Title: MEDICAL USES OF CD38 AGONISTS (ANTIBODIES)

Abstract: Methods and compositions relating to medical (e.g., therapeutic) use of CD38 agonists are provided. In some embodiments, the present invention provides methods and compositions relating to use of a CD38 agonist in the treatment of cancer.
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MEDICAL USES OF CD38 AGONISTS (ANTIBODIES)

BACKGROUND

[01] Monoclonal antibody therapy is rapidly becoming the standard of care for many diseases, disorders, and conditions, including certain cancers. Despite the promising activity of monoclonal antibodies, the response rates among patients with either refractory or advanced cancer often are only partial, less than 25%, due to various factors.

SUMMARY

[02] The present invention demonstrates effective treatment of cancer by agonizing CD38 (i.e., by administration of CD38 agonist therapy, for example comprising administering a CD38 agonist). Prior to the present invention, CD38 expressed by tumor cells had been described as a potential target for inhibitory therapy to treat cancer. According to prior understanding, therefore, agonizing CD38 would be affirmatively undesirable for cancer patients. The present invention, however, establishes that administration of a CD38 agonist can enhance the ability of a subject's immune system to target and destroy cancer cells. In particular, the present invention demonstrates that agonizing CD38 can increase effector cell killing of tumor cells, e.g. via antibody-dependent cellular cytotoxicity (ADCC). In accordance with the present invention, administration of a CD38 agonist can augment the ADCC capability of immune effector cells such as NK cells.

[03] In one aspect, the present invention provides methods of treating cancer by administering to a patient a composition comprising a CD38 agonist. In some embodiments, a CD38 agonist for use in accordance with the present invention binds the extracellular domain of CD38 (e.g., to an epitope within the extracellular domain of CD38). In some embodiments, a CD38 agonist for use in accordance with the present invention binds to CD38 as present on the surface of cells such as immune effector cells). In some embodiments, apoptosis of tumor cells upon administering of a CD38 agonist is increased relative to that observed absent the CD38 agonist. In some embodiments, tumor growth upon administering of a CD38 agonist is reduced relative to that observed absent the CD38 agonist.

[04] In some embodiments, the present invention provides methods of administering CD38 agonist therapy together with agonist therapy directed at one or more inducible immune effector cell surface markers other than CD38.

[05] In some embodiments, methods of the present invention involve determining a CD38 expression level, for example on effector cells (e.g., NK cells) in a subject. Alternatively or additionally, in some embodiments, it may be desirable to determine a CD38 expression
level (and/or an expression level of one or more other tumor antigens) on tumor cells. In some embodiments, CD38 expression level is determined prior to administering (at least one dose of) a CD38 agonist. In some embodiments, CD38 expression level is determined at multiple time points. In some embodiments, CD38 expression level is determined before, substantially simultaneously with, and/or after administration of one or more doses of a CD38 agonist.

[06] In some embodiments, CD38 agonist therapy is not administered unless or until an increase (relative to a relevant prior or reference level) in or particular threshold level of CD38 is detected on effector cells, such as NK cells. In some embodiments, CD38 agonist therapy is not administered if CD38 expression level in tumor cells is above a relevant threshold.

[07] In some embodiments of methods of the present invention, expression levels both of CD38 and of an inducible immune effector cell surface marker other than CD38 are determined. In some embodiments, both such expression levels are determined at the same time. In some embodiments, such expression levels are determined at different times. In some embodiments, expression levels of CD38 and/or of an inducible immune effector cell surface marker other than CD38 may be determined at multiple times, some or all of which may be, but need not be, at the same time.

[08] Those skilled in the art are aware of a variety of techniques for achieving determination of such expression levels (e.g., of CD38, of another inducible immune effector cell surface marker, and/or of another tumor antigen). For example, those skilled in the art are aware that a relevant expression level often may be determined by detecting the relevant marker (i.e., CD38, of another inducible immune effector cell surface marker, and/or of other tumor antigen) itself, and/or by detecting one or more precursors or results of expression of the relevant marker, as a proxy for detecting the marker itself.

[09] In some embodiments, as an alternative or in addition to determination of expression levels of one or more markers (e.g., CD38, another inducible immune effector cell surface marker, and/or another tumor antigen), one or more other steps or types of immune profiling is performed prior to, during, and/or after administration of one or more doses of CD38 agonist in accordance with the present invention. In some embodiments, such immune profiling involves detection or determination of one or more aspects or features of a patient or patient population that correlates with or otherwise predicts likelihood of response to administration of CD38 agonist therapy.

[10] In some embodiments, an expression level (e.g., of CD38, of another inducible immune effector cell surface marker, and/or of another tumor antigen) and/or other immune profiling is determined in a patient sample (e.g., a primary sample or a secondary sample derived by processing a primary sample). In some such embodiments, the patient sample is or
comprises a blood sample. In some embodiments, the patient sample is or comprises a tissue sample. In some embodiments, the patient sample is or comprises a tumor sample (e.g., includes tumor cells). In some embodiments, a patient sample is or comprises circulating, peritumoral, and/or intratumoral immune cell populations. In some embodiments, samples are obtained, prior to, during, and/or after CD38 agonist therapy.

In some embodiments, an inducible immune effector cell surface marker other than CD38 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.

Furthermore, among other things, the present invention provides methods of enhancing antibody-dependent cellular cytotoxicity (ADCC) of effector cells (e.g., NK cells) in a subject, which methods involve administering to the subject a CD38 agonist therapy. In some embodiments, CD38 agonist therapy comprises administration of one or more doses of a CD38 agonist according to a regimen correlated with elevated ADCC of the effector cells (e.g., as compared with a level observed under otherwise comparable conditions lacking such administration). In some embodiments, ADCC is evaluated by a chromium release assay. In some particular embodiments in which the ADCC of effector cells is increased, the degranulation of such effector cells is increased (e.g., relative to that observed under otherwise comparable conditions lacking the CD38 agonist). Alternatively or additionally, in some embodiments, mobilization of CD107a on the surface of such effector cells is increased (e.g., relative to that observed under otherwise comparable conditions lacking the CD38 agonist). In some embodiments, cytokine release from such effector cells is increased (e.g., relative to that observed under otherwise comparable conditions lacking the CD38 agonist).

In some embodiments, subjects to which methods provided by the present invention are applied or administered are suffering from cancer. In some embodiments, the cancer is selected from a group of hematologic malignances including acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, AIDS-related lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma, Langerhans cell histiocytosis, multiple myeloma, and myeloproliferative neoplasms. In some embodiments, the cancer is selected from a group of solid tumors including breast carcinoma, squamous cell carcinoma, colon cancer, head and neck cancer, lung cancer, genitourinary cancer, rectal cancer, gastric cancer, sarcoma, melanoma, and esophageal cancer.
The present invention provides certain CD38 agonist agents and pharmaceutical compositions thereof. In some embodiments, a CD38 agonist is or comprises a non-antibody agent. In some embodiments, such a non-antibody agent is or comprises a peptide, a recombinant protein, or an aptamer that specifically binds to CD38. In some embodiments, a CD38 agonist is or comprises an antibody agent that specifically binds to CD38 (e.g., to CD38 on surfaces of immune effector cells). In some embodiments, such an antibody agent is or comprises an intact antibody. In some embodiments, such an antibody agent is or comprises a monoclonal antibody (mAb). In some embodiments, such an antibody agent is or comprises a humanized or human antibody, or includes antigen binding elements of a human or humanized antibody. In some embodiments, such an antibody agent is or comprises a xenogenic antibody, or includes antigen binding elements thereof. In some embodiments, such an antibody agent is or comprises a chimeric antibody, or includes antigen binding elements thereof. In some embodiments, an antibody agent is a multi-specific agent, such as a bispecific antibody. In some such embodiments, the multi-specific agent binds specifically to CD38 and to an inducible immune effector cell surface marker other than CD38. In some embodiments, the multi-specific agent binds specifically to CD38 and to a tumor antigen. In some embodiments, the multi-specific agent binds specifically to CD38 and to another antigen, which other antigen is not a tumor antigen (so that CD38 and tumor antigen are not simultaneously targeted).

In some embodiments, as described herein, a CD38 agonist may be administered in combination with one or more other agents and/or therapeutic modalities. In some embodiments, for example, a CD38 agonist is administered in combination with therapy that increases immune effector (e.g., NK) cell numbers (e.g., systemic numbers or local numbers, such as intratumoral numbers) relative to that observed absent the therapy. In some embodiments, for example, a CD38 agonist is administered in combination with therapy that increases immune infiltration (e.g., by immune effector cells such as NK cells) relative to that observed absent the therapy. In some embodiments, for example, a CD38 agonist is administered in combination with therapy that increases expression (e.g., cell surface expression) of CD38 on local and/or systemic immune effector cells (e.g., NK cells). In some embodiments, for example, a CD38 agonist is administered in combination with therapy that decreases expression (e.g., cell surface expression) of CD38 on tumor cells (e.g., NK cells), relative to that observed absent the therapy. In some embodiments, for example, a CD38 agonist is administered in combination with therapy that decreases local and/or systemic levels of Treg cells relative to that observed absent the therapy.

Still further, the present invention provides a variety of kits or articles of manufacture containing components relevant to administration of CD38 agonist therapy and/or detection
of CD38 expression, particularly on surfaces of tumor cells and/or immune cells (such as immune effector cells), particularly in samples from patients.

**BRIEF DESCRIPTION OF THE DRAWING**

Figure 1. Agonistic anti-CD38 antibody enhances Natural Killer (NK) cell cytotoxicity against breast tumor cells in vitro. (A) Peripheral blood mononuclear cells (PBMCs) were first activated with HER2-expressing breast cancer cell line HER18 in the presence of Trastuzumab (anti-HER2 antibody, 10 µg/mL) for 24 hours. Cytotoxicity of the activated PBMCs was subsequently measured by an in vitro 51Cr release assay, in which the activated PBMCs were incubated with target (HER18) cells at different effector cell:target cell ratios, in the presence of either media alone (i.e., negative control), or of certain monoclonal antibodies (mAbs), namely: Trastuzumab (labeled "aHER2 mAb"), an agonistic anti-CD38 antibody (labeled "aCD38 (IB4) mAb"), an antagonistic anti-CD38 antibody (labeled "aCD38 (HB7) mAb"), or combinations of these anti-CD38 and anti-HER2 antibodies. An agonistic anti-CD137 antibody was also further combined with the anti-HER2 (Trastuzumab) and agonistic anti-CD38 (IB4) antibodies. (B) Effect of agonistic anti-CD38 antibody on inhibition of breast tumor growth in vivo was evaluated in nu/nu mice that were inoculated with 5 x 10^6 HER2-expressing breast tumor (BT474M1) cells subcutaneously on the right flank. After tumor inoculation, mice received control rat IgG antibody (negative control), anti-HER2 antibody (labeled "aHER2"), or agonistic antibody against either CD38 (labeled "aCD38") or OX40 (labeled "aOX40") on day 3 (d3), day 10 (d10), and day 17 (d17). On day 4 (d4), day 11 (d11), and day 18 (d18), two groups of the mice that had received anti-HER2 antibody further received agonistic antibody against CD38 or OX40. Mice (10 per group) were monitored for tumor growth up to day 60.

Figure 2. Effect of an agonistic anti-CD38 antibody on the inhibition of lymphoma growth in vivo was evaluated in a syngeneic lymphoma BALB/c model that was established by inoculating subcutaneously with 1 x 10^6 CD20-expressing A20 tumor cells. After tumor inoculation, mice received a rat IgG antibody (negative control), an anti-mouse CD20 monoclonal antibody (aCD20; 18B12; 100 µg/dose), or an agonistic anti-mouse CD38 monoclonal antibody (aCD38; NIMR-5; 150 µg/dose) on day 3 (d3), day 10 (d10), and day 17 (d17) as a monotherapy. Mice (10 per group) were monitored for tumor growth up to day 60. The asterisk indicates a p value for statistical significance below 0.001.

Figure 3. Effects of an agonistic anti-CD38 antibody (NIMR-5) as a monotherapy or in combination with an additional antibody (anti-CD4, indicated as aCD4; anti-FR4, indicated as aFR4; anti-CD8, indicated as aCD8) on the inhibition of lymphoma growth in vivo were
tested in a panel of 4 murine cell lines (A20, EL4, H11, and BL3750). Nonspecific mouse IgG (IgG) and the agonistic anti-CD38 antibody in combination with anti-asialo-GM1 (aGM1) were used as control treatments. The data are consolidated in a single graph, showing the actual data on the tumor size as percentage change for each of the 10 mice in every group between Day 0 and Day 14 (each bar indicate a single mouse; black bars indicate growth of tumor size; white bars indicate regression of tumor size.

Figure 4. Effects of an agonistic anti-CD38 antibody (NIMR-5) as a monotherapy or in combination with an additional antibody (anti-CD4, indicated as aCD4; anti-FR4, indicated as aFR4; anti-CD8, indicated as aCD8) on the inhibition of tumor growth in vivo were tested in a panel of 4 murine cell lines for colon cancer (MC38, CT26), lung cancer (LLC1), and sarcoma (Sa1 N). Control treatments, as well as the representation of data on the tumor size, are the same of Figure 3.

DEFINITIONS

Below are provided certain definitions of terms used herein, many or most of which confirm common understanding of those skilled in the art.

**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, intratumoral, etc), 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, subcutaneous, or intratumoral [e.g., particularly when high doses within a tumor are desired] injection).

**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.

[24] **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. An agonist may be direct (in which case it exerts its influence directly upon its target) or 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).

[25] **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.

[26] **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. An antagonist may be direct (in which case it exerts its influence directly upon its target) or 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).
[27] **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; as understood in the art, for example determined according to Kabat numbering scheme) 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 ("SMIps™"), 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®, Centryns®, CoVX bodies, BiCyclic peptides, or Kunitz domain derived antibody constructs. 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.]

[28] 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, humanized 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 ("SMIps™"), 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
come 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 that
is homologous or largely homologous to an immunoglobulin-binding domain.
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.

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, and 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.

Biological Sample, Sample: As used herein, the terms "biological sample" or "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.

[32] **Biomarker.** The term "biomarker" is used herein, consistent with its use in the art, to refer to a 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.

[33] **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.

[34] **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.

[35] **Comparable.** As used herein, the term "comparable" refers to two or more agents, entities, situations, effects, 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, effects, 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, effects, 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, effects, or populations are caused by or indicative of the variation in those features that are varied.

[36] **Composition:** A "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.

[37] **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 proximity, 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.

[38] **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.

[39] **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.

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).

Epitope. As used herein, the term "epitope" refers to a portion of an antigen that is bound by an antibody or T cell receptor (e.g. when presented by an MHC molecule). In some embodiments, where the antigen is a polypeptide, an epitope is conformational in that it is comprised of portions of an antigen that are not covalently contiguous in the antigen but that are near to one another in three-dimensional space when the antigen is in a relevant conformation. For example, for polypeptide antigens, conformational epitopes are those comprised of amino acid residues that are not contiguous in the polypeptide chain; linear epitopes are those comprised of amino acid residues that are contiguous in the polypeptide chain. Those of ordinary skill in the art will appreciate that, in general, an epitope 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, epitopes utilized in accordance with the present invention are provided in a natural or synthetic form. In some embodiments, an epitope is a recombinant polypeptide.

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.

[43] **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.

[44] **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.

[45] **Pharmaceutical Composition.** As used herein, the term "pharmaceutical composition" refers to a composition in which an active agent is 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, intratumoral, 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; transdermal\(^{1}\); or nasally, pulmonary, and to other mucosal surfaces.

[46] **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. In some embodiments, as is understood in the art, the term "resistance" or "resistant" may alternatively be used with respect to specific disease (e.g. tumor) or cells (e.g. cancer cells).

[47] **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 (including cancers arising from transformed cells of mesenchymal origin in tissues such as cancellous bone, cartilage, fat, muscle, vascular, hematopoietic, or fibrous connective tissues), carcinomas (including tumors arising from epithelial cells), lymphomas, mesothelioma, neuroblastoma, retinoblastoma, etc.

[48] **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, a cell type, other biological entity, or medical condition) 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.
[49] **Therapeutically Effective Amount:** As used herein, the term "therapeutically effective amount" means an amount (e.g., of an agent or of a pharmaceutical composition) 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 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 surrogate (e.g., metabolic
and/or 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.

[50] 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.

[51] 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.

[52] **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, linear vs branched, 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**

The present disclosure describes a variety of technologies involving the surprising and beneficial effects of agonizing CD38, particularly in the treatment of cancer. In this section, certain features and particular embodiments of such technologies are discussed in more detail; this discussion is not intended, and should not be construed, to limit the scope of the appended claims, which define the present invention. Rather, it is provided for purposes of illustration and explanation.

**CD38**

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.

CD38 is a marker of immune cell activation, and its expression has been linked to rheumatoid arthritis (see, for example, Fueldner 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).

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.

CD38 expression in tumor cells can be modulated by retinoic acid and/or other retinoids such as all-trans retinoic acid (ATRA). The promoter driving transcription of the CD38 gene is, as other the promoters of other target genes, responsive to a retinoic acid response element (RARE) that are bound by nuclear retinoic acid receptors. It has been proposed that CD38 expression levels might be increased through administration of retinoids or their derivatives, specifically including tamibarotene (Chillemi A, et al., 2013).

Prior to the present disclosure, therapeutic strategies that were targeting CD38 on tumor cell surfaces, and were designed either to neutralize its pro-cancer activities or to orchestrate tumor cell killing, have been extensively studied. These therapies therefore utilize agents that are non-agonistic, and often antagonistic, to CD38 (see, e.g., Tamibarotene; Drach J et al., 1994; Congleton J et al., 2011; Stevenson GT, 2006; Flavell D et al. 2001 ; de Weers M et al., 2011; van der Veer MS et al., 2011; WO2008/035257; WO2008/047242; WO2010/061358; WO2011/154453; WO2012/09261; WO2012/076663; US2012/019662; US2013/0302318).

The present disclosure provides an entirely different approach to targeting CD38 in cancer therapy. Indeed, the present disclosure teaches that, exactly opposite to many teachings in the art, effective antibody therapy can be achieved by agonizing CD38. The present disclosure targets CD38 on immune effector cells, rather than CD38 on tumor cells. As described herein, the present invention provides compositions and methods for agonizing
CD38 activity, including in the treatment of various cancers. The present disclosure specifically demonstrates that agonizing CD38 on surfaces of immune effector cells (e.g., NK cells) can increase ADCC by such cells. Still further, the present disclosure demonstrates that CD38 agonist therapy can desirably and effectively be combined with anti-tumor antibody therapy to enhance killing of tumor cells bound by specific anti-tumor antibody agents (e.g., antibodies that specifically bind to tumor-associated antigens on tumor cell surfaces).

The biology of CD38 and its downstream signaling cascades in immune effector cells has been well studied. When activated, CD38 induces a flux of calcium ions and triggers the phosphorylation of a cascade of intracellular substrates, leading to the secretion of cytokines and the proliferation and enhanced function of lymphocytes. Despite the knowledge of the molecular mechanism of CD38 action, and in contrast to the extensive study of CD38 as a therapeutic target on tumour cell surface, prior to the present disclosure, significant effort had not been directed to exploring the possibility of agonizing CD38 on the immune effector cells to treat cancer in vivo.

Several reasons may explain this apparent lack of interest in CD38 as a target of agonist therapy. Mature resting immune cells, including effector cells such as NK cells, tend to express very low levels of CD38. Moreover, the many studies that, as described above, focused on blocking CD38 on tumour cells did not report negative impacts on anti-tumour immune responses. Still further, and perhaps most importantly, the understanding that activating CD38 promotes survival and proliferation of tumour cells strongly suggested that agonizing CD38 would be affirmatively undesirable, at least for the treatment of CD38-positive immune or blood cell cancers.

Thus, the present invention surprisingly identifies cell surface CD38, in particular on immune effector cells (e.g., NK cells), as a promising therapeutic target, and provides methods of treating cancer by agonizing CD38.

Prior to present invention, however, there were reasons (as discussed above) to doubt the applicability of such therapeutic strategy to a CD38 agonist. In particular, there were concerns that it would be affirmatively undesirable to agonize CD38, at least with regard to CD38-positive immune or blood cancers, because agonist activating CD38 on tumor cells can promote the survival and proliferation of the tumor cells. In some embodiments, provided therapeutic strategies that involve agonizing CD38 (in particular on immune effector cells) may be combined with one or more other therapies. For example, in some embodiments, provided strategies may be combined with a standard of care therapy for a cancer (or, if appropriate, this approach may be applied to a cancer patient who has failed
standard of care therapy) with the scope of enhancing and/or prolonging ADCC response against cancer cells.

Inducible Effector Cell Surface Markers

[64] 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 FcR 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).

[65] One event that happens during the activation of effector cells 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 CD38 (discussed above), CD137, OX40, GITR, CD30, ICOS, etc.

[66] 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.

[67] 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.

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.

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.

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.

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.

**CD38 Agonists**

The present invention, as described herein, provides therapeutic modalities that involve agonizing CD38 on the surface of the effector cells, in particular by binding CD38 extracellular domain. Some agonists of CD38 are known in the art. Others can be identified, generated, and/or characterized as described herein.

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). In some embodiments, therefore, a CD38 agonist can be or comprise the whole or fragments or other variants of the extracellular domain of CD31.
In some embodiments, a CD38 agonist is or comprises an antibody agent (e.g., an intact antibody) specific for human CD38 (e.g., for a linear or conformational epitope thereof, in some embodiments an extracellular epitope). Antibodies that recognize the extracellular domain of CD38 (and in particular of human CD38) have been generated using different approaches but those appropriate for enhancing the ADCC and cancer cell killing are those having comparable features the CD38-specific agonistic properties as originally described in the literature.

For example, the mouse anti-human CD38 monoclonal antibody, named IB4, induces a rapid mobilization of calcium ions, the phosphorylation of intracellular proteins, cytokine secretion (in particular Interleukin 6 and Interferon gamma), and proliferation of human T lymphocytes (Funaro et al., 1990). Since the epitope of IB4 antibody has been mapped in the portion of CD38 corresponding to amino acids 220-241 (Ausiello C et al., 2000; Mallone R et al., 2001), antibodies that are CD38 agonists can be raised in animals and/or selected from libraries of antibodies by using such fragment of CD38 as antigen. Alternatively, other monoclonal anti-CD38 antibodies named as CS/2, clone 90 and NIM-R5 (Santos-Argumedo L et al., 1993; Mayo L et al., 2008; Hara-Yokoyama M et al., 2008) provides similar agonistic activities.

Aside from biological features (such as mobilization of calcium or cytokine secretion) that were described for IB4, the CD38 agonist that is specific for human CD38 may present a series of features that are advantageous for manufacturing, (pre-)clinical testing, and formulation, administration, and/or efficacy (e.g., in particular contexts) for cancer therapy. For example, in some embodiments, such preferable features may include one or more of the following ones: affinity for human CD38 in the micromolar range or lower. In some embodiments, for example the affinity for human CD38 may be in a range defined by an upper bound that may be 100 μM, 10 μM, 1 μM, 0.1 μM, or 0.01 μM, and a lower bound that may in some embodiments be 1000 pM, 100 pM, 10 pM, or 1 pM; cross-reactivity with simian or mouse CD38 (e.g. affinity within 3-10 fold of human CD38, using recombinant, tagged proteins), position of the CD38 epitope, potency in NK cell-based assays (e.g. stimulation, degranulation, in vitro chromium release, and/or cytotoxicity), cell specificity (e.g., CD38 activation to be achieved specifically on NK cells and not on other cells that physiologically, or due to cancer or other disease, also express CD38), safety aspects related to basal activity of human CD38 (e.g. inhibitory effect of the human CD38 agonist on ADP-ribosyl cyclase activity that is below 50%, preferably, below 25%, more preferably below 10%; effect on insulin secretion by pancreatic cells; in vitro cytokine storm assay), reduced propensity to aggregation and/or cross-interaction, high level of thermostability (e.g. at temperature beyond 60 °C, after long exposure at 37 °C, and/or after freeze thaw cycles),
high expression levels in cell lines for recombinant expression of antibodies. One of more
these properties can be compared to the corresponding features for known CD38 agonists
(e.g. the antibodies named as IB4 and NIMR-5) and/or making use of cell lines naturally
expressing CD38 such as the human cell lines Raji HBL6, L540 or CEM (Bolognesi A et al.,
2005), or mouse Ba/F3 cells (Lund F et al., 2006)

[77] In general, an antibody agent that agonizes CD38 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,
such a multi-specific agent binds specifically to CD38 and to a Treg depleting agent. In
some particular embodiments, the multi-specific agent binds specifically to CD38 and to a
tumor antigen. In some particular embodiments, the multi-specific agent binds specifically to
CD38 and to another antigen, which other antigen is neither a Treg depleting agent nor a
tumor antigen (so that CD38 and tumor antigen are not simultaneously targeted.

[78] Additionally, as with other antibody agents described and/or utilized herein, an antibody
agent that agonizes CD38 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. In some other embodiments, a CD38 agonist is or
comprises a non-antibody agent. In some embodiments, such a non-antibody-agent CD38
agonist is or comprises a nucleic acid, saccharide, lipid, small molecule, metal, or a
combination thereof. In some embodiments, a non-antibody-agent CD38 agonist is an
aptamer that specifically binds to CD38.

**CD38 Antagonists**

[79] As described herein, the present invention provides therapeutic modalities that involve using
agents functionally and structurally distinct from CD38 antagonists that are known in the art.
CD38 antagonists consisting of agents that interfere with CD38 activation and/or enzymatic
activity are not considered as being useful and/or characterized according to the present
invention.

**Immune Cells**

[80] As is understood in the art, immune cells are white blood cells that mediate antibody- or cell-
mediated immunity and include, but are not limited to, T cells, B cells, basophils, dendritic
cells, eosinophils, Langerhans cells, mast cells, monocytes and macrophages, neutrophils
and NK cells. In particular, immune effector cells including but not limited to one or more of
natural killer (NK) cells, macrophages, neutrophils, and eosinophils.
Treg deleting agents have been extensively studied for supporting anti-tumor therapies, and have entered clinical trials..

**Tumors**

In some embodiments, a tumor is a hematologic malignancy, including but not limited to, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, AIDS-related lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma, Langerhans cell histiocytosis, multiple myeloma, or myeloproliferative neoplasms.

In some embodiments, a tumor is a solid tumor, including but not limited to breast carcinoma, a squamous cell carcinoma, a colon cancer, a head and neck cancer, a lung cancer, a genitourinary cancer, a rectal cancer, a gastric cancer, or an esophageal cancer.

In some particular embodiments, a tumor is selected from a lymphoma, a breast tumor, a colon tumor, and a lung tumor.

In some embodiments, a tumor is characterized by no or low expression of CD38 on the surface of tumor cells. In some embodiments, a tumor is characterized by significant expression of CD38 on the surface of tumor cells; in some such embodiments, tumor cells express CD38 on their surfaces at levels significantly higher than non-tumor cells (e.g., in the same individual or as typically found in a population). 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 conventional chemotherapy. In some embodiments, a tumor is characterized by a relatively high level of NK cell infiltration (as compared with an appropriate reference). In some embodiments, a tumor is a Treg-driven tumor.

Examples present evidences on how specific cancers that be object of CD38 agonist therapy may be evaluated in animal models, in particular by showing a significant reduction in the growth of tumor size or, preferably, even the regression in tumor size after the administration of CD38 agonist therapy (alone or in combination with other agents, according to the present invention). Furthermore, the CD38 agonist can be administered in patients and/or specific cancer wherein immune profiling is performed and present advantageous features for CD38 agonist therapy. Such advantageous features can be strong CD38 expression on NK cells or correlation between the immune cells profiling data and the results in the tumor animal models with CD38 agonist therapy (e.g. the level of expression of T cells that are negative or positive for one or more combinations of surface markers such as CD3, CD4, CD8, CD1 1b, CD1 1c, CD45, ICOS, Tim3, Lag3, PD-1, Ki-67, and/or FoxP3).
**Anti-Tumor Antigen Antibodies**

[88] Prior strategies, described above, for targeting CD38 make use of CD38 antagonists as part of anti-tumor therapy that are representative of a class of anti-tumor antibody therapies that are rapidly becoming the standard of care for treatment of many tumors.

[89] Antibody agents have been designed or selected to bind to tumor cell antigens in order to kill tumor cells by a) delivering a toxic payload associated with the antibody; b) blocking activity of a tumor cell surface receptor that is thought to be involved in cell proliferation and/or survival; c) agonizing activity of a tumor cell surface receptor that is thought to be involved in triggering apoptosis or cell death; and/or d) displaying bound antibody on tumor cell surface, so that immune mechanisms like complement-dependent cytotoxicity (CDC) and/or antibody-dependent cell toxicity (ADCC) are triggered and directed at the tumor (see, for example, review by Scott AM et al., 2012, including particularly Figure 1 therein). Each of these approaches has been successfully pursued, and several anti-tumor antibody agents are now commercially available for use in cancer therapy.

[90] For example, a steadily increasing number of antibody agents targeting tumor antigens have been approved for use in treating cancers (see, for example, Li G et al., 2013; Scott AM et al., 2012; Sliwkowski M & Mellman I, 2013), and are rapidly becoming standard of care. Indeed, anti-cancer monoclonal antibody therapy can be considered among the most notable scientific advances in the last quarter century for various indications. In particular, the rapid translation of this research towards improved means for targeting molecular targets with a more favorable toxicity profile in comparison with cytotoxic chemotherapy has prolonged the survival of thousands of patients. Monoclonal antibodies against antigens such as CD20, HER2, PD-1, PD-L1, and EGF receptor have become standard of care for patients suffering from aggressive cancers such as B cell lymphomas, breast cancer, colorectal cancer or head and neck cancers.

[91] Moreover, the list of anti-tumor antibodies in clinical trials seems to expand almost daily (see clinicaltrials.gov). Various review articles have been published that describe useful anti-tumor antibody agents (see, for example, Adler & Dimitrov, 2012; Li G et al., 2013; Scott AM et al., 2012; Sliwkowski M & Mellman I, 2013). The below Table presents a non-comprehensive list of certain human antigens targeted by known, available antibody agents, and notes certain cancer indications for which the antibody agents have been proposed to be useful:

<table>
<thead>
<tr>
<th>Human Antigen</th>
<th>Antibody (commercial or scientific name)</th>
<th>Cancer indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>Siplizumab</td>
<td>Non-Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td>CD3</td>
<td>UCHT1</td>
<td>Peripheral or Cutaneous T-cell</td>
</tr>
<tr>
<td>CD4</td>
<td>HuMax-CD4</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>CD19</td>
<td>SAR3419, MEDI-551</td>
<td>Diffuse Large B-cell Lymphoma</td>
</tr>
<tr>
<td>CD19 and CD3 or CD22</td>
<td>Bispecific antibodies such as Blinatumomab, DT2219ARL</td>
<td>Non-Hodgkin's Lymphoma</td>
</tr>
<tr>
<td>CD20</td>
<td>Rituximab, Veltuzumab, Tositumomab, Ofatumumab, Ibritumomab, Obinutuzumab, B cell malignancies (Non-Hodgkin's lymphoma, Chronic lymphocytic leukemia)</td>
<td></td>
</tr>
<tr>
<td>CD22 (SIGLEC2)</td>
<td>Inotuzumab, tetraxetan, CAT-8015, DCDT2980S, Bectumomab</td>
<td>Chemotherapy-resistant hairy cell leukemia, Hodgkin's lymphoma</td>
</tr>
<tr>
<td>CD30</td>
<td>Brentuximab vedotin</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>CD33</td>
<td>Gemtuzumab ozogamicin (Mylotarg)</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>CD37</td>
<td>TRU-016</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>CD38</td>
<td>Daratumumab</td>
<td>Multiple myeloma, hematological tumors</td>
</tr>
<tr>
<td>CD40</td>
<td>Lucatumumab</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>CD52</td>
<td>Alemtuzumab (Campath)</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>CD56 (NCAM1)</td>
<td>Lorivuzumab</td>
<td>Small Cell Lung Cancer</td>
</tr>
<tr>
<td>CD66e (CEA)</td>
<td>Labeluzumab</td>
<td>Breast, colon and lung tumors</td>
</tr>
<tr>
<td>CD70</td>
<td>SGN-75</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>CD74</td>
<td>Milatuzumab</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>CD138 (SYND1)</td>
<td>BT062</td>
<td>Multiple Myeloma</td>
</tr>
<tr>
<td>CD152 (CTLA-4)</td>
<td>Ipilimumab</td>
<td>Metastatic melanoma</td>
</tr>
<tr>
<td>CD221 (IGF1 R)</td>
<td>AVE1642, IMC-A12, MK-0646, R150, CP 751871</td>
<td>Glioma, lung, breast, head and neck, prostate and thyroid cancer</td>
</tr>
<tr>
<td>CD254 (RANKL)</td>
<td>Denosumab</td>
<td>Breast and prostate carcinoma</td>
</tr>
<tr>
<td>CD261 (TRAILR1)</td>
<td>Mapatumumab</td>
<td>Colon, lung and pancreas tumors and haematological malignancies</td>
</tr>
<tr>
<td>CD262 (TRAILR2)</td>
<td>HG5-ETR2, CS-1008</td>
<td>Colon and rectal cancer, malignant ascites, epithelial tumors (breast, colon, lung)</td>
</tr>
<tr>
<td>CD326 (Epcam)</td>
<td>Edrecolomab, 17-1A, IGN101, Catumaxomab, Adecatumumab</td>
<td>Epithelium-derived, gastric solid tumors</td>
</tr>
<tr>
<td>CD309 (VEGFR2)</td>
<td>IM-206, CDP791, Ramucirumab</td>
<td></td>
</tr>
<tr>
<td>CD319 (SLAMF7)</td>
<td>HuLuc63</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>CD340 (HER2)</td>
<td>Trastuzumab, Pertuzumab, Ado-trastuzumab emtansine</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>CAIX (CA9)</td>
<td>cG250</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>EGFR (c-erbB)</td>
<td>Cetuximab, Panitumumab, nimotuzumab and 806</td>
<td>Solid tumors including glioma, lung, breast, colon, and head and neck tumors</td>
</tr>
<tr>
<td>EPHA3 (HEK)</td>
<td>KB004, IIIA4</td>
<td>Lung, kidney and colon tumors, melanoma, glioma and haematological malignancies</td>
</tr>
<tr>
<td>Episialin</td>
<td>Epitumomab</td>
<td>Epithelial ovarian tumors</td>
</tr>
<tr>
<td>FAP</td>
<td>Sibrotuzumab and F19</td>
<td>Colon, breast, lung, pancreas, and head and neck tumors</td>
</tr>
<tr>
<td>HLA-DR beta</td>
<td>Apolizumab</td>
<td>Chronic lymphocytic leukemia, non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>FOLR-1</td>
<td>Farletuzumab</td>
<td>Ovarian tumors</td>
</tr>
<tr>
<td>5T4</td>
<td>Anatumomab</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td>GD3/GD2</td>
<td>3F8, ch14.18, KW-2871</td>
<td>Neuroectodermal and epithelial tumors</td>
</tr>
</tbody>
</table>
In some embodiments, one or more such anti-tumor antibody agents is utilized in the practice of the present invention, for example as part of a bi- or multi-specific antibody agent that also acts as a CD38 agonist as described herein.

In some particular embodiments of the present invention, where a CD20-positive cancer (such as a B cell malignancy) is being treated with a multisppecific (aka heterospecific, such as bispecific, trispecific, etc) antibody, the anti-tumor antigen antibody portion is specific for CD20, (e.g., is or is from an antibody such as Rituximab, Tositumomab, or Ibritumomab).

In some particular embodiments of the present invention, where a CD52-positive cancer (such as leukemia) is being treated with a multispecific, the anti-tumor antigen antibody portion is specific for CD52 (e.g., is or is from an antibody such as Alemtuzumab.

In some particular embodiments of the present invention, where a HER2-positive cancer (such as a solid cancer) is being treated with a multipspecific antibody, the anti-tumor antibody portion is specific for HER2, (e.g., is or is from an antibody such as Trastuzumab.

In some particular embodiments of the present invention, where an EGFR-positive cancer (such as a solid cancer) is being treated with a multipspecific antibody, the anti-tumor antigen antibody portion is specific for EGFR (e.g., is or is from an antibody such as Cetuximab.

In some particular embodiments of the present invention, where a CD326-positive cancer (such as a solid cancer) is being treated with a multipspecific antibody, the anti-tumor
antigen antibody portion is specific for CD326, (e.g., is or is from an antibody such as Edrecolomab.

It is worth noting that, notwithstanding the promising activity of anti-tumor antibody therapy, and the large number of anti-tumor antibody agents currently under development and/or marketed for treatment of cancer, the response rates among patients are often not high. Particularly with refractory or advanced cancers, response rates can be as low as 25% or less. Efforts to enhance the activity of anti-tumor antibody therapy have often focused on combining the anti-tumor antibody therapy with cytotoxic chemotherapy or radiotherapy (Modjtahedi H et al., 2012). However, these approaches largely ignore and may partially antagonize the immunologic mechanism by which monoclonal antibodies function. The present disclosure, by contrast, also suggests effective combination of anti-tumor antigen antibody therapy with CD38 agonist therapy within a multispecific antibody. Combination with other agents or modalities, including cytotoxic chemotherapy or radiotherapy, may be included as well, so long as CD38 agonist therapy is utilized.

**Formats of Antibody Agents**

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 ("SMIPs™"), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, minibodies, BiTE®s, ankyrin repeat proteins or DARPin®s, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®, Centryns®, CoVX bodies, BiCyclic peptides, or Kunitz domain derived antibody constructs.

It is worth noting that masked antibody (e.g., Probody®) formats may be of particular interest for certain antibody agents 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. In some embodiments, use of such a format specifically ensures targeting of CD38 on effector cells in the tumor milieu (e.g., that have infiltrated the tumor).

**Combination**

Those of ordinary skill in the art, reading the present disclosure, will readily appreciate that CD38 agonist therapy, as described herein, may in certain embodiments be combined with
other anti-cancer therapies, including for example administration of chemotherapeutic agents, other immunomodulatory agents (including Treg depleting agents and agonists and/or antagonists of other cell surface markers), radiation therapy, high-frequency ultrasound therapy, surgery, etc.

Thus, in some embodiments, CD38 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. For example, as described herein, in some embodiments, CD38 agonist therapy is combined with anti-tumor antibody therapy

Known 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 (such as Treg depleting agents in the form of regulatory T cell-specific antibodies), vaccines, cell-based therapies (e.g. allogeneic or autologous stem cell transplantation), organ transplantation, radiation therapy, surgery, etc.

Treg Depleting Agents

By “Treg depleting agents” is meant any agent that effectively depletes the function of the regulatory T cell compartment. A subset of T cells, identified as CD4+CD25+ T cells and termed regulatory T cells (herein, Treg), regulate magnitude and specificity of immunity to self antigens. Functional Tregs have been shown to be unfavourable to cancer immunotherapies, whereby the therapeutic scope is to force the immune system to develop a response against the tumor antigen. Depleting these populations of Treg has been demonstrated to improve significantly the clearance of tumor cells, in particular when cancer vaccine are administered (Facciabene A et al., 2012; Oleinika K et al., 2013).

Treg depleting agents may be acting directly by depleting Treg cells or by inactivating Treg cells. Treg cell function is ideally eliminated or impaired by a Treg depleting agent to the maximum degree possible. For example, this may be done by totally depleting the available Treg cell compartment. Preferably, Treg cell function is impaired by at least 25%, more preferably at least 50%, more preferably at least 75%, more preferably at least 90%, even more preferably 100%). Techniques for measurement of the degree of impairment of function of the Treg cell compartment will be known to those of skill in the art and examples are shown herein.

In some embodiments of the present invention, in addition to a CD38 agonist, a Treg depleting agent is administered to cancer patient within combinations to improve the anti-
tumor therapeutic effect of CD38 agonist. Many potentially useful Treg depleting agents are known in the art. Others can be identified, generated, and/or characterized as described herein.

In general, a Treg depleting agent 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, a Treg depleting agent can be provided and/or utilized in a multi-specific (e.g. bi-specific) format that also targets CD38 since containing a CD38 agonist.

Additionally, as with other antibody agents described and/or utilized herein, a Treg depleting agent 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.

Treg depleting agents can be monoclonal antibodies well known in the art that impair the function Treg cells by binding CD25, CTLA-4, or CD4. A further target for Treg depleting agents can be the antigen known in mouse as FR4 (Folate Receptor 4) and in human as Izumol receptor or Juno. Antibodies that are targeting such mouse and human antigen are known in the art (TH6 and OBF13, respectively; Bianchi E et al., 2014; Teng M et al., 2010; Houot R and Levy R, 2009; Yamaguchi T et al., 2007) and can be used for establishing further Treg depleting agents that are effective in combination with CD38 agonist in the treatment of cancer.

Other agonistic antibodies that can exert similar biological functions with respect to Treg are those described in the literature (Baatar D et al., 2007; Grauer O et al., 2007; Bullard Y et al., 2014) that are directed to against cell surface markers such as for OX40 (CD134, having effects on conventional CD4 and CD8 T cells, NK cells, and NKT cells, such as promoting the division, survival, and differentiation, and regulating cytokine production), GITR (TNFSF18, regulating the activity of both conventional and regulatory T cells), CCR4 (CD194), and CXCR4 (CD184).

Still further, in some embodiments, CD38 agonist therapy (and/or other therapy with which it is combined) may be combined with one or more palliative (e.g., pain relieving, anti-nausea, or anti-emesis) 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.

In some embodiments, agents used in combination are administered according to a dosing regimen for which they are approved for individual use. In some embodiments, however, combination with CD38 agonist therapy permits another agent to be administered
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 CD38 agonist 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 without CD38 agonist therapy.

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.

**Dosing and Administration**

Pharmaceutical compositions (e.g., comprising a CD38 agonist, an anti-tumor antibody and/or any other therapeutically active agent) 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 some embodiments, utilized agents (e.g., CD38 agonist, e.g., agonist antibody, anti-tumor antibody, and/or any other therapeutically active agent utilized in accordance with the present invention) 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 (EMEA), e.g., for the relevant indication. In some embodiments, however, use of CD38 agonist therapy (e.g., administration of a CD38 agonist) permits reduced dosing (e.g., lower amount of active in one or more doses, smaller number of doses, and/or reduced frequency of doses) of an approved agent used in combination with the CD38 agonist therapy. Those skilled in the art will be aware, or will readily be able to determine, approved dosing regimens for a variety of agents, including for example, a variety of anti-tumor antigen antibodies.

Those skilled in the art, reading the present disclosure will appreciate various modifications of dosing regimens that are within the scope of the present invention. For example, just to name a few, in some embodiments, CD38 agonist therapy is utilized as monotherapy. In some such embodiments, addition of yet another anti-cancer therapy may be particularly useful.

Moreover, in some embodiments, it may be desirable to tailor dosing regimens, and particularly to design sequential dosing regimens, based on timing and/or threshold expression levels of inducible markers (including CD38), whether for particular types of tumors, particular tumors, particular patient populations (e.g., carrying genetic markers),
and/or particular patients. In some such embodiments, therapeutic dosing regimens may be combined with or adjusted in light of detection methods that assess expression of one or more inducible markers prior to and/or during therapy.

In some embodiments, dosing and administration according to the present invention utilizes active agent having a desired degree of purity combined with one or more physiologically acceptable carriers, excipients or stabilizers in any or variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. In some embodiments, a preferred form may depend on the intended mode of administration and/or therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies.

In some embodiments, ingredient(s) can be prepared with carriers that protect the agent(s) against rapid release and/or degradation, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as polyanhydrides, polyglycolic acid, polyorthoesters, and polylactic acid.

In general, each active agent 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) (e.g., for agents such as antibodies). Pharmaceutical compositions containing active agents 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, transdermal, or other kinds of administration involving physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue).

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.

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.

[124] Factors to be considered when optimizing routes and/or dosing schedule for a given therapeutic regimen may include, for example, the particular cancer being treated (e.g., type, stage, location, etc), the clinical condition of a subject (e.g., age, overall health, 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.

[125] Those skilled in the art will appreciate, for example, that route of delivery (e.g., oral vs intravenous vs subcutaneous vs intratumoral, etc) may impact dose amount and/or required dose amount may impact route of delivery. For example, where particularly high concentrations of an agent within a particular site or location (e.g., within a tumor) are of interest, focused delivery (e.g., in this example, intratumoral delivery) may be desired and/or useful.

[126] Those skilled in the art will further appreciate that some embodiments of combination therapies provided in accordance with the present invention achieve synergistic effects; in some such embodiments, dose of one or more agents utilized in the combination may be materially different (e.g., lower) and/or may be delivered by an alternative route, than is standard, preferred, or necessary when that agent is utilized in a different therapeutic regimen (e.g., as monotherapy and/or as part of a different combination therapy).

[127] 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 or other biological response that is related to the cancer-specific immune response).

[128] In general, type, amount, and frequency of dosing of active agents in accordance with the present invention is 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. 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 the 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 a further agent, can be determined as being sufficient to enhance killing of cancer cells.

A therapeutically effective amount of an active agent or composition comprising it can be readily determined using techniques available in the art including, for example, considering one or more factors such as the disease or condition being treated, the stage of the disease, the age and health and physical condition of the mammal being treated, the severity of the disease, the particular compound being administered, and the like.

In some embodiments, therapeutically effective amount is an effective dose (and/or a unit dose) of an active agent that may be at least about 0.01 Mg/kg body weight, at least about 0.05 Mg/kg body weight; at least about 0.1 Mg/kg body weight, at least about 1 Mg/kg body weight, at least about 2.5 Mg/kg body weight, at least about 5 Mg/kg body weight, and not more than about 100 Mg/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.

Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions
can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The formulation of each agent should desirably be sterile, as can be accomplished by filtration through sterile filtration membranes, and then packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations as discussed herein. Sterile injectable formulations may be prepared using a non-toxic parenterally acceptable diluent or solvent, such as water or 1,3 butanediol, for example. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of biodegradable polymer systems. 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.

Each pharmaceutical composition for use in accordance with the present invention may include 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 compounds 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, boricarbonate, bisulfate, isothionate, lactate, lactobionate, laurate, malate, maleate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, thiothiodioxide, 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; rescorinol; 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 dextrins; 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™, PLURONICS™, or polyethylene glycol (PEG).
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).

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.

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.

To give one exemplary possible protocol for interdigitating associating two intermittent, cycled dosing regimens, 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.

[138] 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).

[139] 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; identity and/or nature of a targeted antigen, identity and/or properties (e.g., pharmacokinetic properties) of the first agent, 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).

[140] 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.

**Articles of Manufacture and Kits**

[141] In another embodiment of the invention, each of first agent, the second agent, and, optionally, the third agent is provided in a separate article of manufacture. In particular, the third agent may target a further antigen on NK cells (such as CD137 and OX40), or further cancer-specific compound selected among chemotherapeutic compounds, cancer vaccines, signal transduction inhibitors, antibodies or other ligands that inhibit tumor growth, and immunomodulatory agents, among many others listed above as a potential third agent.
In another embodiment of the invention, an article of manufacture containing the first agent, the second agent, or, when appropriate, a third agent as described above is provided as a container 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. The 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, the formulation is 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.

The article of manufacture may further comprise a separate container comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. If the second agent and the third agent are simultaneously, the article of manufacture may contain the second agent and the third agent in a single container, or appropriate materials and instructions for reconstituting the second agent and third agent in a single formulation may be provided. For example, the article of manufacture may allow providing each or the agent in an intravenous formulation as a sterile aqueous solution containing a total of 2 mg, 5 mg, 10 mg, 20 mg, 50 mg, 100 mg, or more that are formulated, with appropriate diluents and buffers, at a final concentration of 0.1 mg/ml, 1 mg/ml, 10 mg/ml, or at a higher concentration.

Each of the first agent, second agent, and (when applicable) third agent can be provided within the kits-of-parts in the form of lyophilized is to be reconstituted with any appropriate aqueous solution that provided or not with the kits, or other types of dosage unit using any compatible pharmaceutical carrier. As the article of manufacture, this kits-of-parts is labeled for the treatment of a cancer and it may also contain a third agent, as defined above as a further, separate article of manufacture or within the article of manufacture containing the second agent. One or more unit dosage forms of the each of the first agent, the second agent, and, optionally, the third agent may be 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. The kit-of-parts may further comprise materials and/or devices suitable for measuring expression of the target of the first agent, CD38, the target of the third agent, and/or of a surrogate marker, on NK cells (e.g. a detectable labeled reagent that specifically binds to CD38, the target of the third agent, and/or of a surrogate marker, and references for expression).
order to use correctly such kits-of-parts, it may further comprise buffers, diluents, filters, needles, syringes, and package inserts with instructions for use in the treatment of cancer.

The instructions that are associated to the article of manufacture and/or the kits of the invention 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 other materials in the article of manufacture and/or in the kit. Instructions may be provided together with the article of manufacture and/or in the kit or may be provided separately but with the indication that indications are to be used in association with them.

EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting. Thus, the invention should be construed to encompass any and all variations to the following examples which become evident as a result of the teaching provided herein. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The present Examples surprisingly demonstrate, among other things, that agonizing CD38 can provide effective cancer therapy. Prior to the present disclosure, CD38 expressed by tumor cells had been described as a potential target for inhibitory therapy to treat cancer. According to prior understanding, therefore, agonizing CD38 would be affirmatively undesirable for cancer patients.

The present Examples specifically confirm the relevance and utility of certain animal models for evaluation of CD38 agonist therapy, alone as a monotherapy or in combination with other anti-cancer compounds and treatments. This finding is particularly noteworthy given that, as will be appreciated by those skilled in the art, interactions between certain immune effector cell inducible markers and ligands that bind thereto are sometimes species-specific, or at least not cross-reactive between human and mouse.

Additionally, the present Examples explicitly establish that effect of CD38 agonist therapy (e.g., by administering a anti-CD38 agonist antibody agent) on tumor cell killing, can be enhanced by further administering other compounds in combination with CD38 agonist, including for example by combining therapies that deplete or otherwise interfere with the biological activity of Tregs (e.g., by administering a CD38 agonist in combination with a Treg depleting agent).
Example 1: Agonizing CD38 in Models for Breast Cancer Therapy

Materials & Methods

Human Cell Lines and Culture Conditions. The human breast cancer cell lines BT474M1 (MCF-7 (ATCC® HTB-22™), SKBR3 (ATCC® HTB-30™), and MCF-7/HER2-18 (MCF-7 cells stably overexpressing HER2, also known as HER18; Benz CC et al., 1992.) were kindly provided as a gift from Byron Hann at UCSF (San Francisco, California, USA). BT474M1 is a tumorigenic subclone of BT474 (ATCC® HTB-20™) with increased tumorigenicity was derived from BT474 xenografts selected for maximum growth rate (Park J et al., 2002; Kohrt H et al., 2012). ATCC-indexed cell lines were cultured according to the ATCC guidelines. The BT474M1 cell line was cultured in DMEM medium, and the MCF-7/HER2-18 cell line in DMEM/F12 1:1 medium. All media were purchased from Life Technologies. Cells were grown as adherent cultures at 37°C in 5% CO₂ and passaged after detachment by 0.05% trypsin (Life Technologies). BT474M1, SKBR3, and MCF-7/HER-18 cells express HER2 with specific fluorescence indices (tumor MFI/isotype MFI) between 1.24 and 1.62. MCF7 cells do not express HER2 (tumor MFI/isotype MFI of 0.17). No detectable surface levels of CD38 are observed on these cell lines by flow cytometry assessment.

Murine Cell Lines and Culture Conditions. The murine breast cancer cell lines 4T1 (from BALB/C mice) and EMT6 (from BALB/C mice) are available through ATCC under the number CRL-2539™ and CRL-2755™, respectively. The TUBO-EGFR (from BALB/C mice) cell line was obtained from Yang-Xin Fu at the University of Chicago. 4T1 cells were cultured at 37°C in 5% CO₂ in RPMI 1640 medium with l-glutamine (Cellgro) supplemented with 10% heat-inactivated FCS (HyClone), 100 U/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco), and 50 μM 2-Mercaptoethanol (Gibco), as complete medium (Marabelle A et al., 2013). EMT6 cells were cultured according to the ATCC guidelines. TUBO-EGFR cells were cultured in DMEM supplemented with 10% FCS, 10 mM Hepes, 1% nonessential amino acids, 1% penicillin, and 1% streptomycin (all from Invitrogen Life Technologies). No detectable surface levels of CD38 are observed on these cell lines by flow cytometry assessment.

Mice. For experiments making use of human breast cancer cell lines, five- to six-week-old female athymic (nu/nu) nude Foxn1nu and SCID mice (Prkdc<sup>scid</sup>) were purchased from Harlan and Jackson Laboratories. For experiments making use of murine breast cancer cell lines, five to six-week-old female BALB/c mice were purchased from Jackson Laboratories. Mice were housed at the Laboratory Animal Facility at the Stanford University Medical Center.

Antibodies. Control rat IgG was purchased from Sigma-Aldrich. Human anti-human agonist CD137 monoclonal antibody (BMS-663513, IgG4) was provided by Bristol-Myers Squibb through a Material Transfer Agreement. Trastuzumab (humanized anti-human HER2/neu
receptor, IgG1), was obtained from Genentech through a Material Transfer Agreement. Agonistic anti-CD134 (OX40) antibody was purchased from BioXcell (clone 0X86; Cat. No. BE0031). Mouse anti-human CD38 agonist (IB4) and non-agonistic (HB7) monoclonal antibodies (Funaro A et al., 1990) were kindly donated by Prof. F. Malavasi at Univ. Torino. Rat anti-mouse CD38 agonist monoclonal antibody (NIMR-5; Harada N et al. 1993) was purchased from Abeam (Cat. No. ab25181). Anti-asialo-GM1 antiserum was purchased (Wako Pure Chem. Ind.; Cat. No. 986-10001).

In vitro NK cell cytotoxicity assays. PBMCs were incubated for 24 hours with irradiated (50 Gy) HER2-expressing breast cancer cells (HER18) at a ratio of 1:1 and with Trastuzumab (10 µg/ml). After 24 hours, NK cells were purified by negative magnetic cell sorting using NK cell isolation beads (Miltenyi Biotec) according to the manufacturer’s instructions and to a greater than 90% purity, as defined by CD3-negative and CD56-positive and confirmed by flow cytometry. Activation of NK cells was confirmed by flow cytometry. NK cell cytotoxicity was additionally measured by a chromium release assay: target cancer cells were labeled with 150 µCi 51Cr per 1 x 10^6 cells for 2 hours, and subsequently added to activated PBMCs at variable effector/target cell ratios from 2:1 to 50:1. Percentage of cell lysis was determined after 4 hours of culture in the presence of the media (i.e., alone), anti-HER2 antibody (Trastuzumab, 10 µg/ml), antagonistic anti-CD38 antibody (HB7, 10 µg/ml), agonistic anti-CD38 antibody (IB4, 10 µg/ml), or combinations of this anti-HER2 antibody with an anti-CD38 antibody (each at 10 µg/ml), with or without an agonistic anti-CD137 antibody (10 µg/ml). All assays were performed in triplicate with 3 independent NK cell samples.

Transplantation of breast cancer cells and antibody therapy. In a first set of experiments, HER2-positive BT474M1 breast cancer cells were implanted subcutaneously into 5- to 6-week-old female athymic nu/nu mice at a dose of 5 x 10^6 cells in 50 µl of PBS mixed with 50 µl of Matrigel (BD Biosciences) 1 day after sub-cutaneous implantation of a 0.72 mg/60 d release β-estradiol pellet (Innovative Research of America). After tumor inoculation, mice received by intraperitoneal (i.p.) injection control Rat IgG antibody (150 µg/injection), Trastuzumab (150 µg/injection), or agonistic antibody to CD38 (NIMR-5, 150 µg/injection) or OX40 (150 µg/injection) on day 3, 10 and 17. On day 4, 11, and 18, two groups of the mice that had received Trastuzumab further received by i.p. injection agonistic antibody against CD38 or OX40. The size of tumor mass was measured by caliper twice a week and expressed as the product of length by width in square centimeters. Mice were sacrificed when tumor size reached 4 cm² or when tumor sites ulcerated. All in vivo models were piloted with 5 mice per group and repeated with 10 mice per group. In a second set of experiments, human breast cancer cells (MCF7, BT474M1, SKBR3, and HER18) were implanted subcutaneously into 5- to 6-week-old female athymic nu/nu mice at a dose of 5 x
10^6 cells in 50 µl of PBS mixed with 50 µl of Matrigel (BD Biosciences) 1 day after subcutaneous implantation of a 0.72 mg/60 d release β-estradiol pellet (Innovative Research of America). After tumor inoculation, mice received by intraperitoneal (i.p.) injection control Rat IgG antibody (150 µg/injection) or agonistic antibody to CD38 (NIMR-5, 150 µg/injection) on Day 0, 3, 6 and 9 (where day 0 is determined on the basis of tumor volume reaching 100mm³ when measured by caliper). In a further group, the administration of agonistic antibody to CD38 was combined with the intraperitoneal administration of anti-asialo-GM1 antibody at day 1, 6 and 11 (50 µg/injection). The size of tumor mass was measured by caliper every other day and expressed as the percent change in tumor volume on day 14 compared to baseline at day 0. All in vivo models were performed by using 10 mice per group. In a third set of experiments, murine breast cancer cells (4T1, EMT6, and TUBO-EGFR) were implanted subcutaneously into 5- to 6-week-old female syngeneic mice at a dose of 1 x 10^6 cells in 50 µl of PBS. After tumor inoculation, mice were treated with the same antibodies and analyzed similarly to the previous set of experiments.

Statistics. Prism software (GraphPad) was used to analyze tumor growth and determine statistical significance of differences (including mean ± SEM) between groups by applying a 2-tailed, unpaired Student’s t test or 2-way ANOVA with Bonferroni’s correction for multiple comparisons. P < 0.05 was considered significant. For tumor burdens, comparisons of means were done by ANOVA.

Results

The present invention proposes that a CD38 agonist (such as an anti-CD38 agonist antibody) can achieve a therapeutic effect against cancer cells that may be comparable or superior to that observed with certain anti-tumor antigen antibodies. For example, data presented herein demonstrate effectiveness of CD38 agonist therapy (and specifically anti-CD38 agonist antibody therapy) as compared with administration of an anti-HER2 antibody (Trastuzumab, presently used for breast cancer therapy), an anti-CD137 agonist antibody or an anti-CD134/OX40 agonist antibody as positive control for the effect of CD38 agonist administration.

In a first in vitro model, activated PBMCs (containing CD38-positive NK cells) were incubated with target cells (HER18 breast cancer cells, which strongly express HER2) in the presence of media alone (i.e., negative control), antagonist anti-CD38 antibody (HB7), agonist anti-CD38 antibody (IB4), anti-HER2 antibody (Trastuzumab), combinations of these anti-CD38 and anti-HER2 antibodies, and a combination of anti-HER2 antibody, agonist anti-CD38 antibody, and another agonist antibody targeting CD137.

As can be seen in Figure 1A, activated PBMCs alone showed almost no ability to lyse tumor cells. Some cell lysis was observed in the presence of agonistic anti-CD38 antibody (IB4),
but not in the presence of antagonistic anti-CD38 antibody (HB7). Significant lysis was observed with anti-tumor antibody (i.e., anti-HER2) alone, but the level was dramatically amplified in the presence of agonistic anti-CD38 antibody (and not in the presence of antagonistic anti-CD38 antibody). Agonistic anti-CD137 antibody also amplified the killing observed in the presence of anti-HER2 antibody, though the effect surprisingly appeared to be less dramatic than that observed with agonistic anti-CD38. In the particular experiment shown in Figure 5, Trastuzumab effect may be enhanced by combining the agonistic effects of CD137 or CD38 to Trastuzumab, ADCC while agonizing CD38 alone appears having a limited effect in this model of cell lysis.

These evidences may be confirmed in certain animal models that are commonly used to validate and/or evaluate compounds as valuable tools for cancer therapy. Specifically, a HER2-expressing human cancer cell line was transferred in mice and the growth of tumor was measured in the following weeks during which alternative single compound or combinations treatments were tested. As can be seen in Figure 1B, the regular administration of either an anti-tumor antibody (anti-HER2) or an agonistic antibody that targets an inducible effector cell surface marker (anti-CD38 or anti-OX40) as monotherapy provided some reduction in the growth rate of the tumor in mice (with CD38 agonist providing less statistically significant effects in this specific model). Therapeutic effect of anti-HER2 antibody (Trastuzumab) administration was dramatically enhanced by the administration of agonist antibody against CD38 or OX40 (Figure 1A).

Data on therapeutic effects of CD38 agonist therapy in breast cancer animal models were obtained by using either murine (4T1, TUBO-EGFR, and EMT6) or human (MCF 7, BT474M1, SKBR3, and MCF 7/HER-18) breast cancer cell lines. When these cells are implanted in mice, the progression of tumor growth was reduced by approx. 30-50% compared to control IgG (independently from HER2 high or low expression). These effects in both syngeneic and xenograft mice models were confirmed in experiments that involved different frequency of administration (at days 0, 7, and 14 instead of on Day 0, 3, 6 and 9), using the mouse breast cell line TUBO-EGFR and the human breast cell lines BT474M1 and HER18.

However, if CD38 agonist was administered in combination with an anti-asialo-GM1 antibody (a compound that is used to deplete NK cell activity in vivo; Houot R et al., 2009), the effect of CD38 agonist disappeared. Without wishing to be bound by any particular theory, we note that evidence provided herein suggests that CD38 agonist therapy (e.g., administration of an anti-CD38 agonist antibody agent) effects are mediated, at least in part, by NK cells that express CD38. Moreover, included data suggest that such CD38 agonist therapy may show improved effectiveness relative to therapies that target HER2 on cancer cells and/or may
provide synergistic benefits when combined with such HER2-targeting therapies. Effects of CD38 agonist therapy can be compared in animal models using cell lines for other cancer types presenting cancer antigen other than HER2.

Example 2: Agonizing CD38 in Models for B Cell Malignancy Therapy

Materials & Methods

Antibodies. Control rat IgG, agonistic anti-CD38 antibody (NIMR-5), and anti-asialo-GM1 antibody were obtained as indicated in Example 1. Anti-mouse CD20 monoclonal antibody was described (clone 18B12; Ahuja A et al., 1997). Ascites fluid was harvested from severe combined immunodeficient mice bearing hybridoma GK1.5, 2.43, and TH6 that produce anti-CD4 (rat IgG2b), anti-CD8 (rat IgG2b), and anti-FR4 (rat IgG2a) monoclonal antibodies respectively, as described (Houot R et al., 2009). The ascites were diluted in sterile PBS. TH6 is also commercially available (LifeSpan Biosciences; Cat. No. LS-C148380) or at the International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology as a mouse-rat hybridoma that can produce antibody against mouse Folate Receptor 4 (FR4) under the code FERM BP-10382 in the International Patent Organism Depository (IPOD), National Institute of Technology and Evaluation (Japan).

Murine Cell lines and Culture Conditions. The CD20-positive, aggressive murine B-cell lymphoma cells A20 (BALB/c-related B-cell lymphoma line) and EL4 (C57/BL6-related T-cell lymphoma line) are available from ATCC (TIB208™ and TIB39™, respectively). H11 is a pre-B-cell line in the C57/BL6 background that was generated as described (Goldstein M et al., 2011). The murine CD20-positive B-cell line BL3750, a C57/BL6-cell lymphoma, was developed in a cMyc transgenic mouse as described (Minard-Colin et al. 2008). H11, A20, and EL4 cells were cultured in complete Roswell Park Memorial Institute 1640 medium (cRPMI; Invitrogen) containing 10% fetal bovine serum (FBS; ThermoScientific), 100 U/mL penicillin, 100 µg/mL streptomycin (both from Invitrogen), and 50µM 2-Mercaptoethanol (Sigma-Aldrich). BL3750 was cultured in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 50µM 2-mercaptoethanol (Sigma-Aldrich). Cells were grown as adherent cultures at 37°C in 5% CO2 and passaged after detachment by 0.05% trypsin (Life Technologies). These cells express CD20 with specific fluorescence indices (tumor MFI/isotype MFI) below 1.0. No detectable surface levels of CD38 are observed on these cell lines by flow cytometry assessment.

Human Cell lines and Culture Conditions. The CD20-positive, human B-cell lymphoma cells Raji, Ramos, and DHL4 are available from ATCC (CCL86™, CRL-1596™, and CRL-29577™ respectively). MOLM-13 is an acute myeloid leukemia (AML) human cell line that is available by AddexBio (Cta. No. C0003003). Raji, Ramos, and DHL4 cells were cultured in RPMI
1640 medium (Invitrogen Life Technologies) supplemented with 10% heat-inactivated fetal calf serum (HyClone Laboratories), 100 U/mL penicillin, and 100 µg/mL streptomycin (both from Invitrogen). MOLM-13 was cultured in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 50µM 2-mercaptoethanol (Sigma-Aldrich). Cells were grown as adherent cultures at 37°C in 5% CO₂ and passaged after detachment by 0.05% trypsin (Life Technologies). These cells express CD20 with specific fluorescence indices (tumor MFI/isotype MFI) below 1.0. No detectable surface levels of CD38 are observed on these cell lines by flow cytometry assessment.

**Transplantation of lymphoma cells and antibody therapy.** In a first set of experiments, A20 cells were implanted subcutaneously into BALB/c mice (Jackson Lab.) at a dose of 1 x 10⁶ cells in 50 µL of PBS mixed with 50 µL of Matrigel (BD Biosciences). After tumor inoculation, mice received by intraperitoneal (i.p.) injection control rat IgG antibody (150 µg/injection), anti-mouse CD20 (100 µg/injection), or an agonistic antibody to CD38 (NIMR-5, 150 µg/injection on the indicated days. The size of a tumor mass was measured by caliper twice a week and expressed as the product of length by width in square centimeters. Mice were sacrificed when tumor size reached 4 cm² or when tumor sites ulcerated. All in vivo models used 10 mice per group. In a second set of experiments, human lymphoma cancer cells (MOLM-13, DHL4, Raji, and Ramos) were implanted subcutaneously into 5- to 6-week-old female athymic nu/nu mice at a dose of 5 x 10⁶ cells in 50 µL of PBS mixed with 50 µL of Matrigel (BD Biosciences) and treated with rat IgG antibody, agonistic antibody to CD38 (NIMR-5 alone, or in combination with anti-asialo-GM1 antibody) as indicated in Example 1. In a third set of experiments, murine lymphoma cells (A20, EL4, H11, and BL3750) were implanted subcutaneously into 5- to 6-week-old female syngenic BALB/c or C57/BL6 mice at a dose of 1 x 10⁶ cells in 50 µL of PBS. After tumor inoculation, mice were treated with the same antibodies but in three further groups, the administration of agonistic antibody to CD38 was combined with the intraperitoneal administration of anti-CD4, anti-FR4, or anti-CD8 (500 µg/injection for anti-CD4 and anti-CD8 at day 1, 6 and 11; 100 µg/injection for anti-FR4 at day 1, 5 and 9). Treg depletion conditions were validated by flow cytometry of peripheral blood showing (for example, after anti-FR4 antibody treatment was confirmed by flow cytometry of peripheral blood showing the depletion of approximately 80% of CD4 and CD8 T cells). The analysis of tumor size of tumor mass, the number of mice per group, the data retrieval and statistical analysis in these second and third set of experiments were similar to those applied in Example 1.

**Results**

The experimental approach of Example 1 for demonstrating the in vivo antitumor activity was repeated in appropriate animal models wherein lymphoma cells were injected in a syngeneic
mouse model, thereby potentially improving understanding of how CD38 agonist-based monotherapy or combination therapies provide therapeutic effects. In a first set of experiments that are shown in Figure 2, when compared to placebo (control rat IgG) antibody treatment, the use of an agonistic anti-CD38 antibodies provides a surprisingly, highly statistically relevant effect in reducing the growth of tumor size as a monotherapy when compared with the commonly used anti-tumor antibody treatment for lymphoma that is directed at CD20, a finding that suggest an even higher efficacy of CD38 agonist monotherapy for lymphoma and other B cell or T cell malignancies.

Data demonstrating potent effects of CD38 agonist therapy in breast cancer animal models were obtained by using either murine (A20, EL4, H11, and BL3750) or human (MOLM-13, DHL4, Raji, and Ramos). When human cells were implanted in mice (xenograft model), the progression of tumor growth is reduced by approx. 30-50% compared to control IgG and sensible to anti-asialo-GM1 antibody, as for breast cancer cells.

Effects of CD38 agonist therapy were even much stronger when fully syngeneic, murine models were established (and rat anti-mouse CD38 agonist antibody is used). As shown in Figure 3, the CD38 agonist therapy appeared at least blocking tumor growth and, in a variable number of animals, even to achieve tumor regression. Additionally, the depletion of Regulatory T cells (e.g. by administering a Treg depleting agent, such as an anti-CD4 or an anti-FR4 antibody) in combination with CD38 agonist, further enhance the effect of CD38 agonist therapy against lymphoma cells, with more animals showing an even more significant regression of tumor size. These evidences furthermore suggest that this combination of agents may help overcoming the resistance of such cells (or other lack of efficacy in cancer treatment) with respect to the use of either one or the other category of therapeutic agents.

Treg depletion by using antibodies targeting various antigens has been suggested in the literature as a mean for enhancing cancer therapy and, if the use of an antibody as an anti-CD4 is not applicable since removing any T cell (CD4 T helpers and Tregs), anti-CTLA4, anti-CD25, or anti-FR4 antibodies appear as compounds that can be used in combination with CD38 agonist therapy, as shown for other type of cancer therapies for stimulating potent antitumor immune responses and/or preventing tumor development (Marabelle A et al., 2013; Tian Y et al., 2012; Teng M et al., 2010; Liang S et al., 2013).

**Example 3: Agonizing CD38 in Other Models for Cancer Therapy**

**Materials & Methods**
Antibodies. Control rat IgG, agonistic anti-CD38 antibody (NIMR-5), anti-asialo-GM1 antibody, anti-CD4, anti-CD8, and anti-FR4 were obtained as indicated in Example 1. Anti-EGFR monoclonal antibodies purchased from Stanford Hospital Research Pharmacy.

Cell lines and culture. Mouse cell lines CT26, Renca (from BALB/c mouse; CRL2638™, CRL2947™ respectively), LLC1, B16F0, B16F10 (all from C57/BL6 mouse; CRL1642™, CRL6322™, CRL6475™) and human cell lines T84, HCT16, and SCC4 (CCL248™, CCL247™, and CRL1624™, respectively) are available from ATCC. The human EGFR-expressing cancer cell lines SCC4, SCC6, and PC1 were obtained from John Sunwoo at Stanford University. SCC4 is an upper aerodigestive tract squamous cell carcinoma with mutations in CDKN2A, NF1, and TP53 and wild type KRAS, EGFR, MET, NRAS, and PTEN. SCC6 is an upper aerodigestive tract squamous cell carcinoma with mutations in CDKN2A and wild type NF1, TP53, KRAS, EGFR, NRAS, and PTEN. PC1 is a pancreatic adenocarcinoma with mutations in CDKN2A, KRAS (G12D), and TP53 and wild type EGFR, NRAS, MET, NF1, and PTEN. SCC4, SCC6, and PC1 cell lines were cultured in a 1:1 mixture of DMEM:F12 supplemented with 10% heat-inactivated FCS (HyClone Laboratories), 2% l-glutamine, 1% penicillin, and 1% streptomycin (all from Invitrogen Life Technologies). Human EGFR-positive cancer cell lines T84 (KRAS-WT), HCT16 (KRAS G13D mutant) were cultured in DMEM supplemented with 10% FCS, 10 mM Hepes, 1% nonessential amino acids, 1% penicillin, and 1% streptomycin (all from Invitrogen Life Technologies). No detectable surface levels of CD38 are observed on these cell lines by flow cytometry assessment.

Transplantation of tumor cells and antibody therapy. Human cancer cells were implanted subcutaneously into 5- to 6-week-old female athymic nu/nu mice at a dose of 5 x 10^6 cells in 50 µl of PBS mixed with 50 µl of Matrigel (BD Biosciences) and treated with rat IgG antibody, agonistic antibody to CD38 (NIMR-5 alone, or in combination with anti-asialo-GM1 antibody) as indicated in Example 1. Murine cancer cells were implanted subcutaneously into 5- to 6-week-old female BALB/c, C57BL/6, or A/J-F mice at a dose of 1 x 10^6 cells in 50 µl of PBS. After tumor inoculation, mice were treated with the same antibodies but in three further groups, the administration of agonistic antibody to CD38 was combined with the intraperitoneal administration of anti-CD4, anti-FR4, or anti-CD8 (500 µg/injection for anti-CD4 and anti-CD8 at day 1, 6 and 11; 100 µg/injection for anti-FR4 at day 1, 5 and 9). Treg depletion conditions, analysis of tumor size of tumor mass, the number of mice per group, the data retrieval and statistical analysis in these second and third set of experiments were similar to those applied in Examples 1 and 2.

Results
The experimental approach of Examples 1 and 2 for demonstrating the in vivo antitumor activity was also repeated in animal models wherein EGFR-positive cells were injected in either a xenograft or a syngeneic mouse model. Results further validated CD38 agonist therapy also against this type of cancers. Efficacy of CD38 monotherapy at a level similar to those demonstrated for breast cancer cells in Example 1 was observed for mouse cell lines that are relevant for renal carcinoma (RENCa) and melanoma (B16F0 and B16F10) as well as for human cell lines that are relevant for demonstrating the therapeutic efficacy of compounds against colon (T84 and HCT-116) and head-and-neck (PC1, SCC4, and SCC6) cancers.

Results were comparable or superior to those shown in Example 2, at least for syngeneic mouse models that involve the use of cancer cells that express EGFR and that are relevant for colon cancer (MC38 and CT26), lung carcinoma (LLC1), and sarcoma (Sa1 N). As shown in Figure 4, the use of an agonistic anti-CD38 antibodies provides an effect of therapeutic interest that is highly statistically relevant when compared with control IgG and that is sensible to anti-asialo-GM1 antibody, as for lymphoma cells and confirming the involvement of NK cell activity also when EGFR-expressing tumors are treated with CD38 agonist therapy. Use of a Treg depleting in combination with CD38 agonist therapy, further enhance the effects of CD38 agonist therapy that were observed in these cancer cells.

Those skilled in the art, reading the present disclosure will appreciate various modifications of dosing regimens, etc, that are within the scope of the present invention with respect to the use of a CD38 agonist as monotherapy and/or with specific combination (as with an Treg depleting agent) in cancer therapy.

Still further, those skilled in the art, reading the present disclosure will appreciate various modifications of dosing regimens, etc, that are within the scope of the present invention with respect to the use of a CD38 agonist as monotherapy in cancer.

Thus, it will be appreciated that the demonstrated beneficial effects of CD38 agonism, as documented here, may well also be observed with other CD38 agonists, for example based on other anti-CD38 antibodies (e.g., IB4 or NIM-R5). Useful agonists based on IB4 or NIM-R5 (or other anti-CD38 antibodies or other CD38 agonizing agents, including for example small molecule agents), can be identified and/or characterized as described herein. In some embodiments, such agonists are identified and/or characterized by screening recombinant antibodies or natural libraries of antibodies, such as those identified as CD38-specific agonistic autoantibodies in samples from diabetic patients (Antonelli A et al., 2001) and/or by mapping their CD38 binding features in correlation with calcium mobilization and cytokine release, especially associated with the amino acid 220-241 of human CD38 (Ausiello C et
al., 2000; Mallone R et al., 2001) or other epitopes that can be defined experimentally as characterizing CD38 agonists to be used according to the present invention. Further relevant epitopes of human CD38 can be mapped according to CD38-specific, agonistic effects consequent to their binding to CD38 extracellular extracellular domain with an antibody, peptide, fusion protein, or other recombinant protein, such criteria being one or more of the following ones: level of affinity for human CD38, cross-reactivity across species, position of the CD38 epitope, potency in NK cell-based assays, cell specificity.

EQUIVALENTS AND SCOPE

Those skilled in the art will appreciate that the present invention is defined by the appended claims and not by the Examples or other description of certain embodiments included herein. For example, Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, unless specifically excluded limit in the stated range. Where the stated range includes one or both limits, ranges excluding either or both of those limits are also included in the invention.

Similarly, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

Unless defined otherwise above, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, genetics and protein and nucleic acid chemistry described herein are those well known and commonly used in the art, or according to manufacturer's specifications.

Also, all publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing
date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

REFERENCES

Hara-Yokoyama ME et al., 2008. Int Immunopharmacol. 8: 59-70.
Li G et al., 2013. Drug Discov Ther. 7: 178-84.
Teng M et al., 2010. Cancer Res. 70: 2665-74.
van der Veer MS et al., 2011, Haematologica 96:284-90.
WHAT is CLAIMED IS:

1. A pharmaceutical composition comprising a CD38 agonist.

2. The pharmaceutical composition of claim 1, wherein said CD38 agonist binds the extracellular domain of CD38.

3. The pharmaceutical composition of claim 1, wherein said composition is for use in the treatment of cancer.

4. The pharmaceutical composition of claim 1 wherein the CD38 agonist is an agonist of human CD38.

5. The pharmaceutical composition of claim 1 wherein the CD38 agonist is or comprises an agonistic anti-human CD38 antibody.

6. The pharmaceutical composition of claim 5 wherein the CD38 agonist is monoclonal anti-human CD38 antibody.

7. The pharmaceutical composition of claim 5, wherein the anti-human CD38 antibody is a xenogeneic human antibody.

8. The pharmaceutical composition of claim 5, wherein the anti-human CD38 antibody is a humanized antibody.

9. The pharmaceutical composition of claim 5, wherein the anti-human CD38 antibody is a chimeric antibody.

10. The pharmaceutical composition of claim 1 wherein the CD38 agonist is or comprises a non-antibody agent being a peptide, a recombinant protein, or an aptamer.

11. The pharmaceutical composition of claim 1 wherein the CD38 agonist is or comprises a multi-specific agent.

12. The pharmaceutical composition of claim 11, wherein the CD38 agonist is or comprises an anti-tumor antigen antibody.

13. The pharmaceutical composition of claim 12, wherein the tumor antigen is selected from CD20, HER2, PD-1, PD-L1, or EGF receptor.

14. The pharmaceutical composition of claim 3 wherein the cancer is a B cell malignancy.

15. The pharmaceutical composition of claim 14 wherein the cancer is marginal zone lymphoma, Hodgkins Lymphoma, non-Hodgkins lymphoma, chronic lymphocytic leukemia, acute lymphoblastic leukemia, myelomas, or myeloproliferative disorders.
16. The pharmaceutical composition of claim 3 wherein the cancer is a solid tumor.

17. The pharmaceutical composition of claim 16 wherein the cancer is a breast carcinoma, a squamous cell carcinoma, a colon cancer, a head and neck cancer, a lung cancer, a genitourinary cancer, a rectal cancer, a gastric cancer, sarcoma, melanoma, or an esophageal cancer.
FIGURE 2

- Rat IgG (control) (d3, d10, d17)
- aCD20 (d3, d10, d17)
- aCD38 (d3, d10, d17)
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<td>aCD38</td>
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### FIGURE 4

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- **IgG**
- **aCD38**
- **aCD38+aGM1**
- **aCD38+αFR4**
- **aCD38+aCD4**
- **aCD38+aCD8**
**INTERNATIONAL SEARCH REPORT**

A. **CLASSIFICATION OF SUBJECT MATTER**

**INV.** A61K39/395 C07K16/28

**ADD.**

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)

A61K C07K

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. **DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>WO 00/40265 Al (RES DEV FOUNDATION [US]) 13 July 2000 (2000-07-13) See page 8, lines 7-17, examples 2-6, figure 8, claims</td>
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<td>AU 2013 209 322 Al (SANOFI AVENTIS) 15 August 2013 (2013-08-15) See page 8, lines 7-17, examples 2-6, claims</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

"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) on 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

"A" document member of the same patent family

**Date of the actual completion of the international search**

23 October 2015

**Date of mailing of the international search report**

02/11/2015

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer

Nauche, Stephane
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