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(71) Applicant: **WINDMIL THERAPEUTICS, INC.**
[US/US]; 1812 Ashland Avenue, 1st Floor, Suite 100, Baltimore, MD 21205 (US).

(72) Inventors: **NOONAN, Kimberly, A.**; c/o WindMIL Therapeutics, Inc., 1812 Ashland Avenue, 1st Floor, Suite 100, Baltimore, MD 21205 (US). **BORRELLO, Ivan**; c/o WindMIL Therapeutics, Inc, 1812 Ashland Avenue, 1st Floor, Suite 100, Baltimore, MD 21205 (US).

(74) Agent: **CERMAK, Shelly, Guest et al.**; Cermak Nakajima & McGowan LLP, 127 S. Peyton Street, Suite 200, Alexandria, VA 22314 (US).

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(54) Title: PROSTATE CANCER SPECIFIC MARROW INFILTRATING LYMPHOCYTES AND USES THEREOF

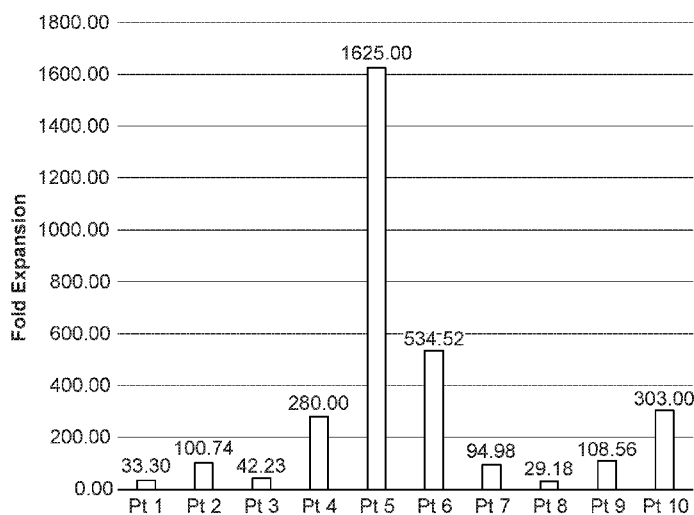


FIG. 1

(57) Abstract: The disclosure provides for compounds comprising prostate cancer specific marrow infiltrating lymphocytes and methods for making and using the same.

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**PROSTATE CANCER SPECIFIC MARROW INFILTRATING
LYMPHOCYTES AND USES THEREOF**

[0001] This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application 62/646,649, filed March 22, 2018, which is hereby incorporated by reference in its entirety.

GENERAL FIELD

[0002] The disclosure generally refers to marrow infiltrating lymphocytes (MILs) specific for treating prostate cancer and methods of use thereof.

BACKGROUND

[0003] Prostate cancer is one of the most commonly diagnosed cancers and is a leading cause of cancer-related deaths, and new therapies remain a clinical priority. Metastatic prostate cancer may be susceptible to autologous cellular immunotherapy. Sipuleucel-T, an autologous cell therapy for patients with mPCa, increased overall survival rates in phase III studies. Chimeric antigen receptor (CAR) T cell therapies directed at PSMA and PSCA are currently being developed in prostate cancer. Although promising, the overall efficacy and feasibility of these therapies remains unknown.

[0004] Marrow infiltrating lymphocytes (MILs) are the product of activating and expanding bone marrow T cells. The bone marrow is a specialized niche in the immune system which is enriched for antigen experienced, central memory T cells. MILs have been shown to confer immunologically measurable clinical benefits in patients with multiple myeloma (See US Patent No. 9,687,510). The bone marrow microenvironment has also been shown to harbor tumor-antigen specific T cells in patients with solid tumors such as breast, pancreatic and ovarian cancers. Therefore, what is needed is prostate cancer specific MILs for use in cancer therapy.

SUMMARY

[0005] Disclosed herein is a method for treating a subject having prostate cancer with marrow infiltrating lymphocytes, the method comprising the steps of: (a) culturing a bone marrow sample obtained from the subject having prostate cancer with an anti-CD3 antibody and an anti-CD28 antibody in a hypoxic environment to produce hypoxic-activated marrow infiltrating lymphocytes; (b) culturing the hypoxic-activated marrow infiltrating lymphocytes in a normoxic environment to produce the therapeutic activated marrow infiltrating lymphocytes; and (c) administering the therapeutic activated marrow infiltrating lymphocytes to the subject having prostate cancer.

[0006] Also disclosed herein is an embodiment of the method as described above, wherein the hypoxic environment has an oxygen content of about 0% to about 5% oxygen.

[0007] Also disclosed herein is an embodiment of the method as described above, wherein the lymphocytes are cultured in the presence of IL-2.

[0008] Also disclosed herein is an embodiment of the method as described above, wherein the culturing the hypoxic-activated marrow infiltrating lymphocytes in a normoxic environment is performed in the presence of IL-2.

[0009] Also disclosed herein is an embodiment of the method as described above, wherein the bone marrow sample is cultured in the hypoxic environment for about 24 hours.

[0010] Also disclosed herein is an embodiment of the method as described above, wherein the bone marrow sample is cultured in the hypoxic environment for about 2 days.

[0011] Also disclosed herein is an embodiment of the method as described above, wherein the bone marrow sample is cultured in the hypoxic environment for about 3 days.

[0012] Also disclosed herein is an embodiment of the method as described above, wherein the bone marrow sample is cultured in the hypoxic environment for about 2 to about 5 days.

[0013] Also disclosed herein is an embodiment of the method as described above, wherein the hypoxic environment is about 1% to about 2% oxygen.

[0014] Also disclosed herein is an embodiment of the method as described above, wherein the hypoxic-activated marrow infiltrating lymphocytes are cultured in the normoxic environment for about 2 to about 12 days.

[0015] Also disclosed herein is an embodiment of the method as described above, wherein the hypoxic-activated marrow infiltrating lymphocytes are cultured in the normoxic environment for about 6 days.

[0016] Also disclosed herein is an embodiment of the method as described above, wherein the hypoxic-activated marrow infiltrating lymphocytes are cultured in the normoxic environment for about 9 days.

[0017] Also disclosed herein is an embodiment of the method as described above, further comprising the step of removing a bone marrow sample from a subject having cancer prior to step (a).

[0018] Also disclosed herein is an embodiment of the method as described above, wherein the anti-CD3 antibody and the anti-CD28 antibody are bound on a bead.

[0019] Also disclosed herein is an embodiment of the method as described above, wherein the prostate cancer is one or more of acinar adenocarcinoma, ductal adenocarcinoma, castrate-resistant, transitional cell cancer, squamous cell cancer, or small cell prostate cancer.

[0020] Also disclosed herein is a method for treating a subject having prostate cancer with therapeutic activated marrow infiltrating lymphocytes, the method comprising the steps of: (a) culturing a bone marrow sample obtained from the subject having prostate cancer with anti-CD3/anti-CD28 beads in a hypoxic environment of about 1% to about 2% oxygen for about 2 to about 5 days to produce hypoxic-activated marrow infiltrating lymphocytes; (b) culturing the hypoxic-activated marrow infiltrating lymphocytes in a normoxic environment of about 21% oxygen for about 2 to about 12 days in the presence of IL-2 to produce the therapeutic activated marrow infiltrating lymphocytes; and (c) administering the therapeutic activated marrow infiltrating lymphocytes to the subject having prostate cancer.

[0021] Also disclosed herein is a method of treating prostate cancer in a subject, the method comprising administering a pharmaceutical composition comprising prostate cancer specific marrow infiltrating lymphocyte to the subject.

[0022] Also disclosed herein is an embodiment of the method as described above, wherein the prostate cancer specific marrow infiltrating lymphocyte is obtained from a subject having prostate cancer.

[0023] Also disclosed herein is an embodiment of the method as described above, wherein the prostate cancer specific marrow infiltrating lymphocyte is autologous to the subject being treated.

[0024] Also disclosed herein is an embodiment of the method as described above, wherein the prostate cancer specific marrow infiltrating lymphocyte is allogeneic to the subject being treated.

[0025] Also disclosed herein is an embodiment of the method as described above, wherein the marrow infiltrating lymphocyte is hypoxic activated.

[0026] Also disclosed herein is an embodiment of the method as described above, wherein the marrow infiltrating lymphocyte is hypoxic activated and normoxic activated.

[0027] Also disclosed herein is an embodiment of the method as described above, wherein the pharmaceutical composition is administered by parenteral administration, intraperitoneal or intramuscular administration.

[0028] Also disclosed herein is an embodiment of the method as described above, wherein the pharmaceutical composition is administered directly into the prostate of the subject.

[0029] Also disclosed herein is an embodiment of the method as described above, wherein the about 75% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.

[0030] Also disclosed herein is an embodiment of the method as described above, wherein the about 80% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.

[0031] Also disclosed herein is an embodiment of the method as described above, wherein the about 85% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.

[0032] Also disclosed herein is an embodiment of the method as described above, wherein the about 90% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.

[0033] Also disclosed herein is an embodiment of the method as described above, wherein the ratio of CD4⁺:CD8⁺ T cells present in the composition or MILs administered to the subject is about 2:1.

[0034] Also disclosed is a composition comprising a population of hypoxic-activated marrow infiltrating lymphocytes isolated from a patient with prostate cancer, wherein about 75% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.

[0035] Also disclosed herein is an embodiment of the method as described above, wherein about 80% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.

[0036] Also disclosed herein is an embodiment of the method as described above, wherein about 85% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.

[0037] Also disclosed herein is an embodiment of the method as described above, wherein about 90% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.

[0038] Also disclosed herein is an embodiment of the method as described above, wherein the ratio of CD4⁺:CD8⁺ T cells present in the composition is about 2:1.

[0039] Also disclosed herein is an embodiment of the method as described above, wherein the cell population is obtainable from a bone marrow sample obtained from a subject having prostate cancer by: (a) culturing the bone marrow sample with an anti-CD3 antibody and an anti-CD28 antibody in a hypoxic environment of about 1% to about 3% oxygen to produce activated marrow infiltrating lymphocytes; and (b) culturing the activated marrow infiltrating lymphocytes in a normoxic environment in the presence of IL-2 to produce the composition.

[0040] Also disclosed herein is an embodiment of the method as described above, wherein the MILs are prostate cancer specific.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] Figure 1 is a graph showing the successful expansion of MILs from metastatic prostate cancer patient bone marrow. The fold expansion expressed as harvested cell #/starting cell number for each patient's bone marrow specimen is shown.

[0042] Figure 2 shows a graph of the percentage of cells staining positively for each T cell marker in each of the 10 metastatic prostate cancer patient bone marrow specimens pre and post expansion.

[0043] Figure 3 shows the quantification of tumor-specific T cells. Autologous antigen-presenting cells (APCs) were pulsed with lysates from prostate cancer cell lines and co-cultured with CFSE-labelled MILs or PBLs.

[0044] Figure 4 shows representative results for a single patient of the quantification of tumor-specific T cells in expanded MILs and PBLs.

[0045] Figure 5 shows the percentages of IFN γ -producing CFSE-lo CD3⁺ T cells measured against each of the tumor cell lysate combinations for each of the expanded MILs.

[0046] Figure 6 shows the percentages of IFN γ -producing CFSE-lo CD3⁺ T cells measured against each of the tumor cell lysate combinations for each of the PBLs that were expanded.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0047] As used herein and unless otherwise indicated, the term “about” is intended to mean \pm 5% of the value it modifies. Thus, about 100 means 95 to 105. Additionally, the term “about” modifies a term in a series of terms, such as “about 1, 2, 3, 4, or 5” it should be understood that the term “about” modifies each of the members of the list, such that “about 1, 2, 3, 4, or 5” can be understood to mean “about 1, about 2, about 3, about 4, or about 5.” The same is true for a list that is modified by the term “at least” or other quantifying modifier, such as, but not limited to, “less than,” “greater than,” and the like.

[0048] As used herein and in the appended claims, the singular forms “a”, “an” and “the” include plural reference unless the context clearly dictates otherwise.

[0049] As used herein, the terms “comprising” (and any form of comprising, such as “comprise”, “comprises”, and “comprised”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”), or “containing” (and any form of containing, such as “contains” and “contain”), are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. Any composition or method that recites the term “comprising” should also be understood to also describe such compositions as consisting, consisting of, or consisting essentially of the recited components or elements.

[0050] As used herein, the terms “treat,” “treated,” or “treating” mean both therapeutic treatments wherein the object is to slow down (lessen) an undesired physiological condition, disorder or disease, or obtain beneficial or desired clinical results. For purposes of the embodiments described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e., not worsening) state of condition, disorder or disease; delay in onset or slowing of condition, disorder or disease progression; amelioration of the condition, disorder or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or

improvement of condition, disorder or disease. Thus, “treatment of cancer” or “treating cancer” means an activity that alleviates or ameliorates any of the primary phenomena or secondary symptoms associated with the cancer or any other condition described herein. In some embodiments, the cancer that is being treated is one of the cancers recited herein.

[0051] As used herein, the term “subject” can be used interchangeably with the term “patient”. The subject can be a mammal, such as a dog, cat, monkey, horse, cow, and the like. In some embodiments, the subject is a human. In some embodiments, the subject has been diagnosed with prostate cancer. In some embodiments, the subject is believed to have prostate cancer. In some embodiments, the subject is suspected of having prostate cancer.

[0052] As used herein, the term “express” as it refers to a cell surface receptor, such as, but not limited to, CD3, CD4, and CD8, can also be referred to as the cell being positive for that marker. For example, a cell that expresses CD3 can also be referred to as CD3 positive (CD3⁺).

[0053] The term “cancer” as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. The term “prostate cancer” as used herein is defined as cancer originating from the prostate, or cancer on or within the prostate. In some embodiments, prostate cancer is one or more of acinar adenocarcinoma, ductal adenocarcinoma, castrate-resistant, transitional cell cancer, squamous cell cancer, or small cell prostate cancer.

[0054] “Effective amount” or “therapeutically effective amount” are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. Such results may include, but are not limited to, the inhibition of virus infection as determined by any means suitable in the art.

[0055] As used herein, “marrow infiltrating lymphocytes” or “MILs” are a subpopulation of immune cells and are described for example in, U.S. Patent No. 9,687,510, which is hereby incorporated by reference in its entirety. MILs significantly differ from peripheral lymphocytes (PBLs). For example, MILs are more easily expanded, upregulate activation markers to a greater extent than PBLs, maintain more of a skewed V β repertoire, traffic to the bone marrow, and most importantly, possess significantly greater tumor specificity. In some embodiments, MILs

can be activated, for example, by incubating them with anti-CD3/anti-CD-28 beads and under hypoxic conditions, as described herein. In some embodiments, growing MILs under hypoxic conditions is also described in U.S. Patent No. 9,687,510, and International Application No. WO2016/037054, both of which are incorporated by reference herein in their entirety.

[0056] Compared to previous methods of using MILs in non-solid tumor type cancers such as multiple myeloma, use of MILs to treat solid tumor cancers require a different and distinct paradigm. Many tumors are known to exploit the PD-1/PD-L1 pathway through upregulation of PD-L1 on tumor cells and other cells to escape T cell-mediated tumor-specific immunity. Inhibiting the interaction between PD-1 and PD-L1 decreases this immunosuppressive signal, allowing tumor-specific cytotoxic T cells to access and kill the tumor cells.

[0057] MILs™ are distinct from two other forms of adoptive cellular therapy. Table 1 compares key characteristics of MILs™ to chimeric antigen receptor (CAR)-T and genetically engineered T cell receptor (eTCR) cell therapies. Most critical is that the efficacy of CAR-T and eTCR cell therapies are dependent upon engagement of the cognate antigen on the tumor cells; selective editing or deletion of that antigen by the tumor will render CAR-T or eTCR therapies non-effective. In contrast, the polyclonal recognition of MILs™ should minimize the risk of generating antigen escape loss tumor variants as a mechanism of disease relapse.

Table 1: Comparison of MILs™ to CAR-T and eTCR cells

Characteristic	CAR-T/eTCR	MILs™
Cell Source	Peripheral Blood	Bone Marrow
Antigen Specificity	Monoclonal (Limited)	Polyclonal
Genetic Modification	Required	Not required
HLA Restricted	No (CAR-T); Yes (eTCR)	No

Abbreviations: MILs™ = marrow infiltrating lymphocytes; CAR-T = chimeric antigen receptor; eTCR = engineered T cell receptor; HLA = human leukocyte antigen

[0058] In some embodiments, methods to prepare MILs may comprise removing cells from the bone marrow, lymphocytes, and/or marrow infiltrating lymphocytes from the subject;

incubating the cells in a hypoxic environment, thereby producing activated MILs. In some embodiments, the subject has prostate cancer. The cells can also be activated in the presence of anti-CD3/anti-CD28 antibodies and cytokines as described herein.

[0059] The collected bone marrow may be frozen or immediately used, for example, to create tumor specific MILs. If the bone marrow is frozen, it is preferably thawed before incubation. The bone marrow may be treated to purify MILs through methods known to one of ordinary skill in the art. The MILs may be activated, for example, with beads, e.g., anti-CD4/CD28 beads. The ratio of beads to cells in the solution may vary; in some embodiments, the ratio is 3 to 1. Similarly, the MILs may be expanded in the presence of one or more antibodies, antigens, and/or cytokines, e.g., in the absence of anti-CD3/CD28 beads. The cell count for the collected bone marrow may be determined, for example, to adjust the amounts of beads, antibodies, antigens, and/or cytokines to be added to the MILs. In some embodiments, MILs are captured using beads specifically designed to collect the cells.

[0060] The collected MILs can be grown in a hypoxic environment for a first period of time. The hypoxic environment may include less than about 7% oxygen, such as less than about 7%, 6%, 5%, 4%, 3%, 2%, or 1% oxygen. For example, the hypoxic environment may include about 0% oxygen to about 7% oxygen, 0% oxygen to about 6% oxygen, such as about 0% oxygen to about 5% oxygen, about 0% oxygen to about 4% oxygen, about 0% oxygen to about 3% oxygen, about 0% oxygen to about 2% oxygen, about 0% oxygen to about 1% oxygen. In some embodiments, the hypoxic environment includes about 1% to about 5% oxygen. In some embodiments, the hypoxic environment is about 1% to about 2% oxygen. In some embodiments, the hypoxic environment is about 0.5% to about 1.5% oxygen. In some embodiments, the hypoxic environment is about 0.5% to about 2% oxygen. The hypoxic environment may include about 7%, 6%, 5%, 4%, 3%, 2%, 1%, or about 0% oxygen, and any fraction thereof in between these amounts.

[0061] Incubating MILs in a hypoxic environment may comprise incubating the MILs, e.g., in tissue culture medium, for at least about 1 hour, such as at least about 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 60 hours, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or even at least about 14 days. Incubating may comprise incubating the MILs for about 1 hour to about 30 days, such as about 1 day to about 20

days, about 1 day to about 14 days, or about 1 day to about 12 days. In some embodiments, incubating MILs in a hypoxic environment comprises incubating the MILs in a hypoxic environment for about 2 days to about 5 days. The method may comprise incubating MILs in a hypoxic environment for about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 day, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days. In some embodiments, the method comprises incubating the MILs in a hypoxic environment for about 3 days. In some embodiments, the method comprises incubating the MILs in a hypoxic environment for about 2 days to about 4 days. In some embodiments, the method comprises incubating the MILs in a hypoxic environment for about 3 days to about 4 days.

[0062] In some embodiments, hypoxic-activated MILs are then cultured in a normoxic environment to produce the therapeutically activated marrow infiltrating lymphocytes. In some embodiments, the normoxic environment may include at least about 7% oxygen. In some embodiments, the normoxic environment may include about, such as about 8% oxygen to about 30% oxygen, 10% oxygen to about 30% oxygen, about 15% oxygen to about 25% oxygen, about 18% oxygen to about 24% oxygen, about 19% oxygen to about 23% oxygen, or about 20% oxygen to about 22% oxygen. In some embodiments, the normoxic environment includes about 21% oxygen.

[0063] In some embodiments, the MILs are cultured in the presence of IL-2 or other cytokines. In some embodiments, the MILs are cultured in normoxic conditions in the presence of IL-2. In some embodiments, the other cytokines can be IL-7, IL-15, IL-9, IL-21, or any combination thereof. In some embodiments, the MILs can be cultured in cell culture medium that comprises one or more cytokines, e.g., such as IL-2, IL-7, and/or IL-15, or any suitable combination thereof. Illustrative examples of suitable concentrations of each cytokine or the total concentration of cytokines includes, but is not limited to, about 25 IU/mL, about 50 IU/mL, about 75 IU/mL, about 100 IU/mL, about 125 IU/mL, about 150 IU/mL, about 175 IU/mL, about 200 IU/mL, about 250 IU/mL, about 300 IU/mL, about 350 IU/mL, about 400 IU/mL, about 450 IU/mL, or about 500 IU/mL or any intervening amount of cytokine thereof. In some embodiments, the cells are cultured in about 100 IU/mL of each of, or in total of, IL-2, IL-1, and/or IL-15, or any combination thereof. In some embodiments, the cell culture medium

comprises about 250 IU/mL of each of, or in total of, IL-2, IL-1, and/or IL-15, or any combination thereof.

[0064] Incubating MILs in a normoxic environment may comprise incubating the MILs, for at least about 1 hour, such as at least about 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 60 hours, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or even at least about 14 days. Incubating may comprise incubating the MILs for about 1 hour to about 30 days, such as about 1 day to about 20 days, about 1 day to about 14 days, about 1 day to about 12 days, or about 2 days to about 12 days.

[0065] In some embodiments, the MILs are obtained by extracting a bone marrow sample from a subject and culturing/incubating the cells as described herein. In some embodiments, the bone marrow sample is centrifuged to remove red blood cells. In some embodiments, the bone marrow sample is not subject to apheresis. In some embodiments, the bone marrow sample does not comprise peripheral blood lymphocytes (“PBLs”) or the bone marrow sample is substantially free of PBLs. These methods select for cells that are not the same as what have become to be known as TILs. Thus, a MIL is not a TIL. TILs can be selected by known methods to one of skill in the art and can be transfected or infected with the nucleic acid molecules described herein such that the TILs can express the chimeric transmembrane protein described herein. In some embodiments, the bone marrow sample contains less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% PBLs as compared to the total of MILs. In some embodiments, the sample is free of PBLs.

[0066] In some embodiments, the cells are also activated by culturing with antibodies to CD3 and CD28. This can be performed, for example by incubating the cells with anti-CD3/anti-CD28 beads that are commercially available or that can be made by one of skill in the art. The cells can then be plated in a plate, flask, or bag. Hypoxic conditions can be achieved by flushing either the hypoxic chamber or cell culture bag for 3 minutes with a 95% Nitrogen and 5% CO₂ gas mixture. This can lead to, for example, 1-2% or less O₂ gas in the receptacle. Examples of such beads and methods of stimulation can be found, for example, in U.S. Patent Nos. 6,352,694, 6,534,055, 6,692,964, 6,797,514, 6,867,041, 6,905,874, each of which are incorporated by reference in its entirety. Alternatives to beads are engineered cells, such as K562 cells, that can be used to stimulate the MILs. Such methods can be found in, for example, U.S. Patent Nos.

8,637,307 and 7,638,325, each of which are incorporated by reference in its entirety. Cells can also be stimulated using other methods, such as those described in U.S. Patent No. 8,383,099, which is incorporated by reference in its entirety.

[0067] In some embodiments, activated MILs and/or therapeutic activated MILs are administered to a subject having, or suspected of having, prostate cancer. In some embodiments, hypoxic-activated MILs and/or therapeutic activated MILs are produced from a bone marrow sample from a subject having or suspected of having prostate cancer, then administering to the same subject to treat prostate cancer. In some embodiments, the MILs are allogeneic to the subject.

[0068] In some embodiments, the MILs can be administered in a pharmaceutical preparation or pharmaceutical composition. Pharmaceutical compositions comprising the prostate cancer specific MILs may further comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions can be formulated for parenteral administration, e.g., intravascular (intravenous or intraarterial), intraperitoneal or intramuscular administration. In some embodiments, the MILs and/or compositions are administered by parenteral administration, e.g., intravascular (intravenous or intraarterial), intraperitoneal or intramuscular administration. The compositions can also be administered directly into the prostate. In some embodiments, the compositions are administered intravenously.

[0069] In some embodiments, compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: DMSO, sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[0070] In some embodiments, the subject can be pre-conditions with cyclophosphamide with or without fludarabine. One such example is provided for in US 9,855,298, which is hereby incorporated by reference. Another non-limiting example is administering fludarabine (30 mg/m² intravenous daily for 4 days) and cyclophosphamide (500 mg/m² intravenous daily for 2 days starting with the first dose of fludarabine). After administration, the MILs can be administered 2 to 14 days after completion of the fludarabine. In some embodiments, the cyclophosphamide is administered or 2-3 days at a dose of about 500 to about 600 mg/m².

[0071] In some embodiments, the pharmaceutical composition that is administered comprises prostate-cancer specific MILs as provided for herein. A composition of such MILs is also provided for herein. In some embodiments, the prostate cancer specific MILs are hypoxic activated. In some embodiments, the prostate cancer specific MILs are hypoxic activated/normoxic activated MILs. A prostate cancer specific MIL is a MIL that can specifically target prostate cancer in a subject.

[0072] In some embodiments, the composition comprises a population of prostate cancer specific MILs that are CD3 positive. In some embodiments, at least about, or at least, 40% of the MILs are CD3 positive. In some embodiments, about, or at least, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, or 89% of MILs are CD3 positive. In some embodiments, at least, or about, 80% of the MILs are CD3 positive. In some embodiments, about 40% to about 100% of the MILs are CD3 positive. In some embodiments, about 45% to about 100%, about 50% to about 100%, about 55% to about 100%, about 60% to about 100%, about 65% to about 100%, about 70% to about 100%, about 75% to about 100%, about 80% to about 100%, about 85% to about 100%, about 86% to about 100%, about 87% to about 100%, about 88% to about 100%, or about 90% to about 100% of the MILs are CD3 positive (express CD3).

[0073] In some embodiments, the composition comprises either a population of MILs that do not express CD3, or a population of MILs that expresses low levels of CD3, for example, relative to the expression level of MILs from the population of MILs that express CD3.

[0074] In some embodiments, the composition comprises a population of MILs that expresses interferon gamma (“IFN γ ”), i.e., wherein each cell in the population of MILs that expresses IFN γ is a marrow infiltrating lymphocyte that expresses IFN γ , e.g., as detected by flow

cytometry. For example, at least about 2% of the cells in the composition may be MILs that express IFN γ , or at least about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, or even at least about 18% of the MILs express IFN γ . In some embodiments, about 2% to about 100% of the MILs express IFN γ , such as about 2% to about 100%, about 3% to about 100%, about 4% to about 100%, about 5% to about 100%, about 6% to about 100%, about 7% to about 100%, about 8% to about 100%, about 9% to about 100%, about 10% to about 100%, about 11% to about 100%, about 12% to about 100%, about 13% to about 100%, about 14% to about 100%, about 15% to about 100%, about 16% to about 100%, about 17% to about 100%, or even about 18% to about 100% of the MILs. In some embodiments, the composition comprises either a population of MILs that do not express IFN γ , e.g., as detected by flow cytometry, or a population of MILs that expresses low levels of IFN γ , i.e., relative to the expression level of MILs from the population of MILs that express IFN γ .

[0075] In some embodiments, the composition comprises a population of MILs that expresses CXCR4. For example, at least about 98% of the MILs express CXCR4, such as at least about 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99.0%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, or even at least about 99.7% of the MILs. In some embodiments, about 98% to about 100% may be MILs that express CXCR4, such as at least about 98.1% to about 100%, about 98.2% to about 100%, about 98.3% to about 100%, about 98.4% to about 100%, about 98.5% to about 100%, about 98.6% to about 100%, about 98.7% to about 100%, about 98.8% to about 100%, about 98.9% to about 100%, about 99.0% to about 100%, about 99.1% to about 100%, about 99.2% to about 100%, about 99.3% to about 100%, about 99.4% to about 100%, about 99.5% to about 100%, about 99.6% to about 100%, or even about 99.7% to about 100% of the MILs in the composition. In some embodiments, the composition comprises either a population of MILs that do not express CXCR4, e.g., as detected by flow cytometry, or a population of MILs that expresses low levels of CXCR4, i.e., relative to the expression level of MILs from the population of MILs that express CXCR4.

[0076] The population of MILs that expresses CD4 may comprise a plurality of MILs that expresses 4-1BB. For example, at least about 21% of the cells in the composition may be MILs from the plurality of MILs that expresses 4-1BB, such as at least about 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%,

42%, or even at least about 43% of the cells in the composition. In some embodiments, about 21% to about 100% of the cells in the composition may be MILs from the plurality of MILs that expresses 4-1BB, such as about 22% to about 100%, about 23% to about 100%, about 24% to about 100%, about 25% to about 100%, about 26% to about 100%, about 27% to about 100%, about 28% to about 100%, about 29% to about 100%, about 30% to about 100%, about 31% to about 100%, about 32% to about 100%, about 33% to about 100%, about 34% to about 100%, about 35% to about 100%, about 36% to about 100%, about 37% to about 100%, about 38% to about 100%, about 39% to about 100%, about 40% to about 100%, about 41% to about 100%, about 42% to about 100%, or even about 43% to about 100% of the cells in the composition.

[0077] The composition may comprise a population of MILs that expresses CD8. The population of MILs that expresses CD8 may comprise a plurality of MILs that expresses CXCR4.

[0078] The population of MILs that expresses CD8 may comprise a plurality of MILs that expresses 4-1BB. For example, at least about 21% of the cells in the composition may be MILs from the plurality of MILs that expresses 4-1BB, such as at least about 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% or even at least about 21% of the cells in the composition. In some embodiments, about 2% to about 100% of the cells in the composition may be MILs from the plurality of MILs that expresses 4-1BB, such as about 8% to about 100%, about 9% to about 100%, about 10% to about 100%, about 11% to about 100%, about 12% to about 100%, about 13% to about 100%, about 14% to about 100%, about 15% to about 100%, about 16% to about 100%, about 17% to about 100%, about 18% to about 100%, about 19% to about 100%, about 20% to about 100%, or even about 21% to about 100% of the cells in the composition.

[0079] In some embodiments, the composition comprises a population of MILs that expresses 4-1BB. For example, at least about 21% of the cells in the composition may be MILs from the population of MILs that expresses 4-1BB, such as at least about 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, or even at least about 43% of the cells in the composition. In some embodiments, about 21% to 100% of the cells in the composition may be MILs from the population of MILs that

expresses 4-1BB, such as about 22% to about 100%, about 23% to about 100%, about 24% to about 100%, about 25% to about 100%, about 26% to about 100%, about 27% to about 100%, about 28% to about 100%, about 29% to about 100%, about 30% to about 100%, about 31% to about 100%, about 32% to about 100%, about 33% to about 100%, about 34% to about 100%, about 35% to about 100%, about 36% to about 100%, about 37% to about 100%, about 38% to about 100%, about 39% to about 100%, about 40% to about 100%, about 41% to about 100%, about 42% to about 100%, or even about 43% to about 100% of the cells in the composition. In some embodiments, the composition comprises either a population of MILs that do not express 4-1BB, e.g., as detected by flow cytometry, or a population of MILs that expresses low levels of 4-1BB, i.e., relative to the expression level of MILs from the population of MILs that express 4-1BB.

[0080] In some embodiments, the composition comprises MILs that express CD4.

[0081] In some embodiments, the composition comprises MILs that express CD8.

[0082] In some embodiments, the composition comprises MILs that express CD4. In some embodiments, the composition comprises MILs that express CD8. In some embodiments, the ratio of CD4⁺:CD8⁺ MILs present in the composition is about 2:1.

[0083] The composition may comprise a population of MILs that expresses CD8. The population of MILs that expresses CD8 may comprise a plurality of MILs that expresses CXCR4.

[0084] In some embodiments, the composition comprises a population of MILs that expresses CD4. The population of MILs that expresses CD4 may comprise a plurality of MILs that expresses CXCR4.

[0085] The MILs may express the different factors or surface receptors as described herein alone or in combination with one another. Thus, for example, a MIL can be CD3⁺, CD4⁺, and CD8⁺. Such cells can also express IFN γ . The cells can also be positive or negative for the various factors or receptors provided for herein.

[0086] In some embodiments, the methods for preventing or treating prostate cancer in a subject are provided. In some embodiments, the methods comprise administering to a subject one of the compositions described herein, such as, but not limited to, prostate-specific MILs as provided herein. In some embodiments, the compositions are administered as provided for herein. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of any one of the compositions described herein. In some embodiments, the method comprises administering to the subject a therapeutically-effective amount of the prostate cancer specific MILs. In some embodiments, the MILs are activated. In some embodiments, the MILs are hypoxic activated as described herein and referenced herein. In some embodiments, the MILs are cultured under hypoxic conditions followed by normoxic conditions as described and referenced herein. In some embodiments, MILs are obtained or extracted from a bone marrow sample obtained from a subject having prostate cancer. In some embodiments, the MILs are allogeneic to the subject being treated. In some embodiments, the methods comprise culturing a bone marrow sample from a subject with an anti-CD3 antibody and an anti-CD28 antibody in a hypoxic environment of about 1% to about 3% oxygen to produce activated marrow infiltrating lymphocytes; and (b) culturing the activated marrow infiltrating lymphocytes in a normoxic environment in the presence of IL-2 to produce the composition. The composition can be then be administered to the subject with prostate cancer.

[0087] The following examples are illustrative, but not limiting, of the compositions and methods described herein. Other suitable modifications and adaptations known to those skilled in the art are within the scope of the following embodiments.

EXAMPLES

Example 1: Producing MILs from subjects with prostate cancer.

[0088] Bone marrow samples were collected from hormone-naïve and castration-resistant prostate cancer patients (n=10) with varying amounts of bone marrow involvement. For a subset of patients (n=4), matched peripheral blood was also collected at the time of bone marrow aspiration.

[0089] Both MILs and peripheral blood lymphocytes (PBLs) were activated and expanded from patient bone marrow and blood samples, respectively, using methods previously described (see U.S. Patents 9,687,510 and 10,172,887, both of which are incorporated by reference). MILs were successfully expanded from bone marrow isolated from ten mPCa patients with an average fold expansion of 315.1 (range: 29.1- 1625). The fold expansion (harvested cell #/ starting cell number) for each patient's bone marrow specimen is shown. See Figure 1.

Example 2: Characterization of T cell phenotypic markers

[0090] The T cell phenotypic markers CD3, CD4, and CD8 were characterized by flow cytometry (FACS) pre- and post-expansion. The percentage of cells staining positively for each T cell marker is shown for each of the ten metastatic prostate cancer patient bone marrow specimens prior to (pre) and following (post) expansion. Pre-expansion, the bone marrow T cell composition was 21.5% (7.8-38.0) CD3⁺, 14.1% (7.5-26.2) CD4⁺, and 6.1% (2.3-11.8) CD8⁺. After activation and expansion, MILs were on average 91.5% (88.6-95.1) CD3⁺ with an ~2.5:1 ratio of CD4⁺:CD8⁺ T cells [66.4% (37.2-88.0) vs. 25.7% (11.9-56.3), respectively]. See figure 2.

Example 3: Quantification of tumor-specific T cells in expanded MILs and PBLs

[0091] Tumor-specific T cells were quantitated in expanded MILs and PBLs using a previously described functional assay. (Noonan KA, Huff CA, Davis J, *et al. Sci Transl Med.* 2015;7(288):288ra78). Briefly, the MILs and PBLs obtained as described in Example 1 were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen), incubated for 10 min at 37°C, and washed per the manufacturer's recommendations. Autologous antigen-presenting cells (APCs) were pulsed with lysates from prostate cancer cell lines and co-cultured with CFSE-labelled MILs or PBLs. APCs pulsed with myeloma cell line lysates or media alone were used as negative controls. After 5 days, tumor specificity was determined by staining the cells with anti-CD3 and IFN- γ (eBioscience) and by analyzing them with flow cytometry. Data were collected on the Gallios flow cytometer (Beckman Coulter) and analyzed with Kaluza

software (Beckman Coulter). Tumor-specific T cells were defined as the IFN γ -producing CFSE-low, CD3⁺ population. See Figure 3.

[0092] Figure 4 shows representative results for a single patient. The percentages of IFN γ -producing CFSE-lo CD3⁺, CD8⁺ and CD4⁺ T cells following stimulation with autologous bone marrow APCs pulsed with no cell lysate (Media Alone), with multiple myeloma lysates as a negative control, or with two of the four different combinations of prostate cancer cell line lysates tested are shown for matched MILs and PBLs expanded from patient 8.

[0093] Figure 5 shows the percentages of IFN γ -producing CFSE-lo CD3⁺ T cells measured against each of the tumor cell lysate combinations for each of the expanded MILs. Prostate tumor-specific T cells were detected in all of the expanded MILs (n=9). On average, 11.1% (1.25-44) of the total T cell repertoire in expanded MILs were tumor specific.

[0094] Figure 6 shows the percentages of IFN γ -producing CFSE-lo CD3⁺ T cells measured against each of the tumor cell lysate combinations for each of the PBLs that were expanded. In contrast, matched PBLs expanded and activated from four patients demonstrated no measurable tumor-specific T cells.

Example 4: Administration of MILs to prostate cancer patients

[0095] Prior to administration of MILs, patients will receive non-myeloablative lymphodepletion with cyclophosphamide (300 mg/m²/day) and fludarabine (30 mg/m²/day) from Days -5 to -3. Lymphodepletion has been shown to increase the overall efficacy of adoptive T cell therapy. 2-mercaptoethane sulfonate sodium (MESNA) may be used to minimize any bleeding in the bladder as required.

[0096] Patients may also receive pembrolizumab (200 mg) administered on Day 1 (approximately 24 hours after MILsTM administration) and again every 3 weeks.

[0097] Patients may be administered MILsTM alone. These subjects will be followed closely for 7 days post MILsTM administration for safety observation.

[0098] The MILs™ will be administered via a central catheter, which could either be a peripherally inserted central catheter (PICC) line or central line. Prior to administering the activated MILs™, the subject will be hydrated with 5% dextrose in water and 50% normal saline (D5W½NS) at a rate of approximately 200 mL per hour for at least one hour. MILs™ will be thawed at the bedside in a 37°C (± 2°C) water bath for approximately 90 seconds (± 30 seconds) per bag prior to being administered on Day 0 (+1 day). Each bag will be removed from the vapor phase liquid nitrogen shipper, one at a time, placed in the waterbath and massaged until there are some small chunks of ice present. Each bag of MILs™ will be infused at a rate of approximately 10 mL per minute and rinsed with saline prior to administering the next bag of MILs™.

[0099] Following MILs™ infusion, the patients will be hydrated with D5W½NS at a rate of approximately 200 mL per hour for 2 hours. Administration information including, but not limited to, date and time of thawing, time of administration, and infusion time, will be recorded for each bag. Dose modification is not applicable as the entire MILs™ product will be administered on at least one day, Day 0 (+1 day).

[0100] Tumor burden in patients will be evaluated by measuring prostate specific antigen ("PSA") using known assays.

[0101] MILs were present and were expanded from all prostate cancer bone marrow samples tested. MILs from all patients contained functionally active tumor-specific T cells. In contrast, the corresponding PBLs failed to show any detectable tumor-specific immune recognition. As such, adoptive T cell therapy with MILs is a surprising and viable novel therapeutic approach for patients with prostate cancer, which until the embodiments provided for herein was not expected to be achievable using adoptive T cell therapy. The results from the prostate-specific MILs were surprising and unexpected.

[0102] This description is not limited to the particular processes, compositions, or methodologies described, as these may vary. The terminology used in the description is for the purpose of describing the particular versions or embodiments only, and it is not intended to limit the scope of the embodiments described herein. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. In some cases, terms with commonly understood meanings are defined herein for

clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. However, in case of conflict, the patent specification, including definitions, will prevail.

[0103] From the foregoing, it will be appreciated that various embodiments of the present disclosure have been described herein for purposes of illustration, and that various modification can be made without departing from the scope and spirit of the present disclosure. Accordingly, the various embodiments disclosed herein are not intended to be limiting. All references cited herein are hereby incorporated by reference in their entireties.

What is claimed:

1. A method for treating a subject having prostate cancer with marrow infiltrating lymphocytes, the method comprising the steps of:
 - (a) culturing a bone marrow sample obtained from the subject having prostate cancer with an anti-CD3 antibody and an anti-CD28 antibody in a hypoxic environment to produce hypoxic-activated marrow infiltrating lymphocytes;
 - (b) culturing the hypoxic-activated marrow infiltrating lymphocytes in a normoxic environment to produce the therapeutic activated marrow infiltrating lymphocytes; and
 - (c) administering the therapeutic activated marrow infiltrating lymphocytes to the subject having prostate cancer.
2. The method of claim 1, wherein the hypoxic environment has an oxygen content of about 0% to about 5% oxygen.
3. The method of claim 1, wherein the lymphocytes are cultured in the presence of IL-2.
4. The method of claim 1, wherein the culturing the hypoxic-activated marrow infiltrating lymphocytes in a normoxic environment is performed in the presence of IL-2.
5. The method of claim 1, wherein the bone marrow sample is cultured in the hypoxic environment for about 24 hours.
6. The method of claim 1, wherein the bone marrow sample is cultured in the hypoxic environment for about 2 days.
7. The method of claim 1, wherein the bone marrow sample is cultured in the hypoxic environment for about 3 days.
8. The method of claim 1, wherein the bone marrow sample is cultured in the hypoxic environment for about 2 to about 5 days.

9. The method of claim 1, wherein the hypoxic environment is about 1% to about 2% oxygen.
10. The method of claim 1, wherein the hypoxic-activated marrow infiltrating lymphocytes are cultured in the normoxic environment for about 2 to about 12 days.
11. The method of claim 1, wherein the hypoxic-activated marrow infiltrating lymphocytes are cultured in the normoxic environment for about 6 days.
12. The method of claim 1, wherein the hypoxic-activated marrow infiltrating lymphocytes are cultured in the normoxic environment for about 9 days.
13. The method of claim 1, further comprising the step of removing a bone marrow sample from a subject having cancer prior to step (a).
14. The method of claim 1, wherein the anti-CD3 antibody and the anti-CD28 antibody are bound on a bead.
15. The method of claim 1, wherein the prostate cancer is one or more of acinar adenocarcinoma, ductal adenocarcinoma, castrate-resistant, transitional cell cancer, squamous cell cancer, or small cell prostate cancer.
16. A method for treating a subject having prostate cancer with therapeutic activated marrow infiltrating lymphocytes, the method comprising the steps of:
 - (a) culturing a bone marrow sample obtained from the subject having prostate cancer with anti-CD3/anti-CD28 beads in a hypoxic environment of about 1% to about 2% oxygen for about 2 to about 5 days to produce hypoxic-activated marrow infiltrating lymphocytes;
 - (b) culturing the hypoxic-activated marrow infiltrating lymphocytes in a normoxic environment of about 21% oxygen for about 2 to about 12 days in the presence of IL-2 to produce the therapeutic activated marrow infiltrating lymphocytes; and

(c) administering the therapeutic activated marrow infiltrating lymphocytes to the subject having prostate cancer.

17. A method of treating prostate cancer in a subject, the method comprising administering a pharmaceutical composition comprising prostate cancer specific marrow infiltrating lymphocyte to the subject.

18. The method of claim 17, wherein the prostate cancer specific marrow infiltrating lymphocyte is obtained from a subject having prostate cancer.

19. The method of claim 17, wherein the prostate cancer specific marrow infiltrating lymphocyte is autologous to the subject being treated.

20. The method of claim 17, wherein the prostate cancer specific marrow infiltrating lymphocyte is allogeneic to the subject being treated.

21. The method of claim 17, wherein the marrow infiltrating lymphocyte is hypoxic activated.

22. The method of claim 17, wherein the marrow infiltrating lymphocyte is hypoxic activated and normoxic activated.

23. The method of claim 17, wherein the pharmaceutical composition is administered by parenteral administration, intraperitoneal or intramuscular administration.

24. The method of claim 17, wherein the pharmaceutical composition is administered directly into the prostate of the subject.

25. The method of any claim 1, wherein the about 75% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.

26. The method of claim 1, wherein the about 80% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.
27. The method of claim 1, wherein the about 85% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.
28. The method of claim 1, wherein the about 90% to about 100% of marrow infiltrating lymphocytes administered to the subject express CD3.
29. The method of claim 1, wherein the ratio of CD4⁺:CD8⁺ T cells present in the composition or MILs administered to the subject is about 2:1.
30. A composition comprising a population of hypoxic-activated marrow infiltrating lymphocytes isolated from a patient with prostate cancer, wherein about 75% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.
31. The composition of claim 30, wherein about 80% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.
32. The composition of claim 30, wherein about 85% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.
33. The composition of claim 30, wherein about 90% to about 100% of the population of the hypoxic activated marrow infiltrating lymphocytes expresses CD3.
34. The composition of claim 30, wherein the ratio of CD4⁺:CD8⁺ T cells present in the composition is about 2:1.
35. The composition of claim 30, wherein the cell population is obtainable from a bone marrow sample obtained from a subjecting having prostate cancer by:

(a) culturing the bone marrow sample with an anti-CD3 antibody and an anti-CD28 antibody in a hypoxic environment of about 1% to about 3% oxygen to produce activated marrow infiltrating lymphocytes; and

(b) culturing the activated marrow infiltrating lymphocytes in a normoxic environment in the presence of IL-2 to produce the composition.

36. The composition of claim 30, wherein the MILs are prostate cancer specific.

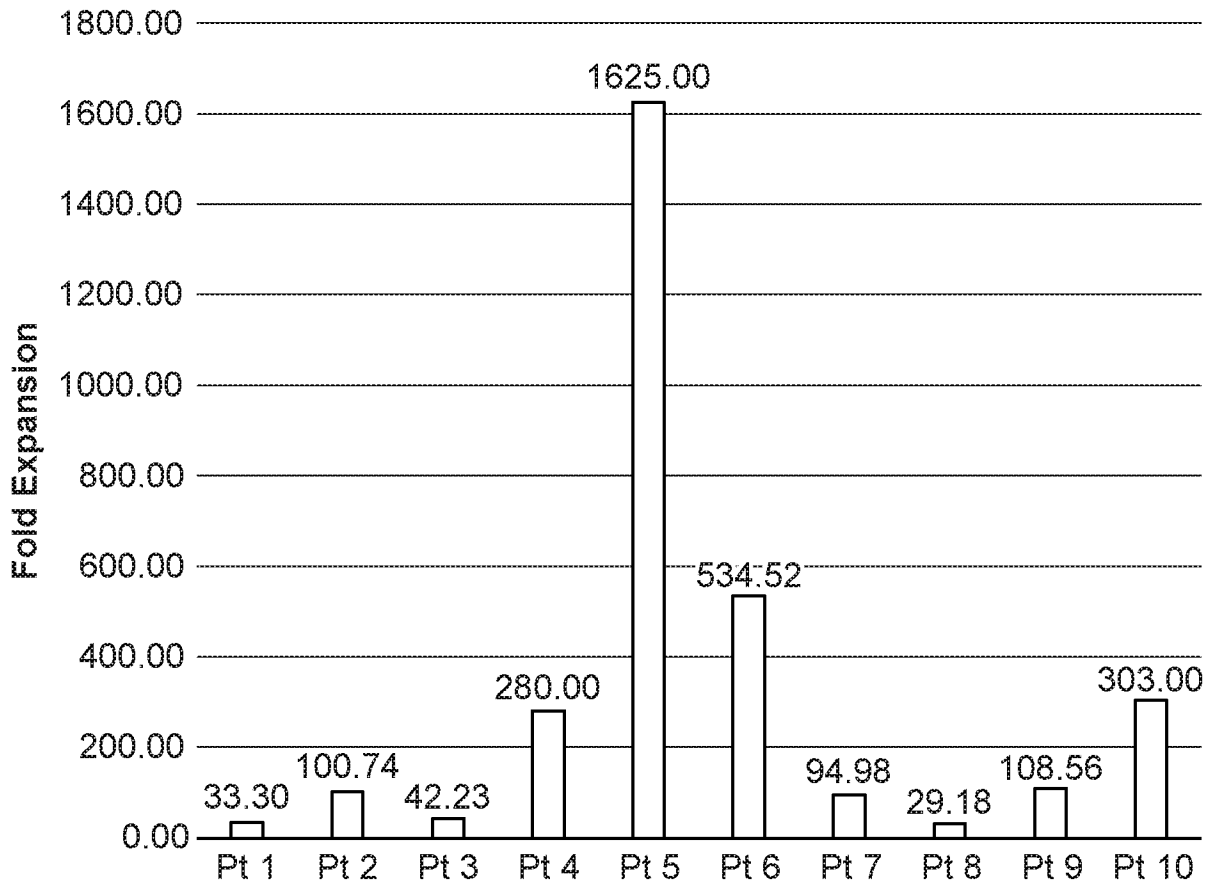


FIG. 1

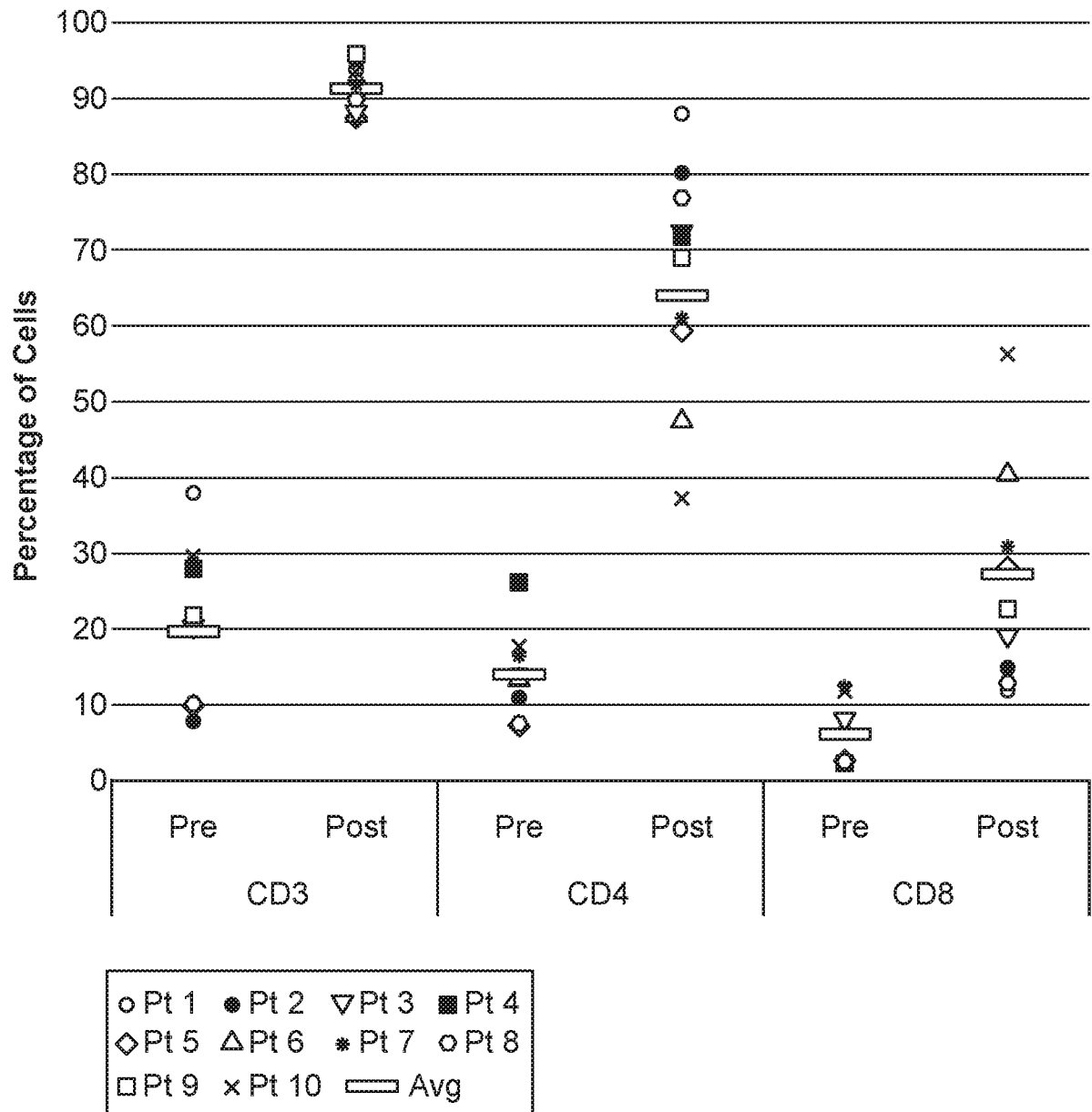


FIG. 2

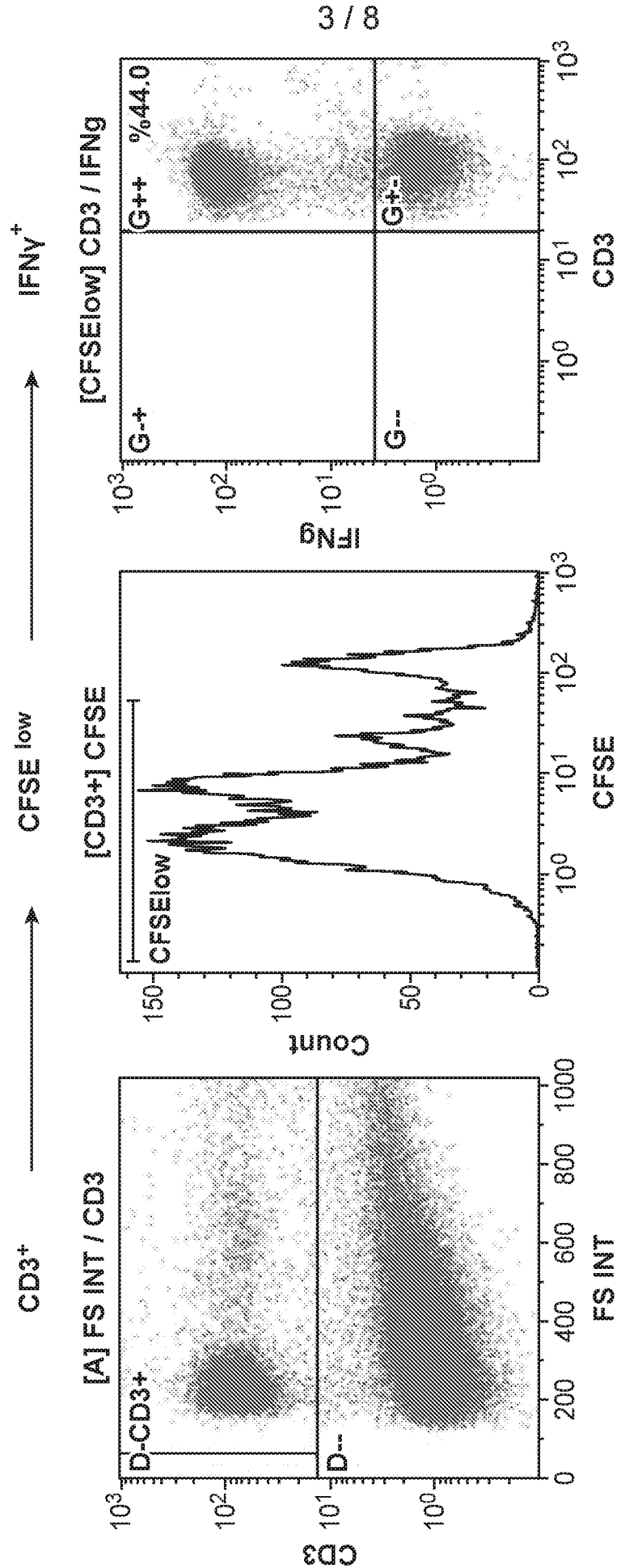


FIG. 3

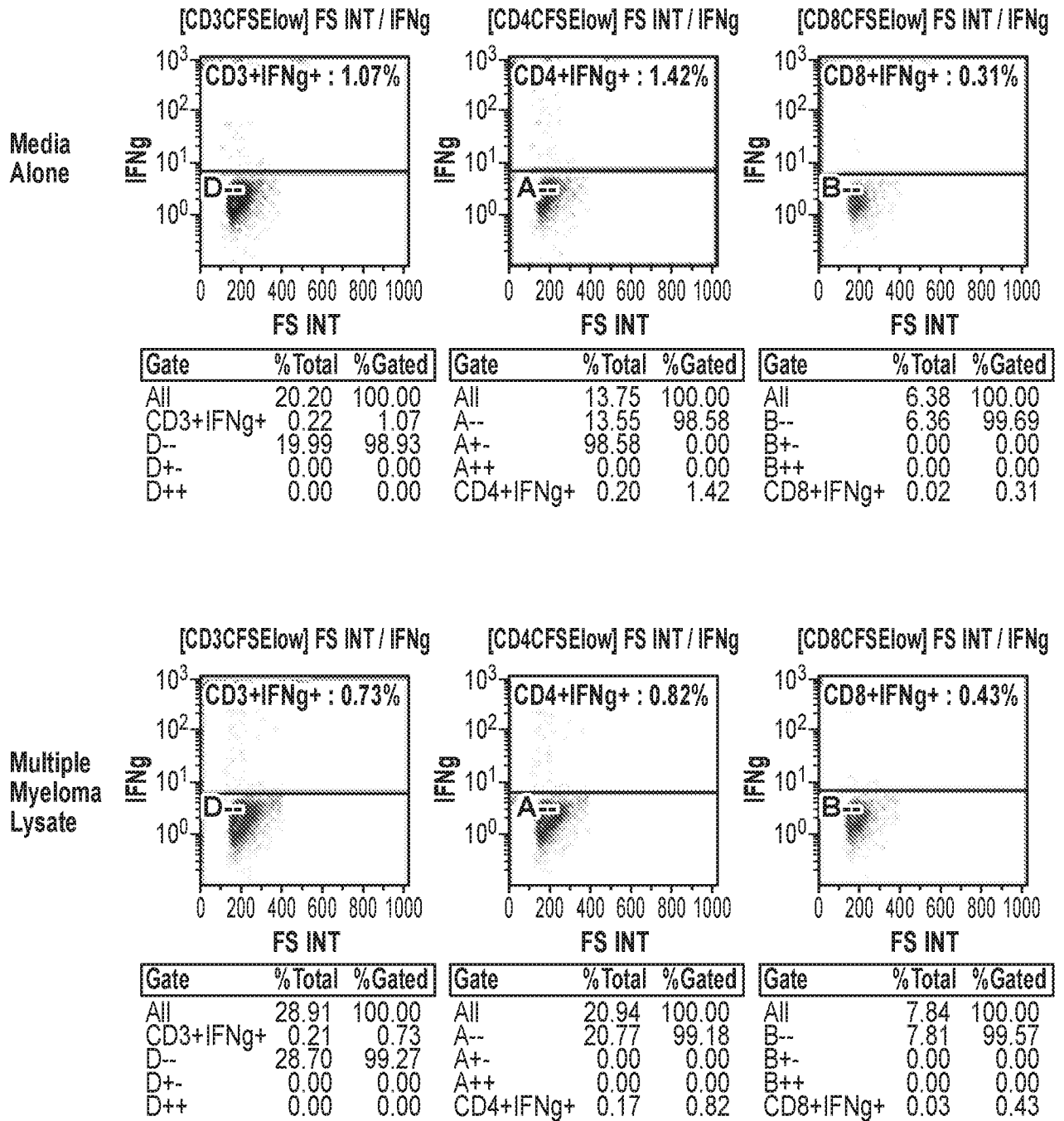


FIG. 4

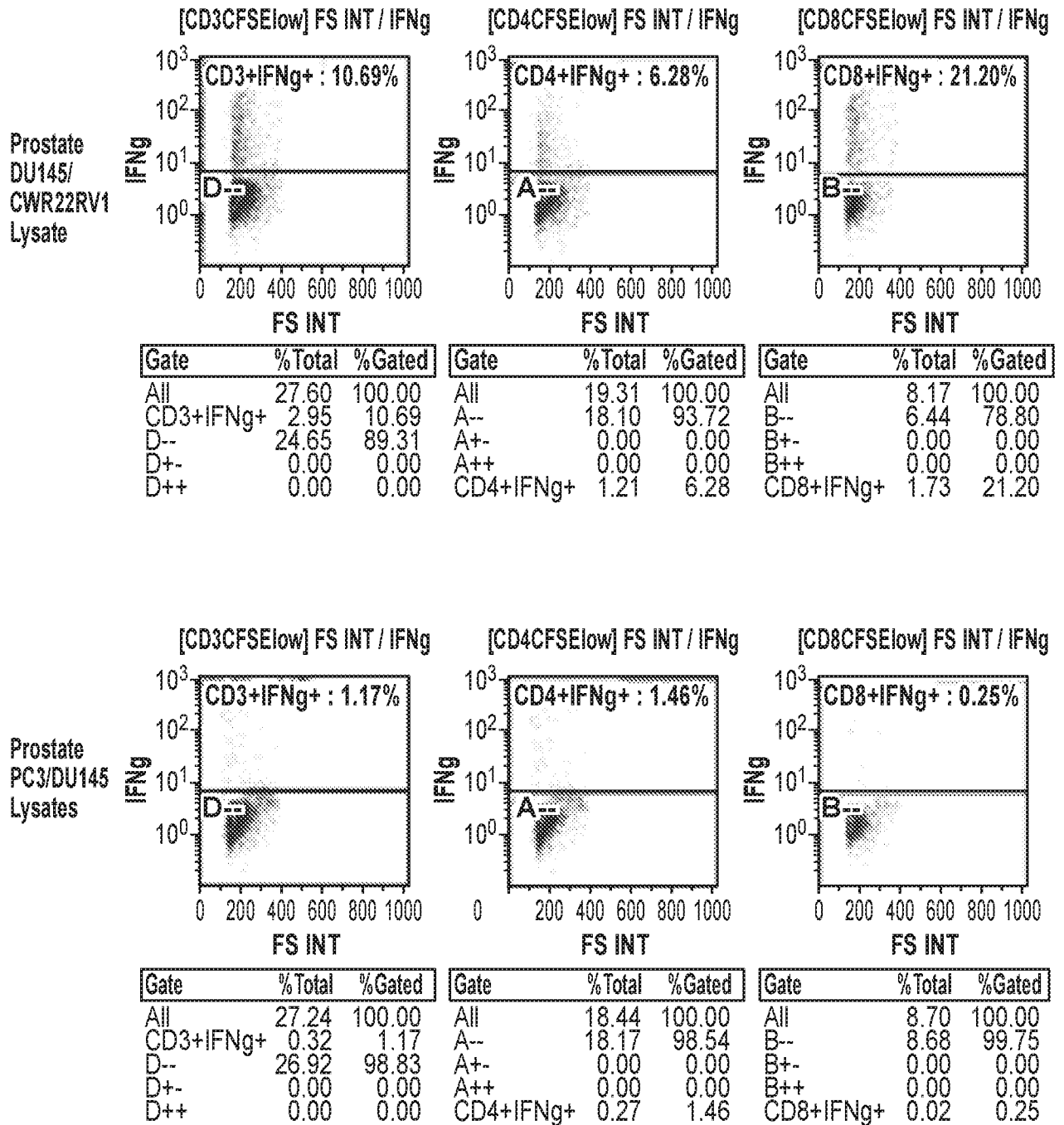


FIG. 4 (Cont. 1)

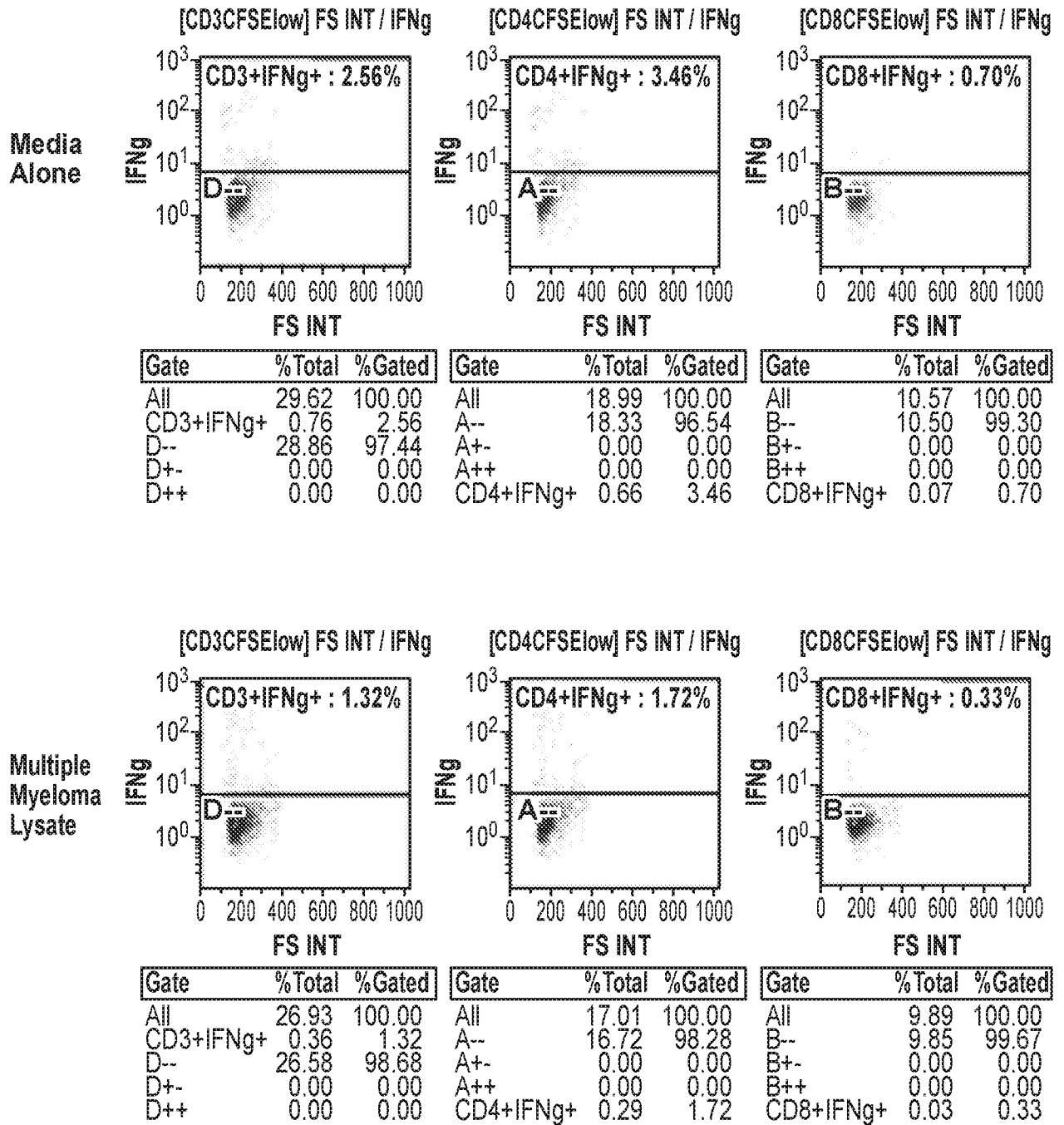


FIG. 4 (Cont. 2)

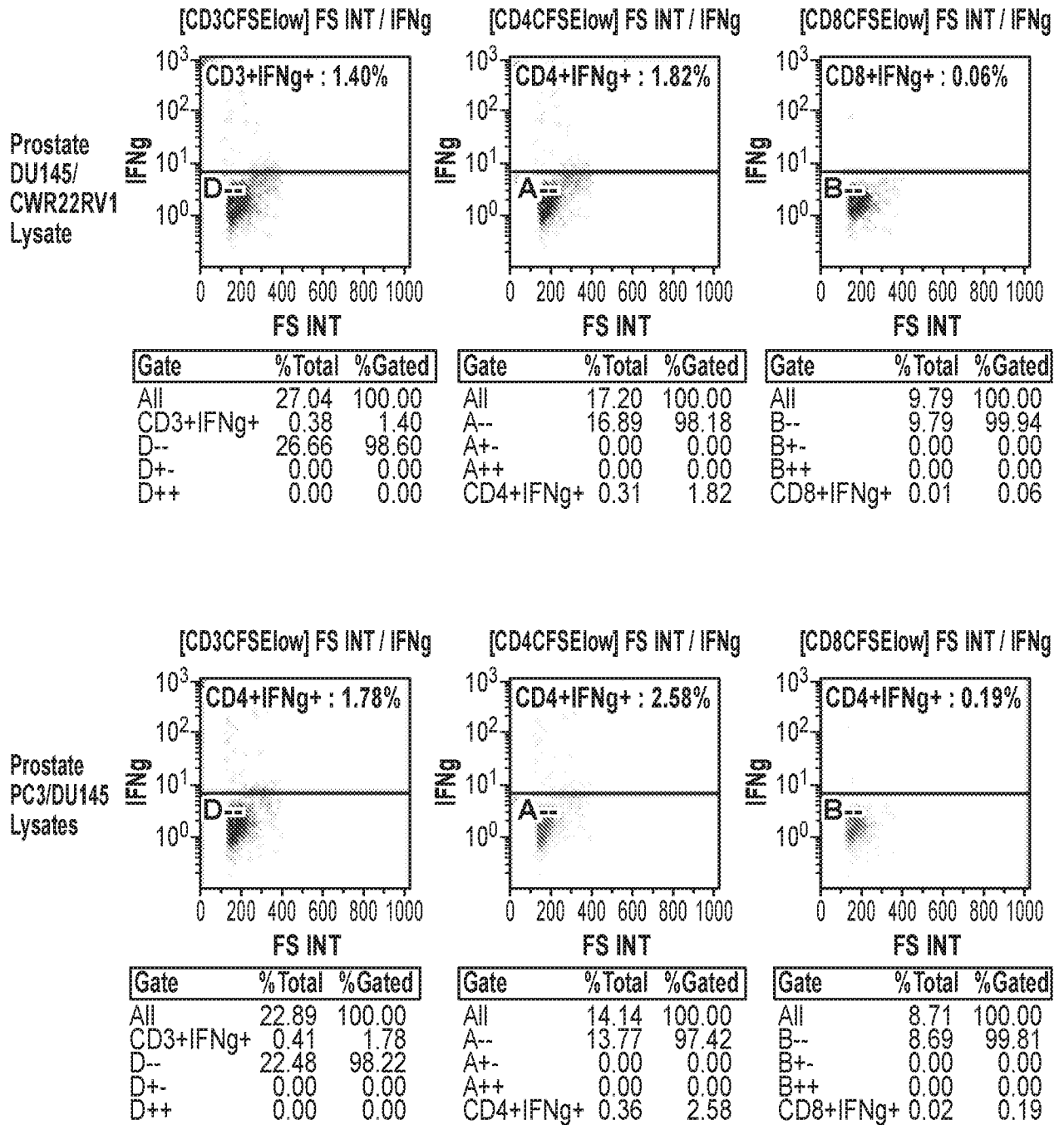


FIG. 4 (Cont. 3)

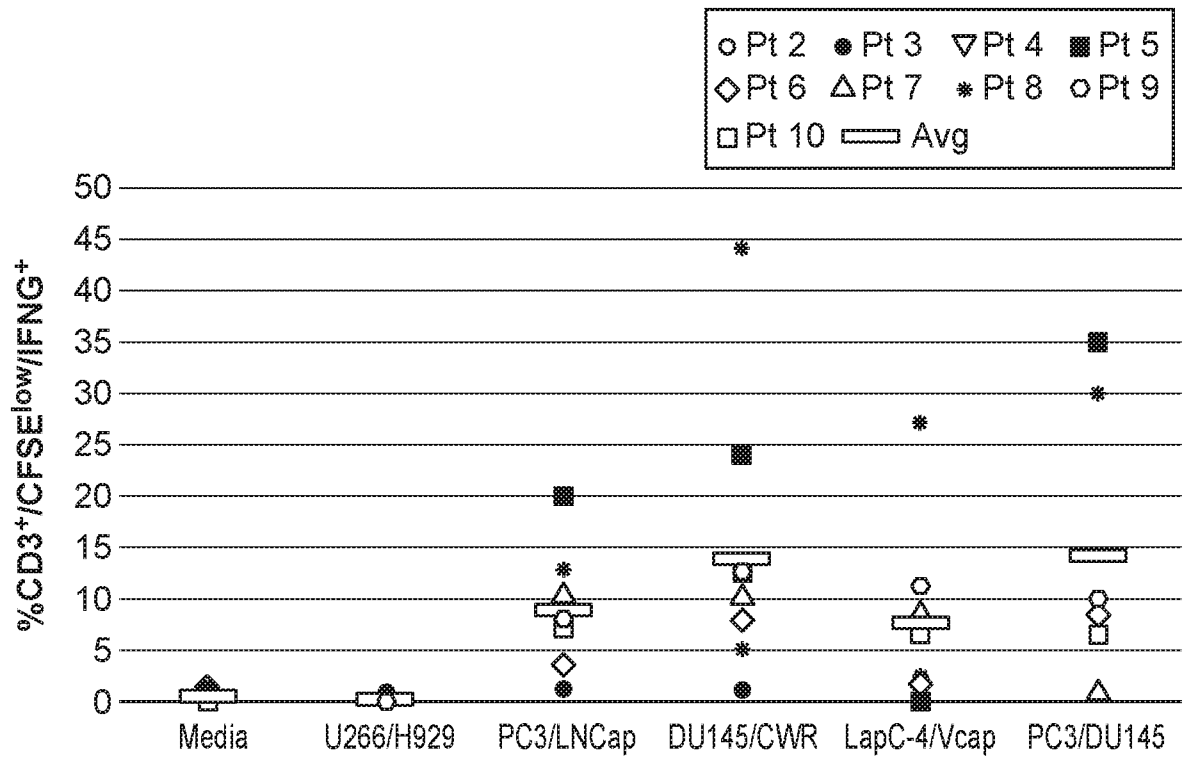


FIG. 5

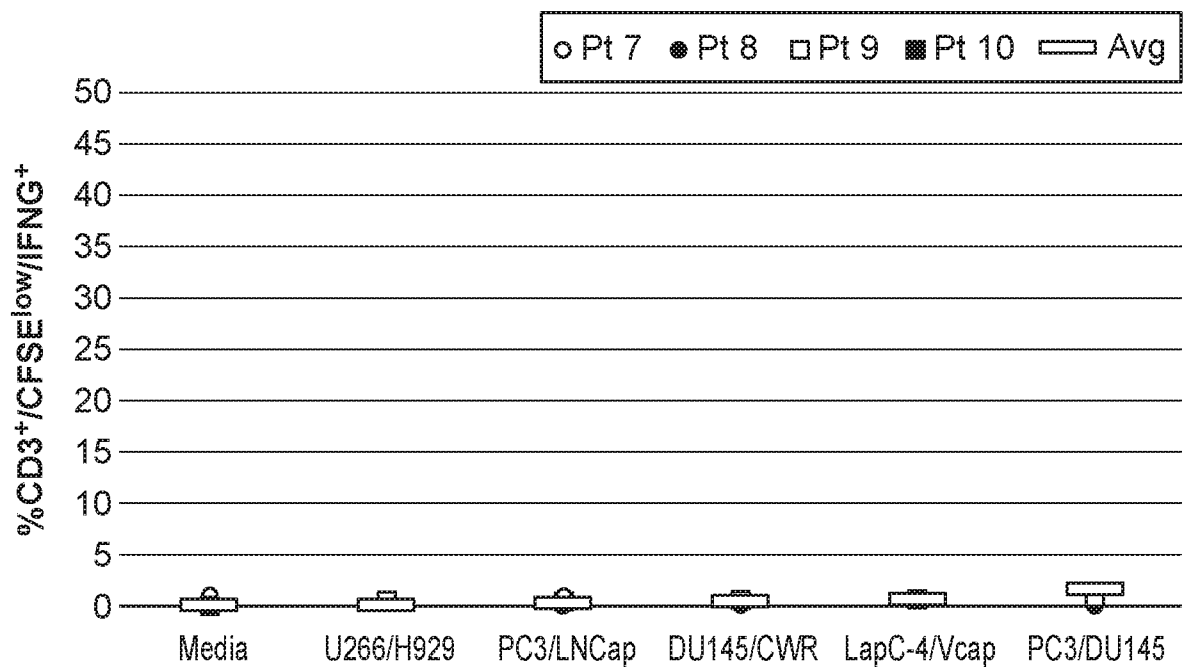


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/23543

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - A61K 35/28; C07K 14/55; C12N 5/10 (2019.01)
 CPC - A61K 35/28; C07K 14/55; C12N 5/0634, 5/10

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/008019 A1 (THE JOHNS HOPKINS UNIVERSITY) 12 January 2017; abstract; page 1, lines 27-28; page 2, lines 1-3; page 3, lines 3-5; page 5, lines 19-24; page 9, lines 19-21; page 18, lines 5-7; page 22, line 25 – page 23, line 2; page 30, lines 12-13; page 31, lines 28-333; page 32, line 30 – page 33, line 10; page 33, lines 8-31; page 34, lines 5-8; page 36, lines 28-29; page 37, lines 21-23; page 38, lines 1-3; page 38, line 30 – page 39, line 2; page 39, lines 16-22	1-14, 16-24 ----- 15, 25-36
Y	WO 2016/037054 A1 (THE JOHNS HOPKINS UNIVERSITY) 10 March 2016; abstract; page 3, lines 3-4; page 4, line 30 – page 5, line 8; page 6, lines 13-17; page 9, lines 3-4; page 12, lines 8-14; page 15, lines 17-18; claim 25	25-36
Y	WO 2017/200492 A1 (ASLAN PHARMACEUTICALS PE LTD) 23 November 2017; page 28, lines 24-26; claim 25	15

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

* Special categories of cited documents:	“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
“A” document defining the general state of the art which is not considered to be of particular relevance	“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
“E” earlier application or patent but published on or after the international filing date	“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	“&” document member of the same patent family
“O” document referring to an oral disclosure, use, exhibition or other means	
“P” document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 9 May 2019 (09.05.2019)	Date of mailing of the international search report 20 JUN 2019
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Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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