CD3	PD-L1 : CD3 & MUC16 : CD28	PD-L1 : CD3 & MUC16 : 41BB
PD-L1 : 41BB & MUC16 : CD3	PD-L1 : 41BB & MUC16 : CD28	PD-L1 : CD28 & MUC16 : 41BB
EGFR : CD28 & MUC16 : CD3	EGFR : CD3 & MUC16 : CD28	EGFR : CD3 & MUC16 : 41BB
EGFR : 41BB & MUC16 : CD3	EGFR : 41BB & MUC16 : CD28	EGFR : CD28 & MUC16 : 41BB
PD-L1 : CD28 & FOLR : CD3	PD-L1 : CD3 & FOLR : CD28	PD-L1 : CD3 & FOLR : 41BB
PD-L1 : 41BB & FOLR : CD3	PD-L1 : 41BB & FOLR : CD28	PD-L1 : CD28 & FOLR : 41BB
EGFR : CD28 & FOLR : CD3	EGFR : CD3 & FOLR : CD28	EGFR : CD3 & FOLR : 41BB
EGFR : 41BB & FOLR : CD3	EGFR : 41BB & FOLR : CD28	EGFR : CD28 & FOLR : 41BB
EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB

FIG. 3B

FOLR : CD28 & MUC16 : CD3	FOLR : CD3 & MUC16 : CD28	FOLR : CD3 & MUC16 : 41BB
FOLR : 41BB & MUC16 : CD3	FOLR : 41BB & MUC16 : CD28	FOLR : CD28 & MUC16 : 41BB
PD-L1 : CD28 & MUC16 : CD3	PD-L1 : CD3 & MUC16 : CD28	PD-L1 : CD3 & MUC16 : 41BB
PD-L1 : 41BB & MUC16 : CD3	PD-L1 : 41BB & MUC16 : CD28	PD-L1 : CD28 & MUC16 : 41BB
ROR1 : CD28 & MUC16 : CD3	ROR1 : CD3 & MUC16 : CD28	ROR1 : CD3 & MUC16 : 41BB
ROR1 : 41BB & MUC16 : CD3	ROR1 : 41BB & MUC16 : CD28	ROR1 : CD28 & MUC16 : 41BB
HER2 : CD28 & MUC16 : CD3	HER2 : CD3 & MUC16 : CD28	HER2 : CD3 & MUC16 : 41BB
HER2 : 41BB & MUC16 : CD3	HER2 : 41BB & MUC16 : CD28	HER2 : CD28 & MUC16 : 41BB
EGFR : CD28 & MUC16 : CD3	EGFR : CD3 & MUC16 : CD28	EGFR : CD3 & MUC16 : 41BB
EGFR : 41BB & MUC16 : CD3	EGFR : 41BB & MUC16 : CD28	EGFR : CD28 & MUC16 : 41BB
PD-L1 : CD28 & FOLR : CD3	PD-L1 : CD3 & FOLR : CD28	PD-L1 : CD3 & FOLR : 41BB
PD-L1 : 41BB & FOLR : CD3	PD-L1 : 41BB & FOLR : CD28	PD-L1 : CD28 & FOLR : 41BB
ROR1 : CD28 & FOLR : CD3	ROR1 : CD3 & FOLR : CD28	ROR1 : CD3 & FOLR : 41BB
ROR1 : 41BB & FOLR : CD3	ROR1 : 41BB & FOLR : CD28	ROR1 : CD28 & FOLR : 41BB
HER2 : CD28 & FOLR : CD3	HER2 : CD3 & FOLR : CD28	HER2 : CD3 & FOLR : 41BB
HER2 : 41BB & FOLR : CD3	HER2 : 41BB & FOLR : CD28	HER2 : CD28 & FOLR : 41BB
EGFR : CD28 & FOLR : CD3	EGFR : CD3 & FOLR : CD28	EGFR : CD3 & FOLR : 41BB
EGFR : 41BB & FOLR : CD3	EGFR : 41BB & FOLR : CD28	EGFR : CD28 & FOLR : 41BB
ROR1 : CD28 & PD-L1 : CD3	ROR1 : CD3 & PD-L1 : CD28	ROR1 : CD3 & PD-L1 : 41BB
ROR1 : 41BB & PD-L1 : CD3	ROR1 : 41BB & PD-L1 : CD28	ROR1 : CD28 & PD-L1 : 41BB
HER2 : CD28 & PD-L1 : CD3	HER2 : CD3 & PD-L1 : CD28	HER2 : CD3 & PD-L1 : 41BB
HER2 : 41BB & PD-L1 : CD3	HER2 : 41BB & PD-L1 : CD28	HER2 : CD28 & PD-L1 : 41BB
EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB
HER2 : CD28 & ROR1 : CD3	HER2 : CD3 & ROR1 : CD28	HER2 : CD3 & ROR1 : 41BB
HER2 : 41BB & ROR1 : CD3	HER2 : 41BB & ROR1 : CD28	HER2 : CD28 & ROR1 : 41BB
EGFR : CD28 & ROR1 : CD3	EGFR : CD3 & ROR1 : CD28	EGFR : CD3 & ROR1 : 41BB
EGFR : 41BB & ROR1 : CD3	EGFR : 41BB & ROR1 : CD28	EGFR : CD28 & ROR1 : 41BB
EGFR : CD28 & HER2 : CD3	EGFR : CD3 & HER2 : CD28	EGFR : CD3 & HER2 : 41BB
EGFR : 41BB & HER2 : CD3	EGFR : 41BB & HER2 : CD28	EGFR : CD28 & HER2 : 41BB

FIG. 3C

MUC16 : CD28 & Mesothelin : CD3	MUC16 : CD3 & Mesothelin : CD28	MUC16 : CD3 & Mesothelin : 41BB
MUC16 : 41BB & Mesothelin : CD3	MUC16 : 41BB & Mesothelin : CD28	MUC16 : CD28 & Mesothelin : 41BB
FOLR : CD28 & Mesothelin : CD3	FOLR : CD3 & Mesothelin : CD28	FOLR : CD3 & Mesothelin : 41BB
FOLR : 41BB & Mesothelin : CD3	FOLR : 41BB & Mesothelin : CD28	FOLR : CD28 & Mesothelin : 41BB
PD-L1 : CD28 & Mesothelin : CD3	PD-L1 : CD3 & Mesothelin : CD28	PD-L1 : CD3 & Mesothelin : 41BB
PD-L1 : 41BB & Mesothelin : CD3	PD-L1 : 41BB & Mesothelin : CD28	PD-L1 : CD28 & Mesothelin : 41BB
ROR1 : CD28 & Mesothelin : CD3	ROR1 : CD3 & Mesothelin : CD28	ROR1 : CD3 & Mesothelin : 41BB
ROR1 : 41BB & Mesothelin : CD3	ROR1 : 41BB & Mesothelin : CD28	ROR1 : CD28 & Mesothelin : 41BB
EGFR : CD28 & Mesothelin : CD3	EGFR : CD3 & Mesothelin : CD28	EGFR : CD3 & Mesothelin : 41BB
EGFR : 41BB & Mesothelin : CD3	EGFR : 41BB & Mesothelin : CD28	EGFR : CD28 & Mesothelin : 41BB
FOLR : CD28 & MUC16 : CD3	FOLR : CD3 & MUC16 : CD28	FOLR : CD3 & MUC16 : 41BB
FOLR : 41BB & MUC16 : CD3	FOLR : 41BB & MUC16 : CD28	FOLR : CD28 & MUC16 : 41BB
PD-L1 : CD28 & MUC16 : CD3	PD-L1 : CD3 & MUC16 : CD28	PD-L1 : CD3 & MUC16 : 41BB
PD-L1 : 41BB & MUC16 : CD3	PD-L1 : 41BB & MUC16 : CD28	PD-L1 : CD28 & MUC16 : 41BB
ROR1 : CD28 & MUC16 : CD3	ROR1 : CD3 & MUC16 : CD28	ROR1 : CD3 & MUC16 : 41BB
ROR1 : 41BB & MUC16 : CD3	ROR1 : 41BB & MUC16 : CD28	ROR1 : CD28 & MUC16 : 41BB
EGFR : CD28 & MUC16 : CD3	EGFR : CD3 & MUC16 : CD28	EGFR : CD3 & MUC16 : 41BB
EGFR : 41BB & MUC16 : CD3	EGFR : 41BB & MUC16 : CD28	EGFR : CD28 & MUC16 : 41BB
PD-L1 : CD28 & FOLR : CD3	PD-L1 : CD3 & FOLR : CD28	PD-L1 : CD3 & FOLR : 41BB
PD-L1 : 41BB & FOLR : CD3	PD-L1 : 41BB & FOLR : CD28	PD-L1 : CD28 & FOLR : 41BB
ROR1 : CD28 & FOLR : CD3	ROR1 : CD3 & FOLR : CD28	ROR1 : CD3 & FOLR : 41BB
ROR1 : 41BB & FOLR : CD3	ROR1 : 41BB & FOLR : CD28	ROR1 : CD28 & FOLR : 41BB
EGFR : CD28 & FOLR : CD3	EGFR : CD3 & FOLR : CD28	EGFR : CD3 & FOLR : 41BB
EGFR : 41BB & FOLR : CD3	EGFR : 41BB & FOLR : CD28	EGFR : CD28 & FOLR : 41BB
ROR1 : CD28 & PD-L1 : CD3	ROR1 : CD3 & PD-L1 : CD28	ROR1 : CD3 & PD-L1 : 41BB
ROR1 : 41BB & PD-L1 : CD3	ROR1 : 41BB & PD-L1 : CD28	ROR1 : CD28 & PD-L1 : 41BB
EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB
EGFR : CD28 & ROR1 : CD3	EGFR : CD3 & ROR1 : CD28	EGFR : CD3 & ROR1 : 41BB
EGFR : 41BB & ROR1 : CD3	EGFR : 41BB & ROR1 : CD28	EGFR : CD28 & ROR1 : 41BB

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FIG. 3D

EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB

FIG. 3E

ROR1 : CD28 & PD-L1 : CD3	ROR1 : CD3 & PD-L1 : CD28	ROR1 : CD3 & PD-L1 : 41BB
ROR1 : 41BB & PD-L1 : CD3	ROR1 : 41BB & PD-L1 : CD28	ROR1 : CD28 & PD-L1 : 41BB
EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB
EGFR : CD28 & ROR1 : CD3	EGFR : CD3 & ROR1 : CD28	EGFR : CD3 & ROR1 : 41BB
EGFR : 41BB & ROR1 : CD3	EGFR : 41BB & ROR1 : CD28	EGFR : CD28 & ROR1 : 41BB

FIG. 3F

EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB

FIG. 3G

EGFR : CD28 & PD-L1 : CD3	EGFR : CD3 & PD-L1 : CD28	EGFR : CD3 & PD-L1 : 41BB
EGFR : 41BB & PD-L1 : CD3	EGFR : 41BB & PD-L1 : CD28	EGFR : CD28 & PD-L1 : 41BB

FIG. 4A

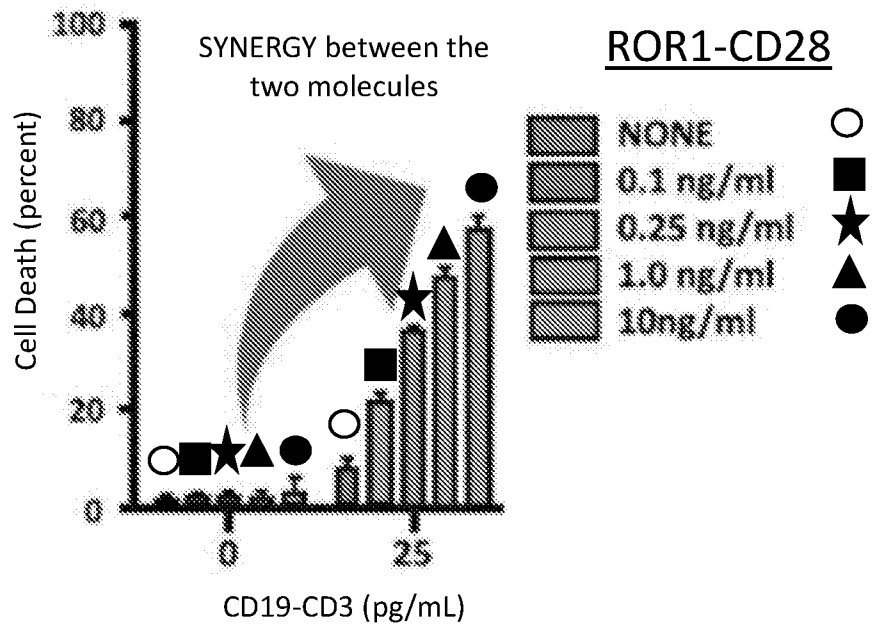
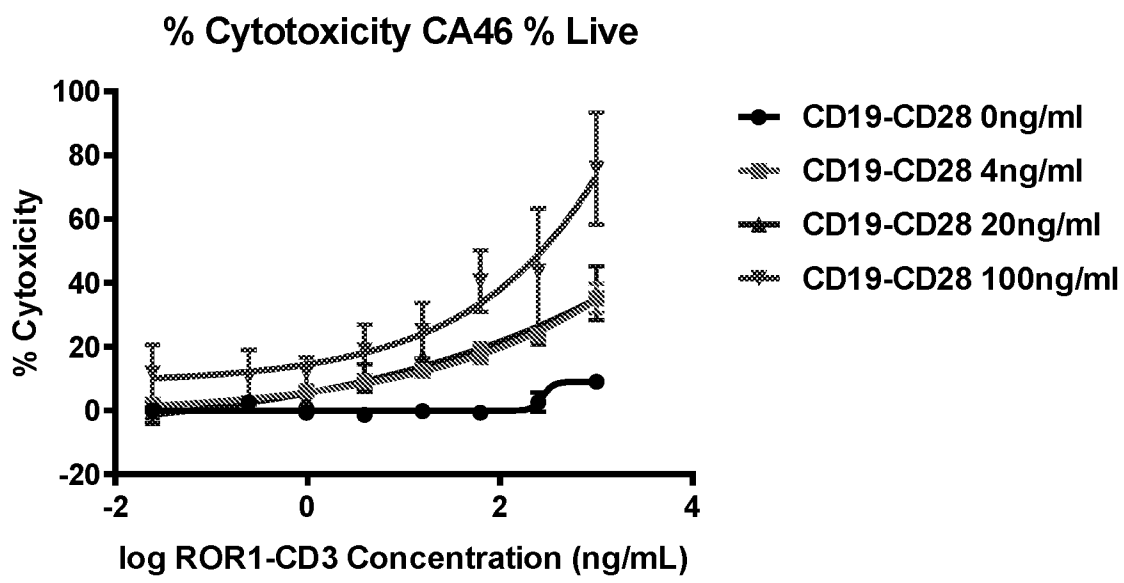
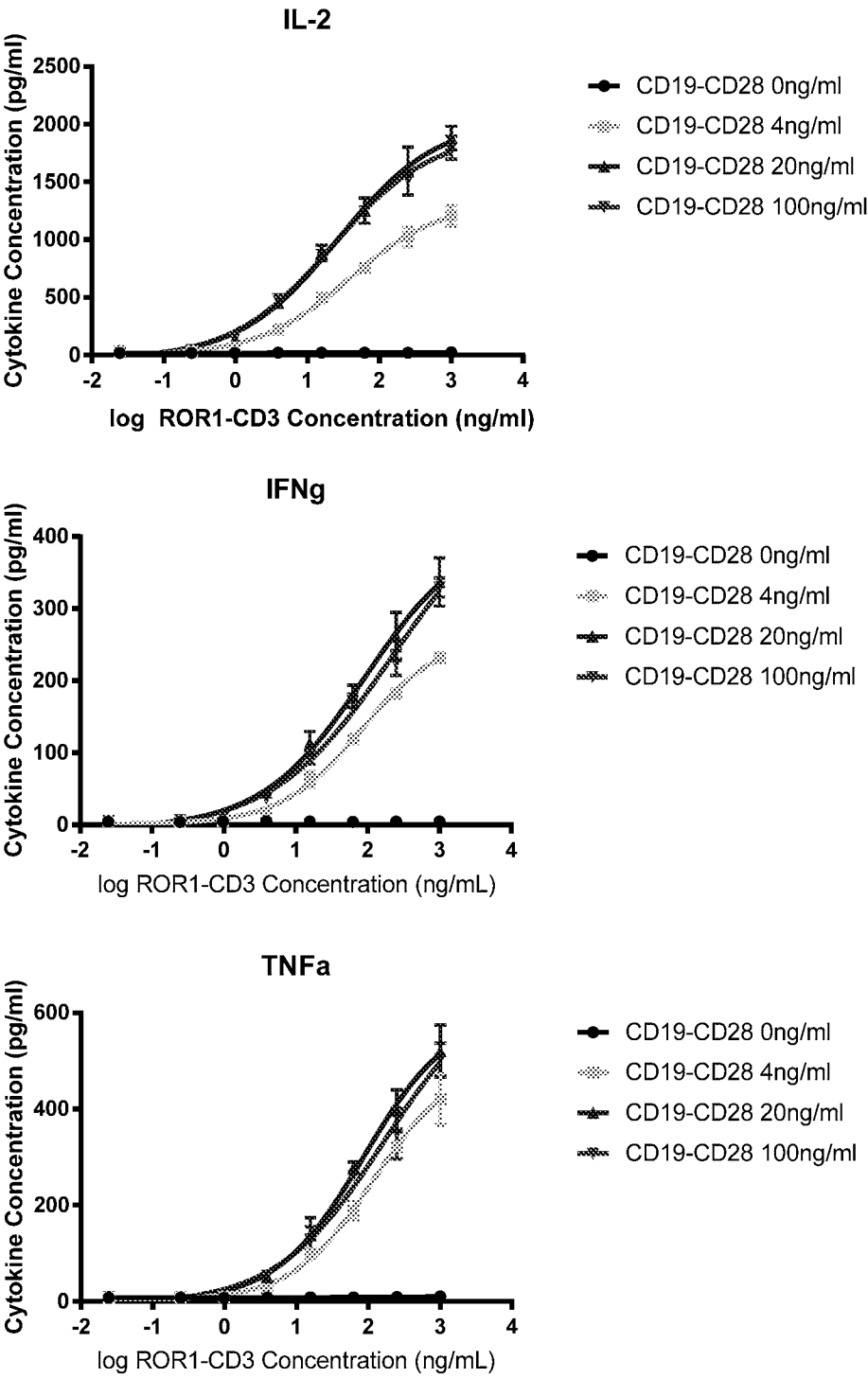


FIG. 4B



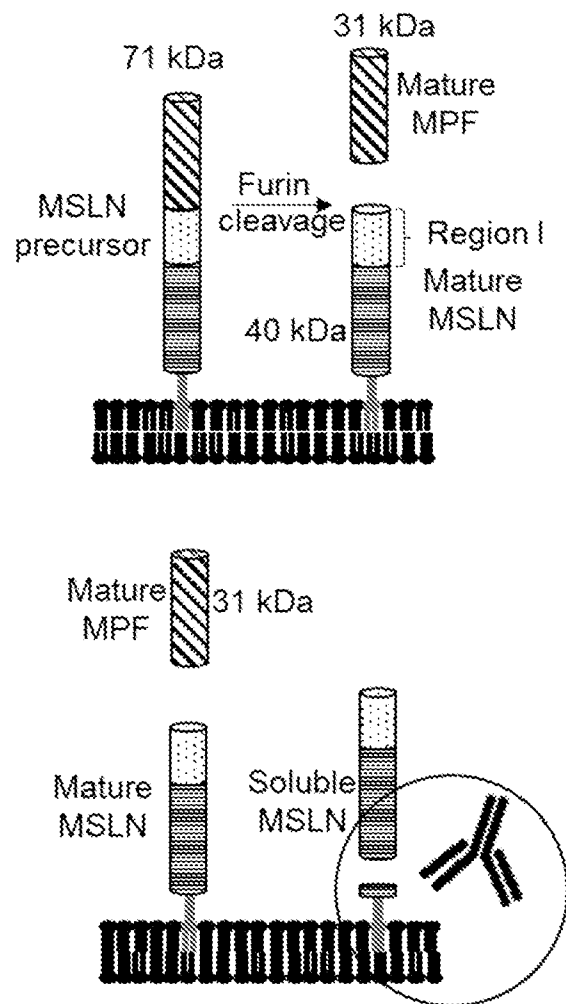
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FIG. 4C



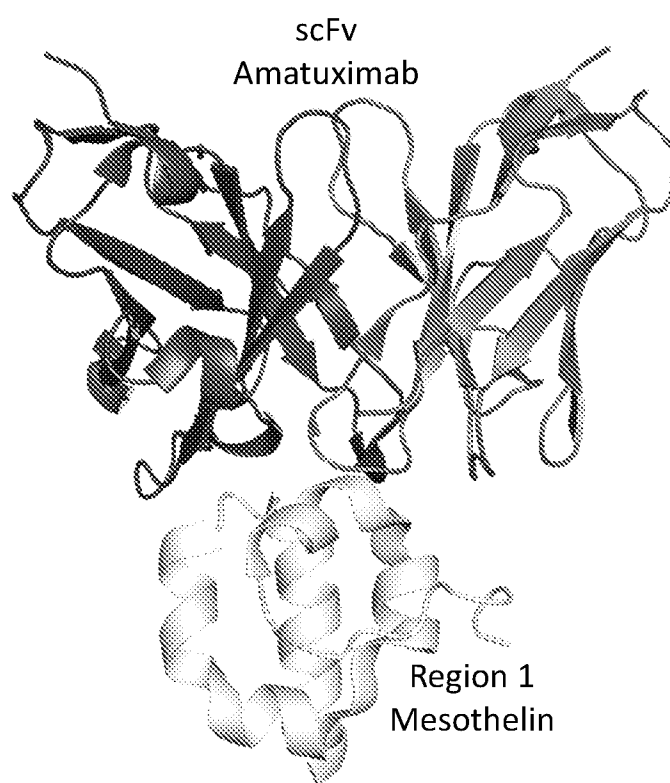
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FIG. 5A



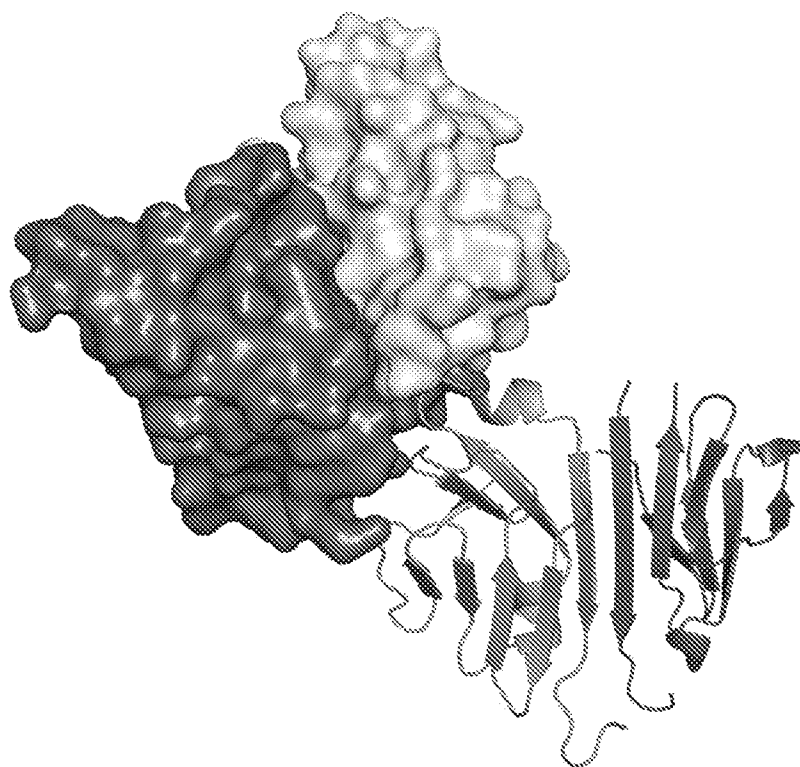
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FIG. 5A (cont'd)



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FIG. 5B



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FIG. 6A

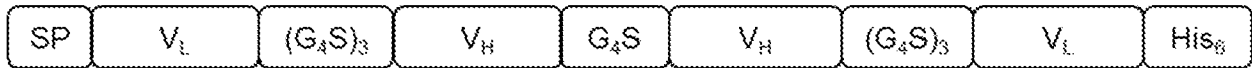


FIG. 6B

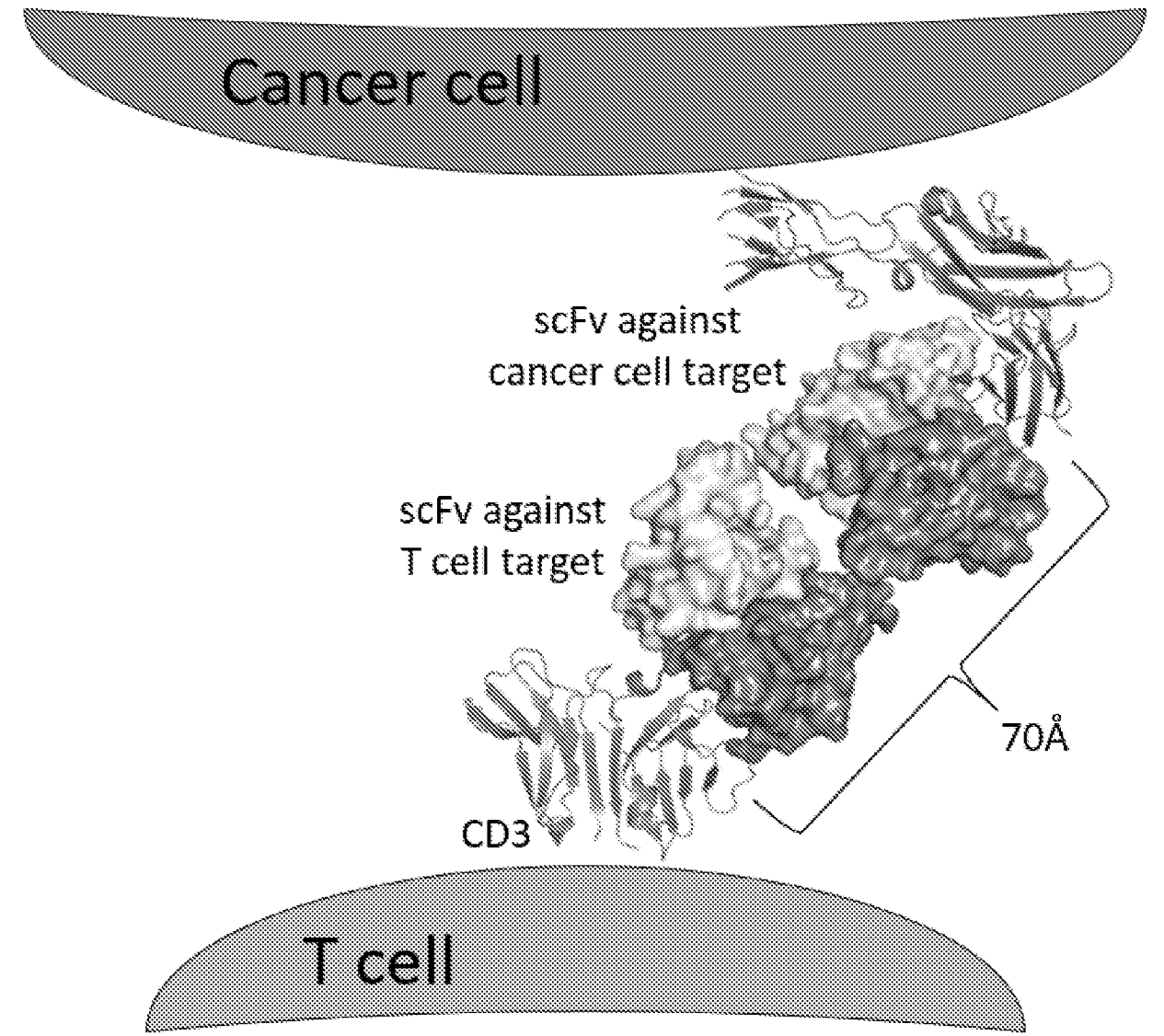
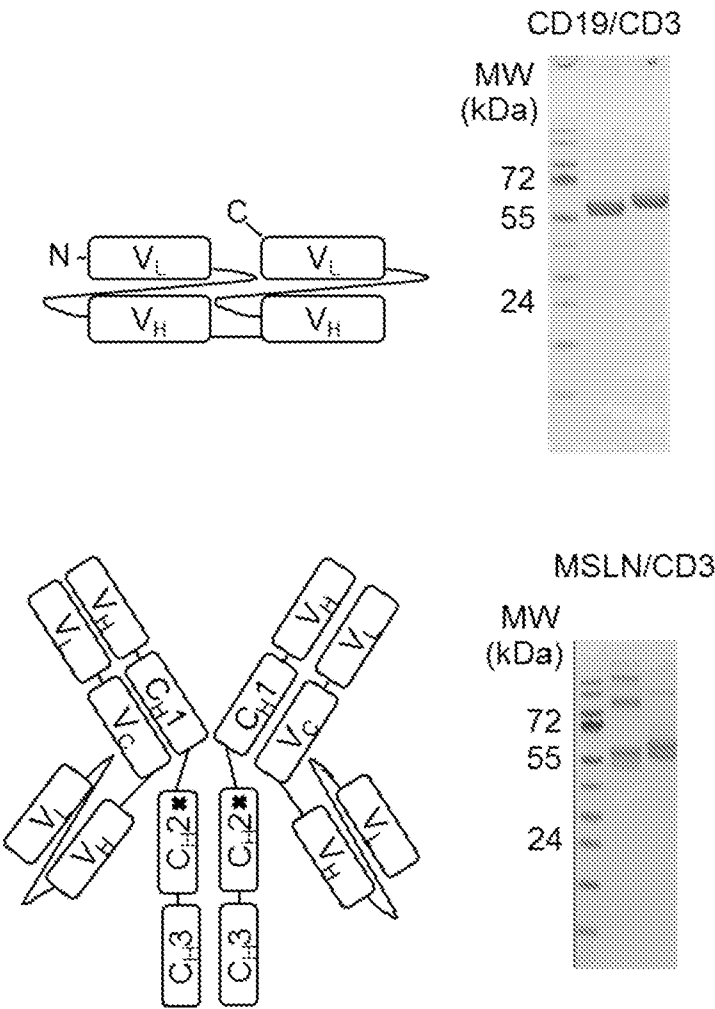


FIG. 6C



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FIG. 6C (cont'd)

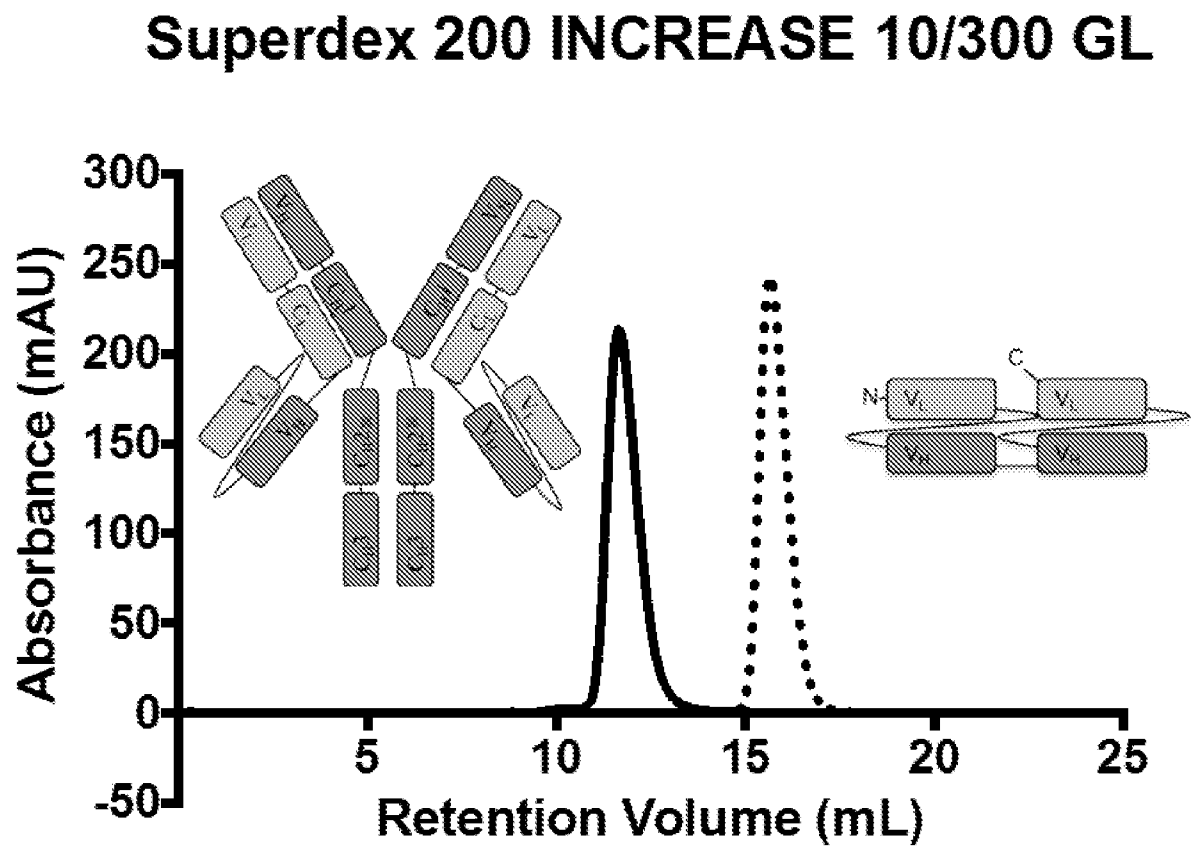


FIG. 6D

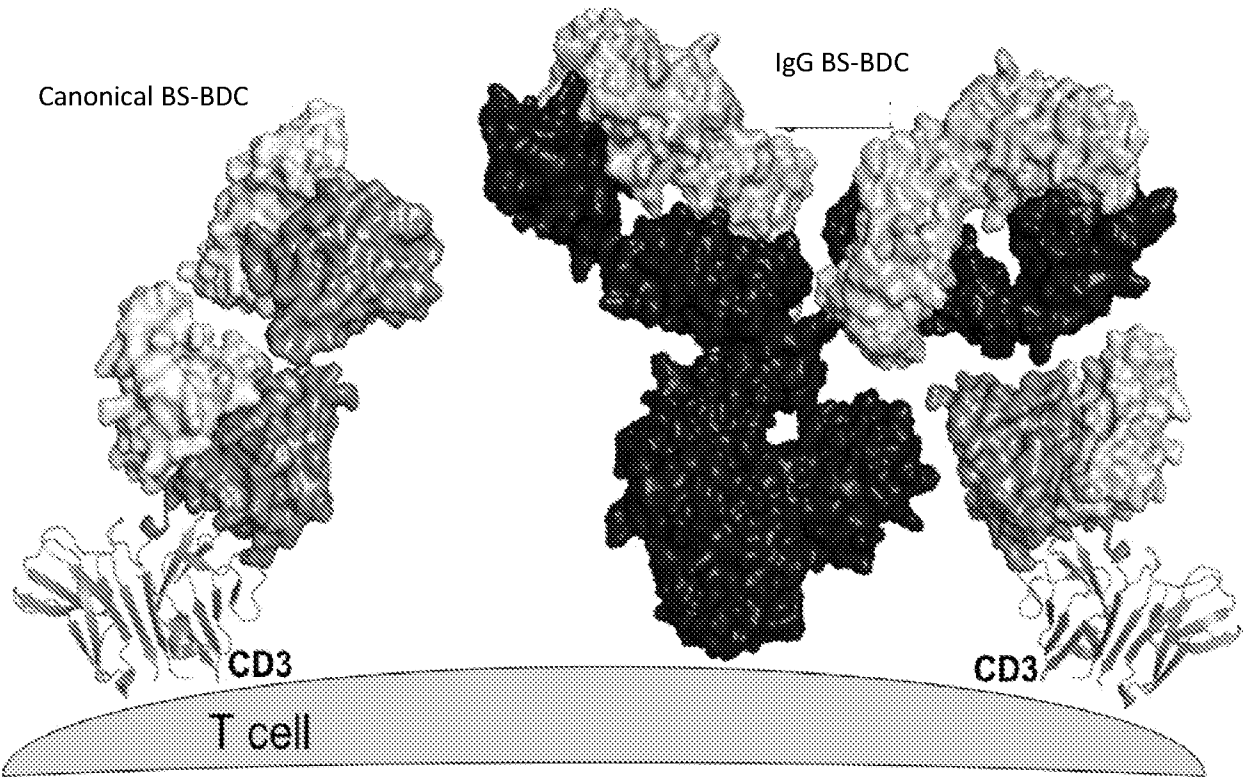


FIG. 6E

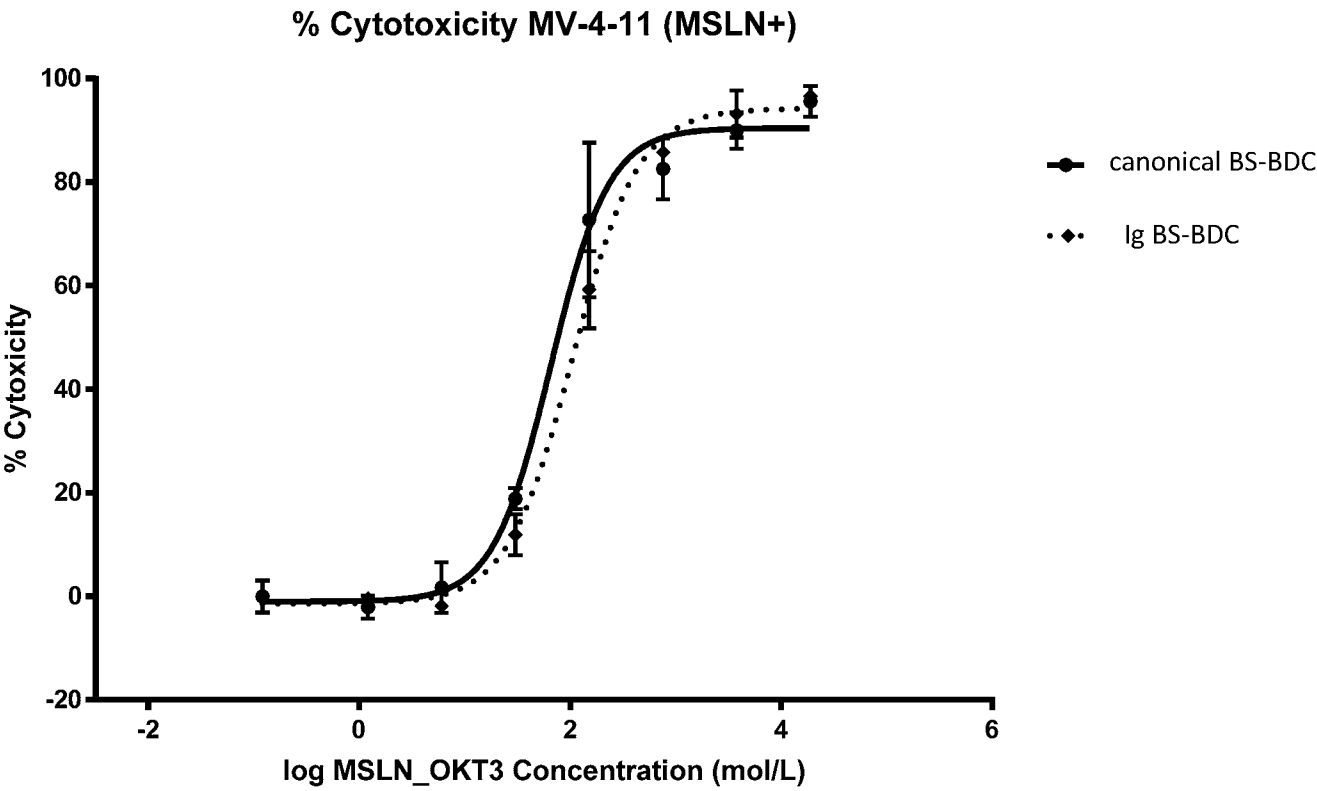


FIG. 6F

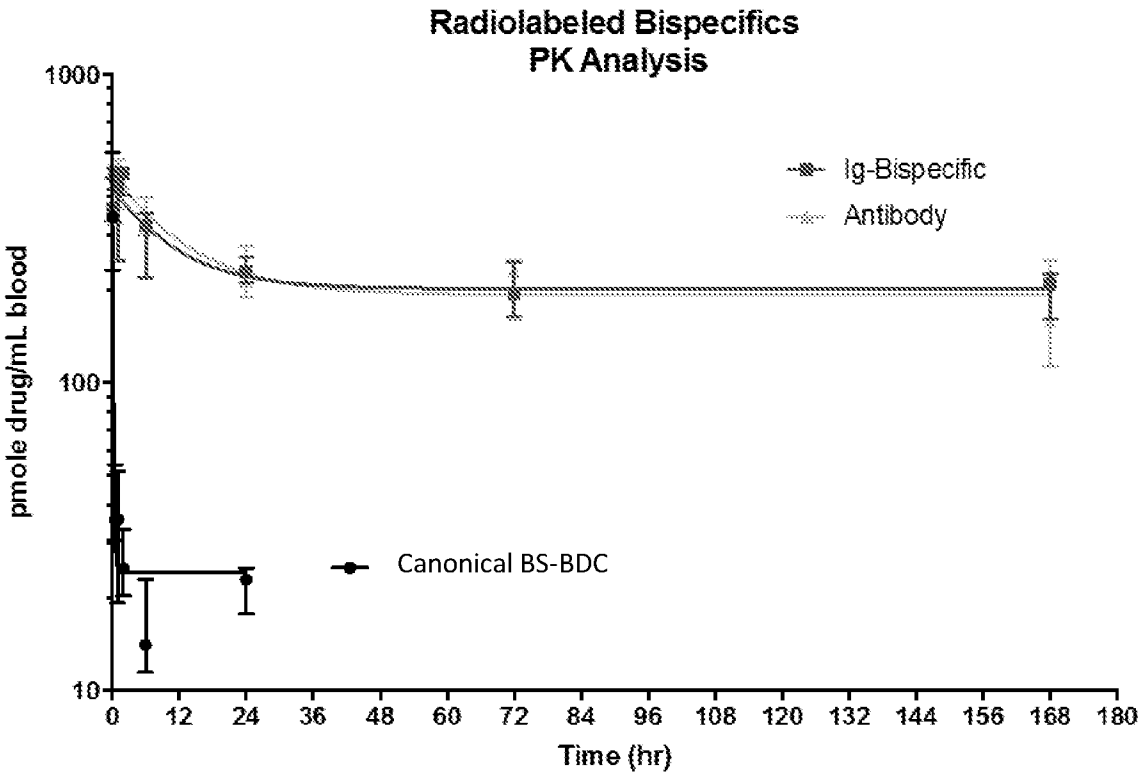


FIG. 6G

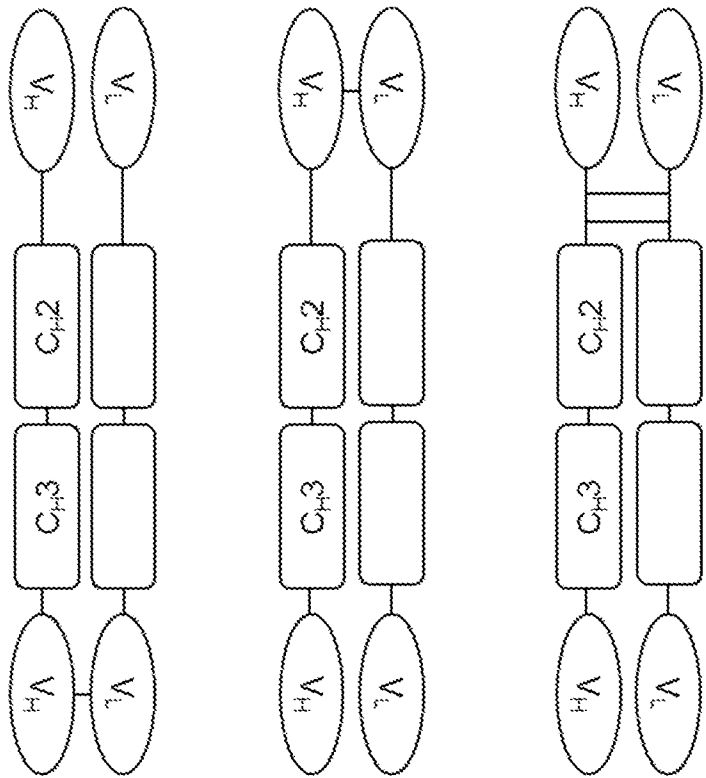
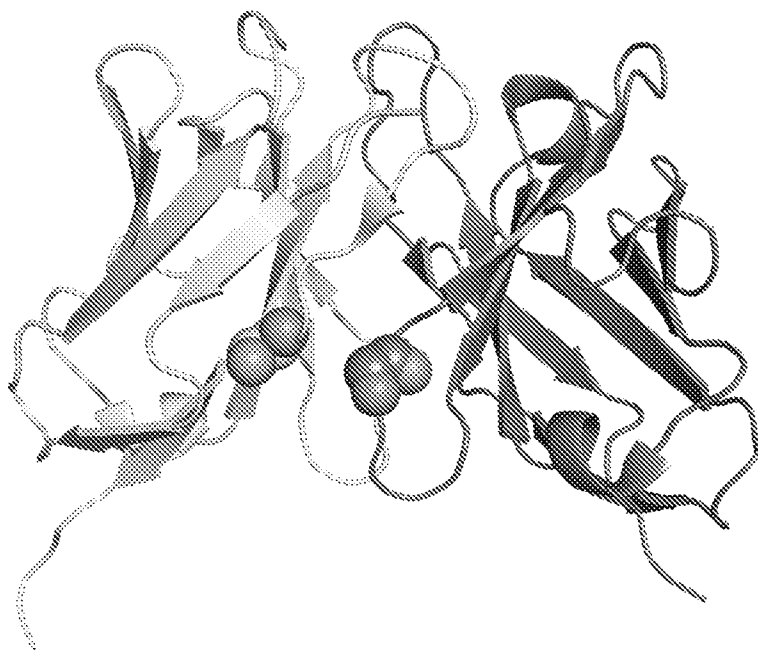


FIG. 6H



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FIG. 7

Mesothelin

MALPTARPLLGSCGTPALGSLLFLLFSLGWWQPSRTLGETGQEAAPLDGVLANPPNISSLSPR
QLLGFPCEVSGLSTERVRELAVALAQKNVKLSTEQLRCLAHRLSEPPEDLDALPLDLLLFLNP
DAFSGPQACTHFFSRITKANVDLLPRGAPERQRLLPAALACWGVRSLLSEADVRALGGLACD
LPGRFVAESADEVLLPRLVSCPGPLDQDQQAARAALQGGGPPYGPPSTWSVSTMDALRGLLP
VLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPRTLPRFRREVEKTACPSGKKAREIDESLIFY
KKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELYPQGYPESVIQHLGYLFLKMSPE
DIRKWNVTSLKALLEVNKGHEMSPQVATLIDRFVKGRGQLDKDTLDTLAFYPGYLCSLSP
EELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEYFVKIQSFLGGAPTEDLK
ALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKLLGPHVEGLKAEERHRPVRDWILRQRQDDLD
TLGLGLQGGIPNGYLVLDLSVQEALSGTPCLLGPVLTVLALLLASTLA (SEQ ID NO: 1)

MUC16 (CA125)

MLKPSGLPGSSSPTRSLMTGSRSTKATPEMDSGLTGATLSPKTSTGAIVVTEHTLPFTSPDKTL
ASPTSSVVGRTTQSLGVMSSALPESTSRGMTHSEQRTSPSLSPQVNGTPSRNYPATSMVSGL
SSPRTRTSSTEGNFTKEASTYTLTVETTSGPVTEKYTVPTETSTTEGDSTETPWDTRYIPVKITS
PMKTFADSTASKENAPVSMTPAETTVTDSHTPGRTNPSFGTLYSSFLDLSPKGTNPNSRGETSL
ELILSTTGYPFSSPEPGSAGHSRISTSAPLSSSASVLDNKISETSIFSGQSLTSPLSPGVPEARAS
TMPNSAIPFSMTLSNAETSAERVSTISSLGTPSISTKQTAETILTFHAFATMDIPSTHIAKTAS
EWLGSPGTGGTSTSAITTTSPSTTLVSEETNTHHSTSGKETEGTLNLSMTPLETSAPGESE
MTATLVPTLGFTTLDKIRSPSQVSSSHPTRELRTTGSTSGRQSSSTAAGSSDILRATTSSTS
KASSWTSESTAQQFSEPQHTQWVETSPSMKTERPPASTSVAAPITTSVPSVSGFTTLKTSST
KGIWLEETSADTLIGESTAGPTTHQFAVPTGISMTGGSSSTRGSQGTTHLLTRATASSETSADLT
LATNGVPVSVSPAVSKTAAGSSPPGGTKPSYTMVSSVIPETSSLQSSAFREGTSLGLTPLNTRH
PFSSPEPDSAGHTKISTSIPLLSSASVLEDKVSATSTFHHKATSSITTGTPEISTKTKPSSAVLSS
MTLSNAATSPERVRNATSPLTHPSPSGEETAGSVLTLSTSAETTDSPNIHPTGTLTSESSESPS
TSLSPSVSGVKTTFFSSSTPSTHLFTSGEETEETSNPSVSQPETSVSRRVTTLASTSVPTPVFPT
MDTWPTRSAQFSSSHLVSELRATSSTSVTNSTGSALPKISHLTGATMSQTNRDTFNDSAAPQ
STTWPETSPRFKTGLPSATTTVSTSATSLSATVMVSKFTSPATSSMEATSIREPSTTILTTETN
GPGSMAVASTNIPIGKYITEGRDTSHPIGTTASSETSMDFTMAKESVSMVSPSQSMDAAG
SSTPGRTSQFVDTFSDDVYHLTSREITIPRDGTSSALTPQMTATHPPSPDPGSARSTWLGLSS
SPSSPTPKVTMSSTFSTQRVTTSMIMDTVETSRWNMPNLPSTTSLTPSNIPTSGAIGKSTLVPL
DTPSPATSLEASEGGLPTLSTYPESTNTPSIHLGAHASSESPSTIKLTMAVVKPGSYTPLTFPSI
ETHIHVSTARMAYSSGSSPEMTAPGETNTGSTWDPTTYITTTDPKDTSSAQVSTPHSVRTLRTT
ENHPKTESATPAAYSGSPKISSPNLTSPATKAWTITDTEHSTQLHYTKLAEKSSGFETQSAP
GPVSVVIPTSPITIGSSTLELTSDVPGEPLVLAPSEQTTITLPMATWLSTSLTEEMASTDLDISSPS
SPMSTFAIFPPMSTPSHELKSEADTSAIRNTDSTTLDQHLGIRSLGRTGDLTTPITPLTTTWT
VIEHSTQAQDTLSATMSPTHVTQSLKDQTSIPASASPSHLTEVYPELGTQGRSSSEATTFWKPS
TDTLSREIETGPTNIQSTPPMDNTTTGSSSSGVTLGIAHLPIGTSSPAETSTNMALERRSSTATV
SMAGTMGLLVTSAPGRSISQSLGRVSSVLSESTTEGVTDSSKGSSPRLNTQGNTALSSSLEPS
YAEGSQMSTSIPLTSSPTTPDVEFIGGSTFWTKEVTTVMTSDISKSSARTESSSATLMSTALGST
ENTGKEKLRTASMDLPSPTPSMEVTPWISLTLSNAPNTTDSLDSLHGVHTSSAGTLATDRSLNT
GVTRASRLENGSDTSSKSLSMGNSTHTSMTYTEKSEVSSSIHPRPETSAPGAETTLTSTPGNR
AISLTLFPSSIPVEEVISTGITSGPDINSAPMTHSPITPPTIVWTSTGTIEQSTQPLHAVSSEKVS
QTQSTPYVNSVAVSASPTHENSVSOGSSSTSSPYSSASLESLDSTISRRNAITSWLWDLTSLPT
TTWPSTSLSEALSSGHSGVSNPSSTTTEFPLFSAASTSAKQRNPETETHGPQNAASTLNTD
ASSVTGLSETPVGASISSEVPLPMAITSRSDVSGLTSESTANPSLGTASSAGTKLRTISLPTSE
SLVSFRMNKDPWTVSIPLGSHPTTNTETSIPVNSAGPPGLSTVASDVIDTPSDGAESIPTVSFSP
SPDTEVTTISHFPEKTHSFRTISSLTHELTSRVTPIPGDWMSSAMSTKPTGASPSITLGERRTIT

FIG. 7 (cont'd)

SAAPTTSPIVLTASFTETSTVSLDNETTVKTS DILDARKTNELPSDSSSSSSDLINTSIASSTMDVTK
TASISPTISISGMTASSSPSLFSSDRPQVPTSTTETNTATSPSVSSNTYSLDGGSNVGGTPSTLP
PFTITHPVETSSALLAWSRPVRTFSTMVSTDTASGENPTSSNSVVTSPVAPGTWTSVGSSTDL
PAMGFLKTSPAGEAHSLLASTIEPATAFTPHLSAAVVTGSSATSEASLLTTSESKAIHSSPQTPT
TPTSGANWETSATPESLLVVTETSDTTLSKILVTDILFSTVSTPPSKFPSTGTLGASFPTLLP
DTPAIPLTATEPTSSLATSFDSTPLVTIASDSLGTVPETTLTMSETSNGDALVLKTVSNPDRSIPGI
TIQGVTESPLHPSSTSPSKIVAPRNTTYEGSITVALSTLPAGTTGSLVFSQSSSENSETTALVDSS
AGLERASVMPLTTGSQGMASGGIRSGSTHSTGKTFSSSLPLTMNPGEVTAMSEITTNRLTAT
QSTAPKGIPVKPTSAESGLLTPVSASSSPSKAFASLTAPPTWGIPQSTLTFFEFSEVPSLDTKSA
SLPTPGQSLNTIPDSASTASSSLSKSPEKNPRARMMTSTKAISASSFQSTGFTETPEGSASPS
MAGHEPRVPTSGTGDPRYASESMSYPDPKASSAMTSTSLASKLTTLFSTGQAARSGSSSSPI
SLSTEKETSFLSPTASTSRKTSFLGPSMARQPNILVHLQTSALTSPSTLNMSQEEPELTSS
QTIAEEEGTTAETQTLTFTPSETPTSLLPVSSPTEPTARRKSSPETWASSISVPAKTSLVETTDG
TLVTTIKMSSQAAQGNSTWPAPAEETGSSPAGTSPGSPEMSTTLKIMSSKEPSISPEIRSTVRN
SPWKTPETTVPMETTVEPVTLQSTALGSGSTSISHLPTGTTSPTKSPTENMLATERVSLSPSP
EAWTNLYSGTPGGTRQSLATMSSVSLESPTARSITGTGQQSSPELVSKTTGMEFSMWHGSTG
GTTGDTHVSLSTSSNILEDPVTPSPNSVSSLTDKSKHKTETWVSTTAIPSTVLNNKIMAAEQQTSR
SVDEAYSSSTSSWSDQTS GSDITLGASPDVTNTLYITSTAQTSLVSLPSGDQGITS LTNPSGGK
TSSASSVTSPSIGLETLRANVSAVKSDIAPTAGHLSQTSSPAEVSILDVTTAPTGPISITTTMTGT
NSISITTPNPEVGMSTMDSTPATERRTTSTEHPSTWSSTAASDSWTVDMTSNLKVARSPTGI
STMHTTSFLASSTELDSMSTPHGRITVIGTSLVTPSSDASAVKTETSTERTLSPSDTTASTPIST
FSRVQRMSISVPDILSTSWTPSSTEAEDVPVSMVSTDHASTKTDPNTPSTFLFDLSLTDWDT
GRSLSSATATTSAPQGATTPQELTLETMISPATSQLPFSIGHITS AVTPAAMARSSGVTF SRPDP
TSKKA EQTSTQLPTTSSAHPGQVPRSAATTLDVIPHAKTPDATFQRQGQTALTTEARATSDS
WNEKEKSTPSAPWITEMMNSVSEDTIKEVTSSSSVLR TLNTLDINLES GTTSSPSWKSSPYERI
APSESTTDKEAIHPSTNTVETTGWVTSSEHASHSTIPAHSAASSKLTSPVTTSTREQAIVSMSTT
TWPESTRARTEPNSTFLTIELRDVSPYMDTSSTTQTSIISPPGSTAITKGPRTEITSSKRISSTFLA
QSMRSDSPSEAITRLSNFPAMTESGGMILAMQTSPPGATSLSAPTLDTSATASWTGTPLATT
QRFTYSEKTTLFSKGPEDTSQPSPPSVEETSSSSSLVPIHATTSPSNILLTSQGHSPSSTPPVTS
VFLSETSGLGKTTDMSRISLEPGTSLPPNLSSTAGEALSTYEASRDTKAIHHSADTAVTNMEAT
SSEYSPIPGHTKPSKATSPLVTSHIMGDITSSTSVFGSSETTEIETVSSVNQGLQERSTSQVASS
ATETSTVITHVSSGDATTHVTKTQATFSSGTSISSPHQFITSTNTFTDVSTNPSTSLIMTESSGV
ITTQTGPTGAATQGPYLLDTSTMPYLTTETPLAVTPDFMQSEKTTLSKGPKDVSWTSPPSVAET
SYPSSLTPFLVTTIPPATSTLQGQHTSSPVSATSVLTSGLVKTTDMLNTSMEPVTNSPQNLNNP
SNEILATLAATTDIETIHPSINKAVTNMGTASSAHVLHSTLPVSSEPSTATSPMVPASSMGDALA
SISIPGSETTDIEGEPTSSLTAGRKENSTLQEMNSTTESNIILSNVSVGAITEATKMEVPSFDATFI
PTPAQSTKFPDIFSVASSRLSNSPPMTISTHMTTQTGSSGATSKIPLALDTSTLETSAGTPSVV
TEGFAHSKITAMNNDVKDVSQTNPPFQDEASSPSSQAPVLVTTLPSSVAFTPQWHSTSSPV
MSSVLTSSLVKTAGKVDTSLETVTSSPQSMSNTLDDISVTS AATTDIETHPSINTVVTNVGTTG
SAFESHSTVSAYPEPSKVTSPNVTTSTMEDTTISRIPKSSKTTTRTETETSSLT PKLRETSISQE
ITSSTETSTVPYKELTGATTEVSRTDVTSSSSTSFPGPDQSTVSLDISTETNTRLSTSPIMTESAE
ITITTQTGPHGATSQDTFTMDPSNTTPQAGIHSAMTHGFSQLDVTTLMSRIPQDVSWTSPPSVD
KTSSPSSFLSSPAMTTPSLISSTLPEDKLSSPMTSLLTSGLVKITDILRTRLEPVTSSLPNFSST
SDKILATSKDSKDTKEIFPSINTEETNVKANNSGHESHSPALADSETPKATTQMVIITTTVGD PAP
STSMPVHGSSETTNIKREPTYFLTPRLRETSTSQESSFPTDTSFLLSKVPTGTITEVSSTGVNSS
SKISTPDHDKSTVPPDFTTGEIPRVFTSSIKTKSAEMTITTQASPPESASHSTLPLDTSTTLSQGG
THSTVTQGFYSEVTTLMGMGPGNVSWMTTPPVEETSSVSSLMSSPAMTSPSPVSSTSPQSI
PSSPLPVTALPTSVLVTTTDLGTTSPESVTSSPPNLSSITHERPATYKDTAHTEAAMHHSTNTA
VTNVGTSGSGHKSQSSVLADSETSKATPLMSTTSTLGDTSVSTSTPNISQTNQIQTEPTASLSP

FIG. 7 (cont'd)

RLRESSTSEKTSSTTETNTAFSYVPTGAIQASRTEISSRSTSISDLDRPTIAPDISTGMITRLFTS
PIMTKSAEMTVTTQTTTPGATSQGILPWDSTTLFQGGTHSTVSQGFPHSEITTLRSRTPGDVS
WMTTPPVEETSSGFSLMSPSMTSPSPVSSTSPESIPSSPLPVTALLTSVLVTTTNVLGTTSP
VTSSPPNLSSPTQERLTTYKDTAHTTEAMHASMHTNTAVANVGTSISGHESQSSVPADSHTSKA
TSPMGITFAMGDTSVSTSTPAFFETRIQTESTSSLIPGLRDRTRTSEEINTVTETSTVLSEVPTTTT
TEVSRTEVITSSRTTISGPDHSMSPYISTETITRLSTFPFVTGSTEMAITNQTGPIGTISQATLTL
DTSSTASWEGTHSPVTQRFPHSEETTTMSRSTKGVSWQSPPSVEETSSPSSPVPLPAITSHSS
LYSAVSGSSPTSALPVTSLTSGRRKTIDMLDTHSELVTSSLPSASSFSGEILTSEASTNTETIHF
SENTAETNMGTTNSMHKLHSSVSIHSQPSGHTPPKVTGSMMEDAIVSTSTPGSPETKNVDRDS
TSPLTPELKEDSTALVMNSTTESNTVFSSVSLDAATEVSRAEVYYDPTFMPASAQSTKSPDIS
PEASSSHSNPPLTISTHKTIATQTGPSGVTSLGQLTLDSTIATSAGTPSARTQDFVDSETTSV
MNNDLNDVLKTSFSAEEANSLSSQAPLLVTTSPSPVTSTLQEHSTSSLVSVTSVPTPTLAKITD
MDTNLEPVTRSPQNLRNTLATSEATTDTHMHPSINTAVANVGTTSSPNEFYFTVSPDSDPYKA
TSAVVITSTSGDSIVSTSMPRSSAMKKIESETTFLIFRLRETSTSQKIGSSSDTSTVFDKAFTAA
TTEVSRTELTSSSRTSIQGTEKPTMSPDTSTRSVTMLSTFAGLTKEERTIATQTGPHRATSQG
TLTWDSITTSQAGTHSAMTHGFSQLDLSTLTSRVPEYISGTSPPSVEKTSSSSSLLSLPAITSP
SPVPTTLPESRPSSPVHLTSLPTSGLVKTTDMLASVASLPPNLGSTSHKIPTTSEDIKDTEKMYP
STNIAVTNVGTTTSEKESYSSVPAYSEPCKVTSPMVTSFNIRDIVSTSMPGSSEITRIEMESTFS
LAHGLKGTSTSQDPIVSTESKSAVLHKLTTGATETSRTEVASSRRTSIPGPDHSTESPDISTEVIPS
LPISLGITESSNMTIITRTGPPLGSTSQGTFTLDTPTTSSRAGTHSMATQEFPHSEMTTVMNKDP
EILSWTIPPSIEKTSFSSSLMPSPAMTSPPVSSSTLPKTIHTTTPSPMTSLLTPSLVMTTDTLGTSP
PTTSSPPNLSSTSHEILTDEDTTAIEAMHPSTSTAATNVETTSSGHGSQSSVLADSEKTKATAP
MDTTSTMGHHTVSTSMSVSSETTKIKRESTYSLTPGLRETSISQNASFSTDTSIVLSEVPTGTTA
EVSRTTEVTSSGRTSIPGPSQSTVLPEISTRMTLRFASPTMTESAEMTIPTQTGPSGSTSQDTLT
LDTSTTKSQAKTHSTLTQRFPHSEM TLM SRGP GDMSWQSSPSLENPSSLP SLLSLPATTSP
PISSTLPVTISSPLPVTSLLTSSPVTTTDM LHTSPELVTSPPKLSHTSDERLTTGKDTTNT EAV
HPSTNTAASNVEIPSSGHESPSSALADSETSKATSPMFITSTQEDTTVAISTPHFLET SRIQKESI
SSLSPKLRETGSSVETSSAIETSAVLSEVSIGATTEISRTEVTSSSRTSISGSAESTMLPEISTTRK
IHKFPTSPILAESSEMTIKTQTSPPGSTSESTFTLDTSTTPSLVITHSTMTQRLPHSEITTLVSRGA
GDVPRPSSLPVEETSPSSQLSLSAMISPPVSSTLPASSHSSASVTSLTTPGQVKTEVLDA
SAEPETSSPPLSSTSVEILATSEVTTDTEKIHFPNTAVTKVGTSSSGHESPSSVLPDSETTKA
TSAMGTISIMGDTSVSTLTPALSNTRKIQSEPASSLTTRLRETSTSEETSLATEANTVL SKVSTGA
TTEVSRTEAISFSRTSMGPEQSTMSQDISGIPRISASSVLTESAKMTIITTQTGPSESTLESTL
NLNTATTPSWWETHSIVIQQGFPHPEMTTSMGRGPGGVSWPSPPFVKETSPSSPLSLPAVTSP
HPVSTTFLAHIPPSPLPVTSLLTSGPATTTDILGTSTEPGTSSSSSLSTTSHERLTTYKDTAHTEA
VHPSTNTGGTNVATTSSGYKSQSSVLADSSPMCTTSTMGDTSVLTSTPAFLETRRIQTELASSL
TPGLRESSGSEGTSSGTKMSTVLSKVPTGATTEISKEDVTSIPGPAQSTISPDISTRVSWFSTS
PVMTESAEITMNTHTSPLGATTQGTSTLDTSSSTSLTMTHSTISQGFHSHSQMSTLMRRGPEDV
SWMSPPLLEKTRPSFSLMSSPATTSPSPVSSTLPESISSSPLPVTSLLTSGLAKTTDMLHKSSEP
VTNSPANLSSTSVEILATSEVTTDTEKTHPSSNRTVTDVGTSSSGHESTSFVLADSQTSKVTSP
MVTSTMEDTSVSTSTPGFFETSRIQTEPTSSLTLGLRKTSSSEGTSLATEMSTVL SGVPTGATA
EVSRTTEVTSSSRTSISGFAQLTVSPETSTETITRLPTSSIMTESAEMMIKTQDPPGSTPESTHT
VDISTTPNWETHSTVTQRFHSEM TLLVSRSPGDMLWPSQSSVEETSSASSLLSLPATTSPS
PVSSSTLVEDFPSASLPVTSLLNPGLVITTD RMGISREPGTSSTSNLSSTSHERLTTLEDTVDTED
MQPSTHTAVTNVRTSISGHESQSSVLSDSETPKATSPMGTTYTMGETSVSISTSDFFETSRIQIE
PTSSLTSGLRETSSSERISSATEGSTVLSEVPSGATTEVSRTEVISSRGTSMSGPDQFTISPDIS
TEAIRLSTSPIMTESAESAITIETGSPGATSEGTTLTLDSTTTTFWSGTHSTASPGFHSSEM TLM
SRTPGDVPWPSLP SVEEASSVSSSLSPAMTSTSFFSTLPESISSSPPHPVTALLTLGPVKTTDM
LRTSSEPETSSPPNLSSTS AEILATSEVTKDREKIHPSNTPVNVGTVIYKHLSPSSVLADLVTT

FIG. 7 (cont'd)

KPTSPMATTSTLGNTSVSTSTPAFPETMMTQPTSSLTSGLREISTSQETSSATERSASLSGMPT
GATTKVSRTEALSLGRTSTPGPAQSTISPEISTETITRISTPLTTTGS AEMTITPKTGHSGASSQG
TFTLDTSSRASWPGTHSAATHRSPHSGMTTPMSRGPEDVSWPSRPSVEKTSPSSSLVLSAV
TSPSPLYSTPSESSHSSPLRVTSLFTPVMMKTTDMLDTSLEPVTTSPPSMNITSDES LATS KAT
METEAIQLSENTAVTQMG TISARQEFYSSYPGLPEPSKVTS PVVTSSTIKDIVSTTIPASSEITRIE
MESTSTLTPTPRETSTSQEIHSATKPSTVPYKALTSATIEDSMTQVMSSSRGPSPDQSTMSQDI
STEVITRLSTSPIKTESTEMTITTQTGSPGATSRGTLTLDSTTFMSGTHSTASQGF SHSQMTAL
MSRTPGDVPWL SHPSVEEASSASFSLSSPVM TSSSPVSSTLPDSIHSSSLPVTSLLTSGLVKTT
ELLGTSSEPETSSPPNLSSTSAEILAITEVTTDTEKLEMTNNVTSGYTHESPSSVLADSVTTKATS
SMGITYPTGDTNVL TSTPAFSDTSRIQTKSKLSLTPGLMETSISEETSSATEKSTVLSSVPTGATT
EVS RTEAISSRTSIPGPAQSTMSSDTSMETITRISTPLTRKESTDMAITPKTGPSGATSQGTFTL
DSSSTASWPGTHSATTQRF PQSVVTT PMSRGPEDVSWPSPLSVEKNSPSSSLVSSSVTSPS
PLYSTPSGSSHSSPVVTS LFTSIMMKATDMLDASLEPETTSAPNMNITSDESLAASKATTETEA
IHVFENTAASHVETTSATEELYSSSPGFSEPTKVISPVVTS SSI RDNMVSTTMPGSSGITRIEIES
MSSLTPGLRETRTSQDITSSTETSTVLYKMPSGATPEVSRTEVMPSSRTSIPGPAQSTM SLDIS
DEVVTRLSTSPIMTESAEITITTQTGYSLATSQVTLPLGTSMTFLSGTHSTMSQGLSHSEMTNLM
SRGPESLSWTS PRFVETTRSSSSLTSLPLTTSLSPV SSTLLDSSPSSPLPVTS LIILPGLVKTT EVL
DTSSEPKTSSSPNLSSTSVEIPATSEIMTDTEKIHPSNTAVAKVRTSSSVHESHSSVLADSETTI
TIPSMGITS AVDDTTVFTSNPAFSETRRIPT EPTFSLTPGFRETSTSEETTSITETSAVLYGVPTS
ATTEVSMTEIMSSNRIHIPDS DQSTMSPDIITEVITRLSSSSMMSESTQMTITTQKSSPGATAQST
LTLATTTAPLARTHSTVPPRFLHSEMTTLM SRSPENPSWKSSLFVEKTSSSSSSLLSPVTTSPS
VSSTLPQSIPSSSF SVTSLLT PGMVKTTDTSTEPGTS LSPNLSGTSVEILAASEVTTDTEKIHPS
SMAVTNVGTTSSGHELYSSSVIHSEPSKATYPVGTPSSMAETSISTSM PANFETTGF EAEPFSH
LTSGFRKTNMSLDTSSVTPTNTPSSPGSTHLLQSSKTDFTSSAKTSSPDWPPASQYTEIPVDIIT
PFNASPSITESTGITSFPESRFTMSVTESTHHLSTDLLPSAETISTGTVMPSLSEAMTSFATTGV
PRAISGSGSPFSRTESGPGDATLSTIAESLP SSTPVPFSSSTFTTTDSSTIPALHEITSSSATPYR
VDTSLGTESSTTEGRLVMVSTLDTSSQPGRTSSSPILDTRMTESVELGTVTSAYQVPSLSTRLT
RTDGIMEHITKIPNEAAHRGTIRPVKGPQTSTSPASPKGLHTGGTKRMETTTTALKTTTALKTT
SRATLTTSVYPTPLGTLTPLNASMQMASTIPTEMMITTPYVFPDVPETTSSLATSLGAETSTALP
RTTPSVFNRESETTASLVSRSGAERSPVIQTL DVSSSEPDTTASWWIHPAETIPTVSKTTPNFFH
SELDTVSSSTATSHGADVSSAIP TNISPEL DALTPLVTISGTDSTTFPTLT KSPHETETRTTWLT
HPAETSS TIPRTIPNFSHHESDATPSIATSPGAETSSAIPIMTVSPGAEDLVTSQVTSSGTD RNM
TIPTLTLS PGEPKTIASLVTHPEAQ TSSAIP TSTISPAVSRLVTSMTVSLAAKTSTTNRALTN SPGE
PATTVSLVTHPAQTSPTVPWTT SIFFHSKSDTTPSMTTSHGAESS SAVPTPTVSTEVPGVVTPL
VTSSRAVISTTIPILT LSPGEPETTPSMATSHGEEASSAIP TPTVSPGVPGVVTSLVTSSRAVTST
TIPILTFSLGEPETTPSMATSHGTEAGSAVPTVLPEVPGMVTS LVASSRAVTSTTLPTLT LSPGE
PETTPSMATSHGAEASSTVPTVSPEVPGVVTSLVTSSSGVNSTSIPTLILSPGELETTPSMATSH
GAEASSAVPTPTVSPGVSGVVTPLVTSSRAVTSTTIPILT LSSSEPETTPSMATSHGVEASSAVL
TVSPEVPGMVTS LVTSRAVTSTTIPTLT ISSDEPETTTSLVTHSEAKMISAIPTLAVSPTVQGLV
TSLVTSSGSETSAF SNLTVASSQPETIDSWAH PGTEASSVPTLT VSTGEPFTNISLVTHPAES
SSTLPRTTSRFSHSELDTMPSTVTSPEAESSSAISTTISPGIPGVLTSLVTSSGRDISATFPTVPE
SPHESEATASWTHPAVTSTTVPRTTPNYSHSEPDTTPSIATSPGAEATSD FPTITVSPDVPDM
VTSQVTSSGTDTSITPTLT LSSGEPETTTSFITYSEHTSSAIP TLPVSPGASKMLTSLVISSGTD
STTTFPTLTETPYEPETTAIQLIHPAETNTMVPRTTPKF SHSKSDTTL PVAITSPGPEASSAVSTT
TISPDMSDLVTS LVPSSGTDSTTFPTLSETPYEPETTATWLTHPAETSTTVSGTIPNFSHRGSD
TAPSMVTSPGVDTRSGVPTTTIPPSIPGVVT SQVTSSATDTSTAIP TLT PSPGEPETTASSATHP
GTQTGFTVPIRTVPSSEPDTMASWTHPPQTSTPVSRTTSSFSHSSPDATPVMATSPRTEASS
AVLTTISPGAPEMVTSQITSSGAATSTTVPTLT HSPGMPETTALLSTHPRTETSKTFPASTVFPQ
VSETTASLTIRPGAETSTALPTQTTSSLFTLLVTGT SRVDLSPTASPGVSAKTAPLSTHPTG TETS

FIG. 7 (cont'd)

TMIPTSTLSLGLLETTGLLATSSSAETSTSTLTTLTVSPAUSGLSSASITTDKPQTVTSWNTETSPS
 VTSVGPPEFSRTVTGTTMTLIPSEMPPTPKTSHGEGVSPTTILRTTMVEATNLATTGSSPTVAKT
 TTTFNTLAGSLFTPLTTPGMSTLASESVTSRTSYNHRSWISTTSSYNRRYWTPATSTPVTSTFS
 PGISTSSIPSSTAATVPFMPVFTLNFTITNLQYEEDMRHPGSRKFNATERELQGLLKPLFRNSSL
 EYLYSGCRLASLRPEKDSSATAVDAICTHRPDPELGLDRERLYWELSNLTNGIQELGPYTLDR
 NSLYVNGFTHRSSMPTTSTPGTSTVDVGTSGTPSSSPSPTTAGPLLMPFTLNFTITNLQYEEDM
 RRTGSRKFNTMESVLQGLLKPLFKNTSVGPLYSGCRLTLRPEKDGAATGVDAICTHRLDPKS
 PGLNREQLYWELSKLTNDIEELGPYTLDRNSLYVNGFTHQSSVSTTSTPGTSTVDLRTSGTPSS
 LSSPTIMAAGPLLVPFTLNFTITNLQYGEDMGHPGSRKFNTTERVLQGLLGPIFKNTSVGPLYSG
 CRLTSRSEKDGAATGVDAICHHLDPKSPGLNRERLYWELSQLTNGIKELGPYTLDRNSLYVN
 GFTHRTSVPTSSSTPGTSTVDLGTSGTPFSLPSPATAGPLLVLFTLNFTITNLQYEEDMRHPGSR
 KFNTTERVLQTLGPMFKNTSVGLLYSGCRLTLRSEKDGAATGVDAICTHRLDPKSPGVDRE
 QLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHWIPVPTSSTPGTSTVDLGSSTPSLPSPTTA
 GPLLVFTLNFTITNLQYEEDMHCPGSRKFNTTERVLQSLGPMFKNTSVGPLYSGCRLTLRS
 EKDGAATGVDAICTHRLDPKSPGVDREQLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHQTS
 APNTSTPGTSTVDLGTSGTPSSLPSPSAGPLLVPFTLNFTITNLQYEEDMHHPGSRKFNTTER
 VLQGLLGPMFKNTSVGLLYSGCRLTLRPEKNGAATGMDAICSHRLDPKSPGLNREQLYWELS
 QLTHGIKELGPYTLDRNSLYVNGFTHRSSVAPTSTPGTSTVDLGTSGTPSSLPSPPTAVPLLVP
 FTLNFTITNLQYGEDMRHPGSRKFNTTERVLQGLLGPLFKNSSVGPLYSGCRLISLRSEKDGA
 TGVDICTHHLNLPQSPGLDREQLYWQLSQMTNGIKELGPYTLDRNSLYVNGFTHRSSGLTTST
 PWTSTVDLGTSGTPSPVPSPTTTGPLLVFTLNFTITNLQYEENMGHPGSRKFNTITESVLQGLL
 KPLFKSTSVGPLYSGCRLTLRPEKDGATRVDAICTHRPDKIPGLDRQQLYWELSQLTHSIT
 ELGPYTLDRDSLYVNGFTQRSSVPTTSTPGTFTVQPETSETPSSLPGPATGPVLLPFTLNFTIT
 NLQYEEDMRHPGSRKFNTTERVLQGLLMPLFKNTSVSSLYSGCRLTLRPEKDGAATRVDAVC
 THRPDPKSPGLDRERLYWKLSQLTHGITELGYPYTLDRHSLYVNGFTHQSSMTTTRTPDTSTMH
 LATSRTPASLSGPMASPLLVFTINFTITNLRYEENMHHPGSRKFNTTERVLQGLLRPVFKNTS
 VGPLYSGCRLTLRPPKDGAAATKVDAICTYRPDPKSPGLDREQLYWELSQLTHSITELGYPYTLDR
 RDSLYVNGFTQRSSVPTTIPGTPTVDLGTSGTPVSKPGPSAASPLLVFTLNFTITNLRYEENM
 QHPGSRKFNTTERVLQGLLRSLFKSTSVGPLYSGCRLTLRPEKDGTATGVDAICTHHPDPKS
 PRLDREQLYWELSQLTHNITELGPYALDNDLSLVNGFTHRSSVSTTSTPGTPTVYLGASKTPAS
 IFGPSAASHLLIFTLNFTITNLRYEENMWPGSRKFNTTERVLQGLLRPLFKNTSVGPLYSGCRL
 TLLRPEKDGEATGVDAICTHRPDPTGPGLDREQLYLELSQLTHSITELGYPYTLDRDSLYVNGFT
 HRSSVPTTSTGVVSEEPFTLNFTINNLRYMADMGGQPGSLKFNTDNVMQHLLSPLFQRSSLGA
 RYTGCRVIALRSVKNGAETRVDLLCTYLQPLSGPLPIKQVFHELSSQTHGITRLGPYSLDKDS
 LYLNQYNEPGPDEPPTPKPATTFPLPSEATTAMGYHLKTLTLNFTISNLQYSPDMGKGSATF
 NSTEGLVQLHLLRPLFQKSSMGPFYLGCLISLRPEKDGAATGVDTTCTYHPDPVGPGLDIQQL
 YWELSQLTHGVTQLGFYVLDRLSLFINGYAPQNLIRGEYQINFHIVNWNLSNPDPSTSEYITLL
 RDIQDKVTTLYKGSQHLDTFRFCLVTNLTMDSVLVTVKALFSSNLDPSLVEQVFLDKTLNASFH
 WLGSTYQLVDIHVTEMESSVYQPTSSSSTQHLYNFTITNLQYSDKAQPGTTNYQRNKRNIED
 ALNQLFRNSSIKSYFSDCQVSTFRSVPNRHHTGVDSLCNFSPLARRVDRVAIYEEFLRMTRNG
 TQLQNFTLDRSSVLVDGYSPNRNEPLTGNSDLPFWAVILIGLAGLLGVITCLICGVLTTRRRKK
 EGEYNVQQQCPGYQSHLDLEDLQ (SEQ ID NO: 2)

Folate receptor [Homo sapiens] GenBank: AAB05827.1

MAQRMTTQLLLLLVWAVVGEAQTRIAWARTELLNVCMAKHHKEKPGPEDKLHEQCRPW
 RKNACCSTNTSQAHKDVSILYRFNWNHCGEMAPACKRHFIQDTCLEYECSPNLGPWQQV
 DQSWRKERVNLNPLCKEDCEQWWEDCRTSYTCKSNWHKGWNWTSGFNKCAVGAACQPFH
 FYFPTPTVLCNEIWTHSYKVSNSYRSGSGRCIQMWFDPAQGNPNEEVARFYAAAMSGAGPWA
 AWPFLSLALMLLWLLS (SEQ ID NO: 3)

FIG. 7 (cont'd)

Folate receptor [Homo sapiens] GenBank: CAA49267.1

MVWKWMPLLLLLVCVATMCSAQDRTDLLNVCMDAKHHKTKPGPEDKLHDQCSPWKKNACC
TASTSQELHKDTSRLYNFNWDHCGKMEPACKRHFQDTCLYECSPNLGPWQQVQNSWRK
ERFLDVPLCKEDCQRWWEDCHTSHTCKSNWHRGWDWTSGVNKCPAGALCRTFESYFPTPA
ALCEGLWSHSYKVSNSYRSGSGRCIQMWFDQAQGNPNEEVARFYAAAMHVNAGEMLHGTGG
LLLSLALMLQLWLLG (SEQ ID NO: 4)

PD-L1 (also known as CD274 or B7-H1, UniProt ID No. Q9NZQ7)

MRIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNII
QFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITV
KVNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFN
VTSTLRINTTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMMDVKKCGIQDTNSKKQSDTHLEET (SEQ ID NO: 5)

ROR1

MHRPRRRGTRPPLLALLAALLAARGAAAQETELSVSAELVPTSSWNISSELNKSYSYLTLDPEM
NNITTSLGQTAEHLCKVSGNPPPTIRWFKNDAPVVQEPRRLSFRSTIYGSRLRIRNLDTTDTGYF
QCVATNGKEVVSSTGVLFVKFGPPPTASPGYSDEYEEDGFCQPYRGIACARFIGNRTVYMESL
HMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCHYAFPYCDETSSVPKPRDLRCRDECEILEN
VLCQTEYIFARSNPMILMRLKLPNCEDLPQPESPEAANCIRIGIPMADPINKNHKCYNSTGVDYR
GTVSVTKSGRQCQPWNSQYPHTHTFTALRFPELNGGHSYCRNPGNQKEAPWCFTLDENFKS
DLCDIPACDSKDSKEKNKMEILYILVPSVAIPLAIALFFFCVCRNNQKSSSAPVQRQPKHVRGQ
NVEMSMNLNAYKPKSKAKELPLSAVRFMEELGECFAFGKIYKGHLYLPGMDHAQLVAIKTLKDYN
NPQQWTEFQQEASLMAELHHPNIVCLLGAVTQEQPVCMLFEYINQGDLEFLIMRSPHSDVGC
SSDEDGTVKSSLDHGDFLHIAIQIAAGMEYLSSHFFVHKDLAARNILIGEQLHVKISDLGLSREIY
SADYYRVQSKSLLPIRWMPPEAIMYGKFSSSDSIWSFGVVLWEIFSFGQLQPYYGFSNQEVIEMV
RKRQLLPCSEDCPPRMYSLMTECWNEIPSRPRFKDIHVRRLRSWEGLSSTSTTPSGGNAT
TQTTSLSASPVSNLSPRYPNYMFPSQGITPQQGIAGFIGPPIPNQRFIPINGYPIPPGYAAFFPA
AHYQPTGPPRVIQHCPPPKSRSPSSASGSTSTGHVTSPLSSGSNQEANIPLLPHMSIPNHPGG
MGITVFGNKSQKPYKIDSKQASLLGDANIHGHTESMISAE (SEQ ID NO: 6)

Her2 UniProtKB/Swiss-Prot: P04626.1

MELAAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLEL
TYLPTNASLSFLQDIQEVQGYVLIHNVQRVPLQRLRIVRGQTQLFEDNYALAVLDNGDPLNNT
TPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSA
CHPCSPMCKGSRCWGESSEDCQSLTRTVACAGGCARCKGPLPTDCCHEQCAAGCTGPKHSD
CLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLV
CPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFL
PESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPLDSVFQNLQVIRGRILHNGAYS
LTLQGLGISWLGLRSLRELGSGLALIHNNHLCFVHTVPWDQLFRNPHQALLHTANRPEDECV
GEGlachQLCARGHCWGPPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHP
ECQPQNGSVTCFGPEADQCVACAHYKDPFFCVARCSGVKPDLSYMPIWKFPDEEGACQPCPI
NCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQKIRKYTMRRLLQET
ELVEPLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLENT
SPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRRGLGSQDLL
NWCMMQIAKGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEHADGGKVPIK
WMALESILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDV
YMIMVKCWMIDSECRPRFRELVSFMRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDM
GDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSSTRSGGGDLTLGLEPSEEEAPRSPLA

FIG. 7 (cont'd)

PSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPE
YVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPEYLTPQ
GGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV (SEQ ID
NO: 7)

Glypican [Homo sapiens] GenBank: AAA98132.1

MAGTVRTACLVVAMLLSLDFPGQAQPPPPPDATCHQVRSFFQRLQPGLKWWPETPVPGSDL
QVCLPKGPTCCSRKMEEKYQLTARLNMEQLLQSASMEKFLIIQNAAVFQEAFEIVVRHAKNYT
NAMFKNNYPSLTPQAFEFVGEFFTDVSLYILGSDINVDDMVNELFDSLFPVIYTQLMNPGLPDS
ALDINECLRGARRDLKVFGNFPKLIMTQVSKSLQVTRIFLQALNLGIEVINTTDHLKFSKDCGRML
TRMWYCSYCQGLMMVKPCGGYCNVVMQGCMAGVVEIDKYWREYILSLEELVNGMYRIYDME
NVLLGLFSTIHDSIQYVQKNAGKLTTTIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVAHVEHEE
TLSSRRRELIQKLKSFISFYALPGYICSHSPVAENDTLCWNGQELVERYSQKAARNGMKNQF
NLHELKMKGPPEPVVSQIIDKLKHINQLLRTMSMPKGRVLDKNLDEEGFESGDCGDDDEDECIGG
SGDGMKVKVKNQLRFLAELAYDLDDVDDAPGNSQQATPKDNEISTFHNLGNVHSPKLKLTSMASIV
VCFFFLVH (SEQ ID NO: 8)

Glypican [Homo sapiens] GenBank: CAA38139.1

MELRARGWWLLCAAAALVACARGDPASKSRSCGEVRQIYGAKGFSLSQAEISGEHLRIPC
QGYTCCTSEMEENLANRSHAELETALRDSSRVLQAMLATQLRSFDDHFQHLLNDSERTLQATF
PGAFGELYTQNARAFRDLYSELRLYYRGANLHLEETLAEFWARLLERLFKQLHPQLLLPDDYLD
CLGKQAEALRPFGEAPRELRLRATRAFAARSFVQGLGVASDVVRKVAQVPLGPECSRAVMK
LVYCAHCLGVPGARPCPDYCRNVLKGCLANQADLDAEWRNLLDSMVLITDKFWGTSGVESVI
GSVHTWLAEAINALQDNRDTLTAKVIQGCNPKVNPQGPPEEKRRRGKLAPRERPPSGTLE
KLVSEAKAQLRDVQDFWISLPGTLCSEKMALSTASDDRCWNGMARGRYLPEVMGDGLANQIN
NPEVEVDITKPDMTIRQQIMQLKIMTNRLRSAYNGNDVDFQDASDDGSGSGSGDGCLDDLCG
RKVSRKSSSSRTPLTHALPGLSEQEGQKTSAASCPQPPTFLLPLLLFLALTVARPRWR (SEQ ID
NO: 9)

GPC2 GenBank: AAH27972.1

MSALRPLLLLLLPLCPGPGPGSEAKVTRSCAETRQVLGARGYSLNLIPPALISGEHLRVCPQ
EYTCCSSETEQRILIRETEATFRGLVEDSGSFLVHTLAARHRKFDEFFLEMLSV AQHSLTQLFSH
SYGRLYAQHALIFNGLFSRLRDFYGESGEGLDDTLADFWAQLLERVFLLHPQYSFPPDYLLCL
SRLASSTDGSLQPFQDSPRRLRLQITRTLVAARAFVQGLETGRNVVSEALKVPVSEGCSQALM
RLIGCPLCRGVPSLMPCQGFCNLNVVRGCLSSRGLEPDWGNLYLDGLLILADKLQGPFSFELTAE
SIGVKISEGLMYLQENSAKVSAQVFQECGPPDPVPARNRRAPPPREEAGRLWSMVTEERPT
TAAGTNLHRLVWELRERLARMRGFWARLSLTVCGDSRMAADASLEAAPCWTGAGRGRYLPP
VVGGSAPAEQVNNPELKVDA SGPDVPTRRRLQLRAATARMKTAALGHDLDDGQDADEDASGS
GGGQQYADDWMAGAVAPPARPPPPYPPRRDGS GGKGGGGSARYNQGRSRSGGASIGFH
TQTILILSLALALLGPR (SEQ ID NO: 10)

HER2 GenBank: AAA75493.1

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVGNNLEL
TYLPTNASLSFLQDIQEVQGYVLIHQNVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNT
TPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTIDTNRSA
CHPCSPMCKGSRWGESSEDCQSLTRTV CAGGCARCKGPLPTDCCHEQCAAGCTGPKHSD
CLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLV
CPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFL
PESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPLDSVFQNLQVIRGRILHNGAYS

FIG. 7 (cont'd)

LTLQGLGISWLGLRSLRELGSSGLALIIHNTLHLCFVHTVPWDQLFRNPHQALLHTANRPEDECV
GEGlachQLCARGHCWGPPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHP
ECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPC
PINCTHSCVDLDDKGCPAEQRASPLTSIVSAVVGILLVVVLGVVFGILIKRRQKKIRKYTMRRLLQ
ETELVEPLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRE
NTSPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRNRRGLGSQ
DLLNWCMIKAGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEHADGGKV
PIKWMALLESILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTI
DVYIMVVKCWMIDSECRPRFRELVSFESRMARDPQRFVVIQNEGLGPASPLDSTFYRSLLEDD
DMGDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSSTRSGGGDLTLGLEPSEEEAPRS
PLAPSEGAGSDVFDGDLGMGAAGKQLSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSP
QPEYVNQPDVRPQPPSPREGPLPAARPAGATLERAKTLSPGKNGVVKDVFAGGAVENPEYL
TPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV (SEQ
ID NO: 11)

EGFR protein [Homo sapiens] GenBank: AAH94761.1

MRPSGTAGAALLALLAALCPASRALEEKVKCQGTSNKLTQLGTFEDHFLSLQRMFNCEVVLG
NLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLNLQIIRGNMYYENSALAVLSNYDANKT
GLKELPMRNLQGGQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRRCRGKSPSDCCHNQC
AAGCTGPRESCLVCRKFRDEATCKDTCPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRN
YVVDHGSVCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKN
CTISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRG
RTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVVISGNKNLCYANTINWKKLFGTSGQKTKIISNR
GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPRFVENSEC
IQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVC
HLCHPNCTYGCTGPGLEGCPNTPKIPSIATGMVGALLLLVVALGIGLFRMRRHIVRKRTLRL
LQERELVEPLTPSGEAPNQALLRILKETEFKIKVLGSGAFGTVYKGLWPEGEKVKIPVAIKELR
EATSPKANKEILDEAYVMASVDNPHVCRLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQY
LLNWCVQIAKGMNYLEDRLVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEKEYHAEGGKV
PIKWMALLESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTID
VYMIMVVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEE
DMDDVDDADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNSTVACIDRNLQSCPIKEDSFLQRY
SSDPTGALTEDSIDDTFLPVPGEWLWVKQSCSSTSSTHSAAASLQCPSQVLPPASPEGETVAD
LQTQ (SEQ ID NO: 12)

FIG. 7 (cont'd)

CD19

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLK
LSLGLPGLGIHMRPLASWLFIFNVSSQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRW
NVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCVPPRDSLNSLSQ
DLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWWMETGLLL
PRATAQDAGKYYCHRGNTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILH
LQRALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWAAGLGG
TAPSYGNPSSDVQADGALGSRPPGVGPEEEEEEGEGYEEPDSEEDSEFYENDSNLGQDQLSQ
DGSYENPEDEPLGPEDEDSFSNAESYENEDDELTQPVARTMDFLSPHGSAWDPSREATSLG
SQSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGGRMGWTWSTR
(SEQ ID NO: 13)

CD3 gamma chain UniProtKB/Swiss-Prot: P09693.1

MEQGKGLAVLILAILLLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLT
EDKKKWNLGSSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATISGFLFAEIVSIFVLA
VGVIYFIAGQDQVRQSRASDKQTLLPNDQLYQPLKDREDDQYSHLQGNQLRRN (SEQ ID NO:
14)

CD3 delta chain UniProtKB/Swiss-Prot: P04234.1

MEHSTFLSGLVLATLLSQVSPFKIPIEELEDVRFVNCNTSITWEGTVGTLLSDITRLDLGKRILD
PRGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLALGVFCFAGHETG
RLSGAADTQALLRNDQVYQPLRDRDDAQYSHLGGNWARNK (SEQ ID NO: 15)

CD3 zeta chain UniProtKB/Swiss-Prot: P20963.2

MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 16)

CD28 [Homo sapiens] GenBank: AAA51945.1

MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSYNEKSNGTIIHVKGKHLCPSPLPFGP
SKPFWVLVVGGVLACYSLLVTVAFIIFWVRSKRSLHSDYMNMTPRRPGPTRKHYPYAPP
RDFAAYRS (SEQ ID NO: 17)

4-1BB, also called CD137 or TNFSF9 (UniProt ID No. Q07011)

MGNSCYNIVATLLLVNLFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCD
ICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQELTKKGCKDCCFGT
FNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQ
IISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGG
CEL (SEQ ID NO: 18)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/13936

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61P 35/00, C07K 16/28, G01N 33/574, A61K 39/00 (2020.01)

CPC - C07K 16/2803, A61K 2039/505, C07K 16/2896, C07K 16/2809, C07K 16/2815, C07K 16/2818, C07K 16/2827, C07K 16/2866, C07K 2317/31, C07K 2317/622, C07K 2317/626, C07K 2317/73, G01N 33/57484

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/014001 A1 (FRED HUTCHINSON CANCER RESEARCH CENTER) 18 January 2018 (18.01.2018) Abstract; Claim 1; Claim 20; Claim 22; Claim 32; Claim 107; para [0007]; para [0009]; para [0011]; para [0017]; para [0019]; para [0024]; para [0053]; para [0118]; para [0127]; para [0179]; para [0189-0191]; para [0207]; para [0214-0215]	1-13, 49
Y	WO 2006/002114 A2 (MANNKIND CORPORATION) 05 January 2006 (05.01.2006) Abstract; Claim 1; para [0034]	1-13, 49
Y	US 2014/0227277 A1 (AMERSDORFFER et al.) 14 August 2014 (14.08.2014) Abstract; para [0088-0089]	10-11

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

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 March 2020

Date of mailing of the international search report

16 APR 2020

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/13936

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 14-48, 50-59
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.