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(71) Applicant: THE BOARD OF TRUSTEES OF THE LE-
LAND STANFORD JUNIOR UNIVERSITY [US/US];
Office of the General Counsel, Building 170, 3rd Floor,
Main Quad P.O. Box 20386, Stanford, California 94305-
2038 (US).

(72) Inventor; and

(71) Applicant : KOHRT, Holbrook [US/US]; 38 Genoa
Place, San Francisco, California 95133 (US).

(74) Agent: SHERWOOD, Pamela J.; Bozicevic, Field &
Francis LLP, 1900 University Avenue, Suite 200, East
Palo Alto, California 94303 (US).

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(54) Title: IMPROVED T-DM1 THERAPY

(57) Abstract: The present disclosure provides improved therapeutic regimens involving a trastuzumab-drug conjugate such as T-DM1.



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IMPROVED T-DM1 THERAPY

BACKGROUND

[01] A remarkably innovative and effective cancer therapy has been described that involves staggered therapy with i) an anti-tumor agent (anti-tumor therapy) and ii) an effector-cell agonist agent (agonist therapy). See Published International Patent Application WO2011/071871 by Kohrt et al., entitled "Methods for Enhancing Anti-Tumor Antibody Therapy"; the "Stanford Patent Application"). Specifically, an antibody agent that targets particular tumor cells is administered and then, a period of time later, an agonist agent that targets an inducible effector cell surface marker is administered. As described in the Stanford Patent Application, and confirmed in subsequent reports, such a staggered therapeutic regimen can be surprisingly effective, even as compared with simultaneous administration of the same agents, in cancer treatment. The present disclosure describes particular usefulness of certain such staggered therapy regimens (e.g., with particular agents and/or in particular therapeutic contexts).

SUMMARY

[02] The present disclosure demonstrates particular effectiveness of staggered therapy regimens in which the anti-tumor agent is or comprises an antibody-drug conjugate, and particularly where the anti-tumor agent is a trastuzumab-drug conjugate, particularly a trastuzumab-chemotherapeutic conjugate such as a trastuzumab-anti-microtubule-stabilizing agent conjugate. Findings of the present disclosure are particularly relevant to staggered therapy regimens in which the anti-tumor agent is ado-trastuzumab emtansine ("T-DM1"). T-DM1 is an antibody-drug conjugate consisting of the monoclonal antibody trastuzumab linked to the cytotoxic agent DM1 and is marketed under the trade name KADCYLA[®]. The present disclosure particularly demonstrates that staggered therapy regimens in which T-DM1 is administered, followed a period of time with an agonist agent as described herein (i.e., that targets an inducible effector cell surface marker, and particularly that targets an inducible effector cell surface marker whose presence or level on effector cell surfaces is increased after the T-DM1 administration as compared with that observed prior to the T-DM1 administration and/or so that it is higher than a relevant threshold level) show excellent effectiveness, and in particular shows increased effectiveness relative to administration of T-DM1 alone.

[03] Furthermore, the present disclosure surprisingly demonstrates that adding an inducible effector cell surface marker agonist (e.g., an agonist antibody to an inducible effector cell surface marker) in a staggered therapy regimen as described herein, can show increased synergy with

T-DM1 therapy relative to that observed when the agonist is added to trastuzumab therapy, even though T-DM1 and trastuzumab target the same tumor antigen and, indeed, the antibody portion of T-DM1 is trastuzumab.

- [04] In particular, the present disclosure documents such surprising increased synergy in treatment of already established tumors (e.g., breast tumors). These findings are both unexpected and useful, as it is common, if not typical, for patients to present with already-established tumors.
- [05] In some embodiments, the present disclosure relates to novel treatment methods of individuals diagnosed with cancer. In some embodiments, an individual diagnosed with cancer is first administered TDM-1. After a period of time, effector cells (e.g., NK cells) which are innate immune effector cells critical for ADCC upregulate expression of an inducible effector cell surface marker such as CD137, OX40, GITR, CD30 or ICOS. Subsequently, a second antibody is administered, which antibody is an agonist antibody targeting the inducible effector cell surface marker on NK cells (including but not limited to anti-CD137, anti-OX40, anti-GITR, anti-CD30 or anti-ICOS). In some embodiments, expression of the aforementioned cell surface marker is evaluated following administration T-DM1, for example in order to determine the optimal time for dosing the second agent. Alternatively, a timing period may be determined empirically, and generally applied. The combination of agents and their sequential administration is shown to provide for a level of tumor-specific, therapeutic synergy that is not observed with administration of the single agents alone. The method specifically enhances the anti-tumor function of monoclonal antibodies directed against tumor antigens. Because the second antibody targets inducible effector cell surface markers that have been inducibly expressed on NK cells by the tumor- directed antibody, this methods allows specific stimulation of NK cells which are implicated in ADCC-mediated killing of the tumor cells, while sparing other NK cells, thereby limiting potential nonspecific side effects.
- [06] In some particular embodiments, the present disclosure provides methods of treating cancer, comprising administering to a patient a composition comprising an agonistic antibody to a molecule whose expression increases on surfaces of natural killer (NK) cells that mediate antibody-dependent cellular cytotoxicity (ADCC) when such cells are exposed to tumor cells bound by anti-tumor antibody T-DM1, which agonistic antibody is characterized as agonistic in that, when the NK cells with the inducible effector cell surface marker on their surface are contacted with the agonistic antibody, their ADCC is increased as compared with that observed absent such contact, the patient having received therapy including T-DM1 a period of time prior to the administering, such that the increase in expression of the inducible effector cell surface marker has occurred.

- [07] In some embodiments, the agonistic antibody is particularly characterized in that, when tumor cells coated with T-DM1 are contacted with NK cells in which the inducible effector cell surface marker is expressed on the surface together with the agonistic antibody, apoptosis of the tumor cells is increased relative to that observed in absence of the agonistic antibody and/or in that, when tumor cells coated with T-DM1 are contacted with NK cells in which the inducible effector cell surface marker is expressed on the surface together with the agonistic antibody, tumor growth is reduced relative to that observed in absence of the agonistic antibody.
- [08] In some embodiments, the agonistic antibody is a monoclonal antibody. In some embodiments, the agonistic antibody is a xenogeneic human antibody. In some embodiments, the agonistic antibody is a humanized antibody. In some embodiments, the agonistic antibody is a chimeric antibody.
- [09] In some embodiments, provided methods may include a step of determining the level of the inducible effector cell surface marker. In some such embodiments, the level of the inducible effector cell surface marker is determined prior to administering T-DM1, and the increase in expression following administration of T-DM1 is determined. Alternatively or additionally, in some such embodiments, the step of determining comprises providing a patient sample; and determining the level in the sample.
- [10] In some embodiments, a patient sample is a blood sample or cellular fraction thereof.
- [11] In some embodiments, the tumor is a solid tumor. In some embodiments the tumor is an adenocarcinoma. In some embodiments, the tumor is a breast tumor, a gastric tumor, or a gastroesophageal tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the tumor is an established tumor, for example in that it has been and/or appears to have been present in the subject for at least 30 days.
- [12] In some embodiments, the inducible effector cell surface marker is a member of the tumor necrosis factor receptor (TNFR) family. In some embodiments, the inducible effector cell surface marker is a member of the CD28 family. In some embodiments, the inducible effector cell surface marker is selected from CD38, CD137, OX40, GITR, CD30 and/or ICOS. In some embodiments, the inducible effector cell surface marker is or comprises CD137.
- [13] In some embodiments, the present disclosure provides an improvement in a method of treating cancer with T-DM1 therapy, the improvement comprising, a period of time after the step of administering T-DM1 therapy (the "first administering step"), performing a second administering step that comprises administering a composition comprising an agonistic antibody that targets a molecule whose expression increases on surfaces of effector NK cells that mediate antibody-

dependent-cellular cytotoxicity (ADCC) when such cells are exposed to tumor cells bound by T-DM1, the period of time being sufficient so that expression of the inducible effector cell surface marker has been increased on such surfaces at the time of the second administering step, so that ADCC is increased.

- [14] In some embodiments, the present disclosure provides a method of enhancing anti-tumor effect(s) of T-DM1 in a patient, which method comprises sequential administration of T-DM1 and an agonistic antibody, the agonistic antibody targeting at least one molecule on NK cells characterized in that its expression is induced on surfaces of NK cells during activation of the NK cells when such cells are exposed to tumor cells bound by an anti-tumor antibody, wherein the agonistic antibody is characterized as agonistic in that, when the NK cells with the inducible effector cell surface marker on their surface are contacted with the agonistic antibody, their ADCC is increased as compared with that observed absent such contact; and wherein the agonistic antibody is administered a period of time after the administration of T-DM1, the period of time being sufficiently long that increased expression of the inducible costimulatory molecule has occurred.

BRIEF DESCRIPTION OF THE DRAWING

- [15] Figure 1A-B shows the quantification of interferon gamma (IFN- γ) secreted from effector cells after co-culture with tumor cells and indicated therapeutic antibodies. Figure 1, Panel A shows results with MCF7 cells; Figure 1, Panel B shows results with HER-18 cells.
- [16] Figure 2A-B shows the quantification of degranulation, as measured by CD107 positivity, of effector cells after co-culture with tumor cells and indicated therapeutic antibodies. Figure 2, Panel A shows results with MCF7 cells; Figure 2, Panel B shows results with HER-18 cells.
- [17] Figure 3A-B shows the quantification of target cell lysis by effector cells after co-culture with tumor cells and indicated therapeutic antibodies. Figure 3, Panel A shows results with MCF7 cells; Figure 3, Panel B shows results with HER-18 cells.
- [18] Figure 4 shows the quantification of tumor size after mice with HER 18 xenografted tumors were treated with indicated therapeutic antibodies.
- [19] Figure 5 shows the rate of survival of mice with HER 18 xenografted tumors after treatment with indicated therapeutic antibodies.

- [20] Figure 6 shows the quantification of tumor size after mice with SU-258 xenografted tumors were treated with indicated therapeutic antibodies.
- [21] Figure 7 shows the rate of survival of mice with SU-258 xenografted tumors after treatment with indicated therapeutic antibodies.

DEFINITIONS

- [22] Below are provided certain definitions of terms used herein, many or most of which confirm common understanding of those skilled in the art.
- [23] *Administration*: As used herein, the term “administration” refers to the administration of a composition to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal. In some embodiments, administration may involve intermittent dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time. As is known in the art, antibody therapy is commonly administered parenterally (e.g., by intravenous or subcutaneous injection).
- [24] *Agent*: The term “agent” as used herein may refer to a compound or entity of any chemical class including, for example, polypeptides, nucleic acids, saccharides, lipids, small molecules, metals, or combinations thereof. As will be clear from context, in some embodiments, an agent can be or comprise a cell or organism, or a fraction, extract, or component thereof. In some embodiments, an agent is or comprises a natural product in that it is found in and/or is obtained from nature. In some embodiments, an agent is or comprises one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. In some embodiments, an agent may be utilized in isolated or pure form; in some embodiments, an agent may be utilized in crude form. In some embodiments, potential agents are provided as collections or libraries, for example that may be screened to identify or characterize active agents within them. Some particular embodiments of agents that may be utilized in accordance with the present invention include small molecules,

antibodies, antibody fragments, aptamers, nucleic acids (e.g., siRNAs, shRNAs, DNA/RNA hybrids, antisense oligonucleotides, ribozymes), peptides, peptide mimetics, etc. In some embodiments, an agent is or comprises a polymer. In some embodiments, an agent is not a polymer and/or is substantially free of any polymer. In some embodiments, an agent contains at least one polymeric moiety. In some embodiments, an agent lacks or is substantially free of any polymeric moiety.

- [25] *Agonist*: As used herein, the term “agonist” refers to an agent whose presence or level correlates with increase in level and/or activity of another agent (i.e., the agonized agent). In general, an agonist may be or include an agent of any chemical class including, for example, small molecules, polypeptides, nucleic acids, carbohydrates, lipids, metals, and/or any other entity that shows the relevant activating activity. In some embodiments, an agonist may be direct (in which case it exerts its influence directly upon its target); in some embodiments, an agonist may be indirect (in which case it exerts its influence by other than binding to its target; e.g., by interacting with a regulator of the target, so that level or activity of the target is altered).
- [26] *Agonist Therapy*: The term “agonist therapy”, as used herein, refers to administration of an agonist that agonizes a particular target of interest to achieve a desired therapeutic effect. In some embodiments, agonist therapy involves administering a single dose of an agonist. In some embodiments, agonist therapy involves administering multiple doses of an agonist. In some embodiments, agonist therapy involves administering an agonist according to a dosing regimen known or expected to achieve the therapeutic effect, for example, because such result has been established to a designated degree of statistical confidence, e.g., through administration to a relevant population.
- [27] *Antagonist*: As used herein, the term “antagonist” refers to an agent whose presence or level correlates with decreased level or activity of another agent (i.e., the antagonized agent, or target). In general, an antagonist may be or include an agent of any chemical class including, for example, small molecules, polypeptides, nucleic acids, carbohydrates, lipids, metals, and/or any other entity that shows the relevant inhibitory activity. In some embodiments, an antagonist may be direct (in which case it exerts its influence directly upon its target); in some embodiments, an antagonist may be indirect (in which case it exerts its influence by other than binding to its target; e.g., by interacting with a regulator of the target, so that level or activity of the target is altered).
- [28] *Antibody*: As used herein, the term “antibody” refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150

kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)—an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y’s stem). A short region, known as the “switch”, connects the heavy chain variable and constant regions. The “hinge” connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another “switch”. Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an “immunoglobulin fold” formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as “complement determining regions” (CDR1, CDR2, and CDR3) and four somewhat invariant “framework” regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present invention include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an “antibody”, whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has

constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc, as is known in the art. Moreover, the term “antibody” as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgG, IgE and IgM, bi- or multi- specific antibodies (e.g., Zybodies®, etc), single chain Fvs, polypeptide-Fc fusions, Fabs, cameloid antibodies, masked antibodies (e.g., Probodies®), Small Modular ImmunoPharmaceuticals (“SMIPsTM”), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®, Centyrins®, and a KALBITOR®. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [e.g., poly-ethylene glycol, etc.]

- [29] *Antibody Agent:* As used herein, the term “antibody agent” refers to an agent that specifically binds to a particular antigen. In some embodiments, the term encompasses any polypeptide or polypeptide complex that includes immunoglobulin structural elements sufficient to confer specific binding. Exemplary antibody agents include, but are not limited to, human antibodies, primatized antibodies, chimeric antibodies, bi-specific antibodies, humanized antibodies, conjugated antibodies (*i.e.*, antibodies conjugated or fused to other proteins, radiolabels, cytotoxins), Small Modular ImmunoPharmaceuticals (“SMIPsTM”), single chain antibodies, cameloid antibodies, and antibody fragments. As used herein, the term “antibody agent” also includes intact monoclonal antibodies, polyclonal antibodies, single domain antibodies (e.g., shark single domain antibodies (e.g., IgNAR or fragments thereof)), multispecific antibodies (*e.g.* bi-specific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. In some embodiments, the term encompasses stapled peptides. In some embodiments, the term encompasses one or more antibody-like binding peptidomimetics. In some embodiments, the term encompasses one or more antibody-like binding scaffold proteins. In some embodiments, the term encompasses monobodies or adnectins. In many embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes one or more structural elements recognized by those skilled in the art as a complementarity determining region (CDR); in some embodiments an antibody agent is or comprises a polypeptide whose amino acid sequence

includes at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR) that is substantially identical to one found in a reference antibody. In some embodiments an included CDR is substantially identical to a reference CDR in that it is either identical in sequence or contains between 1-5 amino acid substitutions as compared with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that it shows at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that it shows at least 96%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that at least one amino acid within the included CDR is deleted, added, or substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that 1-5 amino acids within the included CDR are deleted, added, or substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical to the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that at least one amino acid within the included CDR is substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that 1-5 amino acids within the included CDR are deleted, added, or substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical to the reference CDR. In some embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes structural elements recognized by those skilled in the art as an immunoglobulin variable domain. In some embodiments, an antibody agent is a polypeptide protein having a binding domain which is homologous or largely homologous to an immunoglobulin-binding domain. In some embodiments, an antibody agent is or comprises an antibody-drug conjugate.

- [30] *Antibody-Dependent Cellular Cytotoxicity*: As used herein, the term "antibody-dependent cellular cytotoxicity" or "ADCC" refers to a phenomenon in which target cells bound by antibody are killed by immune effector cells. Without wishing to be bound by any particular theory, we observe that ADCC is typically understood to involve Fc receptor (FcR)-bearing effector cells can recognizing and subsequently killing antibody-coated target cells (e.g., cells that express on their surface specific antigens to which an antibody is bound). Effector cells that mediate ADCC can include immune cells, including but not limited to one or more of natural killer (NK) cells, macrophage, neutrophils, eosinophils.

- [31] *Antigen*: The term “antigen”, as used herein, refers to an agent that elicits an immune response; and/or (ii) an agent that binds to a T cell receptor (e.g., when presented by an MHC molecule) or to an antibody. In some embodiments, an antigen elicits a humoral response (e.g., including production of antigen-specific antibodies); in some embodiments, an antigen elicits a cellular response (e.g., involving T-cells whose receptors specifically interact with the antigen). In some embodiments, an antigen binds to an antibody and may or may not induce a particular physiological response in an organism. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, a polymer (in some embodiments other than a biologic polymer [e.g., other than a nucleic acid or amino acid polymer] etc. In some embodiments, an antigen is or comprises a polypeptide. In some embodiments, an antigen is or comprises a glycan. Those of ordinary skill in the art will appreciate that, in general, an antigen may be provided in isolated or pure form, or alternatively may be provided in crude form (e.g., together with other materials, for example in an extract such as a cellular extract or other relatively crude preparation of an antigen-containing source). In some embodiments, antigens utilized in accordance with the present invention are provided in a crude form. In some embodiments, an antigen is a recombinant antigen.
- [32] *Biological Sample*: As used herein, the term “biological sample” typically refers to a sample obtained or derived from a biological source (e.g., a tissue or organism or cell culture) of interest, as described herein. In some embodiments, a source of interest comprises an organism, such as an animal or human. In some embodiments, a biological sample is or comprises biological tissue or fluid. In some embodiments, a biological sample may be or comprise bone marrow; blood; blood cells; ascites; tissue or fine needle biopsy samples; cell-containing body fluids; free floating nucleic acids; sputum; saliva; urine; cerebrospinal fluid, peritoneal fluid; pleural fluid; feces; lymph; gynecological fluids; skin swabs; vaginal swabs; oral swabs; nasal swabs; washings or lavages such as a ductal lavages or bronchoalveolar lavages; aspirates; scrapings; bone marrow specimens; tissue biopsy specimens; surgical specimens; feces, other body fluids, secretions, and/or excretions; and/or cells therefrom, etc. In some embodiments, a biological sample is or comprises cells obtained from an individual. In some embodiments, obtained cells are or include cells from an individual from whom the sample is obtained. In some embodiments, a sample is a “primary sample” obtained directly from a source of interest by any appropriate means. For example, in some embodiments, a primary biological sample is obtained by methods selected from the group consisting of biopsy (e.g., fine needle aspiration or tissue biopsy), surgery, collection of body fluid (e.g., blood, lymph, feces etc.), etc. In some embodiments, as will be clear from context, the term “sample” refers to a preparation that is obtained by processing (e.g., by removing one

or more components of and/or by adding one or more agents to) a primary sample. For example, filtering using a semi-permeable membrane. Such a "processed sample" may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to techniques such as amplification or reverse transcription of mRNA, isolation and/or purification of certain components, *etc.*

- [33] *Biomarker*: The term "biomarker" is used herein, consistent with its use in the art, to refer to an entity whose presence, level, or form, correlates with a particular biological event or state of interest, so that it is considered to be a "marker" of that event or state. To give but a few examples, in some embodiments, a biomarker may be or comprises a marker for a particular disease state, or for likelihood that a particular disease, disorder or condition may develop. In some embodiments, a biomarker may be or comprise a marker for a particular disease or therapeutic outcome, or likelihood thereof. Thus, in some embodiments, a biomarker is predictive, in some embodiments, a biomarker is prognostic, in some embodiments, a biomarker is diagnostic, of the relevant biological event or state of interest. A biomarker may be an entity of any chemical class. For example, in some embodiments, a biomarker may be or comprise a nucleic acid, a polypeptide, a lipid, a carbohydrate, a small molecule, an inorganic agent (e.g., a metal or ion), or a combination thereof. In some embodiments, a biomarker is a cell surface marker. In some embodiments, a biomarker is intracellular. In some embodiments, a biomarker is found outside of cells (e.g., is secreted or is otherwise generated or present outside of cells, e.g., in a body fluid such as blood, urine, tears, saliva, cerebrospinal fluid, *etc.*
- [34] *Cancer*: The terms "cancer", "malignancy", "neoplasm", "tumor", and "carcinoma", are used interchangeably herein to refer to cells that exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In general, cells of interest for detection or treatment in the present application include precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and non-metastatic cells. The teachings of the present disclosure may be relevant to any and all cancers. To give but a few, non-limiting examples, in some embodiments, teachings of the present disclosure are applied to one or more cancers such as, for example, hematopoietic cancers including leukemias, lymphomas (Hodgkins and non-Hodgkins), myelomas and myeloproliferative disorders; sarcomas, melanomas, adenomas, carcinomas of solid tissue, squamous cell carcinomas of the mouth, throat, larynx, and lung, liver cancer, genitourinary cancers such as prostate, cervical, bladder, uterine, and endometrial cancer and renal cell carcinomas, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular melanoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of

the parathyroid gland, head and neck cancers, breast cancer, gastro-intestinal cancers and nervous system cancers, benign lesions such as papillomas, and the like.

- [35] *Chemotherapeutic Agent*: The term “chemotherapeutic agent”, has used herein has its art-understood meaning referring to one or more pro-apoptotic, cytostatic and/or cytotoxic agents, for example specifically including agents utilized and/or recommended for use in treating one or more diseases, disorders or conditions associated with undesirable cell proliferation. In many embodiments, chemotherapeutic agents are useful in the treatment of cancer. In some embodiments, a chemotherapeutic agent may be or comprise one or more alkylating agents, one or more anthracyclines, one or more cytoskeletal disruptors (e.g. microtubule targeting agents such as taxanes, maytansine and analogs thereof, of), one or more epothilones, one or more histone deacetylase inhibitors HDACs), one or more topoisomerase inhibitors (e.g., inhibitors of topoisomerase I and/or topoisomerase II), one or more kinase inhibitors, one or more nucleotide analogs or nucleotide precursor analogs, one or more peptide antibiotics, one or more platinum-based agents, one or more retinoids, one or more vinca alkaloids, and/or one or more analogs of one or more of the following (i.e., that share a relevant anti-proliferative activity). In some particular embodiments, a chemotherapeutic agent may be or comprise one or more of Actinomycin, All-trans retinoic acid, an Auiristatin, Azacitidine, Azathioprine, Bleomycin, Bortezomib, Carboplatin, Capecitabine, Cisplatin, Chlorambucil, Cyclophosphamide, Curcumin, Cytarabine, Daunorubicin, Docetaxel, Doxifluridine, Doxorubicin, Epirubicin, Epothilone, Etoposide, Fluorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Imatinib, Irinotecan, Maytansine and/or analogs thereof (e.g. DM1) Mechlorethamine, Mercaptopurine, Methotrexate, Mitoxantrone, a Maytansinoid, Oxaliplatin, Paclitaxel, Pemetrexed, Teniposide, Tioguanine, Topotecan, Valrubicin, Vinblastine, Vincristine, Vindesine, Vinorelbine, and combinations thereof. In some embodiments, a chemotherapeutic agent may be utilized in the context of an antibody-drug conjugate. In some embodiments, a chemotherapeutic agent is one found in an antibody-drug conjugate selected from the group consisting of: hLL1-doxorubicin, hRS7-SN-38, hMN-14-SN-38, hLL2-SN-38, hA20-SN-38, hPAM4-SN-38, hLL1-SN-38, hRS7-Pro-2-P-Dox, hMN-14-Pro-2-P-Dox, hLL2-Pro-2-P-Dox, hA20-Pro-2-P-Dox, hPAM4-Pro-2-P-Dox, hLL1-Pro-2-P-Dox, P4/D10-doxorubicin, gemtuzumab ozogamicin, brentuximab vedotin, trastuzumab emtansine, inotuzumab ozogamicin, glembatumomab vedotin, SAR3419, SAR566658, BIIB015, BT062, SGN-75, SGN-CD19A, AMG-172, AMG-595, BAY-94-9343, ASG-5ME, ASG-22ME, ASG-16M8F, MDX-1203, MLN-0264, anti-PSMA ADC, RG-7450, RG-7458, RG-7593, RG-7596, RG-7598, RG-7599, RG-7600, RG-7636, ABT-414, IMGN-853, IMGN-529, vorsetuzumab mafodotin, and lorvotuzumab mertansine. In some embodiments, a chemotherapeutic agent may be one described as

utilized in an antibody-drug conjugate as described or discussed in one or more of Govindan et al, The Scientific World JOURNAL 10:2070, 2010, –2089.

- [36] In some embodiments, a chemotherapeutic agent may be or comprise one or more of farnesyl-thiosalicylic acid (FTS), 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide (CMH), estradiol (E2), tetramethoxystilbene (TMS), δ -tocatrienol, salinomycin, or curcumin.
- [37] *Combination Therapy*: As used herein, the term “combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (e.g., two or more therapeutic agents). In some embodiments, two or more agents may be administered simultaneously; in some embodiments, such agents may be administered sequentially; in some embodiments, such agents are administered in overlapping dosing regimens.
- [38] *Comparable*: As used herein, the term “comparable” refers to two or more agents, entities, situations, sets of conditions, etc., that may not be identical to one another but that are sufficiently similar to permit comparison there between so that conclusions may reasonably be drawn based on differences or similarities observed. In some embodiments, comparable sets of conditions, circumstances, individuals, or populations are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc to be considered comparable. For example, those of ordinary skill in the art will appreciate that sets of circumstances, individuals, or populations are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under or with different sets of circumstances, individuals, or populations are caused by or indicative of the variation in those features that are varied.
- [39] *Composition*: A “composition” or a “pharmaceutical composition” according to this invention refers to the combination of two or more agents as described herein for co-administration or administration as part of the same regimen. It is not required in all embodiments that the combination of agents result in physical admixture, that is, administration as separate co-agents each of the components of the composition is possible; however many patients or practitioners in the field may find it advantageous to prepare a composition that is an admixture of two or more of the ingredients in a pharmaceutically acceptable carrier, diluent, or excipient, making it possible to administer the component ingredients of the combination at the same time.

- [40] *Comprising*: A composition or method described herein as "comprising" one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. To avoid prolixity, it is also understood that any composition or method described as "comprising" (or which "comprises") one or more named elements or steps also describes the corresponding, more limited composition or method "consisting essentially of" (or which "consists essentially of") the same named elements or steps, meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method. It is also understood that any composition or method described herein as "comprising" or "consisting essentially of" one or more named elements or steps also describes the corresponding, more limited, and closed-ended composition or method "consisting of" (or "consists of") the named elements or steps to the exclusion of any other unnamed element or step. In any composition or method disclosed herein, known or disclosed equivalents of any named essential element or step may be substituted for that element or step.
- [41] *Determine*: Many methodologies described herein include a step of "determining". Those of ordinary skill in the art, reading the present specification, will appreciate that such "determining" can utilize or be accomplished through use of any of a variety of techniques available to those skilled in the art, including for example specific techniques explicitly referred to herein. In some embodiments, determining involves manipulation of a physical sample. In some embodiments, determining involves consideration and/or manipulation of data or information, for example utilizing a computer or other processing unit adapted to perform a relevant analysis. In some embodiments, determining involves receiving relevant information and/or materials from a source. In some embodiments, determining involves comparing one or more features of a sample or entity to a comparable reference.
- [42] *Dosage Form*: As used herein, the term "dosage form" refers to a physically discrete unit of an active agent (e.g., a therapeutic or diagnostic agent) for administration to a subject. Each unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or agent administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

- [43] *Dosing Regimen*: As used herein, the term “dosing regimen” refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).
- [44] *Inducible Effector Cell Surface Marker*: As used herein, the term “inducible effector cell surface marker” refers to an entity, that typically is or includes at least one polypeptide, expressed on the surface of immune effector cells, including without limitation natural killer (NK) cells, which expression is induced or significantly upregulated during activation of the effector cells. In some embodiments, increased surface expression involves increased localization of the marker on the cell surface (e.g., relative to in the cytoplasm or in secreted form, etc). Alternatively or additionally, in some embodiments, increased surface expression involves increased production of the marker by the cell. In some embodiments, increased surface expression of a particular inducible effector cell surface marker correlates with and/or participates in increased activity by the effector cell (e.g., increased antibody-mediated cellular cytotoxicity [ADCC]). In some embodiments, an inducible effector cell surface marker is selected from a group consisting of a member of the TNFR family, a member of the CD28 family, a cell adhesion molecule, a vascular adhesion molecule, a G protein regulator, an immune cell activating protein, a recruiting chemokine/cytokine, a receptor for a recruiting chemokine/cytokine, an ectoenzyme, a member of the immunoglobulin superfamily, a lysosomal associated membrane protein. Certain exemplary inducible cell surface markers include, without limitation, CD38, CD137, OX40, GITR, CD30, ICOS, etc. In some particular embodiments, the term refers to any of the above-mentioned inducible cell surface markers other than CD38.
- [45] *Patient*: As used herein, the term “patient” refers to any organism to which a provided composition is or may be administered, e.g., for experimental, diagnostic, prophylactic,

cosmetic, and/or therapeutic purposes. Typical patients include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. In some embodiments, a patient is suffering from or susceptible to one or more disorders or conditions. In some embodiments, a patient displays one or more symptoms of a disorder or condition. In some embodiments, a patient has been diagnosed with one or more disorders or conditions. In some embodiments, the disorder or condition is or includes cancer, or presence of one or more tumors. In some embodiments, the patient is receiving or has received certain therapy to diagnose and/or to treat a disease, disorder, or condition.

- [46] *Pharmaceutically Acceptable*: As used herein, the term "pharmaceutically acceptable" applied to the carrier, diluent, or excipient used to formulate a composition as disclosed herein means that the carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.
- [47] *Pharmaceutical Composition*: As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.
- [48] *Refractory*: The term "refractory" as used herein, refers to any subject or condition that does not respond with an expected clinical efficacy following the administration of provided compositions as normally observed by practicing medical personnel.
- [49] *Solid Tumor*: As used herein, the term "solid tumor" refers to an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign or malignant. Different types of solid tumors are named for the type of cells that form them. Examples of solid

tumors are sarcomas, carcinomas, lymphomas, mesothelioma, neuroblastoma, retinoblastoma, etc.

- [50] *Surrogate Marker*: The term “surrogate marker”, as used herein, refers to an entity whose presence, level, or form, may act as a proxy for presence, level, or form of another entity (e.g., a biomarker) of interest. Typically, a surrogate marker may be easier to detect or analyze (e.g., quantify) than is the entity of interest. To give but one example, in some embodiments, where the entity of interest is a protein, an expressed nucleic acid (e.g., mRNA) encoding the protein may sometimes be utilized as a surrogate marker for the protein (or its level). To give another example, in some embodiments, where the entity of interest is an enzyme, a product of the enzyme’s activity may sometimes be utilized as a surrogate marker for the enzyme (or its activity level). To give one more example, in some embodiments, where the entity of interest is a small molecule, a metabolite of the small molecule may sometimes be used as a surrogate marker for the small molecule.
- [51] *Therapeutically Effective Amount*: As used herein, the term “therapeutically effective amount” means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, stabilizes one or more characteristics of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that the term “therapeutically effective amount” does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. For example, in some embodiments, term “therapeutically effective amount”, refers to an amount which, when administered to an individual in need thereof in the context of inventive therapy, will block, stabilize, attenuate, or reverse a cancer-supportive process occurring in said individual, or will enhance or increase a cancer-suppressive process in said individual. In the context of cancer treatment, a “therapeutically effective amount” is an amount which, when administered to an individual diagnosed with a cancer, will prevent, stabilize, inhibit, or reduce the further development of cancer in the individual. A particularly preferred “therapeutically effective amount” of a composition described herein reverses (in a therapeutic treatment) the development of a malignancy such as a pancreatic carcinoma or helps achieve or prolong remission of a malignancy. A therapeutically effective amount administered to an individual to treat a cancer in that individual may be the same or different from a therapeutically effective amount

administered to promote remission or inhibit metastasis. As with most cancer therapies, the therapeutic methods described herein are not to be interpreted as, restricted to, or otherwise limited to a "cure" for cancer; rather the methods of treatment are directed to the use of the described compositions to "treat" a cancer, i.e., to effect a desirable or beneficial change in the health of an individual who has cancer. Such benefits are recognized by skilled healthcare providers in the field of oncology and include, but are not limited to, a stabilization of patient condition, a decrease in tumor size (tumor regression), an improvement in vital functions (e.g., improved function of cancerous tissues or organs), a decrease or inhibition of further metastasis, a decrease in opportunistic infections, an increased survivability, a decrease in pain, improved motor function, improved cognitive function, improved feeling of energy (vitality, decreased malaise), improved feeling of well-being, restoration of normal appetite, restoration of healthy weight gain, and combinations thereof. In addition, regression of a particular tumor in an individual (e.g., as the result of treatments described herein) may also be assessed by taking samples of cancer cells from the site of a tumor such as a pancreatic adenocarcinoma (e.g., over the course of treatment) and testing the cancer cells for the level of metabolic and signaling markers to monitor the status of the cancer cells to verify at the molecular level the regression of the cancer cells to a less malignant phenotype. For example, tumor regression induced by employing the methods of this invention would be indicated by finding a decrease in any of the pro-angiogenic markers discussed above, an increase in anti-angiogenic markers described herein, the normalization (i.e., alteration toward a state found in normal individuals not suffering from cancer) of metabolic pathways, intercellular signaling pathways, or intracellular signaling pathways that exhibit abnormal activity in individuals diagnosed with cancer. Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated and/or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

- [52] *Subject:* By "subject" is meant a mammal (e.g., a human, in some embodiments including prenatal human forms). In some embodiments, a subject is suffering from a relevant disease, disorder or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

- [53] *Treatment*: As used herein, the term “treatment” (also “treat” or “treating”) refers to any administration of a substance (e.g., anti-receptor tyrosine kinases antibodies or receptor tyrosine kinase antagonists) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition (e.g., cancer). Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.
- [54] *Variant*: As used herein, the term “variant” refers to an entity that shows significant structural identity with a reference entity but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many embodiments, a variant also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a “variant” of a reference entity is based on its degree of structural identity with the reference entity. As will be appreciated by those skilled in the art, any biological or chemical reference entity has certain characteristic structural elements. A variant, by definition, is a distinct chemical entity that shares one or more such characteristic structural elements. To give but a few examples, a small molecule may have a characteristic core structural element (e.g., a macrocycle core) and/or one or more characteristic pendent moieties so that a variant of the small molecule is one that shares the core structural element and the characteristic pendent moieties but differs in other pendent moieties and/or in types of bonds present (single vs double, E vs Z, etc) within the core, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular biological function, a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to one another in linear or three-dimensional space. For example, a variant polypeptide may differ from a reference polypeptide as a result of one or more differences in amino acid sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, etc) covalently attached to the polypeptide backbone. In some embodiments, a variant polypeptide shows an overall sequence identity with a reference polypeptide that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. Alternatively or

additionally, in some embodiments, a variant polypeptide does not share at least one characteristic sequence element with a reference polypeptide. In some embodiments, the reference polypeptide has one or more biological activities. In some embodiments, a variant polypeptide shares one or more of the biological activities of the reference polypeptide. In some embodiments, a variant polypeptide lacks one or more of the biological activities of the reference polypeptide. In some embodiments, a variant polypeptide shows a reduced level of one or more biological activities as compared with the reference polypeptide. In many embodiments, a polypeptide of interest is considered to be a “variant” of a parent or reference polypeptide if the polypeptide of interest has an amino acid sequence that is identical to that of the parent but for a small number of sequence alterations at particular positions. Typically, fewer than 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% of the residues in the variant are substituted as compared with the parent. In some embodiments, a variant has 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substituted residue as compared with a parent. Often, a variant has a very small number (e.g., fewer than 5, 4, 3, 2, or 1) number of substituted functional residues (i.e., residues that participate in a particular biological activity). Furthermore, a variant typically has not more than 5, 4, 3, 2, or 1 additions or deletions, and often has no additions or deletions, as compared with the parent. Moreover, any additions or deletions are typically fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly are fewer than about 5, about 4, about 3, or about 2 residues. In some embodiments, the parent or reference polypeptide is one found in nature. As will be understood by those of ordinary skill in the art, a plurality of variants of a particular polypeptide of interest may commonly be found in nature, particularly when the polypeptide of interest is an infectious agent polypeptide.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Trastuzumab-drug Conjugates

- [55] As described herein, the present disclosure provides therapeutic technologies relating to treatment with certain antibody-drug conjugates, and particularly with trastuzumab-drug conjugates. Teachings of the present disclosure are particularly relevant to trastuzumab conjugates with one or more chemotherapeutic agents, and particularly with one or more microtubule-targeting agents. Indeed, the present disclosure demonstrates surprising attributes of certain methodologies that utilize such conjugates, and specifically of methodologies that utilize a trastuzumab conjugate with the aaytansine analog DM-1 (e.g., the trastuzumab conjugate known as “T-DM1”).

T-DM1

- [56] Ado-trastuzumab emtansine ("T-DM1"). T-DM1 is an antibody-drug conjugate consisting of the monoclonal antibody trastuzumab linked to the cytotoxic agent DM1. T-DM1 is marketed under the trade name KADCYLA[®], and under that name has been approved by the United States Food and Drug Administration (the "FDA") for as a single agent, for the treatment of certain patients with HER2-positive, metastatic breast cancer. The initial approval of KADCYLA[®] has been specifically for patients who previously received trastuzumab and a taxane, separately or in combination. Therapy with KADCYLA[®] is particularly recommended for patients who have either (i) received prior therapy for metastatic disease, or (ii) developed disease recurrence during or within six months of completing adjuvant therapy.
- [57] As noted above, T-DM1 includes the trastuzumab antibody. Trastuzumab is a human monoclonal antibody that targets and antagonizes HER2/neu receptor on tumor cells. Recommended dosing regimens for T-DM1 and trastuzumab are not the same, however. For example, the standard recommended regimen for T-DM1 is 3.6 mg/kg given as an intravenous infusion every 3 weeks (21-day cycle) until disease progression or unacceptable toxicity. Intravenous push is specifically not recommended, as is formulation with Dextrose (5%) solution. Various recommended regimens have been developed for trastuzumab, which is marketed under the trade name HERCEPTIN[®], and has been approved by the FDA under that name for at least (i) the treatment of HER2 overexpressing breast cancer; and (ii) the treatment of HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma.
- [58] Recommended dosing regimens for trastuzumab for adjuvant therapy in treatment of HER2-overexpressing breast cancer include: (i) administer an initial dose of 4 mg/kg over 90 minute IV infusion, then 2 mg/kg over 30 minute IV infusion weekly for 52 weeks; or (ii) administer an initial dose of 8 mg/kg over 90 minutes IV infusion, then 6 mg/kg over 30–90 minutes IV infusion every three weeks for 52 weeks particularly when used as adjuvant therapy in treatment of HER2-overexpressing breast cancer
- [59] Recommended dosing regimens for trastuzumab for treatment of metastatic HER2-overexpressing breast cancer include: (i) administer an initial dose of 4 mg/kg as a 90 minute IV infusion followed by subsequent weekly doses of 2 mg/kg as 30 minute IV infusions.
- [60] Recommended dosing regimens for trastuzumab for treatment of metastatic HER2-overexpressing gastric cancer include: (i) administer an initial dose of 8 mg/kg over 90 minutes IV infusion, followed by 6 mg/kg over 30 to 90 minutes IV infusion every 3 weeks.

- [61] In T-DM1, trastuzumab is conjugated with the cytotoxic agent DM1. DM1 is a synthetic derivative of the microtubule-targeted agent maytansine, which is an ansa macrolide first isolated from the plant *maytenus ovatus* (see, for example, Kupchan, et al, J. Am. Chem. Soc. 94:1354, 1972 and Kupchan et al J. Org. Chem. 42: 2349, 1977, each of which is incorporated herein by reference). DM1 is believed to bind to microtubules, and may inhibit tubulin assembly into such microtubules. It is specifically believe that DM1 binds at the tips of microtubules and suppresses their dynamicity, thereby inducing mitotic arrest and/or cell death. DM1 may share a tubulin binding site with one or more vinca alkaloids. DM1 was specifically developed to overcome systemic toxicity associated with maytansine and to enhance tumor-specific delivery (see for example, Lopus *Cancer Letters* 307:113, 2011, the entire contents of which are incorporated herein by reference). DM1 is amenable to antibody conjugation; typically the antibody-DM1 conjugate cleaves inside cells and releases active DM1 drug, often in a time-dependent manner. The approved dosing regimen for KADCYLA is 3.6 mg/kg given as an intravenous infusion every 3 weeks (21-day cycle) until disease progression or unacceptable toxicity. Intravenous push or bolus is specifically contra-indicated, as is use in dextrose (5%) solution. Doses greater than 3.6 mg/kg are highlighted as to be avoided. See also Barok et al., *Breast Cancer Research* 16:209, 2014; Nandini et al , *Cancer Therapy* Vol. 9:45, 2013 for discussion of T-DM1 and its uses; the entire contents of each of these is incorporated herein by reference.
- [62] Dosing regimens for T-DM1 in combination with one or more other agents have also been described (see, for example, Published United States Patent Application US 2014/0044709 A1 to Hoffman La Roche and Genentech, published February 13, 2014, the entire contents of which are incorporated herein by reference). For example, in at least one case, T-DM1 is administered at a dosage ranging from 2.4-3.6 mg/kg every three weeks, e.g., at a dosage selected from 2.4, 3.0 and 3.6 mg/kg every three weeks, when administered in a combination therapy regimen.
- [63] Highly concentrated, stable pharmaceutical formulation of T-DM1 have been described (see, for example, Published United States Patent Application US 2011/0044977 A1 to Genentech, which was published February 24, 2011 , the entire contents of which are incorporated herein by reference). This formulation is said to be for subcutaneous injection, and to comprise, in addition to a suitable amount of T-DM1, an effective amount of at least one hyaluronidase enzyme as a combined formulation or for use in form of a co-formulation. The formulations is also said to comprise additionally at least one buffering agent, such as e.g. a histidine buffer, a stabilizer or a mixture of two or more stabilizers (e.g. a saccharide, such as e.g. α,α -trehalose dihydrate or sucrose, and optionally methionine as a second stabilizer), a nonionic surfactant

and an effective amount of at least one hyaluronidase enzyme. Methods for preparing such formulations and their uses thereof are also described. In particular embodiments, the formulation is said to comprise or consist of about 50 to 350 mg/ml T-DM1, about 1 to 100 mM of a buffering agent providing a pH of 5.5 ± 2.0 , about 1 to 500 mM of a stabilizer or a mixture of two or more stabilizers, about 0.01 to 0.08% of a nonionic surfactant; and an effective amount of at least one hyaluronidase enzyme.

Inducible Effector Cell Surface Markers

- [64] Both adaptive and innate immune cells participate in surveillance and elimination of cells dynamically expressing cancer antigens. In particular, the interaction between the Fc portion of the antibodies bound to the antigen on the surface of cancer cells with the Fc receptor (FcR) on the surface of immune effector cells mediates cytotoxic effects of and/or phagocytosis of cancer cells by such effector cells.
- [65] Among the effector cells that can destroy tumor cells are natural killer cells (NK cells), which play a major role by releasing small cytoplasmic granules of proteins called perforin and granzyme that cause the target cancer cells to die by apoptosis. NK-cell-mediated lysis of target cells occurs either through spontaneous cytotoxicity, which is modulated by recognition of self versus non-self cell surface markers, or through ADCC. Particularly potent NK-cell-mediated ADCC responses can be triggered by cancer cells to which anti-tumor antibodies (whether naturally generated or administered as part of anti-tumor antibody therapy, for example as described herein) have bound. In fact, in some cases, NK cell-mediated ADCC triggered by Fc γ R engagement with anti-tumor antibodies bound to tumor cell surfaces is one of the primary mechanisms of effective anti-tumor antibody therapy (Weiner GJ, 2007).
- [66] One event that happens during the activation of effector cells upon the FcR engagement with anti-tumor antibodies bound to tumor cell surfaces is the increase of the expression of various inducible effector cell surface markers on the surface of the effector cells. Activation of such inducible effector cell surface markers can enhance the effector cell function, such as increasing ADCC activity. Such inducible surface markers are known to those of skill in the art, and include, without limitation, certain members of the TNFR family, certain members of the CD28 family, certain cell adhesion molecules, certain vascular adhesion molecules, certain G protein regulators, certain immune cell activating proteins, certain recruiting chemokine/cytokines, certain receptors for recruiting chemokine/cytokines, certain ectoenzymes, certain members of the immunoglobulin superfamily, certain lysosomal

associated membrane proteins, and combinations thereof. In some embodiments, inducible effector cell surface markers are selected from CD30, CD38, CD137, GITR, ICOS, OX40, *etc.*

- [67] Many such costimulatory molecules are members of the tumor necrosis factor receptor family (TNFR). TNFR-related molecules do not have any known enzymatic activity and depend on the recruitment of cytoplasmic proteins for the activation of downstream signaling pathways. Members of this receptor family and their structurally related ligands are important regulators of a wide variety of physiologic processes and play an important role in the regulation of immune responses.

CD30

- [68] The transmembrane receptor CD30 (TNFRSF8) and its ligand CD30L (CD153, TNFSF8) are members of the tumor necrosis factor (TNF) superfamily and display restricted expression in subpopulations of activated immune cells. CD30 is a type I transmembrane glycoprotein of the TNF receptor superfamily. The ligand for CD30 is CD30L (CD153). The binding of CD30 to CD30L mediates pleiotropic effects including cell proliferation, activation, differentiation, and apoptotic cell death.

CD38

- [69] CD38 (cluster of differentiation 38), which is also known both as a receptor and a cyclic ADP ribose hydrolase, is a glycoprotein found on the surface of many immune system cells, including CD4-positive T cells, CD8-positive T cells, B cells, and natural killer cells. CD38 catalyzes the synthesis and hydrolysis of cyclic ADP-ribose (cADPR) from NAD⁺ to ADP-ribose, thereby playing a role in regulation of intracellular Ca²⁺ and calcium signaling, as well as in cell adhesion and signal transduction.
- [70] CD38 is a marker of immune cell activation, and its expression has been linked to rheumatoid arthritis (see, for example, Fueeldner et al, 2012), as well as to certain immune and/or blood cell cancers, including diffuse large B-cell lymphoma (DLBCL), acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), follicular lymphoma, mantle cell lymphoma, and multiple myeloma (MM) and chronic lymphocytic leukemia (CLL). for which it has been proposed to be an effective target for anti-tumor antibody therapy (see, for example, Malavasi et al., 2011; Chillemi A et al., 2013). Antagonistic antibodies to CD38 are currently in clinical trials

(sponsored by Genmab [using daratumumab, developed under the mark HuMax[®]-CD38], Sanofi [using SAR650984], and MorphoSys AG [using MOR03087]) for the treatment of multiple myeloma (MM). CD38 has been proposed as a useful target for anti-tumor antibody therapy, both to induce ADCC killing of CD38-positive cancer cells, and to potentially deliver payloads (e.g., cytotoxic moieties) to such CD38-positive cancer cells, though caution has been recommended in order to avoid inducing activation signals on target cells (see Chillemi A, et al., 2013).

- [71] One reported effect of CD38 ligation (as occurs upon binding of ligand or antibody) is down-regulation of miR-193b, known to function as a tumor suppressor miRNA in various cancers including, for example, non-small cell lung carcinoma, breast cancer, prostate carcinoma, melanoma, hepatocellular carcinoma, etc (see, for example, Chillemi A, et al., 2013 and references cited therein). CD38 ligation has also been reported to induce proliferation and immunoblast differentiation of immune tumor cells such as CLL (see, for example, Chillemi A, et al., 2013). Surface levels of CD38 vary on different cell types, whether due to different expression levels, different distribution of forms (e.g., internalized, soluble, etc), or other differences. For example, surface levels tend to be high for myeloma cells; lower for CLL and some other cells.
- [72] CD38 expression is modulated by retinoic acid and other retinoids; the promoter driving transcription of the *CD38* gene is responsive to a retinoic acid response element (RARE). It has been proposed that CD38 expression levels might be increased through administration of retinoids or their derivatives, specifically including tamibarotene (see Chillemi A, et al., 2013). However, those retinoids that have been tested showed only a modest ability to increase surface CD38 when administered to myeloma cells, and no effect at all was observed when administered to CLL.

CD137

- [73] CD137, which may also be referred to as Ly63, ILA or 4-1 BB is a member of the tumor necrosis factor (TNF) receptor family. CD137 is expressed by activated NK cells, T and B lymphocytes and monocytes/macrophages. The gene encodes a 255-amino acid protein with 3 cysteine-rich motifs in the extracellular domain (characteristic of this receptor family), a transmembrane region, and a short N-terminal cytoplasmic portion containing potential phosphorylation sites. Expression in primary cells is strictly activation dependent. The ligand for the receptor is TNFSF9. Human CD137 is reported to bind only to its ligand. Agonists include

the native ligand (TNFSF9), aptamers (see McNamara et al., 2008), and antibodies. Use of T-DM1 in a staggered therapy protocol with a CD137 agonist antibody is exemplified herein, and demonstrates surprising and useful attributes, as described in the Exemplification below.

GITR

- [74] Glucocorticoid-Induced TNFR-Related (GITR) protein belongs to tumor necrosis factor receptor/tumor necrosis factor superfamily and stimulates both the acquired and innate immunity. It is expressed in several cells and tissues, including T and Natural Killer (NK) cells and is activated by its ligand, GITRL, mainly expressed on antigen presenting cells and endothelial cells. GITR/GITRL system participates in the development of autoimmune/inflammatory responses and potentiates response to infection and tumors by mechanisms including NK-cell co-activation

ICOS

- [75] Inducible costimulator (ICOS) is a member of the CD28 family. ICOS expression, may be readily detectable resting, but it upregulated upon activation. ICOS and ICOS-L appear to be a monogamous pair. ICOS activation enhances effector functions.

OX40

- [76] OX40 (CD134) and its binding partner, OX40L (CD252), are members of the tumor necrosis factor receptor/tumor necrosis factor superfamily and are expressed on activated T cells as well as on a number of other lymphoid and non-lymphoid cells. OX40 and OX40L regulate cytokine production from T cells, antigen-presenting cells, natural killer cells, and natural killer T cells, and modulate cytokine receptor signaling.

Agonists of Inducible Effector Cell Surface Markers

- [77] In general, agonists utilized in accordance with the present invention will be direct agonists in that they bind directly to their target (i.e., to an inducible effector cell surface marker). In some embodiments, an agonist may be or comprise a physiological ligand of an inducible effector cell

surface marker. In some embodiments, an agonist may be or comprise an antibody or antibody agent that binds specifically to an inducible effector cell surface marker.

Physiological Ligands

- [78] The physiological ligand for CD30, CD30L (CD153; also, TNFSF8), is a transmembrane glycoprotein with expression restricted to and tightly regulated in immune cells. Activation of CD30 by recombinant CD30L or CD30L-transfectants enhances the activation, proliferation, and various effector functions of both T and B lymphocytes.
- [79] CD31, also known as platelet endothelial cell adhesion molecule-1, PECAM-1, is a CD38 non-substrate ligand that can start the signaling cascade and recapitulates the biological events observed *in vitro* using agonistic monoclonal antibodies (Malavasi F et al., 2008; Chillemi A, et al. 2013).
- [80] The physiological ligand for CD137 (CD137L; also, 4-1BBL, TNFSF9, etc.) is a 50 kDa transmembrane glycoprotein expressed by the professional antigen presenting cells (APCs). The soluble CD137L (sCD137L) released from various APCs is capable of binding and activating the CD137 receptor. CD137L-transfectants were shown to stimulate NK cell activation, proliferation, and cytokine release *in vitro*.
- [81] The physiological ligand for GITR, GITRL (TNFSF18), is a transmembrane protein constitutively expressed on various types of APCs as well as on regulatory T cells. The activation of GITR by GITRL regulates the activity of both conventional and regulatory T cells.
- [82] . The physiological ligand for ICOS, ICOSL (B7-H2), is a transmembrane protein expressed mainly in APCs. Activation of ICOS by ICOSL plays critical role in a variety of lymphocyte activities, including Th2 cell differentiation, T cell proliferation, T helper cell effector function, B cell differentiation, Ig class switch, etc.
- [83] The physiological ligand for OX40 (CD134), OX40L(CD252; also, TNFSF4), is a transmembrane receptor containing 183 amino acids that is expressed on the surface of activated APCs as a trimer allowing it to bind to three OX40 molecules. OX40-OX40L interactions exert several effects on conventional CD4 and CD8 T cells, NK cells, and NKT cells, including promoting division, survival, and differentiation, and regulating cytokine production

Agonist Antibody Agents

- [84] Agonistic antibodies against inducible effector cell surface markers have been shown to exert similar biological functions in immune cells as the physiological ligands. In particular, agonist anti-CD137 mAb (urelumab), agonist anti-OX40 mAbs, and agonist anti-GITR mAb (TRX518) have been extensively studied for their anti-tumor therapeutic effects, and have entered clinical trials.
- [85] In general, an antibody agent that agonizes an inducible effector cell surface marker may be or comprise an intact antibody, or another antibody format (e.g., as known in the art and/or described herein), including for example a single chain format or a multi-specific format. In some particular embodiments, an antibody agent that agonizes an inducible effector cell surface marker is provided and/or utilized in a multi-specific (e.g. bi-specific) format that also targets CD38.
- [86] Additionally, as with other antibody agents described and/or utilized herein, an antibody agent that agonizes an inducible effector cell surface marker may be polyclonal or, preferably, monoclonal and/or may be of non-human origin (e.g., of rodent or camel origin) or, preferably, may be chimeric, humanized or, most preferably, human.

Tumors

- [87] Technologies provided herein are useful in the treatment of tumors targeted by T-DM1 (e.g., any HER2⁺ tumor. In some embodiments, provided technologies are particularly useful in the treatment of tumors that express particularly high levels of HER2, for example above a reference threshold that may be determined by and/or otherwise known to those of ordinary skill in the art.
- [88] In certain embodiments, a tumor is a solid tumor. In certain embodiments, a tumor is an adenocarcinoma. In certain embodiments, a tumor is a breast tumor, a gastric tumor, or a gastroesophageal tumor. In certain embodiments, a tumor is a metastatic tumor. In some particular embodiments, a tumor is a breast tumor, e.g., a metastatic breast tumor.
- [89] In some particular embodiments, a tumor is an advanced tumor, and/or a refractory tumor. In some embodiments, a tumor is characterized as advanced when cancer patients with such tumor are not candidates for one or more other therapies (e.g., therapy with trastuzumab and/or with T-DM1 absent staggered agonist therapy as described herein, and/or one or more

conventional chemotherapy regimens). In some embodiments, a tumor is characterized as refractory when a patient has previously received one or more prior anti-cancer therapies (e.g., therapy with trastuzumab and/or with T-DM1 absent staggered agonist therapy as described herein, and/or one or more conventional chemotherapy regimens) but has not achieved successful resolution of disease.

Formats of Antibody Agents

- [90] A wide variety of formats has been developed for antibody agents, several of which have already progressed into clinical trials (reviewed, for example, in Scott AM et al., 2012). In some embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgG, IgE and IgM, bi- or multi- specific antibodies (e.g., Zybodies®, etc), single chain Fvs, polypeptide-Fc fusions, Fabs, cameloid antibodies, masked antibodies (e.g., Probodies®), Small Modular ImmunoPharmaceuticals ("SMIPsTM"), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®, Centyrins®, and a KALBITOR®.
- [91] It is worth noting that masked antibody (e.g., Probody®) formats may be of particular interest for certain antibody agents (e.g., those targeting CD38; in some embodiments, use of such a format ensures that CD38 targeting occurs substantially or only in the tumor milieu, and not elsewhere in the body).

Dosing and Administration

- [92] In general, each active agent for use in accordance with the present invention is formulated, dosed, and administered in therapeutically effective amount using pharmaceutical compositions and dosing regimens that are consistently with good medical practice and appropriate for the relevant agent(s) and subject. In principle, therapeutic compositions can be administered by any appropriate method known in the art, including, without limitation, oral, mucosal, by-inhalation, topical, buccal, nasal, rectal, or parenteral (e.g. intravenous, infusion, intratumoral, intranodal, subcutaneous, intraperitoneal, intramuscular, intradermal, transfermal, or other kinds of administration involving physical breaching of a tissue of a subject and administration of the therapeutic composition through the breach in the tissue).

- [93] In some embodiments, a dosing regimen for a particular active agent may involve intermittent or continuous (e.g., by perfusion or other slow release system) administration, for example to achieve a particular desired pharmacokinetic profile or other pattern of exposure in one or more tissues or fluids of interest in the subject receiving therapy.
- [94] In some embodiments, different agents administered in combination may be administered via different routes of delivery and/or according to different schedules. Alternatively or additionally, in some embodiments, one or more doses of a first active agent is administered substantially simultaneously with, and in some embodiments via a common route and/or as part of a single composition with, one or more other active agents.
- [95] Factors to be considered when optimizing routes and/or dosing schedule for a given therapeutic regimen may include, for example, the particular indication being treated, the clinical condition of a subject (e.g., age, overall health, prior therapy received and/or response thereto, etc) the site of delivery of the agent, the nature of the agent (e.g. an antibody or other protein-based compound), the mode and/or route of administration of the agent, the presence or absence of combination therapy, and other factors known to medical practitioners. For example, in the treatment of cancer, relevant features of the indication being treated may include, among other things, one or more of cancer type, stage, location, etc.
- [96] In some embodiments, one or more features of a particular pharmaceutical composition and/or of a utilized dosing regimen may be modified over time (e.g., increasing or decreasing amount of active in any individual dose, increasing or decreasing time intervals between doses, etc), for example in order to optimize a desired therapeutic effect or response (e.g., an ADCC response).
- [97] In general, type, amount, and frequency of dosing of active agents in accordance with the present invention are governed by safety and efficacy requirements that apply when relevant agent(s) is/are administered to a mammal, preferably a human. In general, such features of dosing are selected to provide a particular, and typically detectable, therapeutic response as compared with what is observed absent therapy.
- [98] In context of the present invention, an exemplary desirable therapeutic response may involve, but is not limited to, inhibition of and/or decreased tumor growth, tumor size, metastasis, one or more of the symptoms and side effects that are associated with a tumor, as well as increased apoptosis of cancer cells, therapeutically relevant decrease or increase of one or more cell marker or circulating markers and the like. Such criteria can be readily assessed by any of a variety of immunological, cytological, and other methods that are disclosed in the literature. In

particular, the therapeutically effective amount of CD38 agonist, alone or in combination with an third agent, can be determined as being sufficient to enhance ADCC killing of cancer cells targeted by the first agent.

[99] In some embodiments, an effective dose (and/or a unit dose) of an active agent, may be at least about 0.01 µg/kg body weight, at least about 0.05 µg/kg body weight; at least about 0.1 µg/kg body weight, at least about 1 µg/kg body weight, at least about 2.5 µg/kg body weight, at least about 5 µg/kg body weight, and not more than about 100 µg/kg body weight. It will be understood by one of skill in the art that in some embodiments such guidelines may be adjusted for the molecular weight of the active agent. The dosage may also be varied for route of administration, the cycle of treatment, or consequently to dose escalation protocol that can be used to determine the maximum tolerated dose and dose limiting toxicity (if any) in connection to the administration of the first agent, second agent, and/or the third agent at increasing doses. Consequently, the relative amounts of the each agent within a pharmaceutical composition may also vary, for example, each composition may comprise between 0.001 % and 100% (w/w) of the corresponding agent.

Staggered Dosing

[100] As described in the Stanford Patent Application (i.e., International Patent Application WO2011/07187 Kohrt et al., entitled “Methods for Enhancing Anti-Tumor Antibody Therapy”), effective staggered dosing strategies include those in which a subject diagnosed with having a tumor is administered anti-tumor antibody agent that targets (e.g., binds to) a tumor-associated antigen (e.g., a tumor-associated cell surface antigen). Binding of the anti-tumor antibody agent is believed to trigger increased expression (i.e., presence) of certain effector cell surface markers (termed “inducible” effector cell surface markers). A period of time after the anti-tumor antibody is administered, an agonist agent that targets such an inducible effector cell surface marker is administered. The period of time is selected to permit induction (i.e., increased expression) of the inducible effector cell surface marker on surfaces of effector cells (e.g., NK cells).

[101] In some embodiments, administration of the agonist agent is performed when the inducible effector cell surface marker achieves a particular level (e.g., fold or percent) of increase in expression as compared with that observed prior to the administration of the anti-tumor antibody agent. In some embodiments, administration of the agonist agent is performed when the inducible effector cell surface marker achieves a level above a determined threshold.

- [102] In some particular such embodiments, the relevant period of time permits (e.g., is correlated with) increased surface expression of a relevant inducible effector cell surface marker to a level that is at least about 10%, 20%, 50%, 100%, 150%, 200% or more than that observed on the relevant effector cells (e.g., NK cells) prior to (or at the moment of) the administration of the anti-tumor antibody therapy.
- [103] In some embodiments, level of the inducible cell surface marker on relevant effector cell surfaces (e.g., on surfaces of tumor-infiltrating effector cells, and/or on surfaces of NK cells, etc) in the subject is determined prior to administration of the agonist agent to the subject. Alternatively or additionally, in some embodiments, level of the inducible cell surface marker on relevant effector cell surfaces (e.g., on surfaces of tumor-infiltrating effector cells, and/or on surfaces of NK cells, etc) in the subject is determined prior to and/or after administration of the anti-tumor antibody agent. In some embodiments, level of the inducible cell surface marker on relevant effector cell surfaces (e.g., on surfaces of tumor-infiltrating effector cells, and/or on surfaces of NK cells, etc) is determined after administration of the agonist agent to the subject. In some embodiments, level of the inducible cell surface marker on relevant effector cell surfaces (e.g., on surfaces of tumor-infiltrating effector cells, and/or on surfaces of NK cells, etc) is determined at multiple time points during administration of staggered therapy to a subject as described herein.
- [104] For example, in some embodiments, such level may be determined prior to administration of the anti-tumor antibody agent, one or more times between administration of the anti-tumor antibody agent and administration of the agonist agent, and/or after administration of the agonist agent. In some embodiments, surface expression level of the inducible effector cell surface marker is monitored between administration of the anti-tumor antibody therapy and the agonist therapy, for example at (or approximately at) one or more specified time points (e.g., for illustration only, at or approximately at one or more time points such as about 1 hour, 3 hours, 6 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours, etc).
- [105] In some embodiments, effector cell surface marker expression is monitored in assays that utilize human cancer cells, tissues, and/or other biological materials (such as those obtained from biopsies or blood sample of cancer patients). In some embodiments, effector cell surface marker expression is monitored using flow cytometry. In some embodiments, level of inducible effector cell surface marker is determined via detection of a surrogate marker (e.g., an alternative marker of effector cell (e.g., NK cell) activation, rather than of the inducible effector cell surface marker itself.

[106] In some embodiments, administration of the agonist agent is performed a particular period of time (i.e., approximate number of hours, approximate number of days, etc) after administration of the anti-tumor antibody agent, regardless of whether or not level of the inducible cell surface marker on relevant effector cell surfaces is or has been determined in the particular subject. In some embodiments, a particular time period may be selected as one that showed statistically significant correlation with a relevant level (or increase in level) of inducible effector cell surface marker, for example in a particular subject (e.g., as may be or have been determined in prior therapy of the particular subject) and/or in a relevant population (e.g., as may be or have been determined in a clinical trial).

[107] Thus, in some embodiments, the period of time between administration of the anti-tumor antibody agent and the agonist agent is selected through assessment of expression of the targeted inducible effector cell surface marker in the subject; in some embodiments the period of time is selected empirically, and generally applied.

[108] In some embodiments, the period of time may be a period within which administration of the agonist agent occurs may be a period of time that starts at least 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 72 hours, or up to 5 days or more after the administration of anti-tumor antibody agent. In some embodiments, the a time period begins within an hour or so of administration of anti-tumor antibody therapy and lasts at least about 2, about 5, about 11, about 23 hours, about 71 hours or more. In some embodiments, the time period lasts between about 1 hour (or less than hour) and about 24 or more hours or about 72 or more hours. In some embodiments, the time period begins within about 1 hour, about 3 hours, about 6 hours, or about 12 hours of administration of anti-tumor antibody agent; in some embodiments, such a time period lasts until at least about 12 hours, about 24 hours, about 72 hours, or about 5 days or more after administration of anti-tumor antibody agent. In some embodiments, the time period does not last more than about 5 days, about 72 hours, or about 24 hours after administration of anti-tumor antibody. In some embodiments, the time period is a period (i.e., an “elevated display time period” during which the relevant inducible effector cell surface marker is expressed at an elevated level on surfaces of effector cells (e.g., NK cells and/or tumor infiltrating effector cells).

[109] To give but one illustrative example, a given administration of anti-tumor antibody therapy (e.g., of a given dose of anti-tumor antibody agent) may achieve a desired increased in inducible effector cell surface for an elevated display time period that lasts at least about 6 hours, and begins about 12 hours after the administration. In such a circumstance, agonist therapy (i.e., administration of an agonist agent) targeting the induced effector cell surface biomarker may desirably be administered according to a regimen (e.g., in a single dose or

multiple doses) effective to enhance ADCC against tumor cells against which the anti-tumor antibody therapy was targeted, according to a schedule that achieved administration of the agonist therapy, for example, between about 12 hours and about 36 hours (e.g., between about 12 hours and about 24 hours, between about 12 hours and about 18 hours, etc), after the administration of the anti-tumor antibody therapy.

Combination

[110] Those of ordinary skill in the art, reading the present disclosure, will readily appreciate that staggered therapy protocols utilizing T-DM1 and an immune effector cell surface marker agonist as described herein are combination therapy regimens in that the subject's exposure to anti-tumor antibody agent overlaps its exposure to agonist agent. Those of ordinary skill in the art will further appreciate that such staggered therapy protocols may be administered in further combination with one or more other anti-cancer therapies, including for example administration of chemotherapeutic agents, other immunomodulatory agents (including other agonists and/or antagonists of other inducible effector cell surface markers), other anti-tumor antagonist agents (e.g., anti-tumor antibodies), radiation therapy, high-frequency ultrasound therapy, surgery, other etc.

[111] Thus, in some embodiments, staggered TDM1/agonist therapy, as described herein, is utilized in combination with one or more other therapeutic agents or modalities. In some embodiments, the one or more other therapeutic agents or modalities is also an anti-cancer agent or modality; in some embodiments the combination shows a synergistic effect in treating cancer.

[112] For example, as described herein, in some embodiments, provided staggered therapy is combined with anti-tumor anti-body therapy. Alternatively or additionally, in some embodiments, provided staggered therapy is combined with additional immune system agonist therapy (e.g., utilizing one or more agonists that target additional an inducible effector cell surface markers and/or with any other compound or treatment known to show therapeutic efficacy in treating cancer.

[113] To give but a few examples, compounds or treatments that show therapeutic efficacy in treating cancer may include, for example, one or more alkylating agents, anti-metabolites, anti-microtubule agents, topoisomerase inhibitors, cytotoxic antibiotics, angiogenesis inhibitors, immunomodulators, vaccines, cell-based therapies (e.g. allogeneic or autologous stem cell transplantation), organ transplantation, radiation therapy, surgery, etc.

- [114] Still further, in some embodiments, provided staggered therapy (and/or other therapy with which it is combined) may be combined with one or more palliative (e.g., pain relieving, anti-nausea, anti-emesis, etc) therapies, particularly when relieves one or more symptoms known to be associated with the relevant cancer, or with another disease, disorder or condition to which a particular cancer patient is susceptible or from which the particular cancer patient is suffering.
- [115] In some embodiments, one or more agents used in combination in accordance with the present invention are administered according to a dosing regimen for which they are approved for individual use. For example, in some embodiments, one or more utilized agents is administered according to a dosing regimen approved by a regulatory authority such as the United States Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA), e.g., for the relevant indication. In some embodiments, however, combination (e.g., in the context of or in addition to provided staggered therapy) permits another agent to be administered according to a dosing regimen that involves one or more lower and/or less frequent doses, and/or a reduced number of cycles as compared with that utilized when the agent is administered without provided staggered therapy. Alternatively or additionally, in some embodiments, an appropriate dosing regimen involves higher and/or more frequent doses, and/or an increased number of cycles as compared with that utilized when the agent is administered other than in the relevant combination therapy (e.g., not in combination with a provided staggered therapy).
- [116] In some embodiments, one or more doses of agents administered in combination are administered at the same time; in some such embodiments, agents may be administered in the same composition. More commonly, however, agents are administered in different compositions and/or at different times.
- [117] In some embodiments, where two or more active agents are utilized in accordance with the present invention, such agents can be administered simultaneously or sequentially. In some embodiments, administration of one agent is specifically timed relative to administration of another agent. For example, in some embodiments, a first agent is administered so that a particular effect is observed (or expected to be observed, for example based on population studies showing a correlation between a given dosing regimen and the particular effect of interest).
- [118] In some embodiments, desired relative dosing regimens for agents administered in combination may be assessed or determined empirically, for example using ex vivo, in vivo and/or in vitro models; in some embodiments, such assessment or empirical determination is

made in vivo, in a patient population (e.g., so that a correlation is established), or alternatively in a particular patient of interest.

[119] In some embodiments, one or more active agents utilized in practice of the present invention is administered according to an intermittent dosing regimen comprising at least two cycles. Where two or more agents are administered in combination, and each by such an intermittent, cycling, regimen, individual doses of different agents may be interdigitated with one another. In some embodiments, one or more doses of the second agent is administered a period of time after a dose of the first agent. In some embodiments, each dose of the second agent is administered a period of time after a dose of the first agent. In some embodiments, each dose of the first agent is followed after a period of time by a dose of the second agent. In some embodiments, two or more doses of the first agent are administered between at least one pair of doses of the second agent; in some embodiments, two or more doses of the second agent are administered between at least one pair of doses of the first agent. In some embodiments, different doses of the same agent are separated by a common interval of time; in some embodiments, the interval of time between different doses of the same agent varies. In some embodiments, different doses of the different agents are separated from one another by a common interval of time; in some embodiments, different doses of the different agents are separated from one another by different intervals of time.

[120] To give one exemplary possible protocol for interdigitating two intermittent, cycled dosing regimens (e.g., for anti-tumor antibody therapy and inducible effector cell surface marker therapy), a protocol might include:

- a. A first dosing period during which a therapeutically effective amount a first agent is administered to a patient;
- b. A first resting period;
- c. A second dosing period during which a therapeutically effective amount of a second agent and, optionally, a third agent, is administered to the patient; and
- d. A second resting period.

In some embodiments, the first resting period and second resting period may correspond to an identical number of hours or days. Alternatively, in some embodiments, the first resting period and second resting period are different, with either the first resting period being longer than the second one or, preferably, vice versa. In some embodiments, each of the resting periods corresponds to 120 hours, 96 hours, 72 hours, 48 hours, 24 hours, 12 hours, 6 hours, 30 hours,

1 hour, or less. In some embodiments, if the second resting period is longer than the first resting period, it can be defined as a number of days or weeks rather than hours (for instance 1 day, 3 days, 5 days, 1 week, 2, weeks, 4 weeks or more).

[121] If the first resting period's length is determined by existence or development of a particular biological or therapeutic event (e.g., induction of increased surface expression of an inducible effector cell surface marker), then the second resting period's length may be determined on the basis of different factors, separately or in combination. Exemplary such factors may include type and/or stage of a cancer against which anti-tumor antibody therapy (e.g., the first agent) is administered; identity and/or nature of a targeted tumor antigen, identity and/or properties (e.g., pharmacokinetic properties) of the first agent (e.g., of an anti-tumor antibody), and/or one or more features of the patient's response to therapy with the first agent. In some embodiments, length of one or both resting periods may be adjusted in light of pharmacokinetic properties (e.g., as assessed via plasma concentration levels) of one or the other of the administered agents. For example, a relevant resting period might be deemed to be completed with plasma concentration of the relevant agent is below about 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml or 0.001 µg/ml, optionally upon evaluation or other consideration of one or more features of the patient's response (e.g., of degree of cancer reduction and/or magnitude and/or type of induced cancer-specific immune response).

[122] In some embodiments, the number of cycles for which a particular agent is administered may be determined empirically. Also, in some embodiments, the precise regimen followed (e.g., number of doses, spacing of doses (e.g., relative to each other or to another event such as administration of another therapy), amount of doses, etc may be different for one or more cycles as compared with one or more other cycles. Ultimately, patient response is paramount.

Formulations

[123] Therapeutic compositions for use in accordance with the present invention may be prepared for storage and/or delivery using any of a variety of techniques and/or technologies known and/or available to those skilled in the art. In general, dosing and administration according to the present invention utilizes therapeutic compositions, which comprise an active agent (e.g., having a desired degree of purity) combined with one or more physiologically acceptable carriers, excipients or stabilizers in any of a variety of forms. Such forms include, for example, liquid, semi-solid and solid dosage forms, such as liquids, gels, tablets, capsules, powders,

patches, suppositories, etc. In some embodiments, a preferred form may depend on the intended mode of administration and/or therapeutic application.

[124] Typical preferred compositions for administration of antibody agents are in liquid form, suitable for delivery by injection (e.g., intravenous, often by infusion). Often, such agents are prepared and/or stored in dry powder (e.g., lyophilized), and formulated for parenteral delivery prior to administration. KADCYLA®, for example, is distributed as a lyophilized powder in single-use vials containing 100 mg per vial or 160 mg per vial.

[125] Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage.

[126] Those skilled in the art will be aware of a variety of formats for therapeutic compositions that may be utilized in accordance with the present invention. For example, in some embodiments, a therapeutic composition can be formulated as a solution, an emulsion (e.g., a micro- or nano-emulsion), a dispersion, a liposomal preparation, microcrystalline format, a gel format (e.g., a polymer gel format), a gel, a paste, a depot, and/or another dissolving, eroding, or degrading format. etc (e.g., other ordered structure).

[127] In some embodiments, the format may be selected to be suitable for high concentration of active agent. In some embodiments, solutions can be prepared by incorporating active agent (e.g., by dissolving a dry powder form of active agent) in a specified amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. In some embodiments, dispersions are prepared by incorporating the active agent into a sterile vehicle that contains a basic dispersion medium and any other desired or required ingredients.

[128] In many embodiments, therapeutic compositions, particularly of antibody agents, may desirably be prepared and/or utilized as sterile liquid, or otherwise injectable, formulations. In some embodiments, liquid formulations may be sterilized by filtration, radiation, autoclaving, etc.

[129] In some embodiments, therapeutic compositions may be, comprise, or be prepared from solid formats (e.g., powders such as sterile powders) of active agent. In some embodiments, powders may be prepared by vacuum drying and/or freeze drying; typically such approaches yield a powder of the active agent plus any additional desired or required ingredient (e.g., as may have been present with the active agent in the material that was subjected to vacuum and/or freeze drying).

- [130] In some embodiments, desired fluidity of a liquid or otherwise flowable formulation can be achieved and/or maintained, for example, by the use of a coating such as lecithin, by maintenance of a particular particle size (e.g., in the case of dispersion) and/or by use of surfactants (e.g., to stabilize an emulsion).
- [131] In some embodiments, it may be desirable to prepare and/or deliver a therapeutic composition, including a liquid formulation, or an otherwise flowable formulation, in a format that permits prolonged absorption. Those of ordinary skill in the art will be aware of a variety of agents that can be utilized to delay absorption, e.g., after injection, of fluid or otherwise flowable compositions. In some embodiments, such agents may be or include, monostearate salts, gelatin and/or combinations thereof. Alternatively or additionally, in some embodiments, therapeutic compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt. In some particular embodiments, extended delivery is achieved via use of implants, transdermal patches, and/or microencapsulated delivery systems. In some embodiments, and particularly in certain extended release embodiments, biocompatible and or biodegradable polymers can be used, such as but not limited to polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acids, and/or combinations thereof.
- [132] In many embodiments, therapeutic compositions may be prepared and/or distributed in single dose format. In some embodiments, therapeutic compositions may be prepared and/or distributed in a format appropriate for bolus administration. In some embodiments, therapeutic compositions may be prepared and/or distributed in a format appropriate for continuous administration (e.g., by infusion).
- [133] In some embodiments, a therapeutic composition may be associated with packaging. For example, in some embodiments, a therapeutic composition may be provided in an ampule or vial. In some embodiments, a therapeutic composition may be provided in a multi-dose pack.
- [134] In some embodiments, therapeutic compositions for use in accordance with the present invention may include one or more pharmaceutically acceptable dispersing agents, wetting agents, suspending agents, isotonic agents, coatings, antibacterial and antifungal agents, carriers, excipients, salts, or stabilizers are non-toxic to the subjects at the dosages and concentrations employed. A non-exhaustive list of such additional pharmaceutically acceptable agents includes buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; salts containing pharmacologically acceptable anions (such as acetate, benzoate, bicarbonate, bisulfate, isothionate, lactate, lactobionate, laurate,

malate, maleate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, thiethiodode, and valerate salts); preservatives (such as octadecydimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; sodium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or antibodies; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN.TM., PLURONICS.TM. or polyethylene glycol (PEG).

[135] In some particular embodiments, a therapeutic composition (e.g., a sterile and/or injectable composition) may include one or more pharmaceutically acceptable diluent or solvent. To give but a few examples, in some embodiments, an appropriate diluent or solvent may be or include 1,3 butanediol, dextrose solution, phosphate-buffered saline, Ringer's solution, saline, water, and/or etc.

[136] In some embodiments, a therapeutic composition may include one or more preservatives.

Articles of Manufacture and Kits

[137] In some embodiments of the invention, each active agent (optionally in the context of a therapeutic composition) to be administered to a subject is provided in a separate article of manufacture.

[138] In some embodiments, an article of manufacture is provided in which one or more active agents (optionally in the context of a therapeutic composition) is provided in a container, optionally with a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, a container holds a composition that is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). For example, in some embodiments, a formulation may be packaged in clear glass vials with a rubber stopper and an aluminum seal. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice.

- [139] In some embodiments, an article of manufacture may further comprise one or more separate containers comprising, for example, a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and/or dextrose solution.
- [140] Alternatively or additionally, in some embodiments, an article of manufacture may include any of a variety of other materials desirable from a commercial and/or user standpoint, including one or more buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. If two or more agents are to be administered simultaneously, the article of manufacture may contain those agents together in a single container, or appropriate materials and instructions for reconstituting the agents in a single formulation may be provided.
- [141] In some particular embodiments, an article of manufacture may allow providing one or more agents in an intravenous formulation as a sterile aqueous solution containing, for example, a total of 2 mg, 5 mg, 10 mg, 20 mg, 50 mg 100 mg, or more that may be formulated, for example with appropriate diluents and/or buffers, at a desired final concentration, which may for example be about 0.1 mg/ml, about 1 mg/ml, about 10 mg/ml, or at another (e.g., higher) concentration level of interest.
- [142] In some embodiments, separate agents to be administered can be provided in an article of manufacture as described herein in a form (e.g., a dry powder form such as a lyophilized form) suitable for reconstitution, for example with an appropriate aqueous solution, which may or may not be provided in or with the article (or kit).
- [143] In some embodiments, an article of manufacture, or one or more containers included therein, may be labeled for treatment of a particular indication (e.g., cancer or a specific type, grade, etc of cancer).
- [144] In some embodiments, an article of manufacture may include one or more unit dosage forms of one or more active agents. In some embodiments, such unit dosage forms are provided in a pack or dispenser device. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack.
- [145] In some embodiments, an article of manufacture may materials and/or devices suitable for measuring expression of one or more markers of interest (e.g., of one or more inducible effector cell surface markers and/or of one or more tumor-associated antigens), and/or of a surrogate therefor. Alternatively or additionally, in some embodiments an article of manufacture may include one or more reference agents, e.g., useful as controls or references, for example to permit identification and/or comparison of levels or types of markers of interest.

[146] In some embodiments, an article of manufacture may include one or more buffers, diluents, filters, needles, syringes, and package inserts with instructions, for example for use in the treatment of cancer.

[147] In some embodiments, instructions that are associated with an article of manufacture as described herein may be in the form of a label, a leaflet, a publication, a recording, a diagram, or any other means that can be used to inform about the correct use and/or monitoring of the possible effects of the agents, formulations, and/or other materials in the article of manufacture (e.g., kit). Instructions may be provided together with the article of manufacture (e.g., kit) or may be provided separately but with the indication that instructions are to be used in association with the article of manufacture (kit) and/or one or more of its components.

EXEMPLIFICATION

EXAMPLE 1: Inducible effector cell surface marker Agonist Antibody Enhances Cytokine Secretion from T-DM1-Induced Effector Cells

[148] The present Example describes cytokine secretion levels from effector cells after co-culture with tumor cells and certain therapeutic antibodies. Specifically, presented data show interferon-gamma (IFN γ) levels as determined in a 4 hour (1:1) co-culture assay with 20 hour priming prior to administration of an inducible effector cell surface marker agonist antibody. Utilized cells were human breast cancer cell lines MCF-7 and HER-18 (which overexpresses HER2), each of which was separately co-cultured with NK cells. Anti-tumor antibodies rituximab (anti-CD20), trastuzumab (anti-Her2) or T-DM1 (trastuzumab with DM1 payload; each of which can be obtained from, for example, Roche/Genentech) were utilized either alone or in combination (after priming) with an anti-CD137 agonist antibody (which can be obtained from, for example, Bristol-Myers Squibb). These same antibodies were also utilized in studies described below in Examples 2-5.

[149] Figure 1, Panel A shows results with MCF7 cells; Figure 1, Panel B shows results with HER-18 cells. As can be seen, particularly with the HER-18 cells, which overexpress HER2, IFN γ levels were dramatically higher when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1) was utilized alone. Elevated IFN γ levels were observed for both trastuzumab and T-DM1 when agonist antibody was included.

EXAMPLE 2: Inducible effector cell surface marker Agonist Antibody Enhances Degranulation of T-DM1-Induced Effector Cells

[150] The present Example describes degranulation of effector cells after co-culture with tumor cells and certain therapeutic antibodies. Specifically, presented data show percentage of CD107a+ NK cells as determined in a 4 hour (1:1) co-culture assay with 20 hour priming prior to administration of an inducible effector cell surface marker agonist antibody. Utilized cells were human breast cancer cell lines MCF-7 and HER-18 (which overexpresses HER2), each of which was separately co-cultured with NK cells. Anti-tumor antibodies rituximab (anti-CD20), trastuzumab (anti-Her2) or T-DM1 (trastuzumab with DM1 payload) were utilized either alone or in combination (after priming) with an anti-CD137 agonist antibody.

[151] Figure 2, Panel A shows results with MCF7 cells; Figure 2, Panel B shows results with HER-18 cells. As can be seen, particularly with the HER-18 cells, which overexpress HER2, degranulation levels were dramatically higher when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1) was utilized alone. Elevated degranulation levels were observed for both trastuzumab and T-DM1 when agonist antibody was included.

EXAMPLE 3: Inducible effector cell surface marker Agonist Antibody Enhances Target Cell Lysis by T-DM1-Induced Effector Cells

[152] The present Example describes target cell lysis by effector cells after co-culture of the effector cells with target tumor cells and certain therapeutic antibodies. Specifically, presented data show levels of target cell lysis as determined in a 4 hour (1:1) co-culture assay with 20 hour priming prior to administration of an inducible effector cell surface marker agonist antibody. Utilized cells were human breast cancer cell lines MCF-7 and HER-18 (which overexpresses HER2), each of which was separately co-cultured with NK cells. Anti-tumor antibodies rituximab (anti-CD20), trastuzumab (anti-Her2) or T-DM1 (trastuzumab with DM1 payload) were utilized either alone or in combination (after priming) with an anti-CD137 agonist antibody.

[153] Figure 3, Panel A shows results with MCF7 cells; Figure 3, Panel B shows results with HER-18 cells. As can be seen, particularly with the HER-18 cells, which overexpress HER2, target cell lysis levels were dramatically higher when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1)

was utilized alone. Elevated target cell lysis levels were observed for both trastuzumab and T-DM1 when agonist antibody was included.

EXAMPLE 4: Inducible effector cell surface marker Agonist Antibody Synergistically Enhances T-DM1-Efficacy In Vivo

[154] The present Example describes effects of provided therapeutic regimens in a xenograft model in which human Her2-overexpressing breast tumor cells (HER-18 cells) were inoculated by subcutaneous injection into nude mice that were then treated with certain therapeutic antibodies. Specifically, presented data show tumor size (Figure 4) and percent survival (Figure 5) for mice that were inoculated with 1×10^6 HER2⁺ HER18 tumor cells by subcutaneous injection. On day 3, mice were randomized into treatment groups that received either:

- Group 0: control IgG
- Group 1: anti-CD137 agonist only
- Group 2: Trastuzumab only
- Group 3: T-DM1 only
- Group 4: Trastuzumab + anti-CD137 agonist
- Group 5: T-DM1 + anti-CD137 agonist

Antibodies were administered according to the following regimens:

Her-18 Tumor Model:		
1×10^6 HER2 ⁺ HER18 tumor cells injected on day 0 (d0)		
Trastuzumab	5 mg/kg/dose	d3, d10 d17
T-DM1	5 mg/kg/dose	d3, d10 d17
Anti-CD137 agonist	10 mg/kg/dose	d4, d11, d18

Thus, antibody combinations (i.e., Trastuzumab + anti-CD137 agonist and T-DM1 + anti-CD137 agonist) were administered according to staggered combination regimens as described herein.

[155] As can be seen with reference to Figure 4, tumor size was dramatically smaller when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1) was utilized alone. Reduced tumor size was observed for both trastuzumab and T-DM1 when agonist antibody was included.

[156] As can be seen with reference to Figure 5, percent survival was dramatically higher when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1) was utilized alone. Improved survival rates were observed for both trastuzumab and T-DM1 when agonist antibody was included; in fact, 100% survival was observed with both staggered combination regimens.

EXAMPLE 5: Inducible effector cell surface marker Agonist Antibody Synergistically Enhances T-DM1-Efficacy in an In Vivo Model of Established Tumor, with Dramatic Improvement Even Relative to Synergistic Enhancement Observed with Trastuzumab

[157] The present Example describes effects of provided therapeutic regimens in a xenograft model in which human Her2⁺ primary breast tumor cells (SU-258 cells) were inoculated by intramammary injection into SCID mice 24 hours after 200 cYg total body irradiation. The tumors were permitted to establish for 30 days, after which time the mice were treated with certain therapeutic antibodies. Specifically, presented data show tumor size (Figure 6) and percent survival (Figure 7) for mice that were inoculated with 1×10^6 HER2⁺ SU-258 tumor cells by intramammary injection. On day 30, mice were randomized into treatment groups that received either:

Group 0: control IgG

Group 1: anti-CD137 agonist only

Group 2: Trastuzumab only

Group 3: T-DM1 only

Group 4: Trastuzumab + anti-CD137 agonist

Group 5: T-DM1 + anti-CD137 agonist

Antibodies were administered according to the following regimens:

SU-258 Tumor Model:		
1 x 10 ⁶ HER2 ⁺ SU-258 tumor cells injected on day 0 (d0), 24 hours after total body irradiation		
Trastuzumab	5 mg/kg/dose	d30, d37, d44
T-DM1	5 mg/kg/dose	d30, d37, d44
Anti-CD137 agonist	10 mg/kg/dose	d31, d38, d45

Thus, antibody combinations (i.e., Trastuzumab + anti-CD137 agonist and T-DM1 + anti-CD137 agonist) were administered according to staggered combination regimens as described herein.

[158] As can be seen with reference to Figure 6, tumor size was dramatically smaller when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1) was utilized alone. Reduced tumor size was observed for both trastuzumab and T-DM1 when agonist antibody was included.

[159] As can be seen with reference to Figure 7, percent survival was dramatically higher when the agonist antibody (anti-CD137) was included, as compared with when an anti-tumor antibody (specifically trastuzumab or T-DM1) was utilized alone. Improved survival rates were observed for both trastuzumab and T-DM1 when agonist antibody was included. Moreover, Figure 6 documents the surprising finding that the improvement in percent survival achieved when anti-CD137 agonist therapy is added to T-DM1 therapy is dramatically more significant than that observed when anti-CD137 agonist therapy is added to trastuzumab therapy, notwithstanding that T-DM1 and trastuzumab target the same tumor antigen (HER2) and, moreover that T-DM1 includes the identical trastuzumab antibody, differing from trastuzumab in that the antibody is conjugated with DM1.

CLAIMS

1. A method of treating cancer, the method comprising:

administering to a patient a composition comprising an agonistic antibody to a molecule whose expression increases on surfaces of natural killer (NK) cells that mediate antibody-dependent cellular cytotoxicity (ADCC) when such cells are exposed to tumor cells bound by a trastuzumab-drug conjugate, which agonistic antibody is characterized as agonistic in that, when the NK cells with the inducible effector cell surface marker on their surface are contacted with the agonistic antibody, their ADCC is increased as compared with that observed absent such contact;

the patient having received therapy including the trastuzumab-drug conjugate a period of time prior to the administering, such that the increase in expression of the inducible effector cell surface marker has occurred.

2. The method of claim 1, wherein the agonistic antibody is particularly characterized in that, when tumor cells coated with the trastuzumab-drug conjugate are contacted with NK cells in which the inducible effector cell surface marker is expressed on the surface together with the agonistic antibody, apoptosis of the tumor cells is increased relative to that observed in absence of the agonistic antibody.

3. The method of claim 1, wherein the agonistic antibody is particularly characterized in that, when tumor cells coated with the trastuzumab-drug conjugate are contacted with NK cells in which the inducible effector cell surface marker is expressed on the surface together with the agonistic antibody, tumor growth is reduced relative to that observed in absence of the agonistic antibody.

4. The method of claim 1, wherein the agonistic antibody is a monoclonal antibody.

5. The method of claim 1, wherein the agonistic antibody is a xenogeneic human antibody.

6. The method of claim 1, wherein the agonistic antibody is a humanized antibody.

7. The method of claim 1, wherein the agonistic antibody is a chimeric antibody.

8. The method of claim 1, further comprising a step of determining the level of the inducible effector cell surface marker.

9. The method of claim 8, wherein the level of the inducible effector cell surface marker is determined prior to administering the trastuzumab-drug conjugate, and the increase in expression following administration of the trastuzumab-drug conjugate is determined.

10. The method of claim 9 wherein the step of determining comprises:
providing a patient sample; and
determining the level in the sample.

11. The method of claim 10, wherein the patient sample is a blood sample or cellular fraction thereof.

12. The method of claim 1, wherein the tumor is a solid tumor.

13. The method of claim 2, wherein the solid tumor is an adenocarcinoma.

14. The method of claim 1, wherein the tumor is a breast tumor, a gastric tumor, or a gastroesophageal tumor.

15. The method of claim 1, wherein the tumor is a metastatic tumor.

16. The method of claim 1, wherein the tumor is an established tumor.

17. The method of claim 1, wherein the tumor is Her2⁺.

18. The method of claim 14, wherein the tumor is established in that it appears to have been present in the subject for at least 30 days.

19. The method of claim 1, wherein the inducible effector cell surface marker is a member of the tumor necrosis factor receptor (TNFR) family.

20. The method of claim 1, wherein the inducible effector cell surface marker is a member of the CD28 family.

21. The method of claim 1, wherein the inducible costimulatory molecule is selected from CD38, CD137, OX40, GITR, CD30 and/or ICOS.

22. In a method of treating cancer that comprises a step of administering the trastuzumab-drug conjugate therapy, the improvement comprising:

a period of time after the step of administering the trastuzumab-drug conjugate therapy (the "first administering step"), performing a second administering step that comprises administering a composition comprising an agonistic antibody that targets a molecule whose expression increases on surfaces of effector NK cells that mediate antibody- dependent-cellular cytotoxicity (ADCC) when such cells are exposed to tumor cells bound by the trastuzumab-drug conjugate, the period of time being sufficient so that expression of the inducible effector cell surface marker has been increased on such surfaces at the time of the second administering step, so that ADCC is increased.

23. A method of enhancing the anti-tumor effect of the trastuzumab-drug conjugate in a patient, the method comprising sequential administration of the trastuzumab-drug conjugate and an agonistic antibody, the agonistic antibody targeting at least one molecule on NK cells characterized in that its expression is induced on surfaces of NK cells during activation of the NK cells when such cells are exposed to tumor cells bound by an anti-tumor antibody;

the agonistic antibody being characterized as agonistic in that, when the NK cells with the inducible effector cell surface marker on their surface are contacted with the agonistic antibody, their ADCC is increased as compared with that observed absent such contact; and

the agonistic antibody being administered a period of time after the administration of the trastuzumab-drug conjugate, the period of time being sufficiently long that increased expression of the inducible costimulatory molecule has occurred.

24. The method of any one of the above claims, wherein the trastuzumab-drug conjugate is T-DM1.

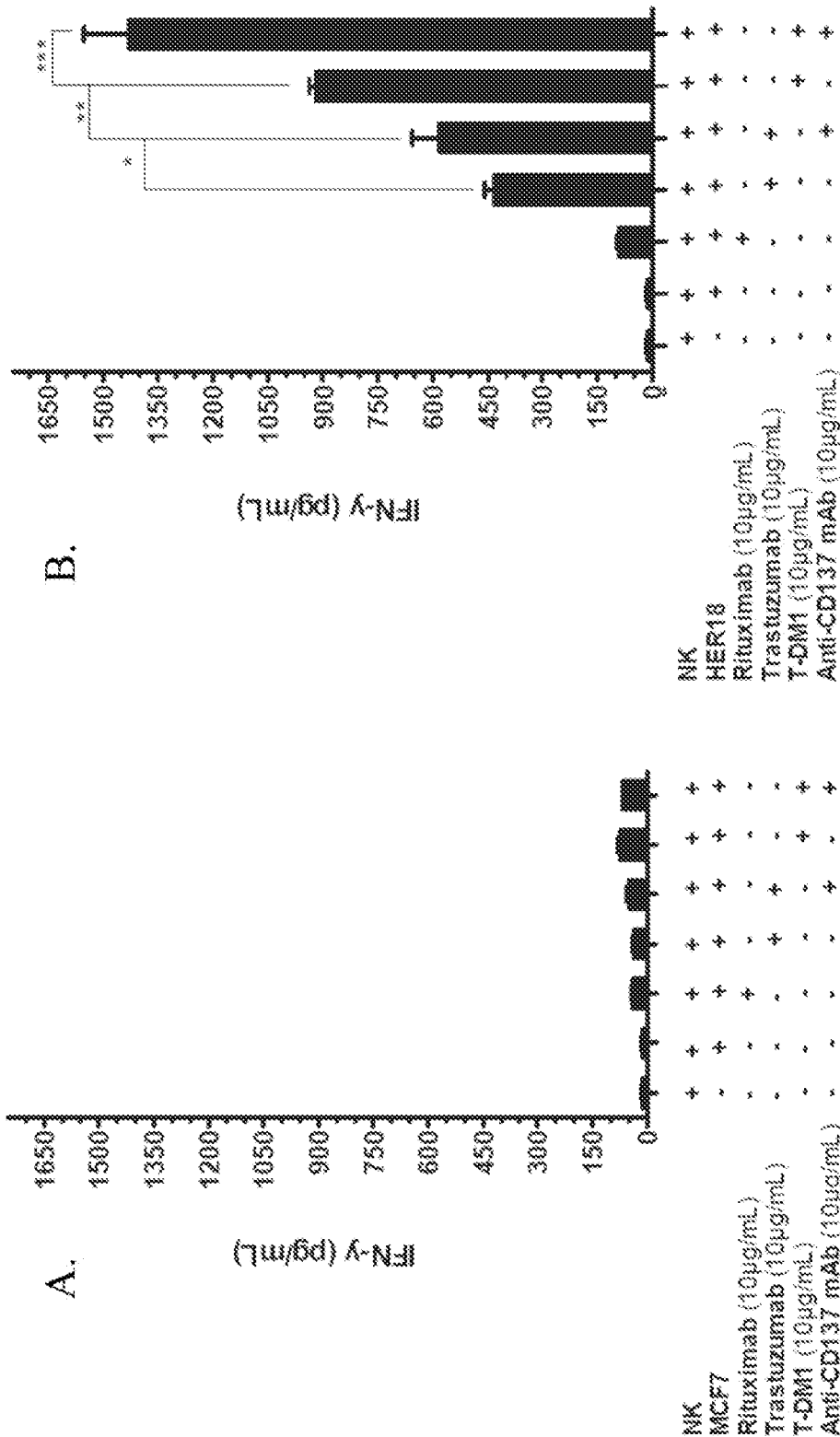


Figure 1

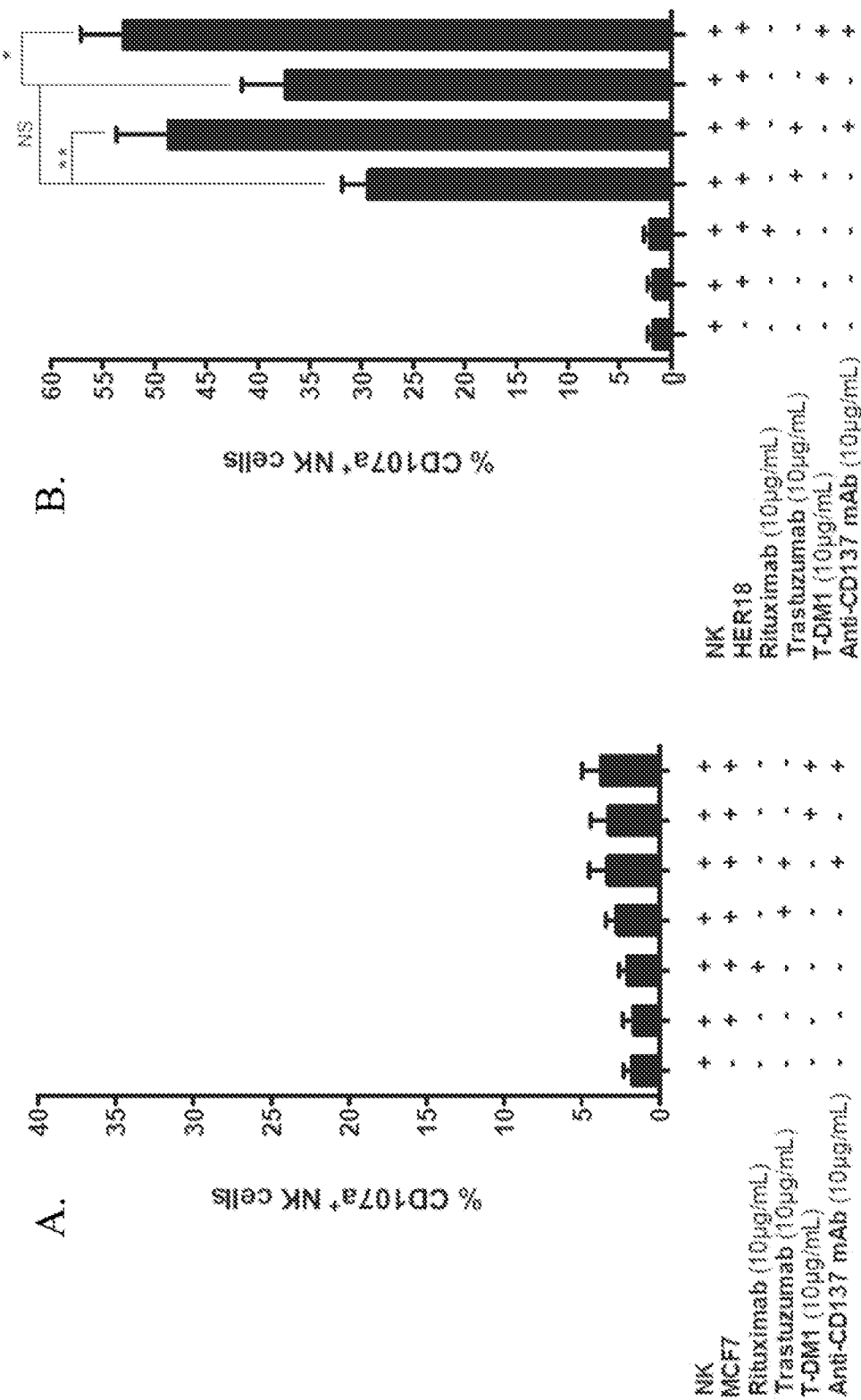


Figure 2

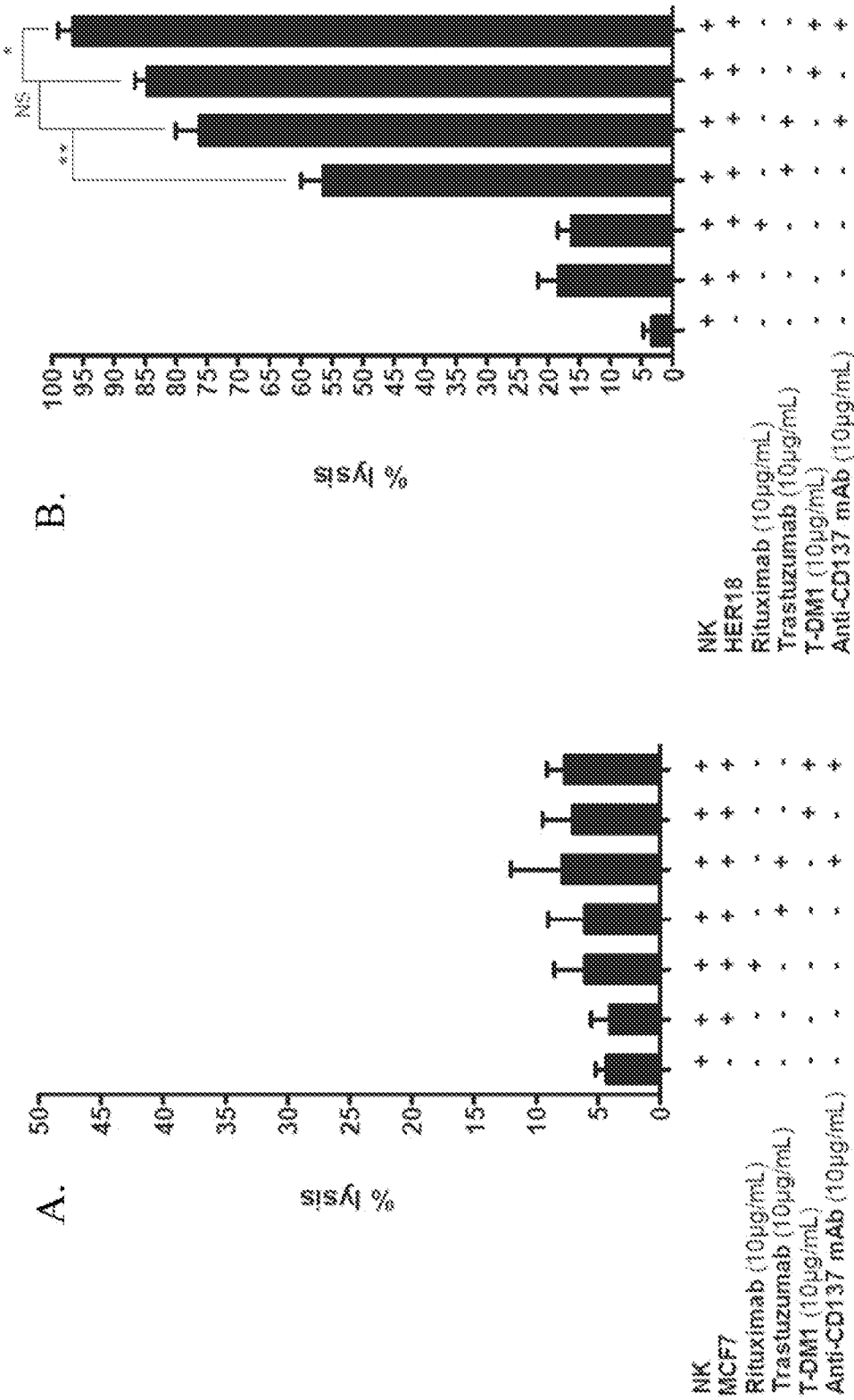


Figure 3

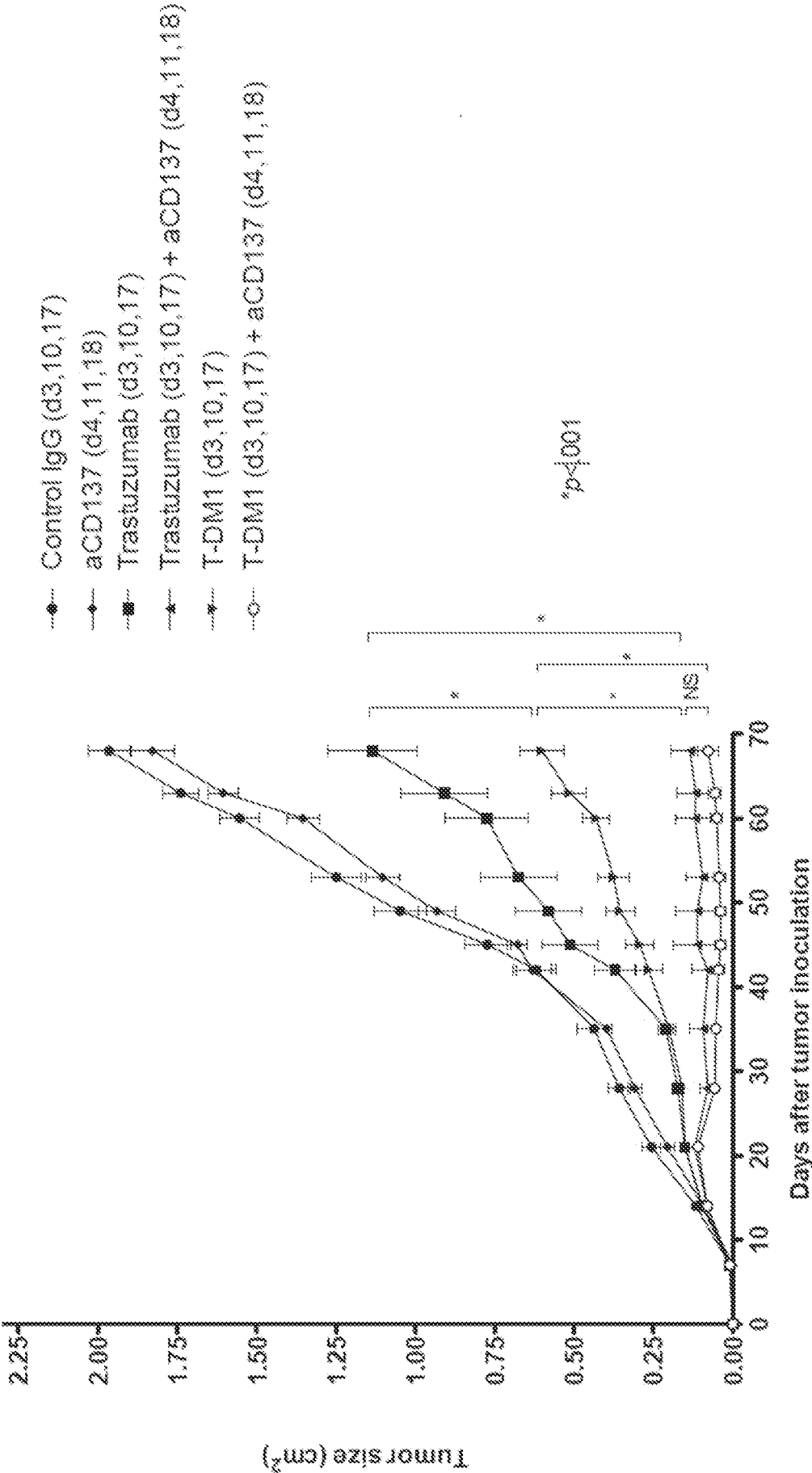


Figure 4

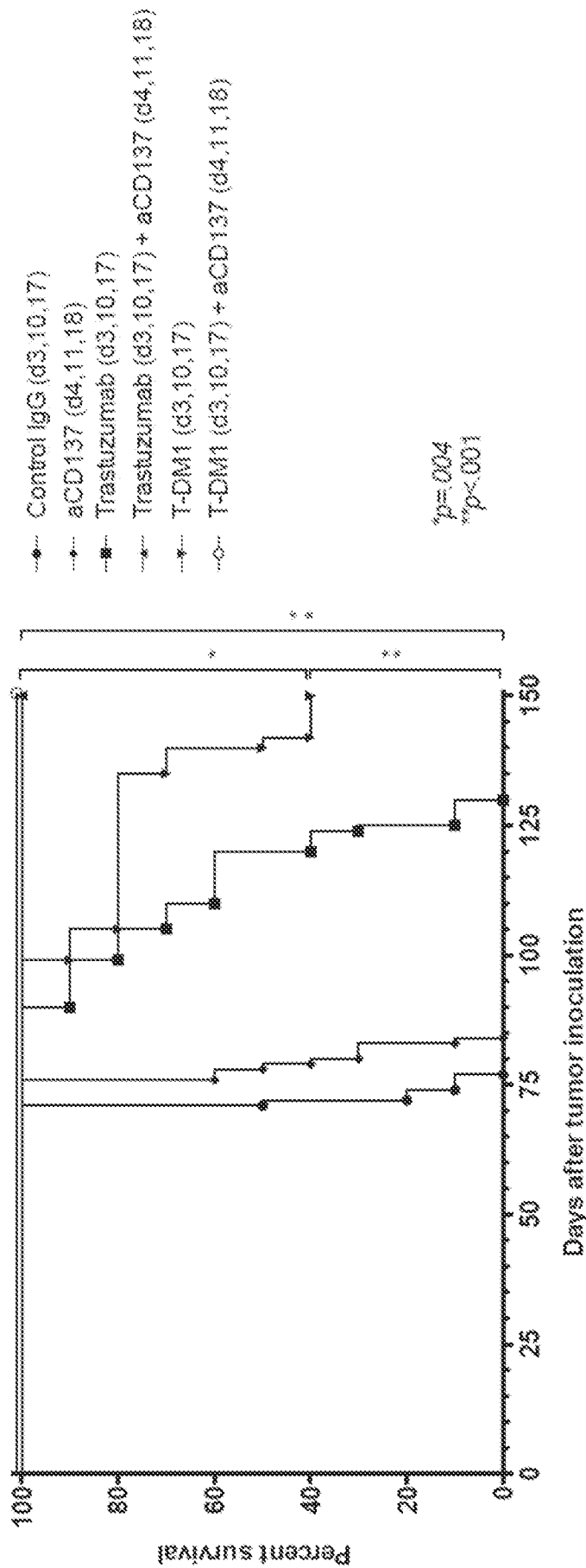


Figure 5

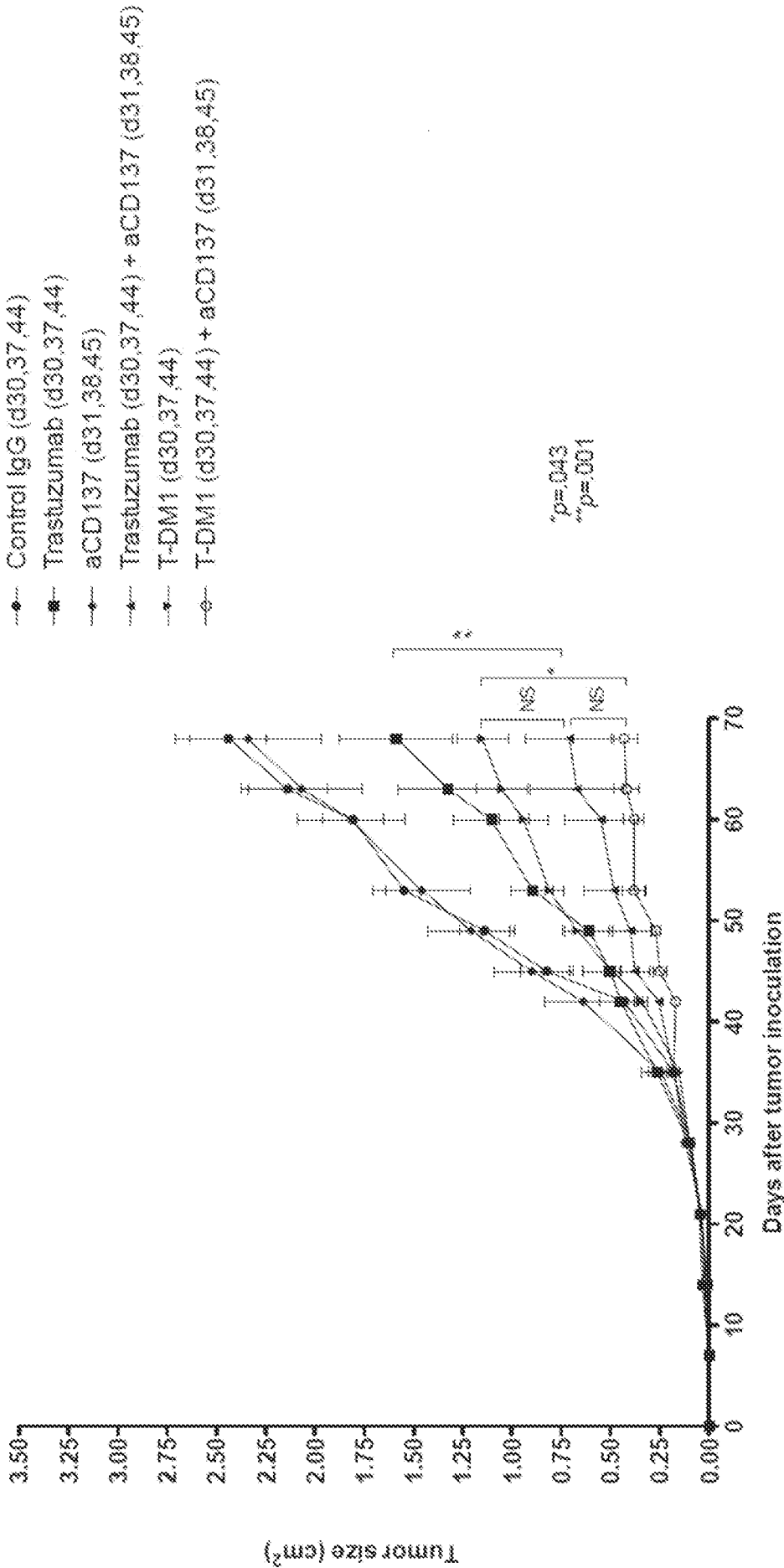


Figure 6

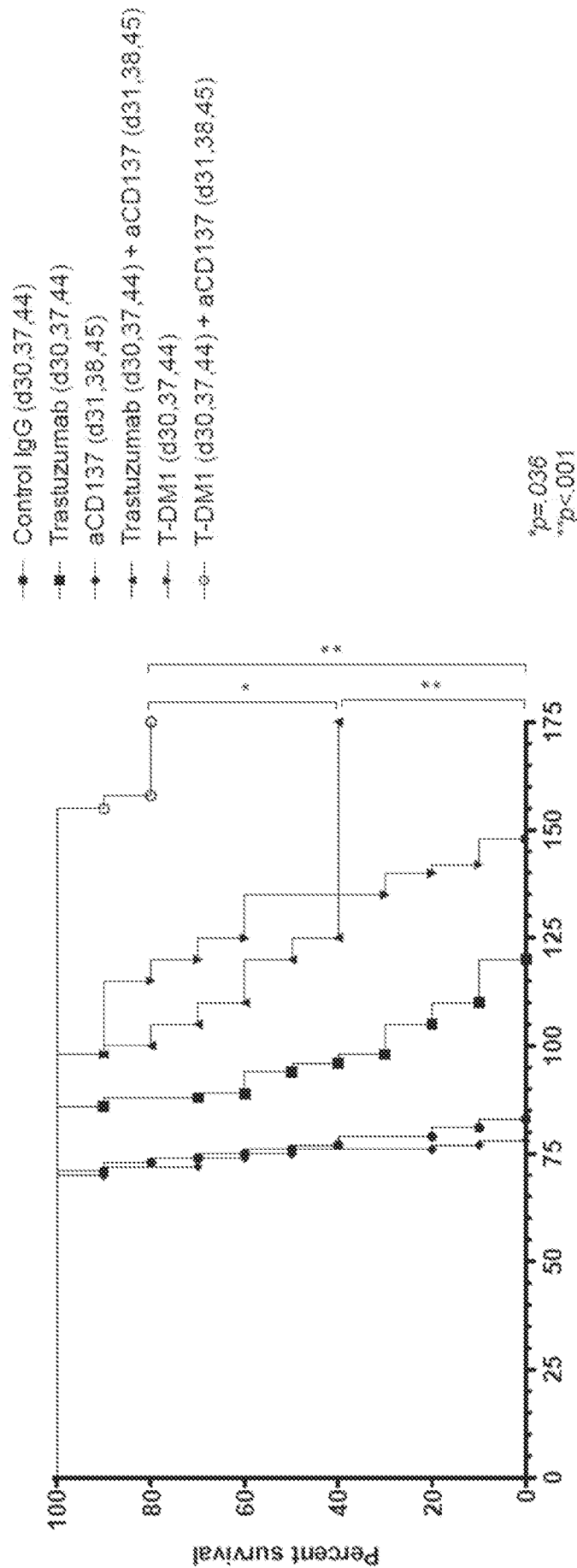


Figure 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/26503

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/00; C12P 21/08; A61K 39/00, 39/385 (2015.01)

CPC - A61K 2039/505; 2039/6043; 47/48407, 38/00, 39/0011; C07K 2317/24, 2319/00, 2319/30

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)

IPC(8): C07K 16/00; C12P 21/08; A61K 39/00, 39/385; CPC: A61K 2039/505; 2039/6043; 47/48407, 38/00, 39/0011; C07K 2317/24, USPC: 424/184.1, 530/387.1, 391.1, 386, 380, 350, 178.1, 181.1, 193.1, 179.1

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

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

PatSeer; Google; Google Scholar; Dialog ProQuest; Pubmed; EBSCO: 'NK cells,' 'surface markers,' 'agonistic antibody,' 'trastuzumab, conjugate,' 'ADCC,' 'apoptosis, cancer, tumor, metastatic,' 'HER2+,' 'CD137,' 'TNF-R,' 'T-DM1,' 'chimeric, xenogenic,' 'CD28'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0321646 A1 (KOHRT, H et al.) December 10, 2012; abstract; figure 7; paragraphs [0006]-[0009], [0014], [0016], [0019], [0043], [0049], [0055], [0056], [0063], [0064], [0072]-[0074], [0077], [0079], [0081], [0086]	1-24
Y	US 2014/0044709 A1 (KLENCKE, B et al.) February 13, 2014; paragraphs [0002], [0006], [0027], [0039]	1-24
A	WO 2014/012479 A1 (SHANGHAI BIRDIE BIOTECH, INC.) January 23, 2014; abstract	1-24



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

"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

07 July 2015 (07.07.2015)

Date of mailing of the international search report

22 JUL 2015

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774