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(54) Title: USE OF A PD-1 ANTAGONIST AND AN ANTI-CCR2 ANTIBODY IN THE TREATMENT OF CANCER

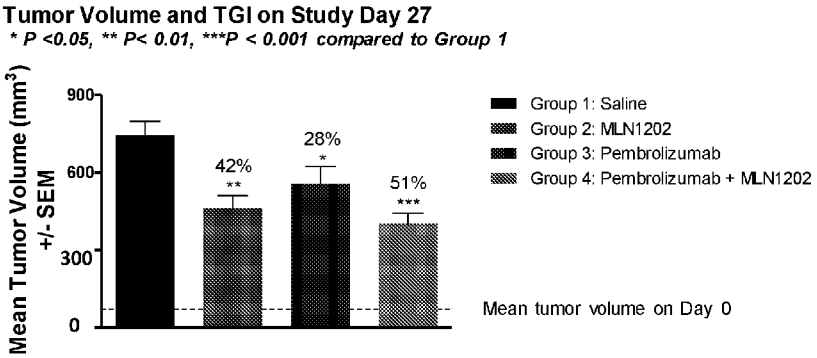

Figure 4: Tumor Growth Inhibition on Study Day 27

(57) Abstract: The present disclosure relates to methods for the treatment of cancers by administering anti-PD-1 antagonists in combination with anti-CCR2 antibodies. In a preferred embodiment, plozalizumab (also known as MLN1202) was used in combination with pembrolizumab (also known as Keytruda) to treat non-small cell lung cancer in humanized NSG mice.

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USE OF A PD-1 ANTAGONIST AND AN ANTI-CCR2 ANTIBODY IN THE TREATMENT OF CANCER

FIELD OF THE DISCLOSURE

[0001] The present disclosure relates to combination therapies useful for the treatment of cancer. In particular, the disclosure relates to a combination therapy which comprises an antagonist of the Programmed Death 1 protein (PD-1) and an anti CC-chemokine receptor 2 (CCR2) antibody.

SUMMARY OF THE DISCLOSURE

[0002] The present disclosure is based, in part, on the finding that concurrent administration of a PD-1 antagonist and an anti-CCR2 antibody to tumor-bearing mice resulted in significantly higher anti-tumor efficacy compared to either agent alone.

[0003] CCR2 is expressed primarily on monocytes as well as a subset of memory T cells, basophils, immature dendritic cells, and some macrophages. In the setting of normal immune surveillance, through interaction with the primary CCR2 ligand MCP-1 (also known as CCL2), CCR2+ cells from the peripheral blood are recruited to sites of active inflammation and are primed to release proinflammatory cytokines, tissue-damaging proteolytic enzymes and reactive oxygen radicals. However, as part of the immune escape mechanism of cancer, tumors secrete factors such as MCP-1 that cause an imbalance in the homing mechanisms of myeloid precursors. Circulating myeloid derived suppressor cells (MDSCs) over express the CCR2 receptor and are preferentially recruited to the tumor microenvironment where they suppress T cell-mediated production of interferon gamma (IFNγ) and effector T-cell proliferation (so called M2-polarization).

[0004] Plozalizumab (also known as TAK202 and MLN1202) is a genetically engineered humanized mAb of the immunoglobulin (IgG1) class that is a potent specific antagonist of cysteine-cysteine chemokine receptor type 2 (CCR2). As a combination therapy, plozalizumab has the potential to block circulating myeloid derived suppressor cells that over express the CCR2-receptor from trafficking to the tumor microenvironment where they suppress T-cell responsiveness. Plozalizumab blocks all CCR2 ligands (MCP-1, 2, 3 and 4) with a high degree of receptor occupancy thought necessary to block chemokine activity sufficiently to produce a pharmacodynamic effect (based on an increase in monocyte chemoattractant protein 1 (MCP-1),

decrease in circulating monocytes and reduction in C-reactive protein (CRP)).

[0005] Thus, in one embodiment, the disclosure provides a method for treating a cancer in an individual comprising administering to the individual a combination therapy which comprises a PD- 1 antagonist and an anti-CCR2 antibody.

[0006] In another embodiment, the disclosure provides a medicament comprising a PD-1 antagonist for use in combination with an anti-CCR2 antibody for treating a cancer.

[0007] Other embodiments provide use of a PD-1 antagonist in the manufacture of medicament for treating a cancer in an individual when administered in combination with an anti-CCR2 antibody and use of an anti-CCR2 antibody in the manufacture of a medicament for treating a cancer in an individual when administered in combination with a PD-1 antagonist.

[0008] In a still further embodiment, the disclosure provides use of a PD-1 antagonist and an anti-CCR2 antibody in the manufacture of medicaments for treating a cancer in an individual. In some embodiments, the medicaments comprise a kit, and the kit also comprises a package insert comprising instructions for using the PD-1 antagonist in combination with an anti-CCR2 antibody to treat a cancer in an individual.

[0009] In all of the above treatment methods, medicaments and uses, the PD-1 antagonist inhibits the binding of PD-L1 and/or PL-L2 to PD-1. In some embodiments of the above treatment methods, medicaments and uses, the PD-1 antagonist is a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to PD-1 or to PD-L1 and blocks the binding of PD-L1 to PD-1. In one embodiment, the PD-1 antagonist is an anti-PD-1 monoclonal antibody selected from the group consisting of nivolumab and pembrolizumab.

[0010] In one embodiment of the treatment methods, medicaments and uses, the anti-CCR2 antibody is plozalizumab.

[0011] In some embodiments of the above treatment methods, medicaments and uses of the disclosure, the individual is a human and the cancer is a solid tumor.

[0012] In other embodiments of the above treatment methods, medicaments and uses of the disclosure, the individual is a human and the cancer is a hematological malignancy.

[0013] Also, in embodiments of any of the above treatment methods, medicaments and uses, the cancer expresses, at elevated or decreased levels, one or both of PD-Ll and PD-L2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIGURE 1. Flow Analysis on Humanized NSG Cohorts: The humanization of NSG mice used for this study was assessed for the levels of human cells in the peripheral blood by flow cytometry starting at 12 weeks post human CD34+ cell injection. In addition to the standard human CD45 and CD3 markers, human CD14 and CD192 (CCR2) were included in the panel of markers analyzed.

[0015] FIGURE 2. Body Weight: No significant difference was observed in body weight between the treatment arms.

[0016] FIGURE 3. Mean Tumor Volume: Plozalizumab and Keytruda monotherapy showed significant tumor growth inhibition. Plozalizumab in combination with Keytruda showed superior efficacy compared to monotherapy.

[0017] FIGURE 4. Tumor Growth Inhibition on Study Day 27

[0018] FIGURE 5A. Flow Cytometry Analysis of Tumor infiltrating lymphocytes (TIL): At day 27 the tumors were removed from the NSG mice and assessed for the levels of human cells by flow cytometry. Tumors were dissociated and stained with human CD45, CD3, CD16, CD19, CD11b, Lin- (CD3/CD19/CD56) antibodies to identify lymphocytes, T cells, NK cells, B cells and myeloid derived suppressor cells respectively. Stacked bar graphs represent the positive cells for each cell population as a % of the total viable CD45 positive cells in each of the four cohorts.

[0019] FIGURE 5B. Evaluation of T cell populations in each of the four cohorts: CD4 and CD8 antibodies were used to identify the % of CD3 T cells that were positive for these two markers.

[0020] FIGURE 5C. Evaluation of myeloid derived suppressor cells in each of the four the cohorts: CD33 and CCR2 antibodies were used to identify the % of myeloid derived suppressor cells (CD11b+Lin-) that were positive for these markers.

[0021] FIGURE 5D. Evaluation of T cells in tumors from saline treated versus MLN1202 treated mice: Flow cytometry dot blots of CD4 and CD8 positive cells within the CD3 positive gate.

[0022] FIGURE 5E. Evaluation of myeloid derived suppressor cells in tumors from saline treated versus MLN1202 treated mice: Flow cytometry dot blots of CD33 and CCR2 positive cells within the CD11b+Lin- positive gate.

DETAILED DESCRIPTION

[0023] The present disclosure relates to new combination therapies for the treatment of cancer. In particular, the present disclosure relates to methods and compositions for treating a subject suffering from a cancer, comprising administering to the subject an anti-CCR2 antibody and a Programmed Death 1 Protein (PD-1) antagonist; wherein the amount of the anti-CCR2 antibody and PD-1 antagonist is such that the combination thereof is therapeutically effective in the treatment of a cancer.

[0024] Both Tumor Associated Macrophages (TAMs) and Myeloid Derived Suppressor Cells (MDSCs) express CCR2 at elevated levels. Various tumor types produce CCL2, which in turn recruits CCR2 expressing immune-suppressive leukocytes including TAMs and MDSCs. TAMs and MDSCs therefore increase in the tumor microenvironment of various tumor types. Both TAMs and MDSCs promote tumor progression by suppressing the activity of CTL and enhancing Treg activity.

[0025] The present disclosure relates to an anti-CCR2 antibody, such as plozalizumab, having the ability to reduce the infiltration of immune-inhibitory macrophages (M2 polarization) by blocking CCR2 signaling. This blockage leads to a downregulation of immune-inhibitory macrophages, thereby allowing the enhancement of the immune-stimulatory activity of PD-1 blocking by the PD-1 antagonist. It is believed that the combinations of the disclosure utilizing an anti-CCR2 antibody and a PD-1 antagonist have advantages over each therapeutic agent administered alone and other combinations involving PD-1 antagonists. Thus, an anti-CCR2 antibody, such as plozalizumab in combination with a PD-1 antagonist can provide a superior clinical benefit with a better safety profile.

[0026] So that the disclosure may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0027] As used herein, including the claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

[0028] "Administration" as it applies to an animal, human, experimental subject, cell, tissue,

organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" also means in vitro and ex vivo treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any organism, preferably an animal, more preferably a mammal (e.g., rat, mouse, dog, cat, rabbit) and most preferably a human.

[0029] As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological or binding activity. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), humanized, fully human antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as humanization of an antibody for use as a human therapeutic.

[0030] In general, the basic antibody structural unit comprises a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the heavy chain may define a constant region primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989).

[0031] The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

[0032] Typically, the variable domains of both the heavy and light chains comprise three

hypervariable regions, also called complementarity determining regions (CDRs), which are located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of Sequences of Proteins of Immunological Interest, Kabat, *et al.;* National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat (1978) Adv. Prot. Chem. 32: 1-75; Kabat, *et al*, (1977) J. Biol. Chem. 252:6609-6616; Chothia, et al, (1987) J Mol. Biol. 196:901-917 or Chothia, et al, (1989) Nature 342:878-883.

[0033] "Variable regions" or "V region" as used herein means the segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.

[0034] As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (i.e. CDRL1, CDRL2 and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and CDRH3 in the heavy chain variable domain). See Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (defining the CDR regions of an antibody by sequence); see also Chothia and Lesk (1987) J. Mol. Biol. 196: 901-917 (defining the CDR regions of an antibody by structure). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

[0035] "Clothia" as used herein means an antibody numbering system described in Al- Lazikani et al, JMB 273:927-948 (1997). "Kabat" as used herein means an immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat ((1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD).

[0036] As used herein, unless otherwise indicated, "antibody fragment" or "antigen binding fragment" refers to antigen binding fragments of antibodies, i.e. antibody fragments that retain the ability to bind specifically to the antigen bound by the full-length antibody, e.g. fragments that retain one or more CDR regions. Examples of antibody binding fragments include, but are

not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

[0037] An antibody that "specifically binds to" a specified target protein is an antibody that exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g. without producing undesired results such as false positives. Antibodies, or binding fragments thereof, useful in the present disclosure will bind to the target protein with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20- times greater, and most preferably at least 100-times greater than the affinity with non-target proteins. As used herein, an antibody is said to bind specifically to a polypeptide comprising a given amino acid sequence, e.g. the amino acid sequence of a mature human PD-1, human PD- LI molecule or human CCR2, if it binds to polypeptides comprising that sequence but does not bind to proteins lacking that sequence.

[0038] "Monoclonal antibody" or "mAb" or "Mab", as used herein, refers to a population of substantially homogeneous antibodies, i.e., the antibody molecules comprising the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of different antibodies having different amino acid sequences in their variable domains, particularly their CDRs, which are often specific for different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler *et al.* (1975) Nature 256: 495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) Nature 352: 624-628 and Marks *et al.* (1991) J. Mol. Biol. 222: 581-597, for example. See also Presta (2005) J. Allergy Clin. Immunol. 1 16:731.

[0039] "Chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in an antibody derived from a particular species (e.g., human) or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in an antibody derived from another species (e.g., mouse) or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

[0040] "Human antibody" refers to an antibody that comprises human immunoglobulin protein sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" or "rat antibody" refer to an antibody that comprises only mouse or rat immunoglobulin sequences, respectively.

[0041] "Humanized antibody" refers to forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The prefix "hum", "hu" or "h" is added to antibody clone designations when necessary to distinguish humanized antibodies from parental rodent antibodies. The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

[0042] The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, leukemia, blastoma, and sarcoma. More particular examples of cancers include but are not limited to, skin cancer, ocular cancer, gastrointestinal cancer, thyroid cancer, breast cancer, ovarian cancer, central nervous system

cancer, laryngeal cancer, cervical cancer, lymphatic system cancer, genitourinary tract cancer, bone cancer, biliary tract cancer, endometrial cancer, liver cancer, lung cancer, prostate cancer, pancreatic cancer and colon cancer. In some embodiments, lung cancer includes non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). In some embodiments, the cancer is selected from skin cancer, ocular cancer, gastrointestinal cancer, thyroid cancer, breast cancer, ovarian cancer, brain cancer, laryngeal cancer, cervical cancer, lymphatic system cancer, genitourinary tract cancer, bone cancer, biliary tract cancer, endometrial cancer, uterine cancer, liver cancer, lung cancer, prostate cancer and colon cancer.

[0043] In some embodiments, the cancer is a hematological malignancy. In some embodiments, the hematological malignancy is selected from acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphoblastic leukemia (CLL), myelodysplastic syndrome, B-cell lymphoma, non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma. In some embodiments, the cancer is NHL. In some embodiments, the NHL is selected from diffused large B cell lymphoma, follicular lymphoma. and multiple myeloma.

[0044] In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is squamous NSCLC. In some embodiments, the cancer is non-squamous NSCLC.

[0045] In some embodiments, the cancer is skin cancer. In some embodiments, the skin cancer is melanoma. In some embodiments, the skin cancer is metastatic melanoma. In some embodiments, the melanoma is B-RAF-mutated melanoma. In some embodiments, the melanoma is N-RAS-mutated melanoma.

[0046] In some embodiments, the cancer is gastrointestinal cancer. As used herein, "gastrointestinal cancer" includes cancer of the esophagus, stomach (also known as gastric cancer), biliary system, pancreas, small intestine, large intestine, rectum and anus. In some embodiments, the gastrointestinal cancer is adenocarcinoma of the esophagus, adenocarcinoma of the gastroesophageal junction or adenocarcinoma of the stomach. In some embodiments, the gastrointestinal cancer is stomach cancer.

[0047] Cancers that may be treated in accordance with the present disclosure include those characterized by elevated or decreased expression of PD-L1, PD-L2 and/or MCP-1 (CCL2) in tested tissue samples in comparison to tissue samples taken from non-cancerous patients.

[0048] "Biotherapeutic agent" means a biological molecule, such as an antibody or fusion protein, that blocks ligand / receptor signaling in any biological pathway that supports tumor maintenance and/or growth or suppresses the anti-tumor immune response.

[0049] "Chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, kinase inhibitors, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topisomerase inhibitors, photosensitizers, anti-estrogens and selective estrogen receptor modulators (SERMs), anti-progesterones, estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, leutinizing hormone-releasing hormone agonists, anti-androgens, aromatase inhibitors, EGFR inhibitors, VEGF inhibitors, anti-sense oligonucleotides that inhibit expression of genes implicated in abnormal cell proliferation or tumor growth. Chemotherapeutic agents useful in the treatment methods of the present disclosure include cytostatic and/or cytotoxic agents.

[0050] "Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (e.g. charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity or other desired property of the protein, such as antigen affinity and/or specificity. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* (1987) Molecular Biology of the Gene, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.)). In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity.

[0051] "Consists essentially of," and variations such as "consist essentially of" or "consisting essentially of", as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, a PD-1 antagonist that consists essentially of a recited amino acid sequence may also include one or more amino acids, including substitutions of one or more amino acid residues, which do not

materially affect the properties of the binding compound.

[0052] "Homology" refers to sequence similarity between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same amino acid monomer subunit, e.g., if a position in a light chain CDR of two different Abs is occupied by alanine, then the two Abs are homologous at that position. The percent of homology is the number of homologous positions shared by the two sequences divided by the total number of positions compared x 100. For example, if 8 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 80% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology. For example, the comparison can be performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences.

[0053] The following references relate to BLAST algorithms often used for sequence analysis: BLAST ALGORITHMS: Altschul, S.F., *et al,* (1990) J. Mol. Biol. 215:403-410; Gish, W., *et al,* (1993) Nature Genet. 3:266-272; Madden, T.L., *et al,* (1996) Meth. Enzymol. 266:131-141; Altschul, S.F., *et al,* (1997) Nucleic Acids Res. 25:3389-3402; Zhang, J., *et al,* (1997) Genome Res. 7:649-656; Wootton, J.C., *et al,* (1993) Comput. Chem. 17: 149-163; Hancock, J.M. *et al,* (1994) Comput. Appl. Biosci. 10:67-70; ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al,* "A model of evolutionary change in proteins." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, Natl. Biomed. Res. Found., Washington, DC; Schwartz, R.M., *et al,* "Matrices for detecting distant relationships." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3." M.O. Dayhoff (ed.), pp. 353-358, Natl. Biomed. Res. Found., Washington, DC; Altschul, S.F., (1991) J. Mol. Biol. 219:555-565; States, D.J., *et al,* (1991) Methods 3:66-70; Henikoff, S., *et al,* (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919; Altschul, S.F., *et al,* (1993) J. Mol. Evol. 36:290-300; ALIGNMENT STATISTICS: Karlin, S., *et al,* (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268; Karlin, S., *et al,* (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877; Dembo, A., *et al,* (1994) Ann. Prob. 22:2022-2039; and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in Theoretical and Computational Methods in Genome Research (S. Suhai, ed.),

(1997) pp. 1-14, Plenum, New York.

[0054] "Isolated antibody" and "isolated antibody fragment" refers to the purification status and in such context means the named molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

[0055] "Patient" refers to any single subject for which therapy is desired or that is participating in a clinical trial, epidemiological study or used as a control, including humans and mammalian veterinary patients such as cattle, horses, dogs, and cats.

[0056] Programmed Death 1 protein (PD-1) is recognized as an important player in immune regulation and the maintenance of peripheral tolerance. PD-1 is moderately expressed on naive T, B and NKT cells and up-regulated by T/B cell receptor signaling on lymphocytes, monocytes and myeloid cells (Sharpe, A.H, *et al.* (2007) Nature Immunology; 8:239-245).

[0057] "PD-1 antagonist" means any chemical compound or biological molecule that serve to regulate the interaction with PD-1 and its cognate ligands expressed on the surface of interacting cell types personified by T cells, cancer cells, macrophages and antigen presenting cells (APCs). PD-1 antagonists include chemical compounds or biological molecules that block binding of PD-L1 and/or PD-L2 expressed on cancer cells or APCs to PD-1 expressed on an immune cell (T cell, B cell or NKT cell). Alternative names or synonyms for PD-1 and its ligands include: PDCD1, PD1, CD279 and SLEB2 for PD-1; PDCD1L1, PDL1, B7H1, B7-4, CD274 and B7-H for PD-Ll; and PDCD1L2, PDL2, B7-DC, Btdc and CD273 for PD-L2. In any of the treatment methods, medicaments and uses of the present disclosure in which a human individual is being treated, the PD-1 antagonist blocks binding of human PD-Ll to human PD-1, and preferably blocks binding of both human PD-Ll and PD-L2 to human PD-1. Human PD-1 amino acid sequences can be found in NCBI Locus No.: NP 005009. Human PD-Ll and PD-L2 amino acid sequences can be found in NCBI Locus No.: NP_054862 and NP_079515, respectively.

[0058] PD-1 antagonists useful in any of the treatment methods, medicaments and uses of the present disclosure include a monoclonal antibody (mAb), or antigen binding fragment thereof,

which specifically binds to PD-1 or PD-Ll, and preferably specifically binds to human PD-1 or human PD-Ll. The mAb may be a human antibody, a humanized antibody or a chimeric antibody, and may include a human constant region. In some embodiments the human constant region is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4 constant regions, and in embodiments, the human constant region is an IgGl or IgG4 constant region. In some embodiments, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, F(ab')2, scFv and Fv fragments.

[0059] Examples of mAbs that bind to human PD-1, and useful in the treatment methods, medicaments and uses of the present disclosure, are described in US 7,521,051, US 8,779,105, US 8,008,449, US 8,900,587, US 8,952,136, US 8,354,509, US 8,735,553, US 9,102,728, US 8,993,731, US 9,102,727, US 9,181,342, US 8,927,697, US 8,945,561, US 7,488,02, US 7,322,582, US 7,524,498 and US 9,205,148. Specific anti-human PD-1 mAbs useful as the PD-1 antagonist in the treatment methods, medicaments and uses of the present disclosure include, but are not limited to: pembrolizumab (formerly MK-3475 and lambrolizumab), marketed in the USA under the tradename KEYTRUDA®, a humanized IgG4 mAb with the structure described in WHO Drug Information, Vol. 27, No. 2, pages 161-162 (2013); and nivolumab (formerly ONO-4538, MDX1106 or BMS-936558), marketed in the USA under the tradename OPDIVO®, a human IgG4 mAb with the structure described in WHO Drug Information, Vol. 27, No. 1, pages 68-69 (2013).

[0060] Examples of mAbs that bind to human PD-L1, and useful in the treatment methods, medicaments and uses of the present disclosure, are described in WO2013/019906, W02010/077634 Al and US 8,383,796. Specific anti-human PD-L1 mAbs useful as the PD-1 antagonist in the treatment methods, medicaments and uses of the present disclosure include, but are not limited to MPDL3280A, BMS-936559, MEDI4736, MSB0010718C and an antibody which comprises the heavy chain and light chain variable regions of SEQ ID NO:24 and SEQ ID NO:21, respectively, of WO2013/019906.

[0061] Other PD- 1 antagonists useful in any of the treatment methods, medicaments and uses of the present disclosure include an immunoadhesin that specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1, e.g., a fusion protein containing the extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region such as

an Fc region of an immunoglobulin molecule. Examples of immunoadhesion molecules that specifically bind to PD-1 are described in WO2010/027827 and WO2011/066342. Specific fusion proteins useful as the PD-1 antagonist in the treatment methods, medicaments and uses of the present disclosure include AMP-224 (also known as B7- DCIg), which is a PD-L2-FC fusion protein and binds to human PD-1.

[0062] In some embodiments, the PD-1 antagonist is selected from nivolumab, pembrolizumab, PDR-001, SHR-1210, AMP-224, REGN-2810, MEDI-0680, BGB-108, PF-06801591, atezolizumab, durvalumab, and BMS-936559, and AMP-224.

[0063] In some embodiments, the PD-1 antagonist is selected from nivolumab, pembrolizumab, PDR-001, SHR-1210, AMP-224, REGN-2810, MEDI-0680, BGB-108, and PF-06801591, and AMP-224.

[0064] In some embodiments, the PD-1 antagonist is selected from pembrolizumab and nivolumab.

[0065] In some embodiments, the PD-1 antagonist is nivolumab.

[0066] In some embodiments, the PD-1 antagonist is pembrolizumab.

[0067] In some embodiments, the PD-1 antagonist is selected from atezolizumab, durvalumab, avelumab and BMS-936559.

[0068] In some embodiments of the treatment methods, medicaments and uses of the present disclosure, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which comprises nivolumab.

[0069] In some embodiments of the treatment methods, medicaments and uses of the present disclosure, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which comprises pembrolizumab.

[0070] As used herein, “CC-chemokine receptor 2” (“CCR-2”) refers to CC-chemokine receptor 2α (CCR-2 α) and/or CC-chemokine receptor 2β (CCR-2 β).

[0071] Antibodies or functional fragments used herein, which can inhibit one or more functions characteristic of a mammalian CCR2, such as a binding activity (e.g., ligand, inhibitor and/or promoter binding), a signaling activity (e.g., activation of a mammalian G protein, induction of a rapid and transient increase in the concentration of cytosolic free calcium [Ca2+]), and/or stimulation of a cellular response (e.g., stimulation of chemotaxis, exocytosis or inflammatory

mediator release by leukocytes, integrin activation) are also encompassed by the present disclosure, such as an antibody which can inhibit binding of a ligand (i.e., one or more ligands) to CCR2 and/or one or more functions mediated by CCR2 in response to a ligand.

[0072] As used herein, a "CCR-2 mediated disorder" refers to a disorder involving the movement, *e.g.*, recruitment from one site to another site, infiltration from one site to another site, proliferation, differentiation, and/or function of cells expressing CCR-2. Cells expressing CCR-2 include, for example, monocytes, dendritic cells, macrophages, T-cells, lymphocytes, basophils, mast cells, endothelial cells and fibroblasts. Examples of CCR-2 mediated disorders include, but are not limited to, i) inflammatory or immune disorders; ii) cardiovascular disorders; iii) proliferative disorders; iv) graft rejections; v) fibrotic diseases; vi) viral infections; vii) neurological disorders; and viii) metabolic disorders.

[0073] Anti-CCR2 antibodies useful in any of the treatment methods, medicaments and uses of the present disclosure include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to CCR-2, and preferably specifically binds to human CCR-2. The mAb may be a human antibody, a humanized antibody or a chimeric antibody, and may include a human constant region. In some embodiments the human constant region is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4 constant regions, and in embodiments, the human constant region is an IgGl or IgG4 constant region. In some embodiments, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, F(ab')2, scFv and Fv fragments.

[0074] Examples of mAbs that bind to human CCR-2, and useful in the treatment methods, medicaments and uses of the present disclosure, are described in US 6,458,353, US 6,312,689, US 6,727,349, US 6,696,550 and US 6,084,075. Specific anti-CCR-2 antibodies useful in the treatment methods, medicaments and uses of the present disclosure include, but are not limited to plozalizumab (formerly MLN1202 and TAK202, also known as humanized mAb 1D9), a humanized IgG1 mAb with the structure described in WHO Drug Information, Vol. 29, No. 2, pages 251-252 (2015); 1D9; 8G2; MCPR-04; MCPR-05; or MCPR-06.

[0075] In some embodiments of the treatment methods, medicaments and uses of the present disclosure, the anti-CCR-2 antibody is a monoclonal antibody, or antigen binding fragment thereof, which comprises plozalizumab.

[0076] The anti-CCR2 antibody as used herein can be a humanized immunoglobulin or antigen-binding fragment thereof for use as described herein having binding specificity for CCR2, said immunoglobulin comprising an antigen binding region of nonhuman origin (e.g., rodent) and at least a portion of an immunoglobulin of human origin (e.g., a human framework region, a human constant region of the gamma type). In one embodiment, the humanized immunoglobulin or fragment thereof described herein can compete with 1D9 for binding to CCR2. In an embodiment, the antigen binding region of the humanized immunoglobulin is derived from monoclonal antibody 1D9. For example, the humanized immunoglobulin or antigen-binding fragment thereof can comprise an antigen binding region comprising at least one complementarity determining region (CDR) of nonhuman origin, and a framework region (FR) derived from a human framework region. In one aspect, the humanized immunoglobulin having binding specificity for CCR2 comprises a light chain comprising at least one CDR derived from an antibody of nonhuman origin which binds CCR2 and a FR derived from a light chain of human origin (e.g., from HF-21/28), and a heavy chain comprising a CDR derived from an antibody of nonhuman origin which binds CCR2 and a FR derived from a heavy chain of human origin (e.g., from 4B4'CL). In another aspect, the light chain comprises three CDRs derived from the light chain of the 1D9 antibody, and the heavy chain comprises three CDRs derived from the heavy chain of the 1D9 antibody.

[0077] The anti-CCR2 antibody as used herein may comprise humanized immunoglobulin light chains and antigen-binding fragments thereof (e.g., comprising CDR1, CDR2 and CDR3 of the light chain of the 1D9 antibody, and a human light chain FR), and humanized immunoglobulin heavy chains and antigen-binding fragments thereof (e.g., comprising CDR1, CDR2 and CDR3 of the heavy chain of the 1D9 antibody, and a human heavy chain FR) for use as described herein.

[0078] The anti-CCR2 antibody as used herein also relates to a humanized immunoglobulin or antigen-binding fragment thereof having binding specificity for CCR2 comprising a heavy chain and a light chain, wherein said light chain comprises at least one complementarity determining region derived from murine monoclonal antibody 1D9 and a framework region derived from the light chain of human antibody HF-21/28, and wherein said heavy chain comprises at least one complementarity determining region derived from murine monoclonal antibody 1D9 and a

framework region derived from the heavy chain of human antibody 4B4'CL for use as described herein. In one embodiment, the light chain comprises three complementarity determining regions derived from the light chain of the 1D9 antibody, and the heavy chain comprises three complementarity determining regions derived from the heavy chain of the 1D9 antibody.

[0079] The anti-CCR2 antibody as used herein preferably also inhibits the interaction of a cell bearing mammalian (e.g., human, non-human primate or murine) CCR2 with a ligand thereof, comprising contacting the cell with an effective amount of an antibody or functional fragment thereof which binds to a mammalian CCR2 or a portion of CCR2. Suitable cells include leukocytes, such as granulocytes, monocytes, macrophages, basophils and eosinophils, mast cells, and lymphocytes including T cells (e.g., CD8+ cells, CD4+ cells, CD25+ cells, CD45RO+ cells), and other cells expressing CCR2 such as a recombinant cell expressing CCR2 (e.g., transfected cells). In a particular embodiment, the antibody is 1D9 or an antibody which can compete with 1D9 for binding to human CCR2 or a portion of human CCR2. In another embodiment, the antibody is plozalizumab or an antibody which can compete with plozalizumab for binding to human CCR2 or a portion of human CCR2.

[0080] The anti-CCR2 antibody as used herein preferably also inhibits the interaction of a cell bearing mammalian CCR2 with a chemokine, comprising contacting said cell with an effective amount of an antibody or functional fragment thereof which binds to CCR2 or a portion of said receptor. In one embodiment of the method, the antibody or functional fragment thereof is any one or more of 1D9, an antigen-binding fragment of 1D9, an antibody or fragment thereof having an epitopic specificity which is the same as or similar to that of 1D9, plozalizumab, an antigen-binding fragment of plozalizumab or an antibody or fragment thereof having an epitopic specificity which is the same as or similar to that of plozalizumab. Furthermore, the disclosure relates to a method of inhibiting a function associated with binding of a chemokine to CCR2, comprising administering an effective amount of an antibody or functional fragment thereof which binds to a mammalian CCR2 protein or a portion of said receptor. In one aspect of the method, the antibody or functional fragment thereof is any one or more of 1D9, an antigen-binding fragment of 1D9, an antibody or fragment thereof having an epitopic specificity which is the same as or similar to that of 1D9, plozalizumab, an antigen-binding fragment of plozalizumab or an antibody or fragment thereof having an epitopic specificity which is the same

as or similar to that of plozalizumab.

[0081] Preferably, the antibodies or fragments can bind CCR2 with an affinity of at least about 0.1 x 10^{-9} M, preferably at least about 1 x 10^{-9} M, and more preferably at least about 3 x 10^{-9} M. In a particular embodiment, antibodies or functional fragments thereof demonstrate inhibition of chemokine-induced (e.g., MCP-1-induced) chemotaxis of cells (e.g., monocytes, macrophages and other PBMCs) at less than about 150 μg/ml, preferably less than about 100 μg/ml, more preferably less than about 50 μg/ml, and even more preferably less than about 20 μg/ml.

[0082] In embodiments of the disclosure, the antibodies or functional fragments thereof can inhibit binding of a CCR2 ligand (e.g., a chemokine) to CCR2 with an IC_{50} of less than about 1.0 μg/ml, preferably less than about 0.05 μg/ml, and more preferably less than about 0.005 μg/ml.

[0083] In an embodiment, the antibodies bind human CCR2, and have an epitopic specificity which is the same as or similar to that of murine 1D9 or 8G2 antibody described herein. Antibodies with an epitopic specificity which is the same as or similar to that of murine 1D9 monoclonal antibody can be identified by their ability to compete with murine 1D9 monoclonal antibody for binding to human CCR2 (e.g., to cells bearing human CCR2, such as transfectants bearing CCR2, CD8+ cells, CD4+ cells, CDR45RO+ cells, CD25+ cells, monocytes, dendritic cells, macrophages and basophils). Similarly, antibodies with an epitopic specificity which is the same as or similar to that of murine 8G2 monoclonal antibody can be identified by their ability to compete with murine 8G2 monoclonal antibody for binding to human CCR2. Using receptor chimeras (Rucker *et al.*, *Cell 87*:437-446 (1996)), the binding site of mAbs 1D9 and 8G2 has been mapped to the amino-terminal domain of human CC-chemokine receptor 2, specifically to an epitope comprising from about amino acid 1 to about amino acid 30 of the protein. Using these or other suitable techniques, antibodies having an epitopic specificity which is the same as or similar to that of an antibody of the present disclosure can be identified. mAbs 1D9 and 8G2 have epitopic specificity for the amino-terminal domain of the CCR2 receptor, e.g., from about amino acid number 1 to about amino acid number 30 of the receptor protein.

[0084] Hybridoma cell lines producing antibodies were deposited on July 17, 1998, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under

Accession Nos. HB-12549 (1D9) and HB-12550 (8G2). The present disclosure also pertains to the hybridoma cell lines deposited under ATCC Accession No. HB-12549 and ATCC Accession No. HB-12550, as well as to the monoclonal antibodies produced by the hybridoma cell lines deposited under ATCC Accession Nos. HB-12549 and HB-12550.

[0085] "PD-Ll" or "PD-L2" expression as used herein means any detectable level of expression of the designated PD-L protein on the cell surface or of the designated PD-L mRNA within a cell or tissue. PD-L protein expression may be detected with a diagnostic PD-L antibody in an IHC assay of a tumor tissue section or by flow cytometry. Alternatively, PD-L protein expression by tumor cells may be detected by PET imaging, using a binding agent (e.g., antibody fragment, affibody and the like) that specifically binds to the desired PD-L target, e.g., PD-Ll or PD-L2. Techniques for detecting and measuring PD-L mRNA expression include RT- PCR and realtime quantitative RT-PCR.

[0086] Several approaches have been described for quantifying PD-Ll protein expression in IHC assays of tumor tissue sections. See, e.g., Thompson, R. H., *et al,* PNAS 101 (49); 17174-17179 (2004); Thompson, R. H. *et al,* Cancer Res. 66:3381-3385 (2006); Gadiot, J., *et al,* Cancer 117:2192-2201 (2011); Taube, J. M. *et al,* Sci Transl Med 4, 127ra37 (2012); and Toplian, S. L. *et al*, New Eng. J Med. 366 (26): 2443-2454 (2012).

[0087] In another approach, PD-Ll expression in the tumor tissue section is quantified in the tumor cells as well as in infiltrating immune cells, which predominantly comprise lymphocytes. The percentage of tumor cells and infiltrating immune cells that exhibit membrane staining are separately quantified as < 5%, 5 to 9%, and then in 10% increments up to 100%. For tumor cells, PD-Ll expression is counted as negative if the score is < 5% score and positive if the score is > 5%. PD-Ll expression in the immune infiltrate is reported as a semi-quantitative measurement called the adjusted inflammation score (AIS), which is determined by multiplying the percent of membrane staining cells by the intensity of the infiltrate, which is graded as none (0), mild (score of 1, rare lymphocytes), moderate (score of 2, focal infiltration of tumor by lymphohistocytic aggregates), or severe (score of 3, diffuse infiltration). A tumor tissue section is counted as positive for PD-Ll expression by immune infiltrates if the AIS is > 5.

[0088] In some embodiments, a level of PD-L1 expression (protein and/or mRNA) by malignant cells and/or by infiltrating immune cells within a tumor is determined to be "over expressed" or

"elevated" based on comparison with the level of PD-L1 expression (protein and/ or mRNA) by an appropriate control. For example, a control PD-L1 protein or mRNA expression level may be the level quantified in nonmalignant cells of the same type or in a section from a matched normal tissue. In some embodiments, PD-L1 expression in a tumor sample is determined to be elevated if PD-L1 protein (and/or PD-L1 mRNA) in the sample is at least 10%, 20%, or 30% greater than in the control.

[0089] "Sustained response" means a sustained therapeutic effect after cessation of treatment with a therapeutic agent, or a combination therapy described herein. In some embodiments, the sustained response has a duration that is at least the same as the treatment duration, or at least 1.5, 2.0, 2.5 or 3 times longer than the treatment duration.

[0090] "Tissue Section" refers to a single part or piece of a tissue sample, e.g., a thin slice of tissue cut from a sample of a normal tissue or of a tumor.

[0091] "Treat" or "treating" a cancer as used herein means to administer a combination therapy of a PD-1 antagonist and an anti-CCR2 antibody to a subject having a cancer, or diagnosed with a cancer, to achieve at least one positive therapeutic effect, such as for example, reduced number of cancer cells, reduced tumor size, reduced rate of cancer cell infiltration into peripheral organs, or reduced rate of tumor metastasis or tumor growth. In some embodiments, the treatment achieved by a therapeutically effective amount is any of progression free survival (PFS), disease free survival (DFS) or overall survival (OS). PFS, also referred to as "Time to Tumor Progression" indicates the length of time during and after treatment that the cancer does not grow, and includes the amount of time patients have experienced a complete response or a partial response, as well as the amount of time patients have experienced stable disease. DFS refers to the length of time during and after treatment that the patient remains free of cancer. OS refers to a prolongation in life expectancy as compared to a control treatment. The dosage regimen of a combination therapy described herein that is effective to treat a cancer patient may vary according to factors such as the disease state, age, and weight of the patient, and the ability of the therapy to elicit an anti-cancer response in the subject. While an embodiment of the treatment method, medicaments and uses of the present disclosure may not be effective in achieving a positive therapeutic effect in every subject, it should do so in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the

chi2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra- test and the Wilcoxon-test.

[0092] "Tumor" as it applies to a subject diagnosed with, or suspected of having, a cancer refers to a malignant or potentially malignant neoplasm or tissue mass of any size, and includes primary tumors and secondary neoplasms. A solid tumor is an abnormal growth or mass of tissue that usually does not contain cysts or liquid areas. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors (National Cancer Institute, Dictionary of Cancer Terms).

[0093] "Tumor burden" also referred to as "tumor load", refers to the total amount of tumor material distributed throughout the body. Tumor burden refers to the total number of cancer cells or the total size of tumor(s), throughout the body, including lymph nodes and bone narrow. Tumor burden can be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., ultrasound, bone scan, computed tomography (CT) or magnetic resonance imaging (MRI) scans.

[0094] The term "tumor size" refers to the total size of the tumor which can be measured as the length and width of a tumor. Tumor size may be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., bone scan, ultrasound, CT or MRI scans.

[0095] “Syngeneic” mouse models, also known as allograft mouse tumor systems, consist of tumor tissues derived from the same genetic background as a given mouse strain. Cancerous cells or solid tumors are transplanted into a host mouse. Because the cancer tissues and the recipient share ancestry, the transplant is not rejected by the host’s immune system. Therapeutic interventions can be performed using these mice and the results can be assessed to understand the treatment potentials. An advantage of syngeneic models, in comparison to xenograft models, is that the host immune system is normal (it has not been altered as in xenograft models), which may more closely represent the real life situation of the tumor’s micro-environment.

[0096] In one aspect of the disclosure, the disclosure provides a method for treating a cancer in

an individual comprising administering to the individual a combination therapy which comprises a PD-1 antagonist and an anti-CCR2 antibody.

[0097] The combination therapy may also comprise one or more additional therapeutic agents. In some embodiments, the combination therapy comprises the additional therapeutic agents is vedolizumab and ipilimumab.

[0098] In certain embodiments, immune oncology treatments can include modulating T-cell co-receptors. In some embodiments, this encompasses agonizing one or more activating receptors or immune modulators including soluble mediators (*e.g.*, CD28, 41BB, OX40, CD27, GITR, CD137, HVEM or a combination thereof), or antagonizing one or more inhibiting receptors or immune modulators including soluble mediators (*e.g.*, CTLA4, BTLA-4, PD-1, PDL-1, PDL-2, IDO, LAG3, ICOS, VISTA, TIM3, CSF-1R or a combination thereof).

[0099] Each therapeutic agent in a combination therapy of the disclosure may be administered either alone or in a medicament (also referred to herein as a pharmaceutical composition) which comprises the therapeutic agent and one or more pharmaceutically acceptable carriers, excipients and diluents, according to standard pharmaceutical practice.

[00100] Each therapeutic agent in a combination therapy of the disclosure may be administered simultaneously (i.e., in the same medicament), concurrently (i.e., in separate medicaments administered one right after the other in any order) or sequentially in any order. Sequential administration is particularly useful when the therapeutic agents in the combination therapy are in different dosage forms (one agent is a tablet or capsule and another agent is a sterile liquid) and/or are administered on different dosing schedules, e.g., a chemotherapeutic that is administered at least daily and a biotherapeutic that is administered less frequently, such as once weekly, once every two weeks, or once every three weeks.

[00101] In some embodiments, the anti-CCR2 antibody is administered before administration of the PD-1 antagonist, while in other embodiments, the anti-CCR2 antibody is administered after administration of the PD-1 antagonist. In one embodiment, the anti-CCR2 may be administered on the same day as the PD-1 antagonist. In another embodiment, the anti-CCR2 antibody may be administered on a different day than the PD-1 antagonist.

[00102] In some embodiments, at least one of the therapeutic agents in the combination therapy is administered using the same dosage regimen (dose, frequency and duration of

treatment) that is typically employed when the agent is used as monotherapy for treating the same cancer. In other embodiments, the patient receives a lower total amount of at least one of the therapeutic agents in the combination therapy than when the agent is used as monotherapy, e.g., smaller doses, less frequent doses, and/or shorter treatment duration.

[00103] Each therapeutic agent in a combination therapy of the disclosure can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal, topical, and transdermal routes of administration.

[00104] A combination therapy of the disclosure may be used prior to or following surgery to remove a tumor and may be used prior to, during or after radiation therapy.

[00105] In some embodiments, a combination therapy of the disclosure is administered to a patient who has not been previously treated with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-naive. In other embodiments, the combination therapy is administered to a patient who failed to achieve a sustained response after prior therapy with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-experienced.

[00106] A combination therapy of the disclosure is typically used to treat a tumor that is large enough to be found by palpation or by imaging techniques well known in the art, such as MRI, ultrasound, or CAT scan. In some embodiments, a combination therapy of the disclosure is used to treat an advanced stage tumor having dimensions of at least about 200 mm^3 300 mm^3, 400 mm^3, 500 mm^3, 750 mm^3, or up to 1000 mm^3.

[00107] A combination therapy of the disclosure can be administered to a human patient who has a cancer that tests positive or negative for PD-L1 expression. In some embodiments, PD-L1 expression is detected using a diagnostic anti-human PD-L1 antibody, or antigen binding fragment thereof, in an IHC assay on an FFPE or frozen tissue section of a tumor sample removed from the patient.

[00108] Selecting a dosage regimen (also referred to herein as an administration regimen) for a combination therapy of the disclosure depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells, tissue or organ in the individual being treated. Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the patient consistent with an acceptable level of side effects. Accordingly, the dose amount and dosing

frequency of each biotherapeutic and chemotherapeutic agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated, and patient characteristics. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available. See, e.g., Wawrzynczak (1996) Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, NY; Bach (ed.) (1993) Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, NY; Baert *et al.* (2003) New Engl. J. Med. 348:601-608; Milgrom *et al.* (1999) New Engl. J. Med. 341: 1966-1973; Slamon *et al.* (2001) New Engl. J. Med. 344:783-792; Beniaminovitz *et al.* (2000) New Engl. J. Med. 342:613-619; Ghosh *et al.* (2003) New Engl. J. Med. 348:24-32; Lipsky *et al.* (2000) New Engl. J. Med. 343: 1594-1602; Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002). Determination of the appropriate dosage regimen may be made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment, and will depend, for example, on the patient's clinical history (e.g., previous therapy), the type and stage of the cancer to be treated and biomarkers of response to one or more of the therapeutic agents in the combination therapy.

[00109] Biotherapeutic agents in a combination therapy of the disclosure may be administered by continuous infusion, or by doses at intervals of, e.g., daily, every other day, three times per week, or one time each week, two weeks, three weeks, monthly, bimonthly, etc. A total weekly dose is generally at least 0.05 μg/kg, 0.2 μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 3.0 mg/kg, 4.0 mg/kg, 8.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. See, e.g., Yang *et al.* (2003) New Engl. J. Med. 349:427-434; Herold *et al.* (2002) New Engl. J. Med. 346: 1692-1698; Liu *et al.* (1999) J. Neurol. Neurosurg. Psych. 67:451-456; Portielji *et al.* (20003) Cancer Immunol. Immunother. 52: 133-144.

[00110] In some embodiments that employ an anti-human PD-1 mAb as the PD-1 antagonist in the combination therapy, the dosing regimen will comprise administering the anti-human PD-1 mAb at a dose of 1, 2, 3, 5 or l0mg/kg at intervals of about 14 days (± 2 days) or about 21 days (± 2 days) or about 30 days (± 2 days) throughout the course of treatment.

[00111] In certain embodiments, a subject will be administered an intravenous (IV)

infusion of a medicament comprising any of the PD-1 antagonists or anti-CCR2 antibodies described herein. In another embodiment, a subject will be administered a medicament comprising any of the PD-1 antagonists or anti-CCR2 antibodies described herein subcutaneously.

[00112] In one embodiment of the disclosure, the PD-1 antagonist in the combination therapy is nivolumab, which is administered intravenously at a dose selected from the group consisting of: 1 mg/kg Q2W, 2 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 10 mg Q2W, 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 5 mg/kg Q3W, and 10 mg Q3W.

[00113] In another embodiment of the disclosure, the PD-1 antagonist in the combination therapy is pembrolizumab, which is administered intravenously at a dose selected from the group consisting of 1 mg/kg Q2W, 2 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 10 mg Q2W, 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 5 mg/kg Q3W, and 10 mg Q3W. In some embodiments, pembrolizumab is administered intravenously which comprises 25 mg/ml pembrolizumab, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80 in 10 mM histidine buffer pH 5.5, and the selected dose of the medicament is administered by IV infusion over a time period of 30 minutes.

[00114] In some embodiments, a liquid medicament comprising the anti-CCR2 antibody is infused into the individual being treated at a dose of between 2 mg/kg and 20 mg/kg. In some embodiments, a liquid medicament comprising the anti-CCR2 antibody is infused into the individual being treated at a dose of 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg or 20 mg/kg.

[00115] In some embodiments, a liquid medicament comprising the anti-CCR2 antibody is infused into the individual being treated at a dose of between 2 mg/kg and 10 mg/kg over a time period of at least 30 minutes on weeks 1, 3 and 5, followed by administration once every four weeks thereafter. Administration therefore occurs on each of days 1, 15, 29 followed by an administration once every four weeks beginning on day 57. In some embodiments, the time period for the IV infusion is longer than 30 minutes.

[00116] In some embodiments, a medicament comprising an anti-PD-1 antibody as the PD-1 antagonist may be provided as a liquid formulation or prepared by reconstituting a

lyophilized powder with sterile water for injection prior to use. WO 2012/135408 describes the preparation of liquid and lyophilized medicaments comprising pembrolizumab that are suitable for use in the present disclosure. In some embodiments, a medicament comprising pembrolizumab is provided in a glass vial which contains about 50 mg of pembrolizumab. In some embodiments, a medicament comprising nivolumab is provided in a glass vial which contains about 40 mg/4mL or 100 mg/10mL of nivolumab.

[00117] The present disclosure also provides a medicament which comprises a plozalizumab compound and a pharmaceutically acceptable excipient. The plozalizumab compound may be formulated as a powder for concentrate in a lyophilized formulation. When reconstituted, each vial of plozalizumab may, in one embodiment, contain 150mg/mL of plozalizumab, formulated with 3% sucrose, 0.9% mannitol, in a 30mM citrate buffer.

[00118] The plozalizumab compound may also be formulated for subcutaneous administration. In one embodiment, the subcutaneous formulation comprises 130mg/mL of plozalizumab, 3% sucrose, 2.25% mannitol at pH 6.8 in a 30mM citrate buffer. In another embodiment, the subcutaneous formulation comprises 130mg/mL of plozalizumab, 3% sucrose, 2.25% mannitol at pH 6.8 in a 75mM histidine buffer. In yet another embodiment, the subcutaneous formulation comprises 90mg/mL of plozalizumab, 3% sucrose, 2.25% mannitol at pH 6.8 in a 30mM citrate buffer.

[00119] Formulation of an antibody or fragment to be administered will vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. An appropriate pharmaceutical composition comprising an antibody or functional fragment thereof to be administered can be prepared in a physiologically acceptable vehicle or carrier. A mixture of antibodies and/or fragments can also be used. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), dextrose solution and glycine. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, *Remington's Pharmaceutical Science*, 16th

Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The antibodies and fragments of this disclosure can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to the skilled artisan, and will depend on the ultimate pharmaceutical formulation desired. For inhalation, the antibody or fragment can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

[00120] The antibody or fragment can be administered in a single dose or multiple doses. The dosage can be determined by methods known in the art and is dependent, for example, upon the antibody or fragment chosen, the subject's age, sensitivity and tolerance to drugs, and overall well-being. Antibodies and antigen-binding fragments thereof, such as human, humanized and chimeric antibodies and antigen-binding fragments can often be administered with less frequency than other types of therapeutics. For example, an effective amount of an antibody can range from about 0.01 mg/kg to about 2, 4, 6, 8, 10, 15 or 20 mg/kg administered daily, weekly, biweekly or monthly.

[00121] Compositions for use in the method of the disclosure may be formulated in unit dosage form for ease of administration and uniformity of dosage. The expression "unit dosage form" as used herein refers to a physically discrete unit of agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present disclosure will be decided by the attending physician within the scope of sound medical judgment. A unit dosage form for parenteral administration may be in ampoules or in multi-dose containers.

[00122] The anti-PD-1 antagonists and anti-CCR2 antibodies described herein may be provided as a kit which comprises a first container and a second container and a package insert. The first container contains at least one dose of a medicament comprising an anti-PD-1 antagonist, the second container contains at least one dose of a medicament comprising an anti-

CCR2 antibody, and the package insert, or label, which comprises instructions for treating a patient for cancer using the medicaments. The first and second containers may be comprised of the same or different shape (e.g., vials, syringes and bottles) and/or material (e.g., plastic or glass). The kit may further comprise other materials that may be useful in administering the medicaments, such as diluents, filters, IV bags and lines, needles and syringes. In some embodiments of the kit, the anti-PD-1 antagonist is an anti-PD-1 antibody. In some embodiments of the kit, the anti-PD-1 antibody is nivolumab. In some embodiments of the kit, the anti-PD-1 antibody is pembrolizumab. In some embodiments of the kit, the anti-CCR2 antibody is plozalizumab.

[00123] These and other aspects of the disclosure, including the exemplary specific embodiments listed below, will be apparent from the teachings contained herein.

EXAMPLES

Pre-clinical Studies

[00124] The objective of this study was to evaluate a novel antibody in LG1306 PDX model for lung cancer using humanized NSG™ mice engrafted with human CD34+ cells.

Mice and Housing

[00125] Seventy-eight (78) Hu-CD34 NSG™ mice that were engrafted with human CD34+ cells and had >25% human CD45+ cells in the peripheral blood at 12 weeks or later post engraftment were used for the study. Hu-CD34 NSG™ mice from two cohorts engrafted with CD34+ cells from donors 8014 and 8904 were used.

[00126] Mice were housed in individually and positively ventilated polysulfone cages with HEPA filtered air at a density of 4-5 mice per cage. The animal room was lighted entirely with artificial fluorescent lighting, with a controlled 12 h light/dark cycle (6 am to 6 pm light). The normal temperature and relative humidity ranges in the animal rooms were 22 ± 4°C and 50 ± 15%, respectively. The animal rooms were set to have 15 air exchanges per hour. Filtered tap water, acidified to a pH of 2.5 to 3.0, and normal rodent chow was provided ad libitum.

Methods and Records

[00127] Mice were implanted subcutaneously on the right flank with tumor fragments from TM00302 (LG1306F130P3). Body weights and clinical observations were recorded 1X weekly. Digital caliper measurements to determine tumor volumes were initiated and performed 1X-2X weekly after the tumors were palpable. Once tumor volumes reached 56–107 mm^3, mice were randomized by tumor volumes into 4 cohorts and dosed according to Table 1. There were multiple enrollments in order to reach the desired group size. Mice not enrolled into study cohorts were euthanized. On Study Day 0, dosing was started according to Table 1.

[00128] Clinical observations, body weight measurements, and caliper tumor volume measurements were performed 2X weekly post dose initiation. All mice had daily cage side observations performed. Eroded tumors were treated with betadine up to 3X weekly.

[00129] Animals with a body condition score ≤ 2, a body weight loss of $\geq 20\%$, a tumor volume >2000 mm^3, or ulcerated tumors were euthanized prior to study terminus. Blood and tissues were collected according to the instructions of the Sponsor. Note: Blood and tissues were not collected from the animals found dead. At study terminus (Study Day 27), mice were euthanized by CO_2 asphyxiation. Total blood was collected by cardiocentesis, processed to serum, and stored frozen. Tissue samples (tumors and spleens) were collected and processed by using either DMEM + 10% FBS or by cutting the tissue samples into 2 pieces and snap freezing one sample and fixing the other sample in FFPE.

Blood Processing Details

[00130] Terminal Blood: Approximately 500µL of blood was collected via cardiocentesis, processed into serum, and stored at -80°C until shipment to Sponsor.

Group	N	Compound	Dose (mg/kg)	Dosing Route	Dosing Frequency*
1	15	Saline	N/A	IP	Q5Dx6
2	16	plozalizumab	10	IP	Q5Dx6
3	16	pembrolizumab	10 (Day 0) 5	IP	Q5Dx6
4	16	plozalizumab +	10	IP	Q5Dx6

		pembrolizumab	10 (Day) 5		Q5Dx6
*Q5Dx6 = once every 5 days on Days 0, 5, 10, 15, 20 and 25.					

Table 1: Treatment protocol

[00131] There were 7 mice euthanized prior to Study Day 27. These mice were euthanized as a result of a reported body condition score ≤2, body weight loss of ≥20%, or ulcerated/necrotic tumor.

Flow cytometry to identify and enumerate different cell types in spleen and tumor

[00132] At day 27 after dosing start, mice were euthanized by CO_2 asphyxiation and spleens and tumors were harvested and placed in DMEM medium plus 10% FBS. Spleens and tumors were then dissociated into single cell suspensions using the gentle MACS dissociator (Miltenyl Biotec). Spleens were processed in autoMACS buffer containing 0.5% BSA and tumors in RPMI-1640 media containing enzymes A, D and R provided in the MACS tumor dissociation kit. After red blood cell removal (eBioscience lysis buffer), cells were washed and suspended at 20x10e6 viable cells/ml. Single cell suspension was distributed into 96 well U bottom plate in PBS for viability staining (Zombie Aqua, Biolegend). Cells were then washed, blocked in Fc block (Biolegend) and subsequently stained using the following conjugated antibodies in 100µl final volume FACS buffer (PBS + 1% BSA). Conjugated antibodies used for staining of CD3, CD4, CD8, CD45, CD19, CD56, CD14, CD16, NKp46, CD11b, CD33, CCR2, Lin- (CD3/CD19/CD56) were purchased from eBioscience, BD Biosciences or Biolegend. After staining, cells were washed and fixed in fixation buffer (eBioscience). Cells were acquired on a Canto II flow cytometer (BD Bioscience). The data was retrieved off the machine and analyzed with FlowJo software (Tree Star, Inc.) and GraphPad Prism (Version 5.04, GraphPad Software, Inc.). Cell populations of interest were determined for each tissue sample. A one-way ANOVA with a post-dunnet's multiple comparison test comparing all groups versus vehicle was done to determine significance. Results are expressed as mean ± standard error mean (SEM). A probability level of $p < 0.05$ was considered significant.

Results

[00133] Flow Analysis on Humanized NSG Cohorts: The humanization of NSG mice used for this study was assessed for the levels of human cells in the peripheral blood by flow cytometry starting at 12 weeks post human CD34+ cell injection. In addition to the standard human CD45 and CD3 markers, human CD14 and CD192 (CCR2) were included in the panel of markers analyzed (refer to Figure 1).

[00134] Body Weight: Body weights (g) were averaged by treatment group. Error bars represent standard error of the mean (SEM) (refer to Figure 2).

[00135] Change in Body Weight: Body weight change was calculated for each mouse by subtracting the body weight on the first day of dosing (baseline) from the body weight on the subsequent day of dosing (final) and calculating the percent change from baseline body weight: [=100*(FinalWeight – BaselineWeight)/BaselineWeight]. Average percent body weight change was then calculated by treatment group.

[00136] Mean Tumor Volume: Tumor volume measurements (mm^3) were averaged by treatment group. Error bars represent standard error of the mean (SEM) (refer to Figure 3).

[00137] Tumor Growth Inhibition on Study Day 27: Tumor volume measurements (mm^3) were averaged by treatment group (Groups 1-4). Tumor growth inhibition (TGI) was calculated for each group using the following method: [100-((TDay27-TDay0)/(CDay27-CDay0)*100)]. T represented the average tumor volume for a drug treatment group and C represented the average tumor volume for Group 1. Error bars represent standard error of the mean (SEM). Numbers above the bars on the graph were the calculated TGI values (refer to Figure 4).

[00138] In the Non Small Cell Lung Cancer PDX model using humanized NSG mice, plozalizumab showed $CD8^+$ T cell increase and $CCR2^+$ MDSC decrease in tumor, whereas pembrolizumab showed $CD8^+$ T cell increase but not $CCR2^+$ MDSC decrease in tumor. These results indicate that plozalizumab and pembrolizumab exhibit anti-tumor activity through distinct mechanisms and suggest the potential therapeutic benefit of combination of these two drugs (refer to Figures 5A-E).

Clinical Studies

[00139] A phase 1b study to evaluate the safety, tolerability, and pharmacodynamics of an

investigational treatment of plozalizumab (a.k.a. MLN1202 or TAK202) in combination with standard of care immune checkpoint inhibitors in patients with advanced melanoma is undertaken. Up to about 52 subjects are enrolled. Approximately 12 subjects are assigned in dose-escalation treatments, with up to 46 subjects in expansion cohorts. Between 2-15 clinical sites support the subjects.

[00140] The subjects are adults, either male or female, with histologically confirmed, unresectable stage III or IV melanoma, according to the AJCC staging system, and with a ECOG performance status of 0-1. Subjects have adequate bone marrow reserve and renal and hepatic function. Subjects with active known or suspected autoimmune disease, or that are undergoing systemic treatment with either corticosteroids (>10mg prednisone or equivalents) or other immunosuppressive medications within 14 days of administration of a study drug administration, are excluded, as are subjects with prior treatment with an anti-PD-1, anti-PDL-1 or anti-PDL-2 antibodies.

[00141] Plozalizumab is administered by IV at a dose of 4 or 8 mg/kg at weeks 1, 3, 5, and Q4W thereafter. In other words, plozalizumab is administered on Week 1, Day 1 and then every 2 weeks on Week 3, Day 15 and Week 5, Day 29. From the fourth dose onwards, the drug is administered every four weeks starting on Week 9, Day 57, until any other discontinuation criterion applies. Administration of plozalizumab may be changed to a lower starting dose (i.e., 2 mg/kg) if the 4 mg/kg dose is not tolerated. Nivolumab is administered IV at a dose of 3 mg/kg Q2W, which is the standard of care. Nivolumab is administered as an IV infusion over 60 minutes. The 250 mL bag of saline with plozalizumab will be administered IV over 30 minutes. On the days of concomitant nivolumab dosing (Week 3, Day 15 and onwards) plozalizumab is administered prior to nivolumab with a 30 minute gap between infusions. Duration of treatment is up to 50 weeks and the period of evaluation is 12 months.

[00142] The primary objective in the dose escalation phase plus Part 1 limited cohort expansion is to determine the recommended Part 2 dose based on the initial safety profile of the combination treatments in each arm when administered to patients with advanced melanoma. The primary objective in Part 2 is to determine the initial antitumor activity of each combination arm.

[00143] The primary endpoint is the frequency of dose limiting toxicities (DLT)(part 1)

and overall response rate as measured by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1(part 2). Secondary safety endpoints for this study are the frequency and severity of treatment emergent adverse events (TEAEs) including serious TEAEs, and TEAEs leading to treatment dioscontinuation. Secondary efficacy endpoints are duration of response (DOR), progression-free survival (PFS) and overall survival (OS). Serial tumor biopsies are used to assess immune cell infiltration in the tumor microenvironment at baseline, post-single agent and post-combination treatment.

[00144] It should be understood that for all numerical bounds describing some parameter in this application, such as "about," "at least," "less than," and "more than," the description also necessarily encompasses any range bounded by the recited values. Accordingly, for example, the description "at least 1, 2, 3, 4, or 5" also describes, *inter alia*, the ranges 1-2, 1-3, 1-4, 1-5, 2-3, 2-4, 2-5, 3-4, 3-5, and 4-5, *et cetera*.

[00145] For all patents, applications, or other reference cited herein, such as non-patent literature and reference sequence information, it should be understood that they are incorporated by reference in their entirety for all purposes as well as for the proposition that is recited. Where any conflict exists between a document incorporated by reference and the present application, this application will control. All information associated with reference gene sequences disclosed in this application, such as GeneIDs or accession numbers (typically referencing NCBI accession numbers), including, for example, genomic loci, genomic sequences, functional annotations, allelic variants, and reference mRNA (including, e.g., exon boundaries or response elements) and protein sequences (such as conserved domain structures), as well as chemical references (e.g., PubChem compound, PubChem substance, or PubChem Bioassay entries, including the annotations therein, such as structures and assays, et cetera), are hereby incorporated by reference in their entirety.

[00146] Headings used in this application are for convenience only and do not affect the interpretation of this application.

[00147] Preferred features of each of the aspects provided by the disclosure are applicable to all of the other aspects of the disclosure *mutatis mutandis* and, without limitation, are

exemplified by the dependent claims and also encompass combinations and permutations of individual features (*e.g.*, elements, including numerical ranges and exemplary embodiments) of particular embodiments and aspects of the disclosure, including the working examples. For example, particular experimental parameters exemplified in the working examples can be adapted for use in the claimed disclosure piecemeal without departing from the disclosure. For example, for materials that are disclosed, while specific reference of each of the various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. Thus, if a class of elements A, B, and C are disclosed as well as a class of elements D, E, and F and an example of a combination of elements A-D is disclosed, then, even if each is not individually recited, each is individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-groups of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this application, including elements of a composition of matter and steps of method of making or using the compositions.

[00148] The forgoing aspects of the disclosure, as recognized by the person having ordinary skill in the art following the teachings of the specification, can be claimed in any combination or permutation to the extent that they are novel and non-obvious over the prior art—thus, to the extent an element is described in one or more references known to the person having ordinary skill in the art, they may be excluded from the claimed disclosure by, *inter alia*, a negative proviso or disclaimer of the feature or combination of features.

Claims:

1. A method for treating a cancer in an individual comprising administering to the individual a combination therapy which comprises an antagonist of a Programmed Death 1 protein (PD-1) and an anti-CCR2 antibody.

2. The method of claim 1, wherein the individual is a human and the PD-1 antagonist is:
(a) a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to human PD-1 and blocks the binding of human PD-L1 and/or PD-L2 to human PD-1; or
(b) a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to human PD-L1 and blocks the binding of human PD-L1 to human PD-1.

3. The method of any one of claims 1 or 2, wherein the PD-1 antagonist is selected from nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab.

4. The method of any one of claims 1 or 2, wherein the anti-CCR2 antibody is plozalizumab.

5. The method of claim 4, wherein plozalizumab is administered at 2mg/kg, 4 mg/kg, 8 mg/kg, 10 mg/kg, 15 mg/kg or 20 mg/kg at weeks 1, 3 and 5 and then once every four weeks thereafter.

6. The method of any of claims 1 to 4, wherein the cancer is a solid tumor.

7. The method of any of claims 1 to 4, wherein the cancer is a hematological malignancy.

8. A medicament comprising an antagonist of a Programmed Death 1 protein (PD-1) for use in combination with an anti-CCR2 antibody.

9. The medicament of claim 8, wherein the PD-1 antagonist is:
(a) a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds

to human PD-1 and blocks the binding of human PD-L1 and/or PD-L2 to human PD-1; or

(b) a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to human PD-L1 and blocks the binding of human PD-L1 to human PD-1.

10. The medicament of any one of claims 8 or 9, wherein the PD-1 antagonist is selected from nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab.

11. The medicament of any one of claims 8 or 9, wherein the anti-CCR2 antibody is plozalizumab.

12. The medicament of any of claims 8 to 11, wherein the cancer is a solid tumor.

13. The medicament of any of claims 8 to 11, wherein the cancer is a hematological malignancy.

14. A kit which comprises a first container, a second container and a package insert, wherein the first container comprises at least one dose of a medicament comprising an antagonist of a Programmed Death 1 protein (PD-1), the second container comprises at least one dose of a medicament comprising an anti-CCR2 antibody, and the package insert comprises instructions for treating an individual for cancer using the medicaments.

15. The kit of claim 14, wherein the PD-1 antagonist is selected from nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab.

16. The kit of claim 14, wherein the anti-CCR2 antibody is plozalizumab.

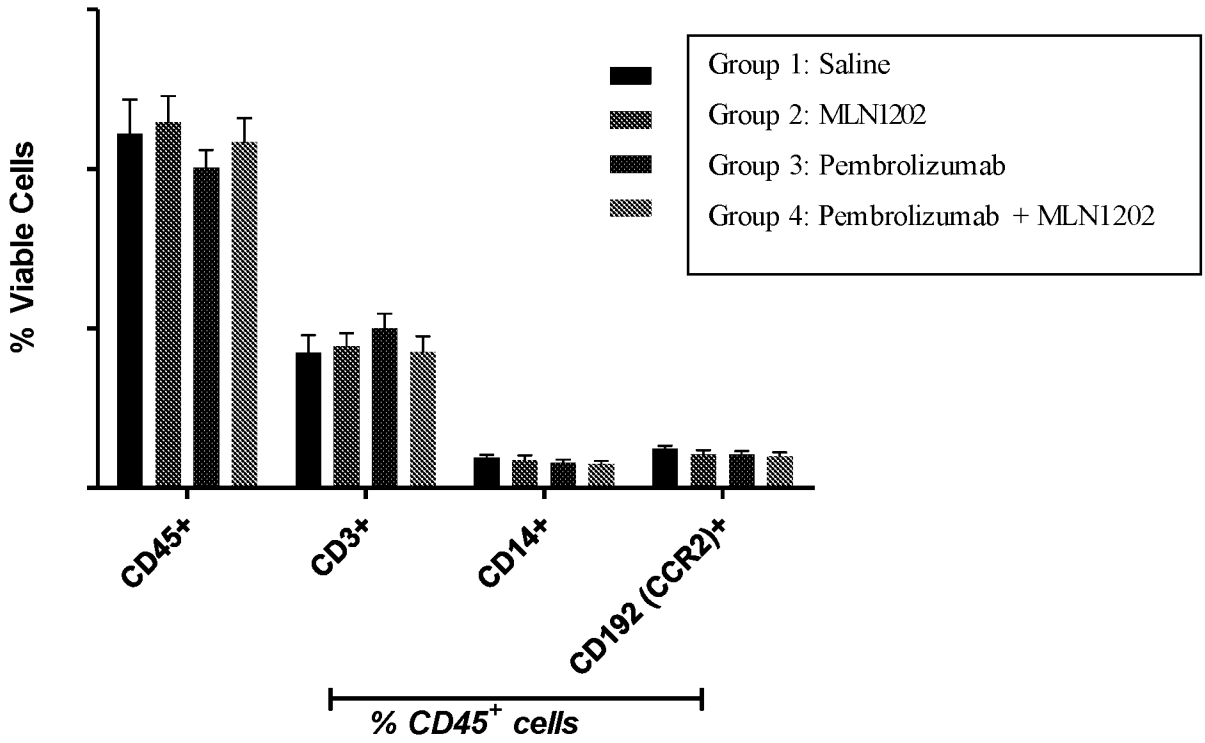

Figure 1: Flow Analysis on Humanized NSG Cohorts

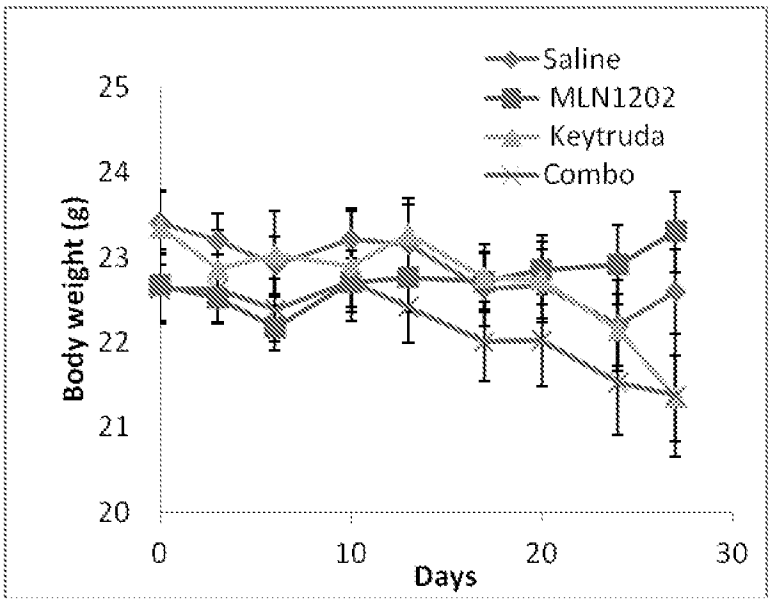

Figure 2: Body Weight

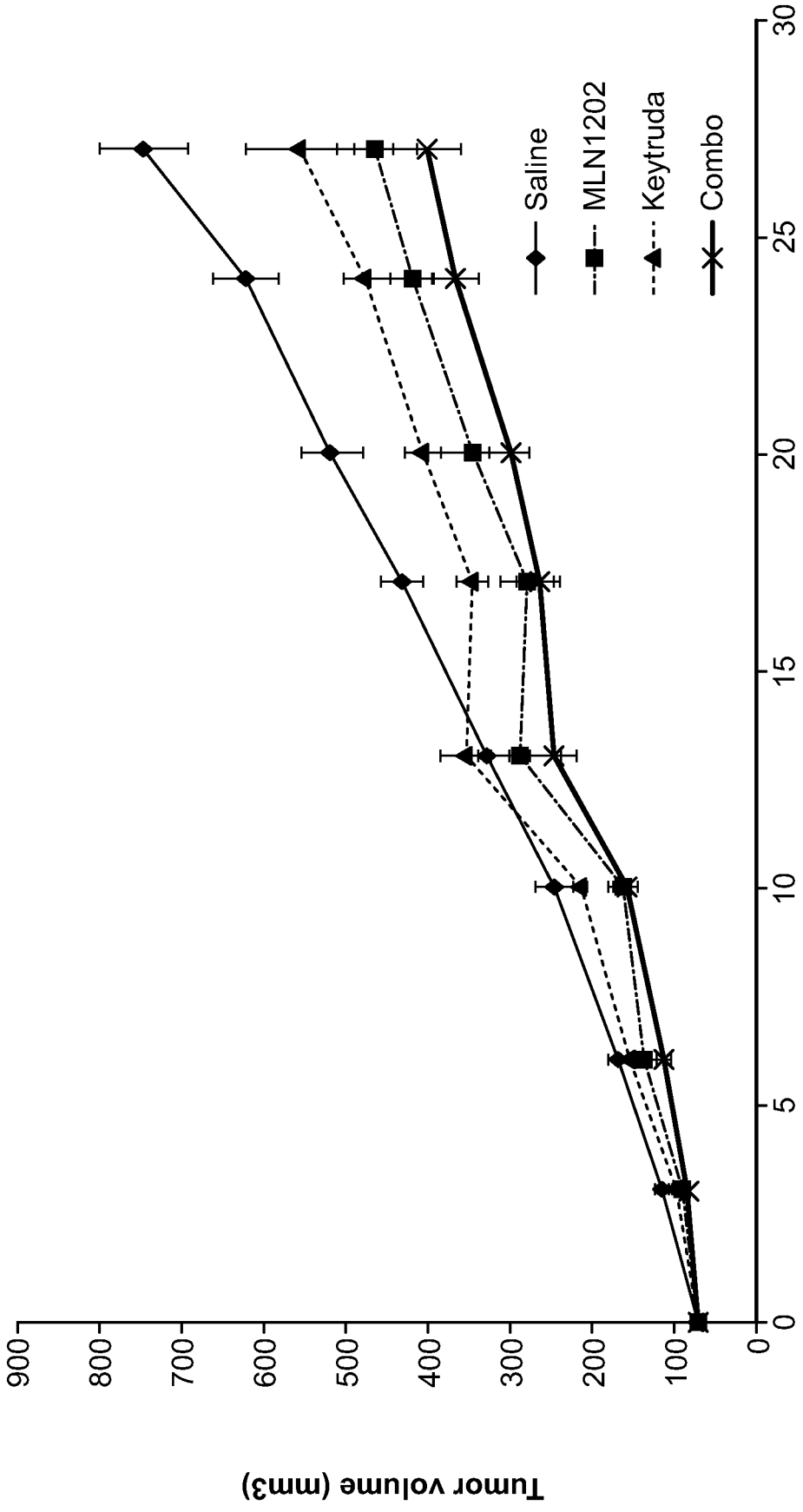

Figure 3: Mean Tumor Volume

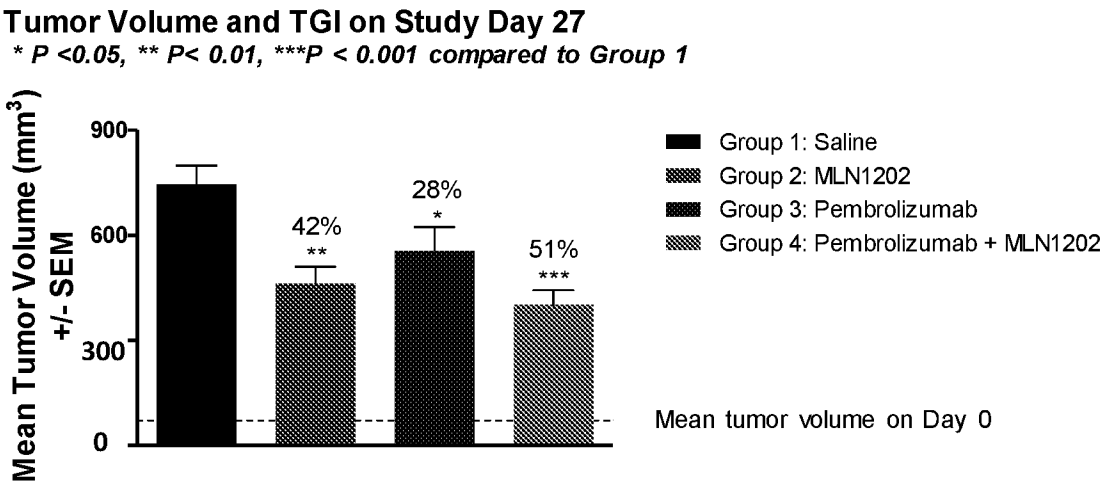

Figure 4: Tumor Growth Inhibition on Study Day 27

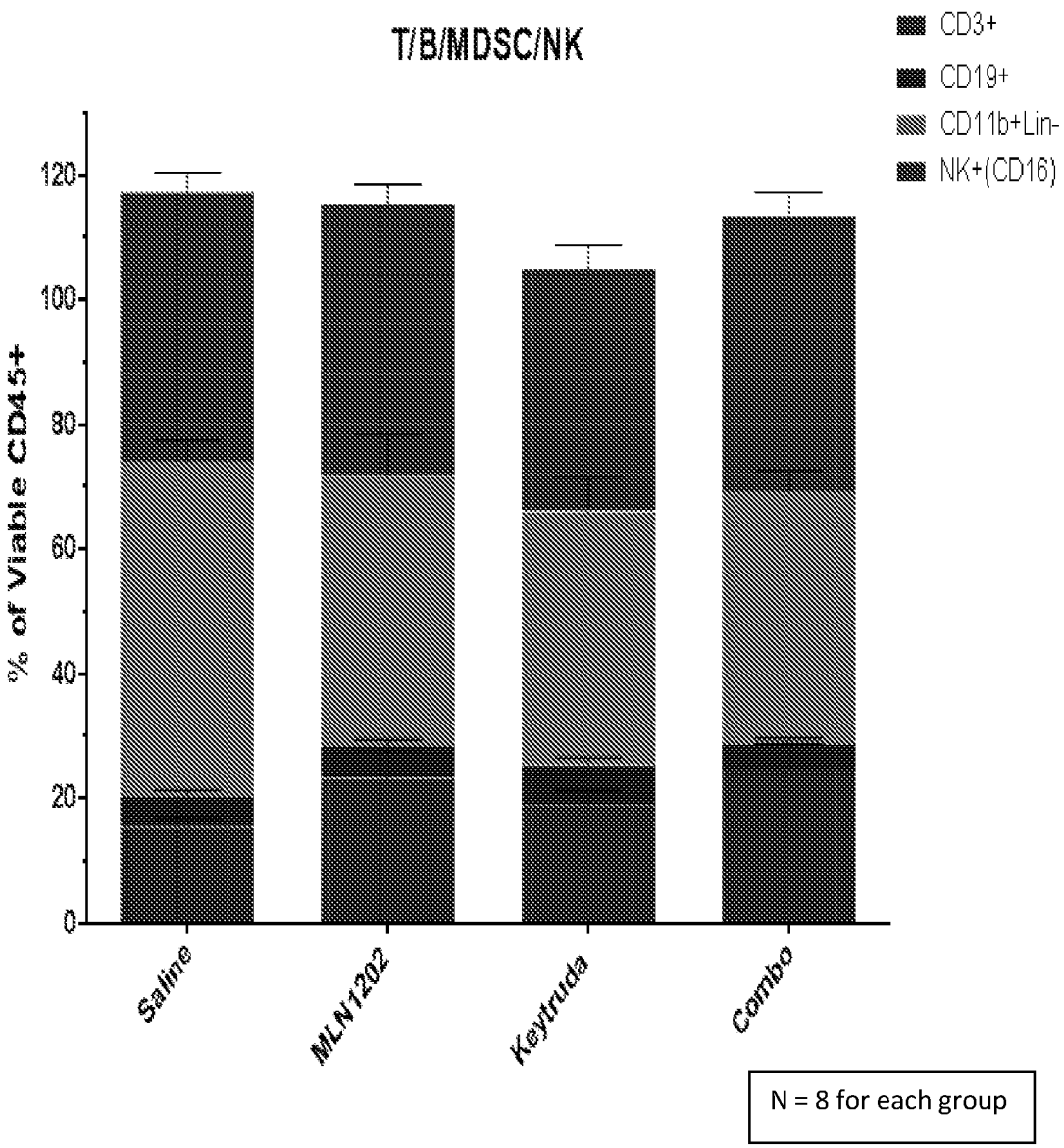

Figure 5A: Immunophenotyping of Tumor infiltrating lymphocyte (TIL) at day 27

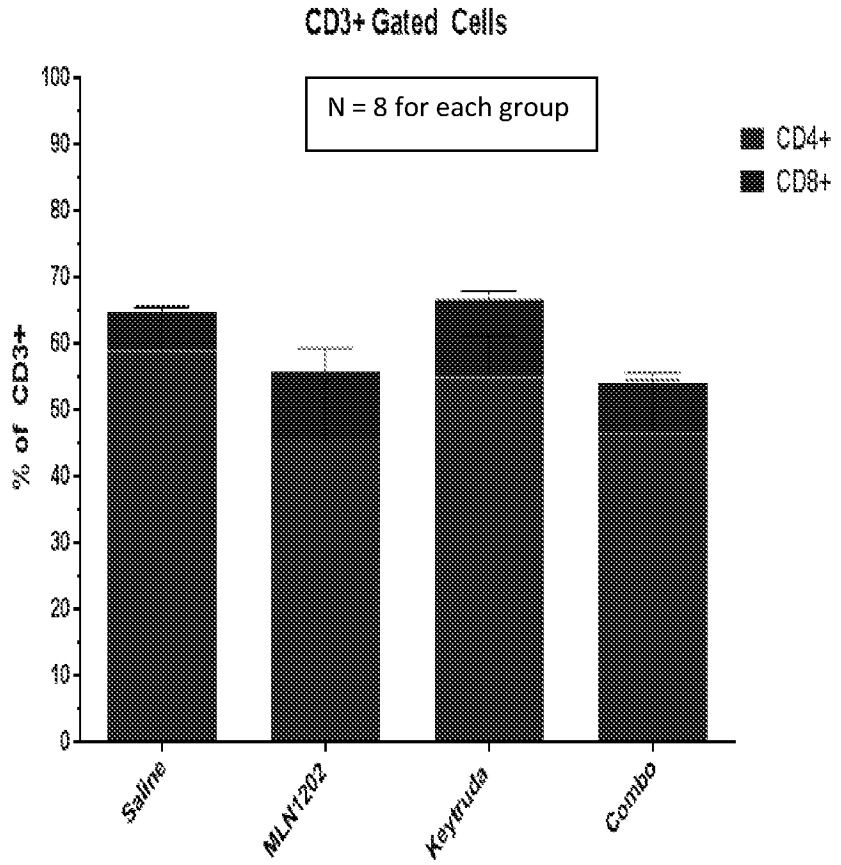

Figure 5B: Immunophenotyping of Tumor infiltrating lymphocyte (TIL) at day 27

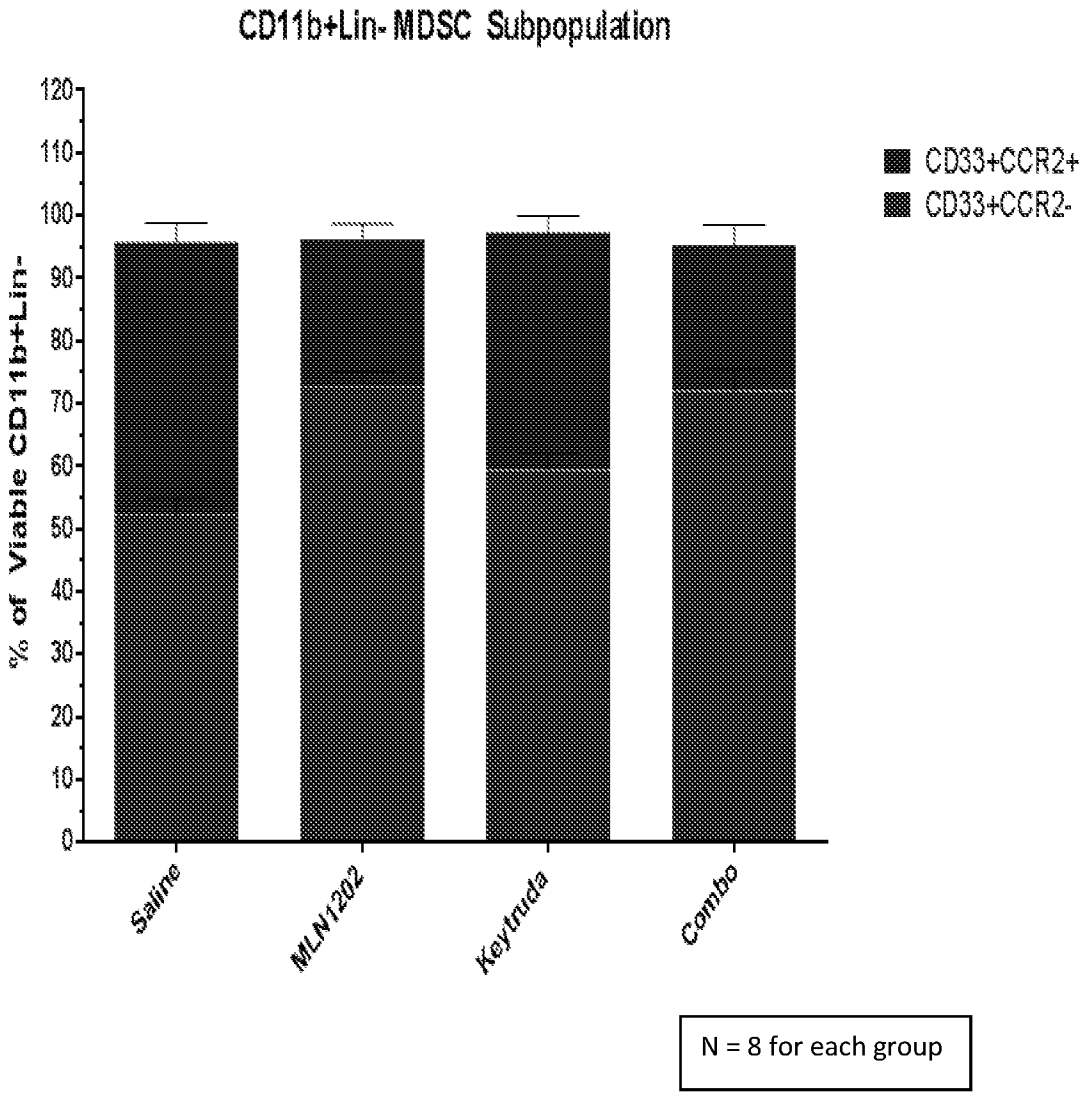

Figure 5C: Immunophenotyping of Tumor infiltrating lymphocyte (TIL) at day 27

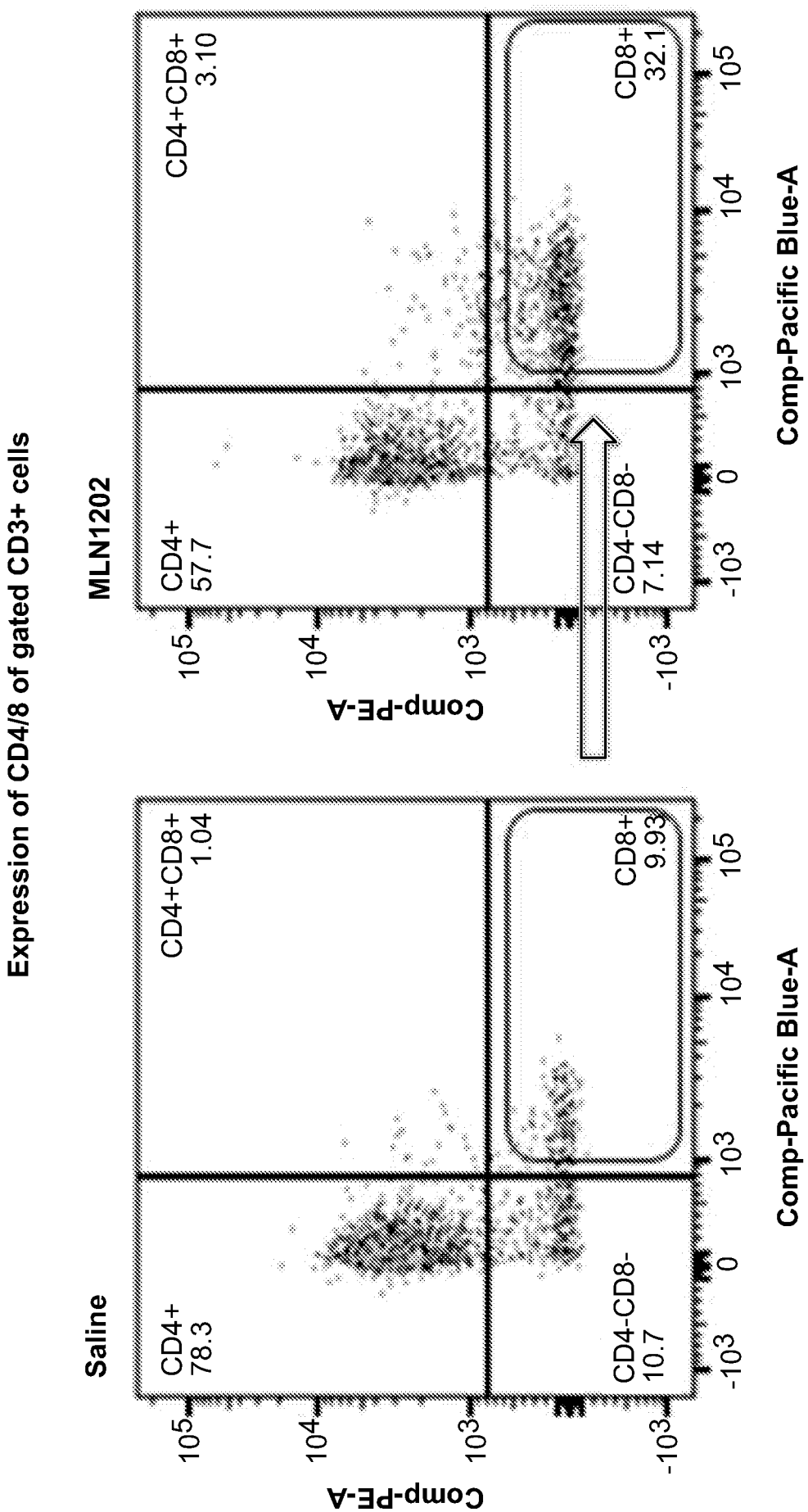

Figure 5D: Expression CD4/8 of gated CD3+ cells

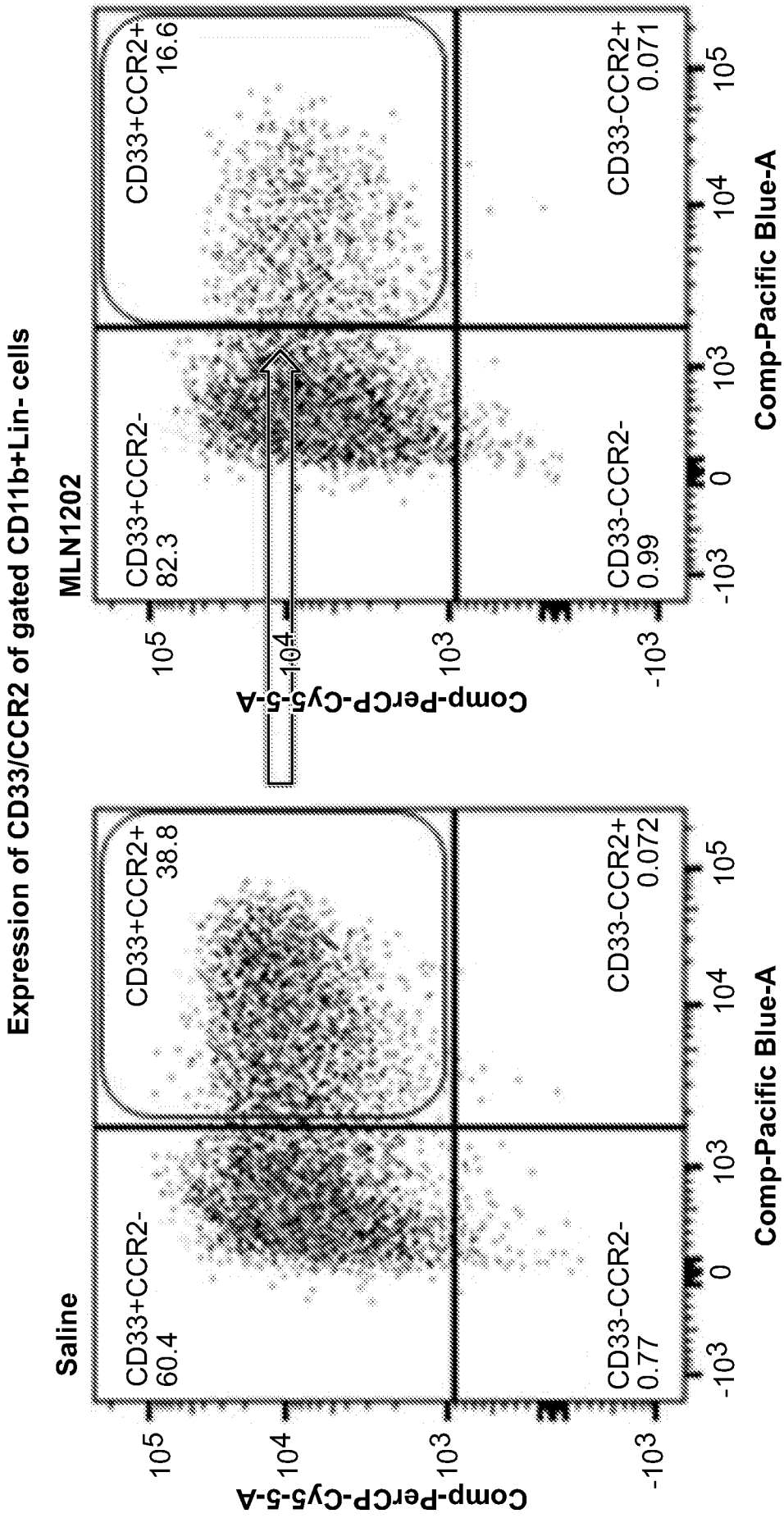

Figure 5E: Expression CD33/CCR2 of gated CD11b+Lin- cells

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/021506

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395 (2006.01) ***A61P 35/00 (2006.01)***

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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)

WPI/EPODOC/FAMPAT/MEDLINE/EMBASE/BIOSIS: PD-1, PD-L1, PD-L2, CCR2, MCP-1-R, pembrolizumab, plozalizumab, cancer, antibodies and related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 105051065 A (SORRENTO THERAPEUTICS INC) 11 November 2015 Paragraph [0136] of the original non-English language document (a machine translation is enclosed **only** for your reference)	1-16
X	US 2015/0166646 A1 (WANG T. ET AL) 18 June 2015 Paragraphs [0004]-[0008], [0110], [0128], [0130], [0148]	1-16
X	WO 2013/149376 A1 (ABBOTT LABORATORIES) 10 October 2013 Page 3, Lines 1-2; Page 6, Lines 2-10; Page 50, Lines 20-22; Tables 1-3	1-16
X	CHUN E. ET AL, CCL2 Promotes Colorectal Carcinogenesis by Enhancing Polymorphonuclear Myeloid-Derived Suppressor Cell Population and Function. *Cell Rep.*, 2 July 2015, Vol. 12, No. 2, pages 244-257 [Retrieved on 2017-06-01] <DOI: 10.1016/J.CELREP.2015.06.024> Abstract, Page 251, Column 2, Paragraph 3; Page 255, Colum 1, Paragraph 1; Figures 5F and 7E	1-16

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

*Special categories of cited documents:

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

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

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

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

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

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/021506

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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