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(54) Title: TREATMENT OF CANCERS WITH GM-CSF ANTAGONISTS

(57) Abstract: The present invention provides, among other things, a method of treating cancer comprising administering a GM-CSF antagonist to the patient in need of treatment, wherein the administration of the GM-CSF antagonist results in inhibition of an immunosuppressive activity of myeloid-derived suppressor cells (MDSCs). The present invention also provides, among other things, a method of inhibiting immunosuppressive activity of myeloid-derived suppressor cells (MDSCs) in a patient suffering from cancer comprising administering a GM-CSF antagonist to the patient.

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TREATMENT OF CANCERS WITH GM-CSF ANTAGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims priority to U.S. provisional patent application Serial No. 62/856,638, filed June 3, 2019, which is hereby incorporated by reference in their entirety for all purposes.

SEQUENCE LISTING

[2] The present specification makes reference to a Sequence Listing (submitted electronically as a .txt file named "KPL-035WO_SL.txt" on June 3, 2020). The .txt file was generated on June 3, 2020 and is 4 KB in size. The entire contents of the Sequence Listing are herein incorporated by reference.

BACKGROUND

[3] Tumor cells express unique antigens that are potentially recognized by the host T cell repertoire and serve as potent targets for tumor immunotherapy. However, tumor cells evade host immunity and express inhibitory cytokines that suppress native antigen presenting effector cell populations. One element in this immunosuppressive milieu is the increased presence of regulatory T cells that are found in the tumor bed, draining lymph nodes, and in the circulation of patients with malignancy. One area of further investigation is the development of therapeutics to reverse tumor-associated anergy and to stimulate effector cells to recognize and eliminate malignant cells.

BRIEF DESCRIPTION OF THE DRAWING

[4] The drawings are for illustration purposes only, not for limitation.

[5] **FIG. 1** is an exemplary bar graph illustrating T cell proliferation assay with CD14+ cells (MDSCs) from blood of patient with pancreatic cancer. T cell proliferation is suppressed following co-culture of healthy donor allogeneic T cells and CD14+ cells sorted from blood of pancreatic cancer patients, compared with those T cells cultured in complete media

alone. T cell proliferation is rescued following culture with an anti-GM-CSFRα antibody and CD14+ cells sorted from blood of pancreatic cancer patients.

[6] **FIG. 2** is an exemplary graph illustrating GM-CSF expression levels in different cancer cell lines.

[7] **FIG. 3** is a series of exemplary bar graphs illustrating that cancer cell-conditioned medium can polarize monocytes to phenotypic MDSCs (CD14+ cells). To generate tumor-conditioned media (CM), four different cell lines were plated and cultured according to methods known in the art. CD14+ monocytes cells were then cultured in the presence of CM for 6 days and analyzed for gene and protein expression. Low levels of HLA-DR biomarker is indicative of MDSC phenotype. An increase in phenotypic MDSCs was observed when CD14+ monocytes were incubated with conditioned medium from GM-CSF-expressing cancer cells, as compared to CD+14 cells that were grown in normal culture medium (control).

[8] **FIG. 4** is a series of exemplary bar graphs illustrating the PD-L1 expression on MDSCs cultured with various media. Data show that cancer cell-conditioned medium (CM) and CM supplemented with recombinant GM-CSF can induce expression of PD-L1 on MDSCs. Additionally, anti-GM-CSFRα antibody (Ab) can reduce PD-L1 expression levels on MDSCs.

[9] **FIG. 5A** and **FIG. 5B** are s a series of exemplary bar graphs illustrating the PD-L1 expression on MDSCs cultured with various media. Data show that cancer cell-conditioned medium (CM) and CM supplemented at Day 1 with recombinant GM-CSF can induce expression of PD-L1 on MDSCs compared to MDSCs that were grown in normal culture medium (Medium). Additionally, anti-GM-CSFRα antibody (Ab) can reduce PD-L1 level on MDSCs grown in CM. Data in **FIG. 5A** show PD-L1 expression when CM and an anti-GM-CSFRα antibody (Ab) are added concurrently. PD-L1 expression was measured after 3 days of treatment. Data in **FIG. 5B** show PD-L1 expression when an anti-GM-CSFRα antibody is added 72 hours after culturing with CM. PD-L1 expression was measured after 24 hours of treatment with the anti-GM-CSFRα antibody.

[10] **FIG. 6** is a series of exemplary bar graphs illustrating T cell proliferation with monocytes treated with conditioned medium from GM-CSF expressing cancer cell lines with and without supplemental human recombinant GM-CSF and/or an anti-GM-CSFRα antibody (Ab). Monocytes were cultured in conditioned medium from GM-CSF expressing cancer cell lines (CM) for three days. T cells ($1x10^5$ cells) were prepared by labeling with 0.1 μM CFSE and stimulation with 10 ng/ mL of IL-2 and 10 uL of soluble CD3/CD28 T cell activator

(ImmunoCult) in IMDM cell culture medium. Then, the stimulated T cells were co-cultured with CM-treated monocytes (at a ratio of 2:1 monocyte: T cell) with or without recombinant GM-CSF (10 ng/mL) and/or anti-GM-CSFRα antibody (100 μg/mL) in a mix lymphocyte reaction (MLR). Stimulated T-cells in IMDM culture medium together with healthy monocytes were used as a control. T-cells were expanded for 5 days, collected and stained for CD4 and CD8, which are markers for helper T and cytotoxic T cells. Cell proliferation was measured by flow cytometry and evaluated by CFSE dilution. Left panel shows the results of the T-cell proliferation assay in terms of % of cells proliferating and right panel shows the results in terms of % of max (MFI) (signal detection of CFSE dilution in CD4+ or CD8+ cells) by flow cytometry.

SUMMARY OF THE INVENTION

[11] The present invention provides, among other things, an improved method for treating cancer based on inhibition of immunosuppressive activity of myeloid-derived suppressor cells using a GM-CSF antagonist. The present invention is based, in part, on a surprising discovery that GM-CSF induces expression of PD-L1 on MDSCs that have immunosuppressive activity and that this expression can be suppressed by antagonizing GM-CSF. The present invention also provides methods for treating cancer using a GM-CSF antagonist in combination with other cancer therapies as further described herein.

[12] In some aspects, the present invention provides a method of treating cancer comprising administering a GM-CSF antagonist to the patient in need of treatment, wherein the administration of the GM-CSF antagonist results in inhibition of an immunosuppressive activity of myeloid-derived suppressor cells (MDSCs).

[13] In some aspects, the present invention provides a method of inhibiting immunosuppressive activity of myeloid-derived suppressor cells (MDSCs) in a patient suffering from cancer comprising administering a GM-CSF antagonist to the patient.

[14] In some aspects, the present invention provides a method of enhancing immune response for cancer treatment comprising administering a GM-CSF antagonist to a patient receiving a cancer treatment, wherein the immune response is increased as compared to a control.

[15] In some embodiments, the immune response is a percentage of T cell proliferation. In some embodiments, T cells are CD8 positive (CD8+). In some embodiments, T cells are CD4 positive (CD4+). In some embodiments, T cells are double-positive for CD8 and CD4 (CD8+/CD4+).

[16] In some embodiments, the control is indicative of the immune response level in the patient prior to the administration of GM-CSF antagonist. In some embodiments, the control is a reference immune response level in a control patient receiving the cancer treatment without GM-CSF antagonist or a reference immune response level based on historical data.

[17] In some embodiments, the cancer therapy is an immunotherapy.

[18] In some embodiments, the administering the GM-CSF antagonist increases the efficacy of the immunotherapy.

[19] In one aspect, the present invention provides, among other things, a method of suppressing PD-L1 in a cancer patient comprising administering a GM-CSF antagonist to a patient in need of treatment as compared to a control.

[20] In some embodiments, the administering the GM-CSF antagonist decreases a level of PD-L1 in a cancer patient.

[21] In some embodiments, the control is indicative of the PD-L1 level in the patient prior to the administration of GM-CSF antagonist.

[22] In some embodiments, the control is a reference PD-L1 level in a control patient receiving the cancer treatment without GM-CSF antagonist or a reference PD-L1 level based on historical data.

[23] In some embodiments, the level of PD-L1 in the patient is decreased by at least 10% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 15% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 20% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 30% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 40% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 45% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 50% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 60% as compared to the control. In some embodiments, the level of PD-

L1 in the patient is decreased by at least 70% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 75% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 80% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 85% as compared to the control. In some embodiments, the level of PD-L1 in the patient is decreased by at least 90% as compared to the control.

[24] In some embodiments, the PD-L1 is expressed on MDSCs. In some embodiments, the PD-L1 is expressed on circulating MDSCs. In some embodiments, the PD-L1 is expressed on plasma-derived MDSCs. In some embodiments, the PD-L1 is expressed on tumor cells. In some embodiments, PD-L1 is expressed on tumor-infiltrating immune cells.

[25] In some embodiments, the patient has circulating myeloid derived suppressor cells (MDSCs).

[26] In some embodiments, the patient suffers from a cancer with a low level of infiltrating T cells.

[27] In some embodiments, the patient suffers from an immune checkpoint inhibitor (ICI) refractory cancer.

[28] In some embodiments, the patient suffers from a late stage or metastatic cancer.

[29] In some embodiments, the patient suffers from a cancer selected from breast cancer, colorectal cancer (CRC), prostate cancer, melanoma, bladder carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, hepatocellular carcinoma, gastric cancer, non-small cell lung cancer (NSCLC), , small cell lung cancer (SCLC), head and neck squamous cell carcinoma, non-Hodgkin lymphoma, cervical cancer, gastrointestinal cancer, urogenital cancer, brain cancer, mesothelioma, renal cell cancer, gynecological cancer, ovarian cancer, endometrial cancer, lung cancer, gastrointestinal cancer, pancreatic cancer, oesophageal cancers, hepatocellular cancer, cholangiocellular cancer, brain cancers, mesothelioma, malignant melanoma, Merkel Cell Carcinoma, multiple myeloma, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome or acute lymphoblastic leukemia.

[30] In some embodiments, the patient suffers from a cancer selected from Stage IV breast cancer, Stage IV colorectal cancer (CRC), prostate cancer, or melanoma.

[31] The method of any one of the preceding claims, wherein the method further comprises administering at least one other cancer therapy to the patient.

[32] In some embodiments, the at least one other cancer therapy is chemotherapy, MDSC-targeted therapy, immunotherapy, radiation therapy and combinations thereof.

[33] In some embodiments, the GM-CSF antagonist and the other cancer therapy are administered concurrently.

[34] In some embodiments, the GM-CSF antagonist and the other cancer therapy are administered sequentially.

[35] In some embodiments, the patient has received a treatment with the other cancer therapy prior to the administration of the GM-CSF antagonist.

[36] In some embodiments, the patient has received a treatment with the GM-CSF antagonist prior to the administration of the other cancer therapy.

[37] In some embodiments, the other cancer therapy is an ICI.

[38] In some embodiments, the ICI antagonizes the activity of PD-1, CTLA-4, B7, BTLA, HVEM, TIM-3, GAL-9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK1, CHK2, or A2aR.

[39] In some embodiments, the ICI is selected from an anti-PD-1 antibody (optionally pembrolizumab, nivolumab, cemiplimab), an anti-PD-L1 antibody (optionally atezolizumab, avelumab, durvalumab), an anti-CTLA-4 antibody (optionally ipillimumab), an anti-PD-L2 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-BTLA antibody, an anti-HVEM antibody, an anti-TIM-3 antibody, an anti-GAL-9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-2B4 antibody, an anti-CD160 antibody, an anti-CGEN-15049 antibody, an anti-CHK1 antibody, an anti-CHK2 antibody, an anti-A2aR antibody, an anti-B-7 antibody, and combinations thereof.

[40] In some embodiments, the ICI is an anti-PD-L1 antibody. In some embodiments, the ICI is an anti-PD-L1 antibody.

[41] In some embodiments, the method further comprises administering a chemotherapy agent to the patient.

[42] In some embodiments, the MDSC-targeted therapy is selected from an anti-CFS-1R antibody, an anti-IL-6 antibody, all-trans retinoic acid, axitinib, entinostat, gemcitabine, or phenformin, and combinations thereof.

[43] In some embodiments, the immunotherapy is selected from a monoclonal antibody, cytokine, cancer vaccine, T-cell engaging therapies, and combinations thereof .

[44] In some embodiments, the monoclonal antibody is selected from an anti-CD3 antibody, an anti-CD52 antibody, an anti-PDl antibody, an anti-PD-Ll antibody, an anti-CTLA4 antibody, an anti-CD20 antibody, an anti- BCMA antibody, bi-specific antibodies, or bispecific T-cell engager (BiTE) antibodies, and combinations thereof.

[45] In some embodiments, the cytokines are selected from IFNa, IFNp, IFNy, IFN , IL-2, IL-7, IL-15, IL-21, IL-11, IL-12, IL-18, hGM- CSF, TNFa, or any combination thereof.

[46] In some embodiments, the GM-CSF antagonist is an anti-GM-CSF antibody or a fragment thereof.

[47] In some embodiments, the GM-CSF antagonist is a soluble GM-CSF receptor.

[48] In some embodiments, the GM-CSF antagonist is an anti-GM-CSF receptor antibody or a fragment thereof.

[49] In some embodiments, the anti-GM-CSF receptor antibody or a fragment thereof is an anti- GM-CSFRα antibody or a fragment thereof.

[50] In some embodiments, the anti- GM-CSFRα antibody or a fragment thereof is a monoclonal antibody specific for human GM-CSFRα.

[51] In some embodiments, the anti-GM-CSFRα antibody is human or humanized IgG4 antibody.

[52] In some embodiments, the anti-GM- CSFRα antibody is mavrilimumab.

[53] In some embodiments, the anti-GM- CSFRα antibody a fragment thereof comprises a light chain complementary-determining region 1 (LCDR1) defined by SEQ ID NO: 6, a light chain complementary-determining region 2 (LCDR2) defined by SEQ ID NO: 7, and a light chain complementary-determining region 3 (LCDR3) defined by SEQ ID NO: 8; and a heavy chain complementary-determining region 1 (HCDR1) defined by SEQ ID NO: 3, a heavy chain complementary-determining region 2 (HCDR2) defined by SEQ ID NO: 4, and a heavy chain complementary-determining region 3 (HCDR3) defined by SEQ ID NO: 5.

[54] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in reduced level of MDSCs in the patient as compared to a control.

[55] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in reduced level of MDSC-mediated immunosuppressive activity in the patent as compared to a control.

[56] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in reduced percentage of Lin-CD14+HLA-DR-M-MDSCs in the peripheral blood of the patient as compared to a control.

[57] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in increased percentage of mature MDSC cells in the patient as compared to a control.

[58] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in a reduced level of Treg cells, macrophages, and/or neutrophils as compared to a control.

[59] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in a decreased level of an inhibitory cytokine.

[60] In some embodiments, the inhibitory cytokine is selected from IL-10 and TGFβ.

[61] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in a decreased level of an immune suppressive factor.

[62] In some embodiments, the immune suppressive factor is selected from arginase 1, inducible nitric oxide synthase (iNOS), peroxynitrite, nitric oxide, reactive oxygen species, tumor associated macrophages, and combinations thereof.

[63] In some embodiments, the administration of the GM-CSF antagonist and/or the ICI results in an increased level of CD4+ T effector cells as compared to a control.

[64] In some embodiments, the control is a pre-treatment level or percentage in the patient, or a reference level or percentage based on historical data.

[65] In some aspects, the present invention provides a pharmaceutical composition for treating cancer comprising a GM-CSF antagonist and an ICI.

[66] In some embodiments, the GM-CSF antagonist is an anti-GM-CSF antibody or a fragment thereof.

[67] In some embodiments, the GM-CSF antagonist is a soluble GM-CSF receptor.

[68] In some embodiments, the GM-CSF antagonist is an anti-GM-CSF receptor antibody or a fragment thereof.

[69] In some embodiments, the anti-GM-CSF receptor antibody or a fragment thereof is an anti- GM-CSFRα antibody or a fragment thereof.

[70] In some embodiments, the anti-GM-CSFRα antibody or a fragment thereof is a monoclonal antibody specific for human GM-CSFRα.

[71] In some embodiments, the anti-GM-CSFRα antibody is human or humanized IgG4 antibody.

[72] In some embodiments, the anti-GM-CSFRα antibody is mavrilimumab.

[73] In some embodiments, the anti-GM-CSFRα antibody a fragment thereof comprises a light chain complementary-determining region 1 (LCDR1) defined by SEQ ID NO: 6, a light chain complementary-determining region 2 (LCDR2) defined by SEQ ID NO: 7, and a light chain complementary-determining region 3 (LCDR3) defined by SEQ ID NO: 8; and a heavy chain complementary-determining region 1 (HCDR1) defined by SEQ ID NO: 3, a heavy chain complementary-determining region 2 (HCDR2) defined by SEQ ID NO: 4, and a heavy chain complementary-determining region 3 (HCDR3) defined by SEQ ID NO: 5.

[74] In some embodiments, the ICI antagonizes the activity of PD-1, CTLA-4, B7, BTLA, HVEM, TIM-3, GAL-9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK1, CHK2, A2aR, and combinations thereof.

[75] In some embodiments, the ICI is selected from an anti-PD-1 antibody (optionally pembrolizumab, nivolumab, cemiplimab), an anti-PD-L1 antibody (optionally atezolizumab, avelumab, durvalumab), an anti-CTLA-4 antibody (optionally ipillimumab), an anti-PD-L2 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-BTLA antibody, an anti-HVEM antibody, an anti-TIM-3 antibody, an anti-GAL-9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-2B4 antibody, an anti-CD160 antibody, an anti-CGEN-15049 antibody, an anti-CHK1 antibody, an anti-CHK2 antibody, an anti-A2aR antibody, an anti-B-7 antibody, and combinations thereof.

[76] In some aspects, the present invention provides a kit for treating cancer comprising a pharmaceutical composition comprising a GM-CSF antagonist and pharmaceutical composition comprising at least one other cancer therapy selected from a chemotherapy, MDSC-targeted therapy, immunotherapy, radiation therapy and combinations thereof.

[77] In some embodiments, the immunotherapy is an ICI selected from an anti-PD-1 antibody (optionally pembrolizumab, nivolumab, cemiplimab), an anti-PD-L1 antibody (optionally atezolizumab, avelumab, durvalumab), an anti-CTLA-4 antibody (optionally ipillimumab), an anti-PD-L2 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-BTLA antibody, an anti-HVEM antibody, an anti-TIM-3 antibody, an anti-GAL-9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-2B4 antibody, an anti-CD160 antibody, an anti-CGEN-15049 antibody, an anti-CHK1 antibody, an anti-CHK2 antibody, an anti-A2aR antibody, an anti-B-7 antibody, and combinations thereof.

DEFINITIONS

[78] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

[79] *Antibody*: As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that binds (immunoreacts with) an antigen. By "binds" or "immunoreacts with" is meant that the antibody reacts with one or more antigenic determinants of the desired. Antibodies include, antibody fragments. Antibodies also include, but are not limited to, polyclonal, monoclonal, chimeric dAb (domain antibody), single chain, Fab, Fab', F(ab')2 fragments, scFvs, and Fab expression libraries. An antibody may be a whole antibody, or immunoglobulin, or an antibody fragment.

[80] *Amino acid*: As used herein, term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some embodiments, an amino acid is an l-amino acid. "Standard amino acid" refers to any of the twenty standard l-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, "synthetic amino

acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxyl- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (*e.g.*, methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, *etc.*). The term "amino acid" is used interchangeably with "amino acid residue," and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[81] *Amelioration*: As used herein, the term "amelioration" is meant the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes, but does not require complete recovery or complete prevention of a disease condition. In some embodiments, amelioration includes increasing levels of relevant protein or its activity that is deficient in relevant disease tissues.

[82] *Approximately or about*: As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[83] *Delivery*: As used herein, the term "delivery" encompasses both local and systemic delivery.

[84] *Improve, increase, inhibit or reduce*: As used herein, the terms "improve," "increase" "inhibit" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein, e.g., a subject who is administered a placebo. A

"control subject" is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[85] *"Inhibition" or "inhibiting"*: As used herein "inhibition" or "inhibiting," or grammatical equivalents, means reduction, decrease or inhibition of biological activity. *Neutralization*: As used herein, neutralization means reduction or inhibition of biological activity of the protein to which the neutralizing antibody binds, in this case GM-CSFRα, e.g. reduction or inhibition of GM-CSF binding to GM-CSFRα, or of signaling by GM-CSFRα e.g. as measured by GM-CSFRα-mediated responses. The reduction or inhibition in biological activity may be partial or total. The degree to which an antibody neutralizes GM-CSFRα is referred to as its neutralizing potency.

[86] *Patient:* As used herein, the term "patient" refers to any organism to which a provided composition may be administered, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre- and post-natal forms.

[87] *Pharmaceutically acceptable*: The term "pharmaceutically acceptable" as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[88] *Substantial identity:* The phrase "substantial identity" is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially identical" if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLAS TN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J Mal. Biol.*,215(3): 403-410, 1990; Altschul, *et al., Methods in Enzymology;* Altschul *et al., Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis *et al., Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins,* Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to

identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80,85,90,95, 100,125,150,175,200,225,250,275,300,325,350,375,400,425,450,475, 500 or more residues.

[89] *Subject*: As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[90] *Substantially*: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[91] *Systemic distribution or delivery*: As used herein, the terms "systemic distribution," "systemic delivery," or grammatical equivalent, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body's circulation system, e.g., blood stream. Compared to the definition of "local distribution or delivery."

[92] *Therapeutically effective amount*: As used herein, the term "therapeutically effective amount" of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[93] *Treating*: As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

DETAILED DESCRIPTION

[94] The present invention provides, among other things, method of treating cancer by inhibiting immunosuppressive activity of myeloid-derived suppressor cells (MDSCs) in a patient in need of treatment using a GM-CSF antagonist. In some embodiments, a GM-CSF antagonist is used in combination with an immune checkpoint inhibitor. It is contemplated that the present invention is particularly effective in treating immune checkpoint inhibitory (ICI) refractory or resistant cancers, or late stage or metastatic cancers.

[95] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Myeloid-derived suppressor cells (MDSCs)

[96] MDSC are a heterogenous group of immune cells from the myeloid lineage. MDSCs strongly expand in pathological situations such as chronic infections and cancer and are distinguished from other myeloid cell types in which they possess strong immunosuppressive activities, rather than immunostimulatory properties. Monocytes that have diminished or no HLA-DR expression, called $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes are grouped into MDSCs and can alter adaptive immunity and produce immunosuppression.

[97] MDSCs accumulate in the peripheral blood, lymphoid organ, spleen, and tumor sites in cancer, infection, chronic inflammation, transplantation, and autoimmunity. The specific pathways by which tumors recruit, expand, and activate MDSCs remain unknown, but increasing evidence exists for the involvement of interleukin (IL-1β), IL-6, cyclooxyhenase 2 (COX2)-generated PGE_2, high concentrations of GM-CSF, M-CSF, vascular endothelial growth factor

(VEGF), IL-10, transforming growth beta (TGFβ), indoleamine 2, 3-dioxyhenase (IDO), FLT3 ligand, and stem cell factor.

[98] Co-culture of immune competent cells with tumor cell lines as been shown to induce tolerogenic DC or MDSC. Previous studies also suggest that tumor cells release GM-CSF, which induces granulocyte ROS production to inhibit T-cell function. Additionally, the expression of programmed death ligand 1 (PD-L1) is increased on the surface of MDSC in murine tumor models, though the role of this in MDSC-mediated suppression remains unclear.

Programmed death ligand 1 (PD-L1)

[99] Programmed death ligand 1 (PD-L1; also known as CD274) is an immune checkpoint protein that binds to its receptor PD-1. PD-L1 is widely expressed on various cell types, mainly in tumor cells, MDSCs, monocytes, macrophages, natural killer (NK) cells, dendritic cells (DCs), and activated T cells and also on immune-privileged sites such as the brain, cornea, and retina. In normal physiological conditions, the activation of the PD-1/PD-L1 signaling pathway is closely related to the induction and maintenance of peripheral tolerance, maintenance of T cell immune homeostasis, avoiding hyperactivation and protecting against immune-mediated tissue damage. In disease states, PD-L1 interacts with its receptor programmed death 1 (PD-1), transmitting a negative signal to control a series of processes of T cell-mediated cellular immune responses, including priming, growth, proliferation and apoptosis, and functional maturation, leading to immune escape.

[100] Immune checkpoint inhibitors (ICIs) have changed the treatment landscape of many tumors, inducing durable responses in some cases, Tumor mutational load, $CD8^+$ T cell density and PD-L1 expression have each been proposed as distinct biomarkers of response to PD-1/-L1 antagonists. One of the main challenges for immune checkpoint blockade antibodies lies in malignancies with limited T-cell responses or immunologically “cold” tumors. These cold tumors contain few infiltrating T cells and are not recognized and do not provoke a strong response by the immune system, making them difficult to treat with current immunotherapies. Present inventors surprisingly found that GM-CSF upregulates PD-L1, contributing to immunosuppressive activity. Converting a “cold” tumor to a “hot” tumor is one of the milestones in cancer treatment.

GM-CSF Antagonists

GM-CSF signaling

[101] GM-CSF is a type I proinflammatory cytokine which enhances survival and proliferation of a broad range of hematopoietic cell types. It is a growth factor first identified as an inducer of differentiation and proliferation of myeloid cells (e.g., neutrophils, basophils, eosinophils, monocytes, and macrophages) (Wicks IP and Roberts AW. Nat Rev Rheumatol. 2016, 12(1):37-48). Studies using different approaches have demonstrated that with GM-CSF overexpression, pathological changes almost always follow (Hamilton JA et al., Growth Factors. 2004, 22(4):225-31). GM-CSF enhances trafficking of myeloid cells through activated endothelium of blood vessels and can also contribute to monocyte and macrophage accumulation in blood vessels during inflammation. GM-CSF also promotes activation, differentiation, survival, and proliferation of monocytes and macrophages as well as resident tissue macrophages in inflamed tissues. It regulates the phenotype of antigen-presenting cells in inflamed tissues by promoting the differentiation of infiltrating monocytes into M1 macrophages and monocyte-derived dendritic cells (MoDCs). Moreover, the production of IL-23 by macrophages and MoDCs, in combination with other cytokines such as IL-6 and IL-1, modulates T-cell differentiation.

[102] Together with M-CSF (macrophage-colony stimulating factor), GM-CSF regulates the number and function of macrophages. Macrophages activated by GM-CSF acquire a series of effector functions, all of which identify them as inflammatory macrophages. GM-CSF-activated macrophages produce proinflammatory cytokines, including TNF, IL-1β, IL-6, IL-23 and IL-12 and chemokines, such as CCL5, CCL22, and CCL24, which recruit T cells and other inflammatory cells into the tissue microenvironment.

[103] The GM-CSF receptor is a member of the haematopoietin receptor superfamily. It is heterodimeric, consisting of an alpha and a beta subunit. The alpha subunit is highly specific for GM-CSF, whereas the beta subunit is shared with other cytokine receptors, including IL-3 and IL-5. This is reflected in a broader tissue distribution of the beta receptor subunit. The alpha subunit, GM-CSFRα, is primarily expressed on myeloid cells and non-haematopoietic cells, such as neutrophils, macrophages, eosinophils, dendritic cells, endothelial cells and respiratory epithelial cells. Full length GM-CSFRα is a 400 amino acid type I membrane glycoprotein that belongs to the type I cytokine receptor family and consists of a 22 amino acid signal peptide (positions 1-22), a 298 amino acid extracellular domain (positions 23-320), a transmembrane

domain from positions 321-345 and a short 55 amino acid intra-cellular domain. The signal peptide is cleaved to provide the mature form of GM-CSFRα as a 378 amino acid protein. Complementary DNA (cDNA) clones of the human and murine GM-CSFRα are available and, at the protein level, the receptor subunits have 36% identity. GM-CSF is able to bind with relatively low affinity to the α subunit alone (Kd 1-5 nM) but not at all to the β subunit alone. However, the presence of both α and β subunits results in a high affinity ligand-receptor complex (Kd~100 pM). GM-CSF signaling occurs through its initial binding to the GM-CSFRα chain and then cross-linking with a larger subunit the common β chain to generate the high affinity interaction, which phosphorylates the JAK-STAT pathway. This interaction is also capable of signaling through tyrosine phosphorylation and activation of the MAP kinase pathway.

[104] Pathologically, GM-CSF has been shown to play a role in exacerbating inflammatory, respiratory and autoimmune diseases. Neutralization of GM-CSF binding to GM-CSFRα is therefore a therapeutic approach to treating diseases and conditions mediated through GM-CSFR. Accordingly, the invention relates to a binding member that binds human GM-CSF or GM-CSFRα, or inhibits the binding of human GM-CSF to GM-CSFRα, and/or inhibits signaling that results from GM-CSF ligand binding to the receptor. Upon ligand binding, GM-CSFR triggers stimulation of multiple downstream signaling pathways, including JAK2/STAT5, the MAPK pathway, and the PI3K pathway; all relevant in activation and differentiation of myeloid cells. The binding member may be a reversible inhibitor of GM-CSF signaling through the GM-CSFR.

GM-CSF Antagonists

[105] A GM-CSF antagonist suitable for the present invention includes those therapeutic agents that can reduce, inhibit or abolish one or more GM-CSF mediated signaling including those described herein. For example, a suitable GM-CSF antagonist according to the invention includes, but is not limited to an anti-GM-CSF antibody or a fragment thereof, a soluble GM-CSF receptor and variants thereof including fusion proteins such as a GM-CSF soluble receptor-Fc fusion protein, an anti-GM-CSF receptor antibody or a fragment thereof, to name but a few.

[106] In some embodiments, a suitable GM-CSF antagonist is an anti- GM-CSFRα antibody. Exemplary anti-GM-CSFRα monoclonal antibodies include those described in the international application PCT/GB2007/001108 filed on 03-27-2007 which published as WO2007/110631, the EP application 120770487 filed on 10-10-2010, US Application 11/692,008 filed on 03-27-2007, US Application 12/294,616 filed on 09-25-2008, US Application 13/941,409 filed on 07-12-2013, US Application 14/753,792 filed on 11/30/2010, international application PCT/EP2012/070074 filed on 10-10-2012, which published as WO/2013/053767, international application PCT/EP2015/060902 filed on 05-18-2015, which published as WO2015/177097, international application PCT/EP2017/062479, filed on 05-23-2017, each of which are hereby incorporated by reference in their entirety. In one embodiment, the anti-GM-CSFRα monoclonal antibody is mavrilimumab. WO2007/110631 reports the isolation and characterization of the anti-GM-CSFRα antibody mavrilimumab and variants of it, which share an ability to neutralize the biological activity of GM-CSFRα with high potency. The functional properties of these antibodies are believed to be attributable, at least in part, to binding a Tyr-Leu-Asp-Phe-Gln motif at positions 226 to 230 of human GM-CSFRα, thereby inhibiting the association between GM-CSFRα and its ligand GM-CSF. Mavrilimumab is a human IgG4 monoclonal antibody designed to modulate macrophage activation, differentiation and survival by targeting the GM-CSFRα. It is a potent neutralizer of the biological activity of GM-CSFRα and, was shown to exert therapeutic effects by binding GM-CSFRα on leukocytes within the synovial joints of RA patients, leading to reduced cell survival and activation. The safety profile of the GM-CSFRα antibody mavrilimumab for *in vivo* use to date has been established in a Phase II clinical trial for rheumatoid arthritis (RA).

[107] In certain embodiments, the antibody is comprised of two light chains and two heavy chains. The heavy chain variable domain (VH) comprises an amino acid sequence identified in SEQ ID NO: 1. The light chain variable domain (VL) comprises an amino acid sequence identified in SEQ ID NO: 2. The heavy and light chains each comprise complementarity determining regions (CDRs) and framework regions in the following arrangement:

FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

[108] The mavrilimumab antibody heavy chain comprises CDRs: HCDR1, HCDR2, HCDR3 as identified by the amino acid sequences in SEQ ID NO: 3, 4 and 5 respectively. The light chain comprises CDRs: LCDR1, LCDR2, LCDR3 as identified by the amino acid sequences in SEQ ID NO: 6, 7 and 8 respectively.

Anti-GM-CSFRα Heavy Chain Variable Domain Amino Acid Sequence

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSIHWVRQAPGKGLEWM
GGFDPEENEIVYAQRFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCAI
VGSFSPLTLGLWGQGTMVTVSS **(SEQ ID NO: 1)**

Anti-GM-CSFRα Light Chain Variable Domain Amino Acid Sequence

QSVLTQPPSVSGAPGQRVTISCTGSGSNIGAPYDVSWYQQLPGTAPKLLIY
HNNKRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCATVEAGLSGSVF
GGGTKLTVL **(SEQ ID NO: 2)**

Anti-GM-CSFRa Heavy Chain Variable Domain CDR1 (HCDR1) Amino Acid Sequence

ELSIH **(SEQ ID NO: 3)**

Anti-GM-CSFRα Heavy Chain Variable Domain CDR2 (HCDR2) Amino Acid Sequence

GFDPEENEIVYAQRFQG **(SEQ ID NO: 4)**

Anti-GM-CSFRα Heavy Chain Variable Domain CDR3 (HCDR3) Amino Acid Sequence

VGSFSPLTLGL **(SEQ ID NO: 5)**

Anti-GM-CSFRα Light Chain Variable Domain CDR 1 (LCDR1) Amino Acid Sequence

TGSGSNIGAPYDVS **(SEQ ID NO: 6)**

Anti-GM-CSFRα Light Chain Variable Domain CDR 2 (LCDR2) Amino Acid Sequence

HNNKRPS **(SEQ ID NO: 7)**

Anti-GM-CSFRa Light Chain Variable Domain CDR3 (LCDR3) Amino Acid Sequence

ATVEAGLSGSV **(SEQ ID NO: 8)**

[109] In some embodiments the anti-GM-CSFRα antibody for cancer treatment is a variant of mavrilimumab, selected from the GM-CSFα binding members disclosed in the application WO2007/11063 and WO2013053767, which is incorporated by reference in its entirety.

[110] In some embodiments the anti-GM-CSFRα antibody for cancer treatment comprises CDR amino acid sequences with at least 75%, 80%, 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity with one or more of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

[111] In some embodiments the anti-GM-CSFRα antibody comprises a light chain variable domain having an amino acid sequence at least 90% identical to SEQ ID NO: 2 and a heavy chain variable domain having an amino acid sequence at least 90% identical to SEQ ID NO: 1. In some embodiments of the invention, an anti-GM-CSFRα antibody has a light chain variable domain amino acid sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NO: 2 and a heavy chain variable domain amino acid sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NO: 1. In some embodiments of the invention, an anti-GM-CSFRα antibody comprises a light chain variable domain that has the amino acid sequence set forth in SEQ ID NO: 2 and a heavy chain variable domain that has the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments of the invention, a heavy chain constant region of an anti-GM-CSFRα antibody comprises CH1, hinge and CH2 domains derived from an IgG4 antibody fused to a CH3 domain derived from an IgG1 antibody. In some embodiments of the invention, a heavy chain constant region of an anti-GM-CSFRα antibody is, or is derived from, an IgG1, IgG2 or IgG4 heavy chain constant region. In some embodiments of the invention, a light chain constant region of an anti-GM-CSFRα antibody is, or is derived from, a lambda or kappa light chain constant region.

[112] In some embodiments, the anti-GM-CSFRα inhibitor is a fragment of mavrilimumab antibody. In some embodiments the inhibitor comprises a single chain variable fragment (ScFv) comprising at least any one of the CDR sequences of SEQ ID NO: 3, 4, 5, 6, 7, or 8. In some embodiments the inhibitor is a fusion molecule comprising at least any one of the

CDR sequences of SEQ ID NO: 3, 4, 5, 6, 7, or 8. In some embodiments, the anti-GM-CSFRα inhibitor sequence is a bispecific antibody comprising at least one of the CDR sequences of SEQ ID NO: 3, 4, 5, 6, 7, or 8.

[113] In other embodiments, a suitable GM-CSF antagonist is an anti-GM-CSF antibody. Exemplary anti-GM-CSF monoclonal antibodies include those described in the international application PCT/EP2006/004696 filed on 05-17-2006 which published as WO2006/122797, international application PCT/EP2016/076225 filed on 10-31-2016, which published as WO2017/076804, and international application PCT/US2018/053933 filed on 10-2-2018, which published as WO/2019/070680 each of which are hereby incorporated by reference in their entirety. In one embodiment, the anti-GM-CSF monoclonal antibody is otilimab.

[114] An anti-GM-CSFRα or anti-GM-CSF antibody of the present disclosure may be multispecific, e.g., bispecific. An antibody of the may be mammalian (e.g., human or mouse), humanized, chimeric, recombinant, synthetically produced, or naturally isolated. Exemplary antibodies of the present disclosure include, without limitation, IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA (e.g., IgA1, IgA2, and IgAsec), IgD, IgE, Fab, Fab′, Fab′2, F(ab′)2, Fd, Fv, Feb, scFv, scFv-Fc, and SMIP binding moieties. In certain embodiments, the antibody is an scFv. The scFv may include, for example, a flexible linker allowing the scFv to orient in different directions to enable antigen binding. In various embodiments, the antibody may be a cytosol-stable scFv or intrabody that retains its structure and function in the reducing environment inside a cell (see, e.g., Fisher and DeLisa, J. Mol. Biol. 385(1): 299-311, 2009; incorporated by reference herein). In particular embodiments, the scFv is converted to an IgG or a chimeric antigen receptor according to methods known in the art. In embodiments, the antibody binds to both denatured and native protein targets. In embodiments, the antibody binds to either denatured or native protein.

[115] In most mammals, including humans, whole antibodies have at least two heavy (H) chains and two light (L) chains connected by disulfide bonds. Each heavy chain consists of a heavy chain variable region (VH) and a heavy chain constant region (CH). The heavy chain constant region consists of three domains (CH1, CH2, and CH3) and a hinge region between CH1 and CH2. Each light chain consists of a light chain variable region (VL) and a light chain constant region (CL). The light chain constant region consists of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed

framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen.

[116] Antibodies include all known forms of antibodies and other protein scaffolds with antibody-like properties. For example, the anti-GM-CSFRα antibody can be a monoclonal antibody, a polyclonal antibody, human antibody, a humanized antibody, a bispecific antibody, a monovalent antibody, a chimeric antibody, or a protein scaffold with antibody-like properties, such as fibronectin or ankyrin repeats. The antibody can have any of the following isotypes: IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA (e.g., IgA1, IgA2, and IgAsec), IgD, or IgE.

[117] An antibody fragment may include one or more segments derived from an antibody. A segment derived from an antibody may retain the ability to specifically bind to a particular antigen. An antibody fragment may be, e.g., a Fab, Fab′, Fab′2, F(ab′)2, Fd, Fv, Feb, scFv, or SMIP. An antibody fragment may be, e.g., a diabody, triabody, affibody, nanobody, aptamer, domain antibody, linear antibody, single-chain antibody, or any of a variety of multispecific antibodies that may be formed from antibody fragments.

[118] Examples of antibody fragments include: (i) a Fab fragment: a monovalent fragment consisting of VL, VH, CL, and CH1 domains; (ii) a F(ab′)2 fragment: a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment: a fragment consisting of VH and CH1 domains; (iv) an Fv fragment: a fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment: a fragment including VH and VL domains; (vi) a dAb fragment: a fragment that is a VH domain; (vii) a dAb fragment: a fragment that is a VL domain; (viii) an isolated complementarity determining region (CDR); and (ix) a combination of two or more isolated CDRs which may optionally be joined by one or more synthetic linkers. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, e.g., by a synthetic linker that enables them to be expressed as a single protein, of which the VL and VH regions pair to form a monovalent binding moiety (known as a single chain Fv (scFv)). Antibody fragments may be obtained using conventional techniques known to those of skill in the art, and may, in some instances, be used in the same manner as intact antibodies. Antigen-binding fragments may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact immunoglobulins. An antibody fragment may

further include any of the antibody fragments described above with the addition of additional C-terminal amino acids, N-terminal amino acids, or amino acids separating individual fragments.

[119] An antibody may be referred to as chimeric if it includes one or more antigen-determining regions or constant regions derived from a first species and one or more antigen-determining regions or constant regions derived from a second species. Chimeric antibodies may be constructed, e.g., by genetic engineering. A chimeric antibody may include immunoglobulin gene segments belonging to different species (e.g., from a mouse and a human).

[120] An antibody may be a human antibody. A human antibody refers to a binding moiety having variable regions in which both the framework and CDR regions are derived from human immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from a human immunoglobulin sequence. A human antibody may include amino acid residues not identified in a human immunoglobulin sequence, such as one or more sequence variations, e.g., mutations. A variation or additional amino acid may be introduced, e.g., by human manipulation. A human antibody of the present disclosure is not chimeric.

[121] An antibody may be humanized, meaning that an antibody that includes one or more antigen-determining regions (e.g., at least one CDR) substantially derived from a non-human immunoglobulin or antibody is manipulated to include at least one immunoglobulin domain substantially derived from a human immunoglobulin or antibody. An antibody may be humanized using the conversion methods described herein, for example, by inserting antigen-recognition sequences from a non-human antibody encoded by a first vector into a human framework encoded by a second vector. For example, the first vector may include a polynucleotide encoding the non-human antibody (or a fragment thereof) and a site-specific recombination motif, while the second vector may include a polynucleotide encoding a human framework and a site-specific recombination complementary to a site-specific recombination motif on the first vector. The site-specific recombination motifs may be positioned on each vector such that a recombination event results in the insertion of one or more antigen-determining regions from the non-human antibody into the human framework, thereby forming a polynucleotide encoding a humanized antibody.

[122] In certain embodiments, an antibody is converted from scFv to an IgG (e.g., IgG1, IgG2, IgG3, and IgG4). There are various methods in the art for converting scFv fragments to

IgG. One such method of converting scFv fragments to IgG is disclosed in US patent application publication number 20160362476, the contents of which are incorporated herein by reference.

Combination Therapy

Immune Checkpoint Inhibitors (ICIs)

[123] In some embodiments, the method of treating cancer according to the present invention comprises administering to a subject in need thereof a GM-CSF antagonist in combination with ICI.

[124] In some embodiments, the ICI is a biologic therapeutic or a small molecule. In some embodiments, the ICI is a monoclonal antibody, a humanized antibody, a fully human antibody, a fusion protein or a combination thereof.

[125] In some embodiments, the ICI inhibits a checkpoint protein which may be CTLA-4, PD-L1, PD-L2, PD-1, B7-H3, B7-H4, BTLA, HVEM, TIM-3, GAL-9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK1, CHK2, A2aR, B-7 family ligands or a combination thereof. In some embodiments, the ICI interacts with a ligand of a checkpoint protein which may be CTLA-4, PD-L1, PD-L2, PD-1, B7-H3, B7-H4, BTLA, HVEM, TIM-3, GAL-9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, B-7 family ligands or a combination thereof.

[126] In some embodiments, the ICI is an anti-CTLA-4 antibody. In some embodiments, the ICI is an anti-PD-L1 antibody. In some embodiments, the ICI is an anti-PD-L2 antibody. In some embodiments, the ICI is an anti-PD-1 antibody. In some embodiments, the ICI is an anti-B7-H3 antibody. In some embodiments, the ICI is an anti-B7-H4 antibody. In some embodiments, the ICI is an anti-BTLA antibody. In some embodiments, the ICI is an anti-HVEM antibody. In some embodiments, the ICI is an anti-TIM-3 antibody. In some embodiments, the ICI is an anti-GAL-9 antibody. In some embodiments, the ICIis an anti-LAG-3 antibody. In some embodiments, the ICI is an anti-VISTA antibody. In some embodiments, the ICI is an anti-KIR antibody. In some embodiments, the ICI is an anti-2B4 antibody. In some embodiments, the ICI is an anti-CD160 antibody. In some embodiments, the ICI is an anti-CGEN-15049 antibody. In some embodiments, the ICI is an anti-CHK1 antibody. In some embodiments, the ICI is an anti-CHK2 antibody. In some embodiments, the ICI is an anti-A2aR antibody. In some embodiments, the checkpoint inhibitor is an anti-B-7 antibody.

[127] In some embodiments, the PD-1 antibody is pembrolizumab. In some embodiments, the PD-1 antibody is nivolumab. In some embodiments, the PD-1 antibody is cemiplimab. In some embodiments, the PD-L1 antibody is atezolizumab. In some embodiments, the PD-L1 antibody is avelumab. In some embodiments, the PD-L1 antibody is durvalumab. In some embodiments, the CTLA-4 antibody is ipillimumab.

Additional Therapeutic Agents

[128] In some embodiments, the method of treating cancer according to the present invention comprises administering to a subject in need thereof a GM-CSF antagonist in combination with an additional therapeutic agent. In certain embodiments, the additional agent is a cancer therapy comprising chemotherapy and/or radiation therapy. In certain embodiments, the additional therapeutic agent comprises a recombinant protein or monoclonal antibody. In certain embodiments, the recombinant protein or monoclonal antibody comprises Etaracizumab (Abegrin), Tacatuzumab tetraxetan, Bevacizumab (Avastin), Labetuzumab, Cetuximab (Erbitux), Obinutuzumab (Gazyva), Trastuzumab (Herceptin), Clivatuzumab, Trastuzumab emtansine (Kadcyla), Ramucirumab, Rituximab (MabThera, Rituxan), Gemtuzumab ozogamicin (Mylotarg), Pertuzumab (Omnitarg), Girentuximab (Rencarex), or Nimotuzumab (Theracim, Theraloc).

[129] In certain embodiments, the GM-CSF antagonist comprises an immunomodulator that targets a checkpoint inhibitor as described in the *Checkpoint Inhibitors* section above. In certain embodiments, the immunomodulator comprises Nivolumab, Ipilimumab, Atezolizumab, or Pembrolizumab. In certain embodiments, the additional therapeutic agent is a chemotherapeutic agent. In certain embodiments, the chemotherapeutic agent is an alkylating agent (e.g., cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, or temozolomide), an anthracycline (e.g., doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (e.g., paclitaxel or docetaxel), a histone deacetylase inhibitor (e.g., vorinostat or romidepsin), an inhibitor of topoisomerase (e.g., irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (e.g., bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (e.g., azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (e.g., actinomycin or bleomycin), a platinum-based agent (e.g, cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (e.g., vincristine, vinblastine, vinorelbine, vindesine,

podophyllotoxin, paclitaxel, or docetaxel). In some embodiments, the chemotherapeutic agent is a nucleoside analog. In some embodiments, the chemotherapeutic agent is gemcitabine. In certain embodiments, the additional therapeutic agent is radiation therapy.

Treatment of Cancer

[130] The present invention may be used to treat various cancers, in particular, those immune checkpoint inhibitory (ICI) refractory or resistant cancers, or late stage or metastatic cancers.

[131] In some embodiments, the cancer is any solid tumor or liquid cancers, including urogenital cancers (such as prostate cancer, renal cell cancers, bladder cancers), gynecological cancers (such as ovarian cancers, cervical cancers, endometrial cancers), lung cancer, gastrointestinal cancers (such as non-metastatic or metastatic colorectal cancers, pancreatic cancer, gastric cancer, oesophageal cancers, hepatocellular cancers, cholangiocellular cancers), head and neck cancer (e.g. head and neck squamous cell cancer), brain cancers including malignant gliomas and brain metastases, malignant mesothelioma, non-metastatic or metastatic breast cancer (e.g. hormone refractory metastatic breast cancer), malignant melanoma, Merkel Cell Carcinoma or bone and soft tissue sarcomas, and haematologic neoplasias, such as multiple myeloma, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia. In a some embodiment, the disease is non-small cell lung cancer (NSCLC), breast cancer (e.g. stage IV breast cancer, hormone refractory metastatic breast cancer), head and neck cancer (e.g. head and neck squamous cell cancer), metastatic colorectal cancers, hormone sensitive or hormone refractory prostate cancer, colorectal cancer (e.g. stage IV colorectal cancer), ovarian cancer, hepatocellular cancer, renal cell cancer, soft tissue sarcoma, or small cell lung cancer.

[132] As used herein, the term “cancer” refers to the broad class of disorders characterized by hyperproliferative cell growth, either in vitro (e.g., transformed cells) or in vivo. Conditions which can be treated or prevented by the compositions and methods of the invention include, e.g., a variety of neoplasms, including benign or malignant tumors, a variety of hyperplasias, or the like. Compounds and methods of the invention can achieve the inhibition and/or reversion of undesired hyperproliferative cell growth involved in such conditions.

[133] Examples of cancer include Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Adrenocortical

Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor, Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood: Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primary; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma. Childhood Brain Stem; Glioma. Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer,

Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS-Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's; Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non-Hodgkin's, Childhood; Lymphoma, Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Malignant Thymoma; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Neurofibroma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood', Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland' Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous

Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T-Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma, Malignant; Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor.

Pharmaceutical compositions and administration

[134] The antibodies or agents of the invention (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the antibody or agent and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[135] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl

parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[136] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[137] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[138] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in

the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[139] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[140] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[141] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[142] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to

methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[143] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[144] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

EXAMPLES

[145] The present invention is further illustrated, but not limited, by the slides accompanying this specification. While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

Example 1. An anti-GM-CSFRα antibody rescues T cell proliferation

[146] The study in this example illustrates that suppressive potential of myeloid populations on T cell proliferation can be rescued by GM-CSF antagonist.

[147] T cell proliferation assay with CD14+ cells with or without treatment of anti-GM-CSFRα antibody was performed. Briefly, CD14+ (MDSC) cells were isolated from the blood samples (PBMCs) obtained from pancreatic cancer patients according to methods known in the art. The isolated CD14+ cells were treated with anti-GM-CSFRα antibodies for 48 hours. Next, the CD3+ T-cells labeled with carboxy-fluorescein diacetate succinimidyl ester (CFSE) were co-cultured with anti-GM-CSFRα antibody-treated or untreated CD14+ cells for 96 hours and

proliferation was determined by CFSE dilution (divided cells). T cells without co-culture were used as a negative control.

[148] As shown in FIG.1, T cell proliferation is suppressed significantly following culture with the untreated CD14+ cells. On the other hand, anti-GM-CSFRα antibody-treated CD14+ cells showed an increased T cell proliferation, suggesting that an addition of anti-GM-CSFRα antibody rescued T cell proliferation and prevented suppressive potential of MDSCs.

Example 2. Cancer cell conditioned medium polarize monocytes to phenotypic MDSCs

[149] In this study, various cancer cell lines were used to illustrate an increase in phenotypic MDSCs (HLA-DR^{low}) when CD+14 monocytes were incubated with conditioned medium from GM-CSF expressing cancer cells.

[150] Cancer cell lines were analyzed for expression of GM-CSF. In this particular study, two colorectal carcinoma (HCT116 and SW-480), two pancreatic carcinoma (Panc-1 and Capan-1), cervical adenocarcinoma (HeLa), and malignant melanoma (A375) cell lines were measured for GM-CSF expression. As shown in **FIG. 2**, cancer cell lines express GM-CSF at different levels. In particular, SW480 and Capan-1 have relatively high expression of GM-CSF, whereas HeLa cells have relatively low expression of GM-CSF.

[151] To generate tumor-conditioned media (CM), cell lines were plated and cultured according to methods known in the art. CD14+ cells were then cultured in the presence of CM for 6 days and analyzed for gene and protein expression. Low levels of HLA-DR biomarker is indicative of MDSC phenotype. **FIG. 3** shows an increase in phenotypic MDSCs when CD14+ monocytes were incubated with conditioned medium from GM-CSF expressing cancer cells, as compared to CD+14 cells that were grown in normal culture medium. Results show that CM from cancer cell lines with high GM-CSF expression have high induction of MDSCs, suggesting that GM-CSF contributes to polarization of monocytes to phenotypic MDSCs.

Example 3. An anti-GM-CSFRα antibody blocks PD-L1 upregulation in MDSCs

[152] The study in this example illustrates a surprising finding by the present inventors that GM-CSF induces expression of PD-L1 on phenotypic MDSCs. Notably, treatment with a

GM-CSF antagonist is sufficient to represses the expression of PD-L1 on monocytes treated with conditioned medium (CM) from GM-CSF expressing cancer cell lines.

[153] One of the main challenges for immune checkpoint blockade antibodies lies in malignancies with limited T-cell responses or immunologically "cold" tumors. These cold tumors contain few infiltrating T cells and are not recognized and do not provoke a strong response by the immune system, making them difficult to treat with current immunotherapies. Present inventors surprisingly found that GM-CSF upregulates PD-L1, a checkpoint protein on the surface of MDSCs that contributes to immunosuppressive activity. The study in this example shows that an anti-GM-CSFRα antibody can be used to convert a "cold" tumor to a "hot" tumor, possibly increasing effectiveness and sensitivity of immunotherapy.

[154] In this study, various cancer cell lines were used to evaluate changes in PD-L1 expression levels on MDSCs when CD14+ monocytes were incubated with conditioned medium from GM-CSF expressing cancer cells. As shown in **FIG. 4**, adding conditioned medium from GM-CSF expressing cancer cell lines to the CD14+ monocytes increased the level of PD-L1 expression as compared to the baseline (culture medium only). HeLa cell lines, which exhibited low levels of GM-CSF expression (see **FIG. 2**) did not upregulate PD-L1 expression. Adding recombinant GM-CSF in combination with CM (CM + GM-CSF) increased the expression of PD-L1, indicating that GM-CSF induces expression of PD-L1 on phenotypic MDSCs. The spike in PD-L1 was more pronounced in cell lines that had low baseline PD-L1 expression with CM only (e.g., Panc-1 and HeLa cells). The effect of an anti-GM-CSFRα antibody on PD-L1 expression was also examined. Treating the MDSCs with anti-GM-CSFRα antibody in CM, in the absence or in presence of recombinant GM-CSF (CM+Ab and CM+GM-CSF+Ab, respectively) resulted in a markedly decreased level of PD-L1 as compared to CM only or CM + GM-CSF, respectively. These data show that an anti-GM-CSFRα antibody suppresses PD-L1 upregulation in CD14+ monocytes (MDSCs) treated with condition medium conditioned by from GM-CSF expressing cancer cell lines.

Example 4. An anti-GM-CSFRα antibody represses PD-L1 expression in MDSCs

[155] The study in this example shows that a GM-CSF antagonist is able to suppress PD-L1 expression on MDSCs treated with condition medium from GM-CSF expressing cancer cell lines (CM), whether the GM-CSF antagonist is added concurrently with, or after the CM treatment when PD-L1 levels on the MDSCs are already increased.

[156] As shown in **FIG. 5A**, conditioned medium from GM-CSF cancer cell line (CM) with or without recombinant GM-CSF (at 10 ng/mL) and anti-GM- CSFRα antibody (at 100 μg/mL) (as shown in **Table 1**) were added to CD14+ monocytes (MDSCs) at day 1. After three days of incubation, the MDSC cells were analyzed for expression of PD-L1.

Table 1.

Sample	Content
A	Control (Culture medium)
B	CM
C	CM + anti-GM-CSFRα antibody
D	CM + GM-CSF
E	CM + GM-CSF + anti-GM-CSFRα antibody

[157] Consistent with example 3, adding conditioned medium from cancer cell lines expressing GM-CSF to the MDSCs (B; CM) or CM with recombinant GM-CSF (D; CM + GM-CSF) increased the level of PD-L1 expression as compared to the baseline (A; culture medium only). Moreover, when an anti-GM-CSFRα antibody was added concurrently with CM or CM + GM-CSF, as shown in samples C and E of **FIG. 5A**, respectively, a decrease in PD-L1 was observed, suggesting that the anti-GM-CSFRα antibody blocks upregulation of PD-L1 on MDSCs.

[158] Next, the effect of adding an anti-GM-CSFRα antibody after incubation of MDSCs with conditioned medium to examine the effect of GM-CSF blockade on PD-L1 expression levels when elevated level of PD-L1 on MDSCs was already established. In this particular setup, MDSCs were cultured in conditioned medium with or without recombinant GM-CSF (samples B-E of **FIG. 5B**) for 48 hours. Then, an anti-GM-CSFRα antibody was added at day 3 to samples C and E of **FIG. 5B** (after 48 hours). MDSCs in sample A were incubated in culture medium for three days as a control. Phenotypic analysis of MDSCs was performed on day 4. As shown in **FIG. 5B**, treatment of MDSCs with an anti-GM-CSFRα antibody after MDSCs were cultured with conditioned medium from GM-CSF expressing cancer cell lines (samples C and E) repressed the expression level of PD-L1 on MDSCs. Notably, PD-

L1 expression in sample C and E were decreased as compared to samples B and D, respectively, after just 24 hours of treatment with an anti-GM-CSFRα antibody.

Example 5. An anti-GM-CSFRα antibody reduces MDSC mediated T-cell suppression

[159] The study in this example further illustrates that suppressive potential of myeloid populations on T cell proliferation can be repressed by a GM-CSF antagonist.

[160] In this particular experiment, monocytes treated with conditioned medium from GM-CSF expressing cancer cell lines were used in T cell proliferation assay. Briefly, monocytes were cultured in conditioned medium from GM-CSF expressing cancer cell lines (CM) for three days (CM-treated monocytes). T cells ($1x10^5$ cells) were prepared by labeling with 1 μM CFSE and stimulation with 10 ng/ mL of IL-2 and 10 uL of soluble CD3/CD28 T cell activator in IMDM cell culture medium. Then, the stimulated T cells were co-cultured with CM-treated monocytes (at a ratio of 2:1 monocyte:T cell) with or without supplemental human recombinant GM-CSF (10 ng/mL) and/or anti-GM-CSFRα antibody (100 μg/mL) in a mix lymphocyte reaction (MLR) as shown in **FIG. 6**. Stimulated T-cells in IMDM culture medium together with healthy monocytes were used as a control. T-cells were expanded for 5 days, collected and stained for CD4 and CD8, which are markers for helper T and cytotoxic T cells. Cell proliferation was measured by flow cytometry and evaluated by CFSE dilution.

[161] **FIG. 6** shows the results of the T-cell proliferation assay in terms of % of cells proliferating (left panel) and % of max (MFI) (signal detection of CFSE dilution in CD4+ or CD8+ cells) by flow cytometry (right panel). As shown in **FIG. 6**, CM-treated monocytes suppressed T-cell proliferation as compared to the control and addition of recombinant GM-CSF further suppressed T cell proliferation. Treatment with an anti-GM-CSFRα antibody (Ab) reduced the MDSC-mediated T cell suppression.

EQUIVALENTS

[162] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

CLAIMS

We claim:

1. A method of treating cancer comprising administering a GM-CSF antagonist to the patient in need of treatment, wherein the administration of the GM-CSF antagonist results in inhibition of an immunosuppressive activity of myeloid-derived suppressor cells (MDSCs).

2. A method of inhibiting immunosuppressive activity of myeloid-derived suppressor cells (MDSCs) in a patient suffering from cancer comprising administering a GM-CSF antagonist to the patient.

3. A method of enhancing immune response for cancer treatment comprising administering a GM-CSF antagonist to a patient receiving a cancer treatment, wherein the immune response is increased as compared to a control.

4. The method of claim 3, wherein the immune response is a percentage of T cell proliferation.

5. The method of claim 3 or 4, wherein the control is indicative of the immune response level in the patient prior to the administration of GM-CSF antagonist.

6. The method of claim 3 or 4, wherein the control is a reference immune response level in a control patient receiving the cancer treatment without GM-CSF antagonist or a reference immune response level based on historical data.

7. The method of any one of claims 3-6, wherein the cancer treatment is an immunotherapy.

8. The method of claim 7, wherein the administering the GM-CSF antagonist increases the efficacy of the immunotherapy.

9. A method of suppressing PD-L1 in a cancer patient comprising administering a GM-CSF antagonist to a patient in need of treatment as compared to a control.

10. The method of claim 9, wherein the administering the GM-CSF antagonist decreases a level of PD-L1 in a cancer patient.

11. The method of claim 9 or 10, wherein the control is indicative of the PD-L1 level in the patient prior to the administration of GM-CSF antagonist.

12. The method of claim 9 or 10, wherein the control is a reference PD-L1 level in a control patient receiving the cancer treatment without GM-CSF antagonist or a reference PD-L1 level based on historical data.

13. The method of any one of claims 10-12, wherein the level of PD-L1 in the patient is decreased by at least 10%, 20%, 30%, 50%, 60%, 70%, 80% or 90% as compared to the control.

14. The method of any one of claims 9-13, wherein the PD-L1 is expressed on MDSCs.

15. The method of claim 14, wherein the PD-L1 is expressed on circulating MDSCs.

16. The method of claim 14, wherein the PD-L1 is expressed on plasma-derived MDSCs.

17. The method of any one of preceding claims, wherein the patient has circulating myeloid derived suppressor cells (MDSCs)

18. The method of any one of preceding claims, wherein the patient suffers from a cancer with a low level of infiltrating T cells.

19. The method of any one of preceding claims, wherein the patient suffers from an immune checkpoint inhibitor (ICI) refractory cancer.

20. The method of any one of the preceding claims, wherein the patient suffers from a late stage or metastatic cancer.

21. The method of any one of the preceding claims, wherein the patient suffers from a cancer selected from breast cancer, colorectal cancer (CRC), prostate cancer, melanoma, bladder carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, hepatocellular carcinoma, gastric cancer, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell carcinoma, non-Hodgkin lymphoma, cervical cancer, gastrointestinal cancer, urogenital cancer, brain cancer, mesothelioma, renal cell cancer, gynecological cancer, ovarian cancer, endometrial cancer, lung cancer, gastrointestinal cancer, pancreatic cancer, oesophageal cancers, hepatocellular cancer, cholangiocellular cancer, brain cancers, mesothelioma, malignant melanoma, Merkel Cell Carcinoma, multiple myeloma, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome or acute lymphoblastic leukemia.

22. The method of any one of the preceding claims, wherein the patient suffers from a cancer selected from Stage IV breast cancer, Stage IV colorectal cancer (CRC), prostate cancer, or melanoma.

23. The method of any one of the preceding claims, wherein the method further comprises administering at least one other cancer therapy to the patient.

24. The method of claim 23, wherein the at least one other cancer therapy is chemotherapy, MDSC-targeted therapy, immunotherapy, radiation therapy and combinations thereof.

25. The method of claim 23 or claim 24, wherein the GM-CSF antagonist and the other cancer therapy are administered concurrently.

26. The method of claim 23 or claim24, wherein the GM-CSF antagonist and the other cancer therapy are administered sequentially.

27. The method of claim 23 or claim 24, wherein the patient has received a treatment with the other cancer therapy prior to the administration of the GM-CSF antagonist.

28. The method of claim 23 or claim 24, wherein the patient has received a treatment with the GM-CSF antagonist prior to the administration of the other cancer therapy.

29. The method of any one of claims 23-28, wherein the other cancer therapy is an ICI.

30. The method of any one of claims 29, wherein the ICI antagonizes the activity of PD-1, CTLA-4, B7, BTLA, HVEM, TIM-3, GAL-9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK1, CHK2, or A2aR.

31. The method of claim 29 , wherein the ICI is selected from an anti-PD-1 antibody (optionally pembrolizumab, nivolumab, cemiplimab), an anti-PD-L1 antibody (optionally atezolizumab, avelumab, durvalumab), an anti-CTLA-4 antibody (optionally ipillimumab), an anti-PD-L2 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-BTLA antibody, an anti-HVEM antibody, an anti-TIM-3 antibody, an anti-GAL-9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-2B4 antibody, an anti-CD160 antibody, an anti-CGEN-15049 antibody, an anti-CHK1 antibody, an anti-CHK2 antibody, an anti-A2aR antibody, an anti-B-7 antibody, and combinations thereof.

32. The method of claim 29, wherein the ICI is an anti-PD-L1 antibody.

33. The method of any one of claims 24-32, wherein the method further comprises administering a chemotherapy agent to the patient.

34. The method of any one of claims 24-28, wherein the MDSC-targeted therapy is selected from an anti-CFS-1R antibody, an anti-IL-6 antibody, all-trans retinoic acid, axitinib, entinostat, gemcitabine, or phenformin, and combinations thereof.

35. The method of any one of claims 24-28, wherein the immunotherapy is selected from a monoclonal antibody, cytokine, cancer vaccine, T-cell engaging therapies, and combinations thereof .

36. The method of claim 35, wherein the monoclonal antibody is selected from an anti-CD3 antibody, an anti-CD52 antibody, an anti-PDl antibody, an anti-PD-Ll antibody, an anti-

CTLA4 antibody, an anti-CD20 antibody, an anti- BCMA antibody, bi-specific antibodies, or bispecific T-cell engager (BiTE) antibodies, and combinations thereof.

37. The method of claim 35, wherein the cytokines are selected from IFNa, IFNp, IFNy, IFN , IL-2, IL-7, IL-15, IL-21, IL-11, IL-12, IL-18, hGM- CSF, TNFa, or any combination thereof.

38. The method of any one of the preceding claims, wherein the GM-CSF antagonist is an anti-GM-CSF antibody or a fragment thereof.

39. The method of any one of claims 1-37, wherein the GM-CSF antagonist is a soluble GM-CSF receptor.

40. The method of any one of claims 1-37, wherein the GM-CSF antagonist is an anti-GM-CSF receptor antibody or a fragment thereof.

41. The method of claim 40, wherein the anti-GM-CSF receptor antibody or a fragment thereof is an anti- GM-CSFRα antibody or a fragment thereof.

42. The method of claim 41, wherein the anti- GM-CSFRα antibody or a fragment thereof is a monoclonal antibody specific for human GM-CSFRα.

43. The method of claim 42, wherein the anti-GM-CSFRα antibody is human or humanized IgG4 antibody.

44. The method of claims 42 or 43, wherein the anti-GM- CSFRα antibody is mavrilimumab.

45. The method of any of claims 41-44, wherein the anti-GM- CSFRα antibody a fragment thereof comprises a light chain complementary-determining region 1 (LCDR1) defined by SEQ ID NO: 6, a light chain complementary-determining region 2 (LCDR2) defined by SEQ ID NO: 7, and a light chain complementary-determining region 3 (LCDR3) defined by SEQ ID NO: 8; and a heavy chain complementary-determining region 1 (HCDR1) defined by SEQ ID NO: 3, a heavy chain complementary-determining region 2

(HCDR2) defined by SEQ ID NO: 4, and a heavy chain complementary-determining region 3 (HCDR3) defined by SEQ ID NO: 5.

46. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in reduced level of MDSCs in the patient as compared to a control.

47. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in reduced level of MDSC-mediated immunosuppressive activity in the patent as compared to a control.

48. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in reduced percentage of $Lin^{-}CD14^{+}HLA\text{-}DR^{-}$M-MDSCs in the peripheral blood of the patient as compared to a control.

49. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in increased percentage of mature MDSC cells in the patient as compared to a control.

50. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in a reduced level of Treg cells, macrophages, and/or neutrophils as compared to a control.

51. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in a decreased level of an inhibitory cytokine.

52. The method of claim 51, wherein the inhibitory cytokine is selected from IL-10 and TGFβ.

53. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in a decreased level of an immune suppressive factor.

54. The method of claim 53, wherein the immune suppressive factor is selected from arginase 1, inducible nitric oxide synthase (iNOS), peroxynitrite, nitric oxide, reactive oxygen species, tumor associated macrophages, and combinations thereof.

55. The method of any one of the preceding claims, wherein the administration of the GM-CSF antagonist and/or the ICI results in an increased level of $CD4^+$ T effector cells as compared to a control.

56. The method of any one of claims 46-55, wherein the control is a pre-treatment level or percentage in the patient, or a reference level or percentage based on historical data.

57. A pharmaceutical composition for treating cancer comprising a GM-CSF antagonist and an ICI.

58. The pharmaceutical composition of claim 57, wherein the GM-CSF antagonist is an anti-GM-CSF antibody or a fragment thereof.

59. The pharmaceutical composition of claim 57, wherein the GM-CSF antagonist is a soluble GM-CSF receptor.

60. The pharmaceutical composition of claim 57, wherein the GM-CSF antagonist is an anti-GM-CSF receptor antibody or a fragment thereof.

61. The pharmaceutical composition of claim 60, wherein the anti-GM-CSF receptor antibody or a fragment thereof is an anti- GM-CSFRα antibody or a fragment thereof.

62. The pharmaceutical composition of claim 61, wherein the anti-GM-CSFRα antibody or a fragment thereof is a monoclonal antibody specific for human GM-CSFRα.

63. The pharmaceutical composition of claim 62, wherein the anti-GM-CSFRα antibody is human or humanized IgG4 antibody.

64. The pharmaceutical composition of claims 60-63, wherein the anti-GM-CSFRα antibody is mavrilimumab.

65. The pharmaceutical composition of any of claims 60-63, wherein the anti-GM-CSFRα antibody a fragment thereof comprises a light chain complementary-determining region 1 (LCDR1) defined by SEQ ID NO: 6, a light chain complementary-determining region 2 (LCDR2) defined by SEQ ID NO: 7, and a light chain complementary-determining region 3 (LCDR3) defined by SEQ ID NO: 8; and a heavy chain complementary-determining region 1 (HCDR1) defined by SEQ ID NO: 3, a heavy chain complementary-determining region 2 (HCDR2) defined by SEQ ID NO: 4, and a heavy chain complementary-determining region 3 (HCDR3) defined by SEQ ID NO: 5.

66. The pharmaceutical composition of any one of claims 57-65, wherein the ICI antagonizes the activity of PD-1, CTLA-4, B7, BTLA, HVEM, TIM-3, GAL-9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK1, CHK2, A2aR, and combinations thereof.

67. The pharmaceutical composition of any one of claims 57-63, wherein the ICI is selected from an anti-PD-1 antibody (optionally pembrolizumab, nivolumab, cemiplimab), an anti-PD-L1 antibody (optionally atezolizumab, avelumab, durvalumab), an anti-CTLA-4 antibody (optionally ipillimumab), an anti-PD-L2 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-BTLA antibody, an anti-HVEM antibody, an anti-TIM-3 antibody, an anti-GAL-9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-2B4 antibody, an anti-CD160 antibody, an anti-CGEN-15049 antibody, an anti-CHK1 antibody, an anti-CHK2 antibody, an anti-A2aR antibody, an anti-B-7 antibody, and combinations thereof.

68. A kit for treating cancer comprising a pharmaceutical composition comprising a GM-CSF antagonist and pharmaceutical composition comprising at least one other cancer therapy selected from a chemotherapy, MDSC-targeted therapy, immunotherapy, radiation therapy and combinations thereof.

69. The kit of claim 68, wherein the immunotherapy is an ICI selected from an anti-PD-1 antibody (optionally pembrolizumab, nivolumab, cemiplimab), an anti-PD-L1 antibody

(optionally atezolizumab, avelumab, durvalumab), an anti-CTLA-4 antibody (optionally ipillimumab), an anti-PD-L2 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-BTLA antibody, an anti-HVEM antibody, an anti-TIM-3 antibody, an anti-GAL-9 antibody, an anti-LAG3 antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-2B4 antibody, an anti-CD160 antibody, an anti-CGEN-15049 antibody, an anti-CHK1 antibody, an anti-CHK2 antibody, an anti-A2aR antibody, an anti-B-7 antibody, and combinations thereof.

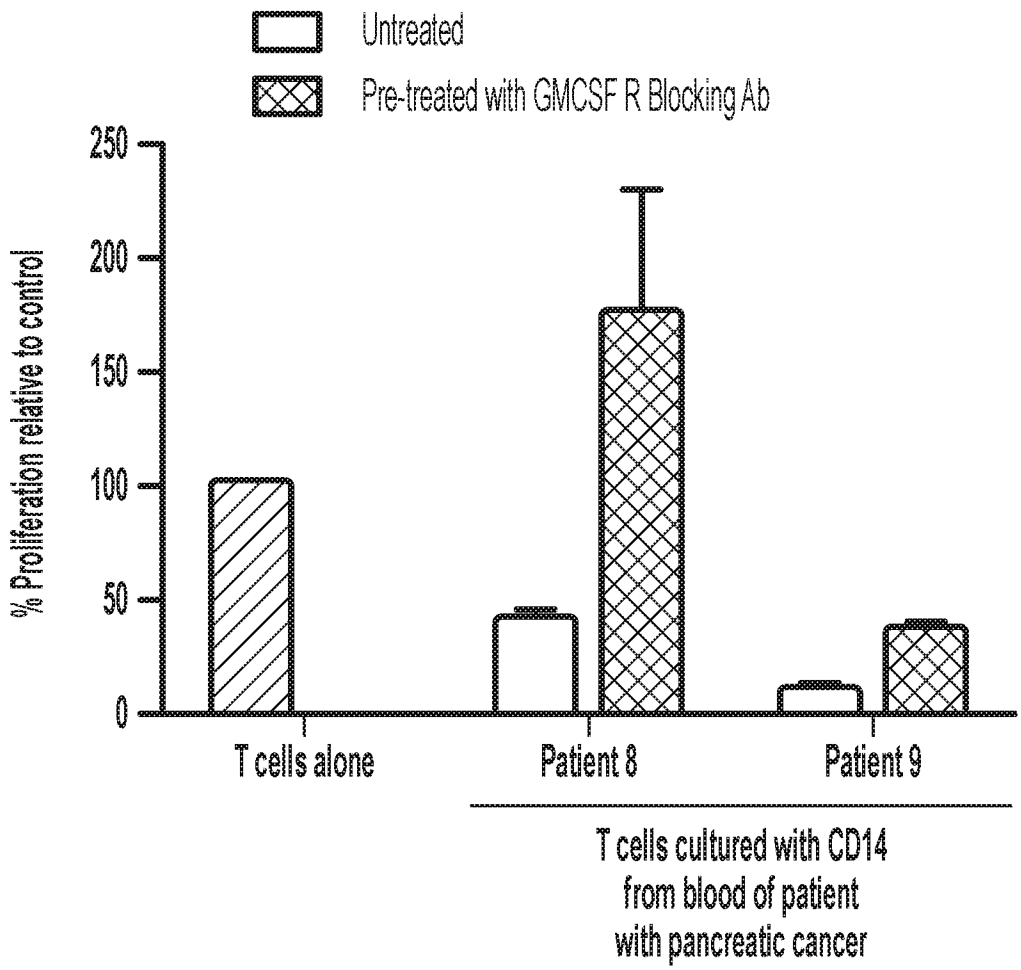

Untreated
Pre-treated with GMCSF R Blocking Ab
% Proliferation relative to control
250
200
150
100
50
0
T cells alone
Patient 8
Patient 9
T cells cultured with CD14
from blood of patient
with pancreatic cancer

FIG. 1

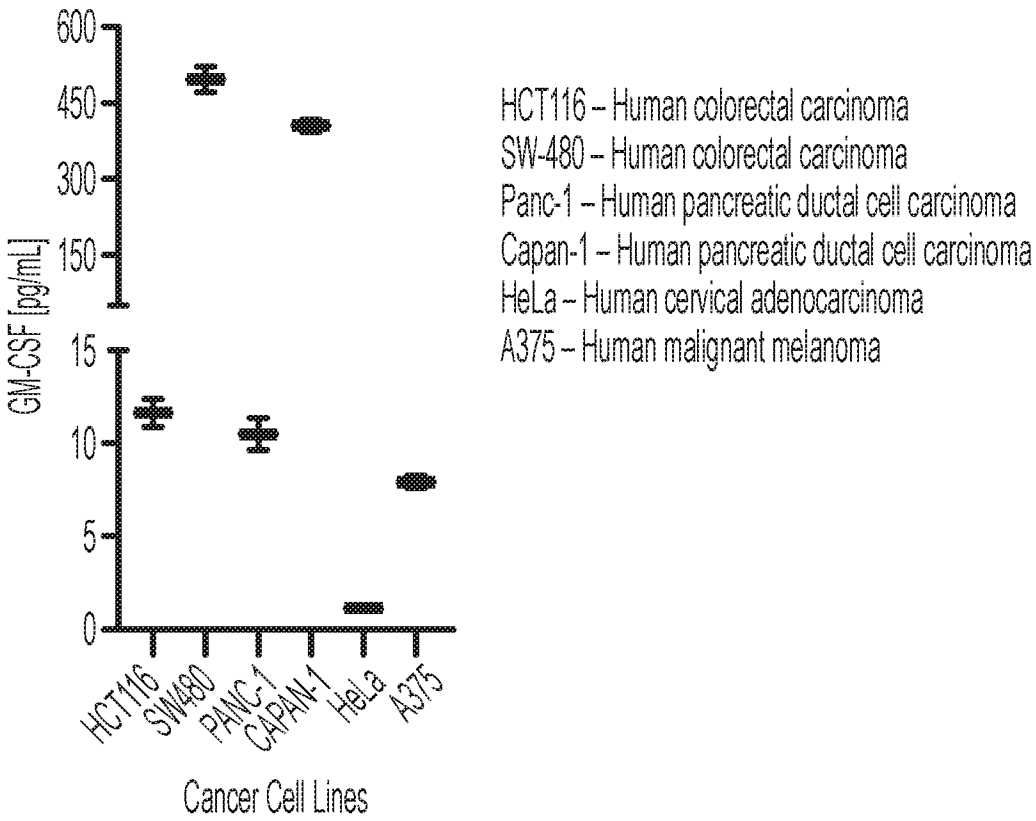

600
450
300
150
15
10
5
0
GM-CSF [pg/mL]
HCT116
SW480
PANC-1
CAPAN-1
HeLa
A375
Cancer Cell Lines
HCT116 – Human colorectal carcinoma
SW-480 – Human colorectal carcinoma
Panc-1 – Human pancreatic ductal cell carcinoma
Capan-1 – Human pancreatic ductal cell carcinoma
HeLa – Human cervical adenocarcinoma
A375 – Human malignant melanoma

FIG. 2

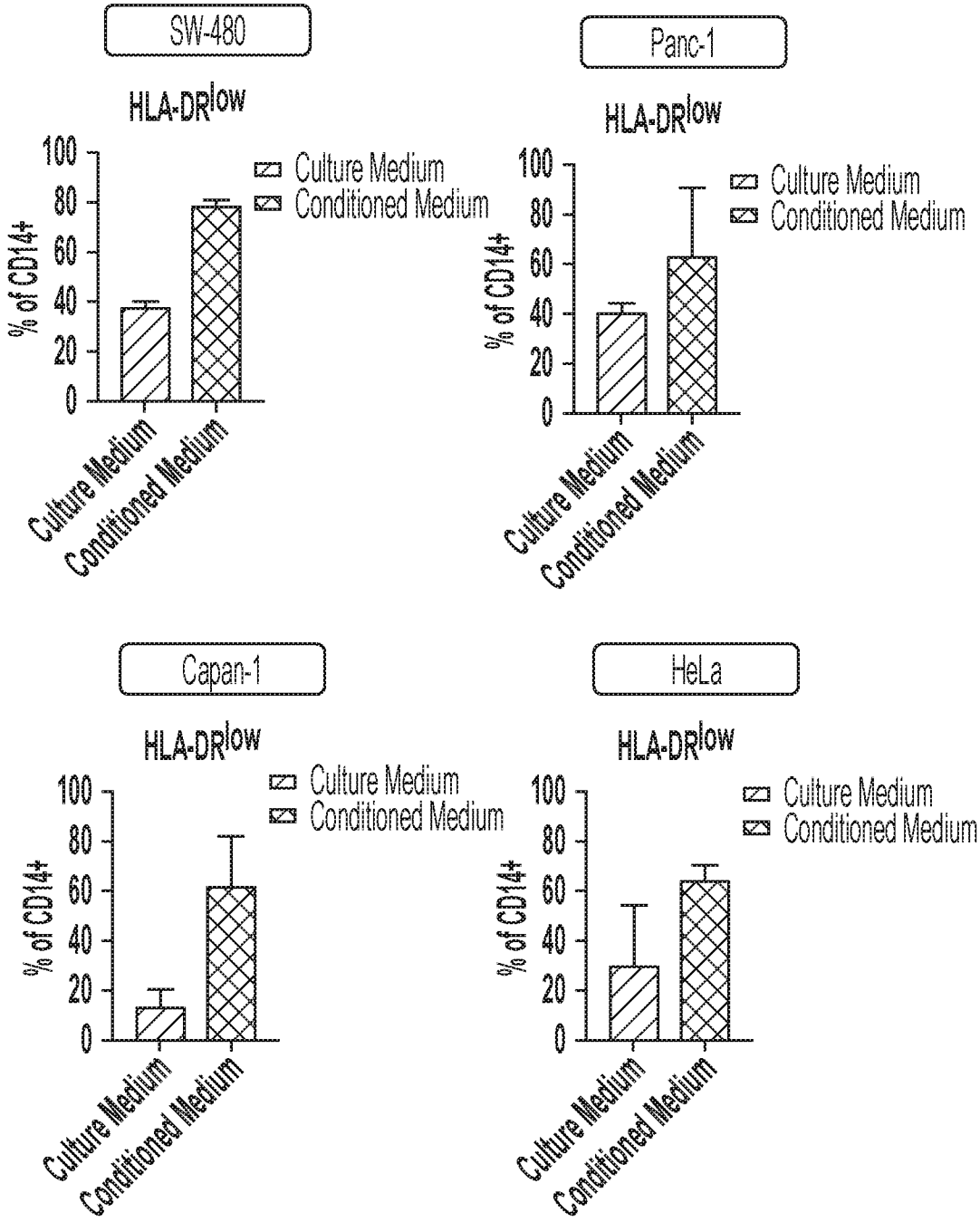

SW-480
HLA-DRlow
% of CD14+
100
80
60
40
20
0
Culture Medium
Conditioned Medium
Culture Medium
Conditioned Medium
Panc-1
HLA-DRlow
% of CD14+
Culture Medium
Conditioned Medium
Capan-1
HLA-DRlow
% of CD14+
Culture Medium
Conditioned Medium
HeLa
HLA-DRlow
% of CD14+
Culture Medium
Conditioned Medium

FIG. 3

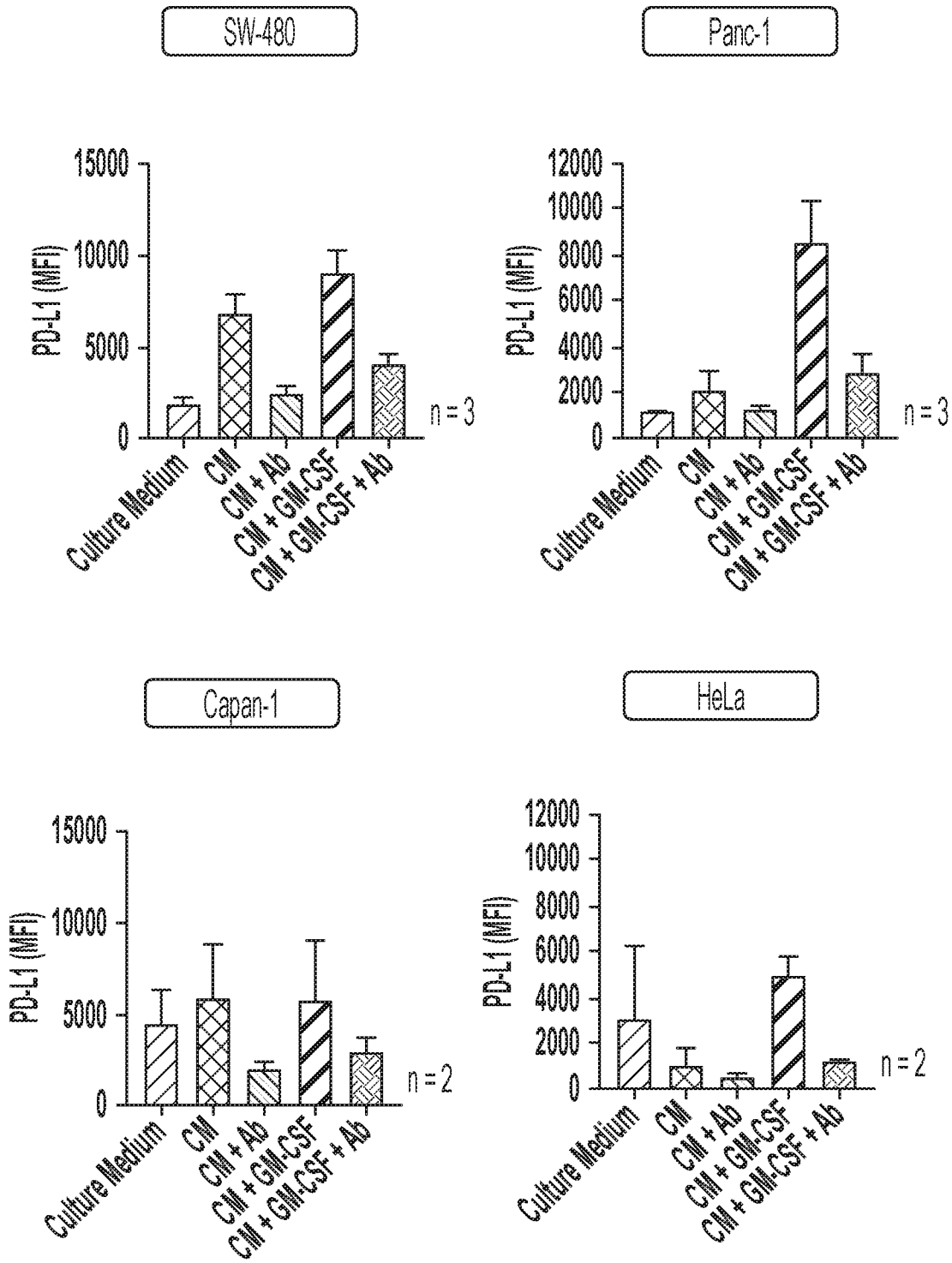

SW-480
PD-L1 (MFI)
15000
10000
5000
0
n = 3
Culture Medium
CM
CM + Ab
CM + GM-CSF
CM + GM-CSF + Ab
Panc-1
PD-L1 (MFI)
12000
10000
8000
6000
4000
2000
0
n = 3
Culture Medium
CM
CM + Ab
CM + GM-CSF
CM + GM-CSF + Ab
Capan-1
PD-L1 (MFI)
15000
10000
5000
0
n = 2
Culture Medium
CM
CM + Ab
CM + GM-CSF
CM + GM-CSF + Ab
HeLa
PD-L1 (MFI)
12000
10000
8000
6000
4000
2000
0
n = 2
Culture Medium
CM
CM + Ab
CM + GM-CSF
CM + GM-CSF + Ab

FIG. 4

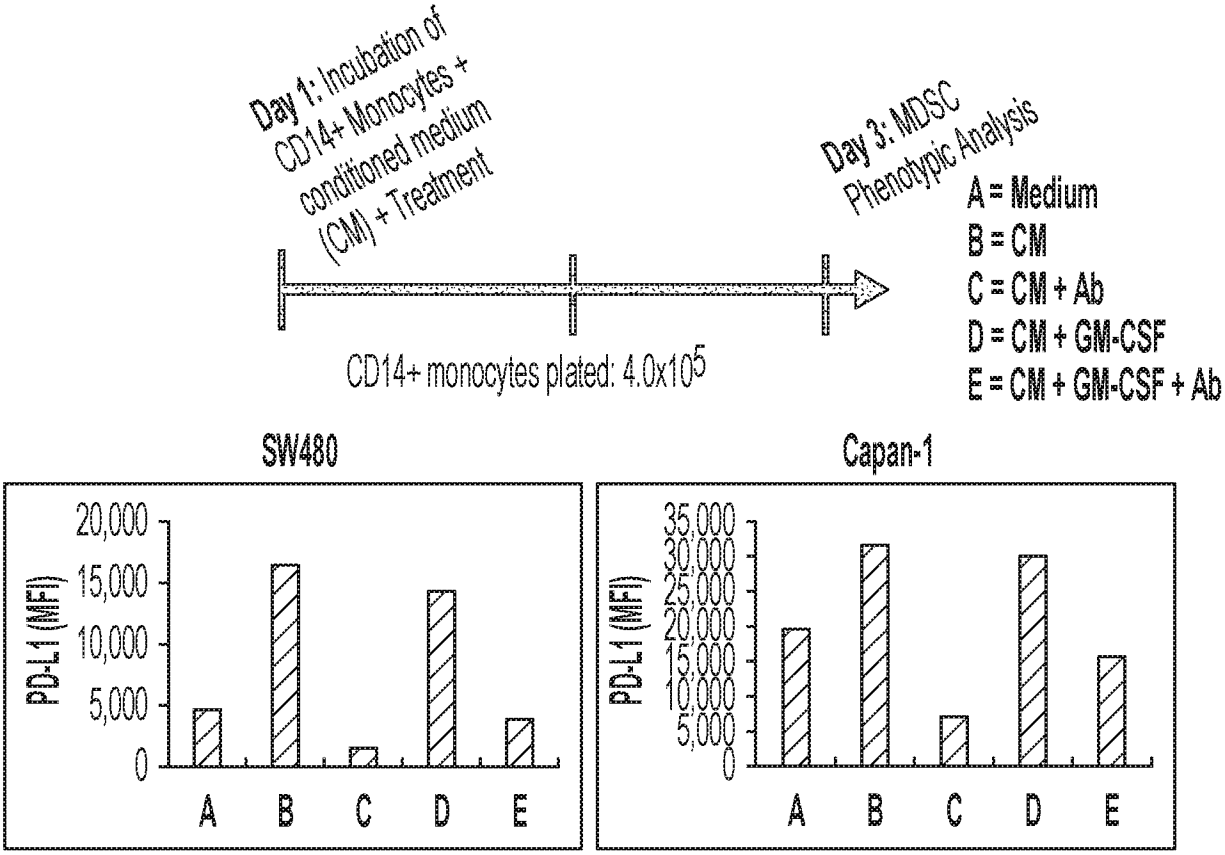

Day 1: Incubation of CD14+ Monocytes + conditioned medium (CM) + Treatment
Day 3: MDSC Phenotypic Analysis
A = Medium
B = CM
C = CM + Ab
D = CM + GM-CSF
E = CM + GM-CSF + Ab
CD14+ monocytes plated: 4.0x10^5
SW480
Capan-1
PD-L1 (MFI)
20,000
15,000
10,000
5,000
0
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
A
B
C
D
E

FIG. 5A

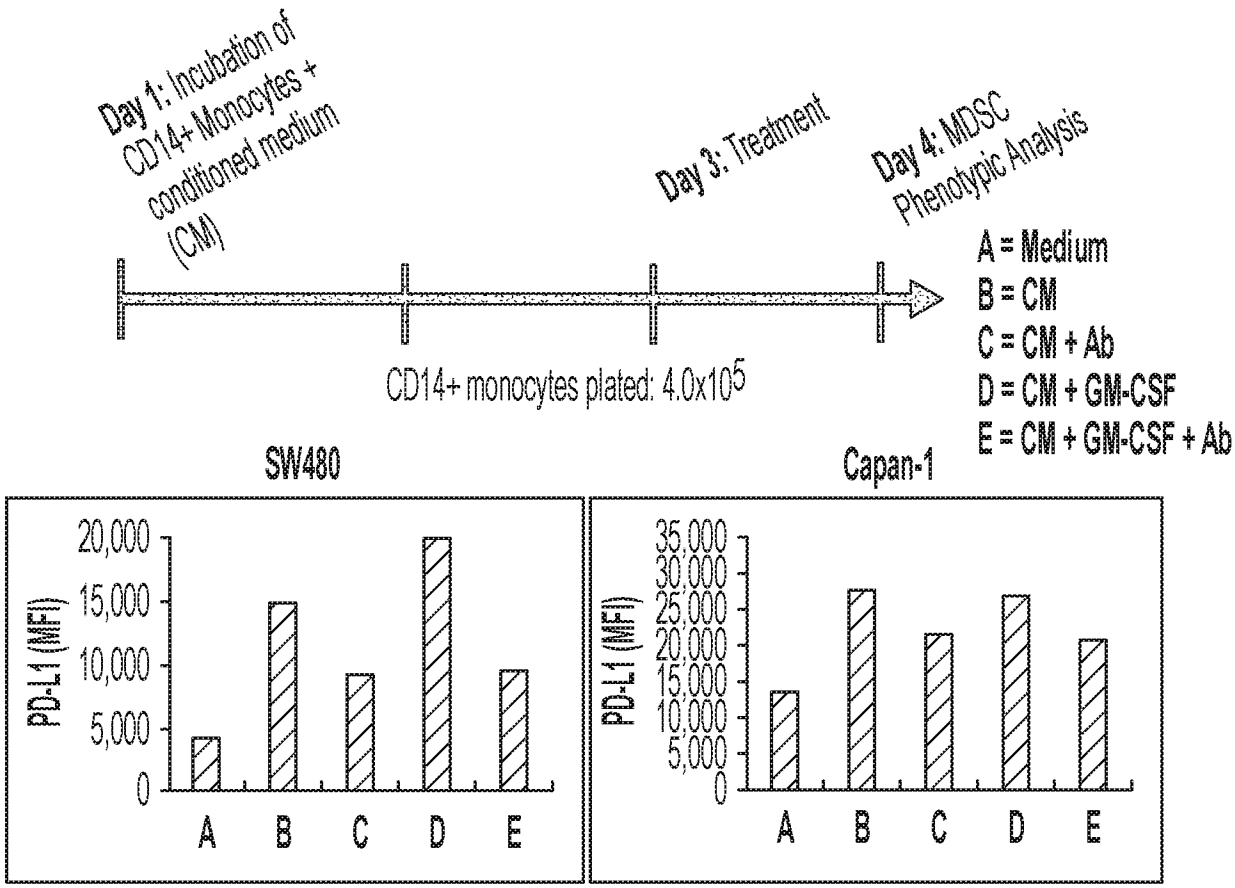
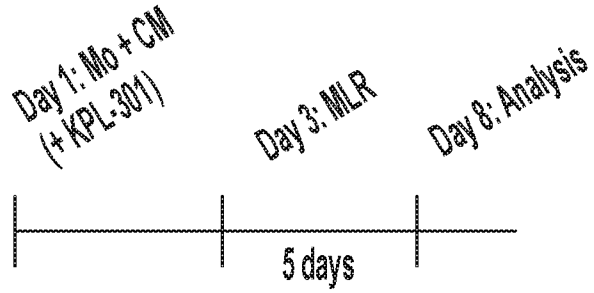
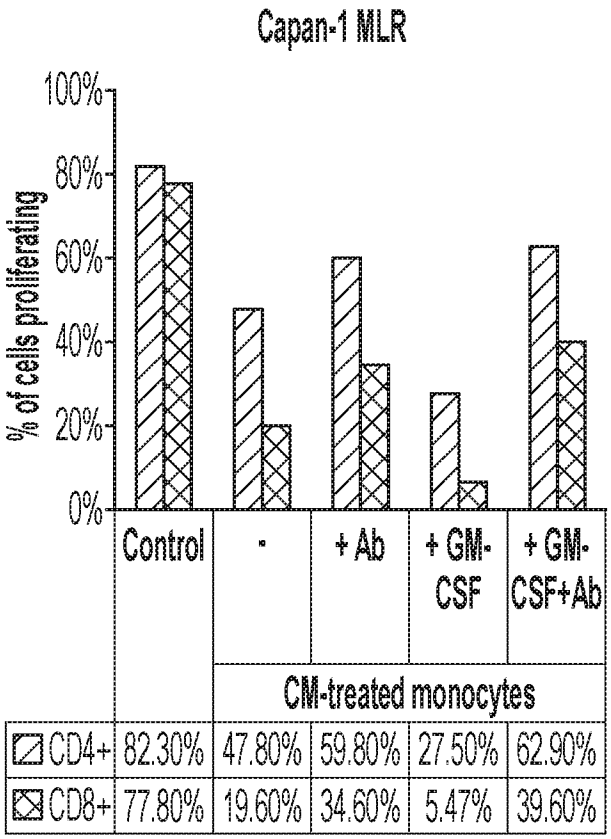
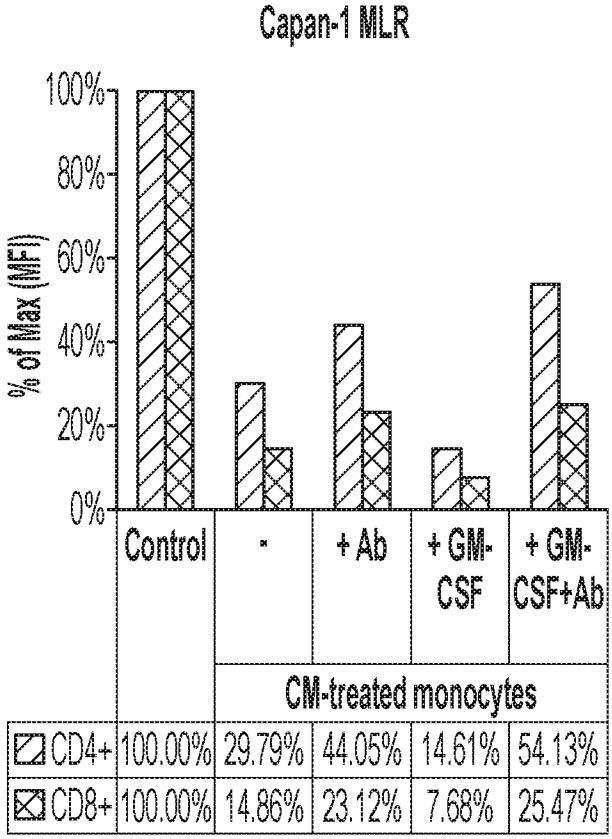

Day 1: Incubation of CD14+ Monocytes + conditioned medium (CM)
Day 3: Treatment
Day 4: MDSC Phenotypic Analysis
A = Medium
B = CM
C = CM + Ab
D = CM + GM-CSF
E = CM + GM-CSF + Ab
CD14+ monocytes plated: 4.0x10^5
SW480
Capan-1
PD-L1 (MFI)
20,000
15,000
10,000
5,000
0
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
A
B
C
D
E

FIG. 5B





		CM-treated monocytes			
	Control	-	+ Ab	+ GM-CSF	+ GM-CSF+Ab
CD4+	82.30%	47.80%	59.80%	27.50%	62.90%
CD8+	77.80%	19.60%	34.60%	5.47%	39.60%



		CM-treated monocytes			
	Control	-	+ Ab	+ GM-CSF	+ GM-CSF+Ab
CD4+	100.00%	29.79%	44.05%	14.61%	54.13%
CD8+	100.00%	14.86%	23.12%	7.68%	25.47%

FIG. 6

SEQUENCE LISTING

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<120> TREATMENT OF CANCERS WITH GM-CSF ANTAGONISTS

<130> KPL-035WO

<150> 62/856,638
<151> 2019-06-03

<160> 8

<170> PatentIn version 3.5

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<211> 120
<212> PRT
<213> Artificial Sequence

<220>
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<400> 1

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1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
20 25 30

Ser Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Gly Phe Asp Pro Glu Glu Asn Glu Ile Val Tyr Ala Gln Arg Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ile Val Gly Ser Phe Ser Pro Leu Thr Leu Gly Leu Trp Gly Gln
100 105 110

Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 2
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Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln

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Arg Val Thr Ile Ser Cys Thr Gly Ser Gly Ser Asn Ile Gly Ala Pro
            20                  25                  30

Tyr Asp Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35                  40                  45

Leu Ile Tyr His Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
    50                  55                  60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Val Glu Ala Gly
                85                  90                  95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
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<220>
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1               5
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<211> 17
<212> PRT
<213> Artificial Sequence

<220>
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<400> 4

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1               5                   10                  15

Gly
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<210> 6
<211> 14
<212> PRT
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<400> 7

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1 5

<210> 8
<211> 11
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<220>
<223> Synthetic polypeptide

<400> 8

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1 5 10