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(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; P.O. Box 4000, Route 206 and ProvinceLine Road, Princeton, New Jersey 08543-4000 (US).

(72) Inventors: **SELBY, Mark**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US). **KORMAN, Alan**; c/o Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, New Jersey 08543 (US).

(74) Agents: **FRANK, Eve** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Route 206 & Province Line Road, Princeton, New Jersey 08543-4000 (US).

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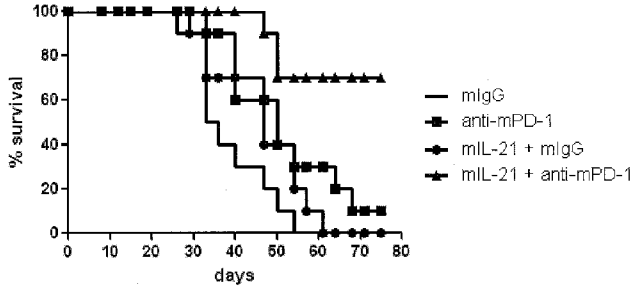
(54) Title: METHODS OF TREATING CANCER USING AN IL-21 POLYPEPTIDE AND AN ANTI-PD-1 ANTIBODY

FIG. 3



(57) Abstract: The present invention provides a method for treating cancer in a subject involving administering an IL-21 polypeptide and an anti-PD-1 antibody.

WO 2013/169693 A1

METHODS OF TREATING CANCER USING AN IL-21 POLYPEPTIDE AND AN ANTI-PD-1 ANTIBODY

FIELD OF THE INVENTION

[0001] The present invention relates generally to immunotherapy in the treatment of human disease. More specifically, the present invention relates to combination immunotherapy, involving the combination of an IL-21 polypeptide and an anti-PD-1 antibody, to treat cancer.

BACKGROUND OF THE INVENTION

[0002] IL-21, a member of the common gamma chain cytokine family, is produced by activated CD4+ T cells and natural killer T (NKT) cells (Parrish-Novak, J. et al., *Nature*, 408:57-63 (2000)). IL-21 stimulates expansion and cytotoxicity of CD8+ T cells, enhances T cell-dependent B cell proliferation and antibody production, facilitates differentiation and activation of NK cells, and reduces regulatory T (Treg) cells in tumors (Moroz, A. et al., *J. Immunol.*, 173:900-909 (2004); Parrish-Novak, J. et al., *J. Leukoc. Biol.*, 72:856-863 (2002); Peluso, I. et al., *J. Immunol.*, 178:732-739 (2007); Li, Y. et al., *Blood*, 111:229-235 (2008); and Kim-Schulze, S. et al., *Mol. Ther.*, 17:380-388 (2009)). Treatment with recombinant IL-21 therapy has been shown to produce antitumor activity in nonclinical and clinical studies (Hashmi, M.H. et al., *Exp. Opin. Biol. Ther.*, 10(5):807-817 (May 2010); and Petersen, C.C., et al., *Cytokine*, 49(1):80-88 (Jan 2010).

[0003] PD-1 (or CD279), a 55-kD type 1 transmembrane protein, is a member of the CD28 family of T cell co-stimulatory receptors that include immunoglobulin superfamily member CD28, CTLA-4, inducible costimulator (ICOS), and B and T lymphocyte attenuator (BTLA). PD-1 is highly expressed on activated T cells and B cells. PD-1 expression can also be detected on memory T-cell subsets with variable levels of expression. Two ligands specific for PD-1 have been identified: programmed death-ligand 1 (PD-L1, also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD-1 in both mouse and human systems (Freeman, G.J. et al., *J. Exp. Med.*, 192(7):1027-1034 (2000); Latchman, Y. et al., *Nat. Immunol.*, 2(3):261-268 (2001); and Carter, L.L. et al., *Eur. J. Immunol.*, 32(3):634-643 (2002)). The interaction of PD-1 with

its ligands, PD-L1 and PD-L2, which are expressed on antigen-presenting cells (APCs) and dendritic cells (DCs), transmits negative regulatory stimuli to down-modulate the activated T cell immune response. Blockade of PD-1 suppresses this negative signal and amplifies T cell responses.

[0004] Loss of effective immune response to antigens expressed by tumors may be a significant factor in tumor progression. PD-L1 expression has been found on a number of tumors, and may also be a mechanism by which tumors can directly engage PD-1+ immune cells to evade an effective antitumor immune response. Additionally, PD-L1 expression on tumor-associated APCs could enable PD-1 engagement on T cells by PD-L1+ APCs or PD-L1+ tumor cells in the tumor microenvironment to prematurely limit effective immune responses (Dong, H. et al., *Nat. Med.*, 8(8):793-800 (2002); Wintterle, S. et al., *Cancer Res.*, 63(21):7462-7467 (2003); and Dong, H. et al., *J. Mol. Med.*, 81(5):281-287 (2003)). Immunotherapy of tumors rests on the premise that tumors can be recognized as foreign rather than as self, and effectively attacked. Many tumors express tumor-specific antigens and ongoing immune surveillance is believed to abort the progression of many tumors as they arise. Tumor progression may depend upon acquisition of mechanisms to evade an effective immune response. Studies in multiple tumor models using a chimeric rat/mouse anti-mouse PD-1 antibody demonstrate that PD-1 blockade has antitumor activity and that this activity may be independent of the expression of PD-L1 on the tumor itself (Iwai, Y. et al., *Int. Immunol.*, 17(2):133-144 (2005)). Thus, blocking PD-1 in PD-L1+ tumors may reverse the inactivation of tumor-specific effector T cells at the tumor site and activate antitumor responses that are limited by PD-L1 expression on "host" DC or APC. The antitumor effects of PD-1 blockade observed in several mouse models suggest that both PD-L1+ and PD-L1- tumors may be targeted using this approach. In addition, in several tumor models in which anti-mouse-PD-1 mAb has proved ineffective, PD-1 blockade can be combined with vaccines or other immunomodulatory antibodies for improved therapeutic efficacy (Hirano, F. et al., *Cancer Res.*, 65(3):1089-1096 (2005); Li, B. et al., *Clin. Cancer Res.*, 15:1507-1509 (2009); and Curran, M.A. et al., *Proc. Natl. Acad. Sci.*, 107(9):4275-4280 (2010)).

[0005] Combination therapy with immunomodulatory agents is emerging as an improved option for the treatment and management of cancer. In particular, combination of agents with complementary immunomodulatory effects have the potential to elicit

potent and durable immune responses that may translate into enhanced therapeutic benefit. An increased understanding of the mechanisms that regulate antitumor immune responses has led to the development of several immune-based approaches for the treatment of cancer. One such approach is blockade of programmed death-1 (PD-1), a key regulatory receptor that down-regulates T cell responses (Jin, H.T. et al., *Curr. Top. Microbiol. Immunol.*, 350:17-37 (2011)). Other approaches have applied the use of cytokines involved in regulating immune function, such as interleukin 21 (IL-21) (Fewkes, N.M. et al., *Cancer J.*, 16(4):392-398 (2010)).

[0006] Accordingly, combination therapy comprising IL-21 polypeptide and blockade of PD-1 is desired.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention provides a method for treating cancer in a subject, comprising administering an Il-21 polypeptide to the subject and administering an anti-PD-1 antibody or antigen-binding portion thereof to the subject. The present invention provides a method for treating cancer in a subject comprising administering to a subject a composition comprising an IL-21 polypeptide and a pharmaceutically acceptable carrier and a composition comprising an anti-PD-1 antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier. The present invention also provides a method for treating cancer in a subject, comprising administering an IL-21 polypeptide comprising the amino acid sequence of SEQ ID NO: 5 and a human anti-PD-1 antibody (5C4) to the subject. The present invention further provides a method for treating cancer in a subject, comprising administering to a subject a composition comprising an IL-21 polypeptide comprising the amino acid sequence of SEQ ID NO: 5 and a pharmaceutically acceptable carrier and administering a composition comprising 5C4 and a pharmaceutically acceptable carrier.

[0008] In some embodiments, the IL-21 polypeptide and the anti-PD-1 antibody or antigen-binding portion thereof are administered sequentially. In other embodiments, the IL-21 polypeptide and the anti-PD-1 antibody or antigen-binding portion thereof are administered concurrently. In some embodiments, the IL-21 polypeptide is administered before the anti-PD-1 antibody or antigen-binding portion thereof. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered before the IL-

21 polypeptide. In some embodiments, the IL-21 polypeptide and the anti-PD-1 antibody or antigen-binding portion thereof are admixed as a single composition and administered concurrently. In some embodiments, the cancer is selected from the group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer. In other embodiments, the subject is a human.

[0009] In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a human antibody. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a monoclonal antibody. In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain variable region comprising amino acids having the sequence set forth in SEQ ID NO: 7 and a light chain variable region comprising amino acids having the sequence set forth in SEQ ID NO: 8. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 9; a heavy chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 10; a heavy chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 11; a light chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 12; a light chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 13; and a light chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 14. In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is 5C4. In some embodiments, the dose of the anti-PD-1 antibody is 3 mg/kg or 1 mg/kg.

[0010] In some embodiments, the IL-21 polypeptide has 95% sequence identity with the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. In other embodiments, the IL-21 polypeptide comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, the IL-21 polypeptide comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the dose of the IL-21 polypeptide is selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg.

[0011] In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered every other week and the IL-21 polypeptide is administered weekly during weeks 1-4 of a 6-week cycle. In other embodiments, the anti-PD-1

antibody or antigen-binding portion thereof is administered every other week and the IL-21 polypeptide is administered 3 times per week during weeks 1 and 3 of a 6-week cycle. In some embodiments, the administration of IL-21 polypeptide 3 times per week during weeks 1 and 3 occurs on Monday, Wednesday and Friday.

[0012] In some embodiments, the anti-PD-1 antibody is 5C4, the dose of 5C4 is 3 mg/kg, and the 5C4 is administered every other week, while the IL-21 polypeptide is rIL-21, the dose of rIL-21 is selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg, and the rIL-21 is administered weekly during weeks 1-4 of a 6 week cycle. In other embodiments, the anti-PD-1 antibody is 5C4, the dose of 5C4 is 3 mg/kg, and the 5C4 is administered every other week, while the IL-21 polypeptide is rIL-21, the dose of rIL-21 is selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg, and the rIL-21 is administered 3 times per week during weeks 1 and 3 of a 6-week cycle. In some embodiments, the administration of IL-21 polypeptide 3 times per week during weeks 1 and 3 occurs on Monday, Wednesday and Friday.

BRIEF DESCRIPTION OF THE FIGURES

[0013] Figure 1 shows the mean and median antitumor activity of mouse IL-21 (mIL-21) and mouse PD-1 (mPD-1) mAb alone or in combination (MC38 Study 1408-226).

[0014] Figure 2 shows individual mouse tumor volume data (MC38 Study 1408-226).

[0015] Figure 3 shows survival of mice treated with mIL-21 and mPD-1 mAb alone or in combination (MC38 Study 1408-226).

[0016] Figure 4 shows mean and median tumor activity of mIL-21 and mPD-1 alone or in combination (MC38 Study 1106-248).

[0017] Figure 5 shows individual mouse tumor volume data (MC38 Study 1106-248).

[0018] Figure 6 shows survival of mice treated with mIL-21 and mPD-1 mAb alone or in combination in the MC38 tumor model (MC38 Study 1106-248).

[0019] Figure 7 shows median tumor volume in mice treated with mIL-21 and mPD-1 mAb, alone or in combination, in the EMT-6 established tumor model (EMT-6 Study #39).

[0020] Figure 8 shows tumor volumes measured in individual mice (EMT-6 Study #39).

[0021] Figure 9 shows median tumor volume in mice treated with PBS, mIL-21, or mPD-1, alone or in combination, in the subcutaneous (SC) B16-F10 mouse model (TGM Study 1104)

[0022] Figures 10A-D show tumor volume in individual mice treated with PBS, mIL-21 or mPD-1 mAb, alone or in combination, in the SC B16-F10 mouse model (TGM Study 1104).

[0023] Figure 11 shows survival analysis for mice treated with PBS, mIL-21, or mPD-1 mAb, alone or in combination, in the SCB16-F10 mouse model.

[0024] Figure 12 shows the number of lung metastases in mice treated with PBS, mIL-21 or mPD-1 mAb, alone or in combination, in the intravenous (IV) B16-F10 mouse model (TGM Study 1108) (mean + SD).

[0025] Figure 13 shows change in body weight (BW) in mice treated with PBS, mIL-21 or mPD-1 mAb, alone or in combination, in the IV B16-10 mouse model (TGM Study 1108) (mean ± SD).

[0026] Figure 14 shows median tumor volume in mice treated with PBS, mIL-21, or mPD-1 mAb, alone or in combination, in the SC B16-F10 mouse model (TGM Study 1109).

[0027] Figures 15A-D show tumor volume in individual mice treated with PBS, mIL-21, or mPD-1 mAb, alone or in combination, in the SC B16-F10 mouse model (TGM Study 1109).

[0028] Figure 16 shows survival analysis for mice treated with PBS, mIL-21, or mPD-1 mAb, alone or in combination, in the SC B16-F10 mouse model.

[0029] Figure 17 shows a schematic of rIL-21 and anti-PD-1 antibody, 5C4, administration.

BRIEF DESCRIPTION OF THE TABLES

[0030] Table 1 shows the study design for MC38 Study 1408-226.

[0031] Table 2 shows percent mean and median tumor growth inhibition and tumor-free mice (MC38 Study 1408-226).

[0032] Table 3 shows the study design for MC38 Study 1106-248.

[0033] Table 4 shows percent mean and median tumor growth inhibition and percent tumor-free mice (MC38 Study 1106-248).

[0034] Table 5 shows the study design for EMT-6 Study #39.

[0035] Table 6 shows antitumor activity of mIL-21 in combination with mPD-1 mAb in the EMT-6 mammary carcinoma tumor model (EMT-6 Study #39).

[0036] Table 7 shows the study design for TGM Study 1104 (SC B16-F10).

[0037] Table 8 shows the study design for TGM Study 1108 (IV B16-F10).

[0038] Table 9 shows the study design for TGM Study 1109 (SC B16-F10).

[0039] Table 10 shows a summary of nonclinical tumor model studies evaluating combination treatment of mIL-21 and mPD-1 mAb.

[0040] Table 11 shows dose escalation schedule for rIL-21 and anti-PD-1 antibody, 5C4, (Arm A and Arm B).

[0041] Table 12 shows a summary of results for the clinical evaluation of the combination of recombinant IL-21 and anti-PD-1 antibody (5C4) in virally-associated tumors.

BRIEF DESCRIPTION OF THE CHARTS

[0042] Chart 1 shows individual tumor measurements (mm^3) for MC38 Study 1408-226.

[0043] Chart 2 shows individual mouse body weights (grams) for MC38 Study 1408-226.

[0044] Chart 3 shows individual tumor measurements (mm^3) for MC38 Study 1106-248.

[0045] Chart 4 shows individual mouse body weights (grams) for MC38 Study 1106-248.

[0046] Chart 5 shows group body weights (grams) for EMT-6 Study #39.

[0047] Chart 6 shows individual tumor measurements (mm^3) for SC B16-F10 TGM Study 1104.

[0048] Chart 7 shows individual mouse weights (grams) for SC B16-F10 TGM Study 1104.

[0049] Chart 8 shows individual metastasis counts at termination (day 20) for IV B16-10 TGM Study 1108.

[0050] Chart 9 shows individual mouse weights (grams) for IV B16-10 TGM Study 1108.

[0051] Chart 10 shows individual tumor measurements (mm^3) for SC B16-F10 TGM Study 1109.

[0052] Chart 11 shows individual mouse weights (grams) for SC B16-F10 TGM Study 1109.

DETAILED DESCRIPTION OF THE INVENTION

[0053] The invention provides a method for treating cancer in a subject involving administering an IL-21 polypeptide and an anti-PD-1 antibody. It is based upon the discovery that administration of mouse IL-21 polypeptide in combination with an anti-PD-1 antibody (a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc) results in synergistic anti-tumor activity and/or anti-tumor activity that is more potent than administration of IL-21 polypeptide or anti-PD-1 antibody alone.

[0054] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[0055] The term "therapeutically effective amount" is defined as an amount of an IL-21 polypeptide, an anti-PD-1 antibody or an IL-21 polypeptide in combination with an anti-PD-1 antibody that preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0056] The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0057] The term "antibody" as referred to herein includes whole antibodies and any antigen-binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L 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 V_H and V_L 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. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0058] The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PD-1). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 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 consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., *Nature*, 341:544-546 (1989)), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird et al., *Science*, 242:423-426 (1988); and Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883 (1988)). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0059] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0060] The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0061] The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

[0062] The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0063] As used herein, an antibody that "specifically binds to human PD-1" is intended to refer to an antibody that binds to human PD-1 with a K_D of $1x10^{-7}$ M or less, more preferably $5x10^{-8}$ M or less, more preferably $1x10^{-8}$ M or less, more preferably $5x10^{-9}$ M or less, more preferably $1x10^{-10}$ M or less.

[0064] The term "k_{assoc}" or "k_a" as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "k_{dis}" or "k_d", as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of k_d to k_a (*i.e.*, k_d/k_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a BIACORE® system.

[0065] The term "treatment" or "therapy" refers to administering an active agent with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect a condition (*e.g.*, a disease), the symptoms of the condition, or to prevent or delay the onset of the symptoms, complications, biochemical indicia of a disease, or otherwise arrest or inhibit further development of the disease, condition, or disorder in a statistically significant manner.

[0066] As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, although mammals are preferred, such as non-human primates, sheep, dogs, cats, cows and horses. Preferred subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting the T-cell mediated

immune response. In a particular embodiment, the methods are particularly suitable for treatment of cancer cells *in vivo*.

[0067] As used herein, the terms "concurrent administration" or "concurrently" mean that administration occurs on the same day. The terms "sequential administration" or "sequentially" mean that administration occurs on different days.

[0068] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

[0069] As used herein, "about" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" can mean a range of up to 20%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values are provided in the application and claims, unless otherwise stated, the meaning of "about" should be assumed to be within an acceptable error range for that particular value.

IL-21 Polypeptide

[0070] Human IL-21 (SEQ ID NO: 1 and SEQ ID NO: 2) was originally designated zalpha11 Ligand, and is described in commonly-owned U.S. Patent Nos. 6,307,024 and 6,686,178, and PCT Publication No. WO 05/113001 which are incorporated herein by reference. The IL-21 receptor, (previously designated zalpha11) now designated IL-21R, and heterodimeric receptor IL-21R/IL-2R-gamma are described in commonly-owned PCT Publication Nos. WO 00/17235 and WO 01/77171, which are incorporated herein by reference. As described in these publications, IL-21 was isolated from a cDNA library generated from activated human peripheral blood cells (hPBCs), which were selected for CD3. CD3 is a cell surface marker unique to cells of lymphoid origin, particularly T cells.

[0071] The amino acid sequence for the IL-21R indicated that the encoded receptor belonged to the Class I cytokine receptor subfamily that includes, but is not limited to, the receptors for IL-2, IL-4, IL-7, IL-15, EPO, TPO, GM-CSF and G-CSF (for a review see,

Cosman, *Cytokine*, 5(2):95-106 (1993)). The IL-21 receptor has been identified on NK cells, T cells and B cells, indicating that IL-21 acts on hematopoietic lineage cells, in particular lymphoid progenitor cells and lymphoid cells. Other known four-helical-bundle cytokines that act on lymphoid cells include IL-2, IL-4, IL-7, and IL-15. For a review of four-helical-bundle cytokines, see, Nicola et al., *Advances in Protein Chemistry*, 52:1-65 (1999) and Kelso, A., *Immunol. Cell Biol.*, 76:300-317 (1998).

[0072] For IL-21, a secretory signal sequence comprises amino acids 1-29 of SEQ ID NO: 2 (SEQ ID NO: 3) and a mature polypeptide comprises amino acids 30-162 of SEQ ID NO: 2 (SEQ ID NO: 4). In some embodiments, the mature polypeptide comprises one or more additional amino acids on the amino-terminus or carboxyl-terminus. The amino acid can be any amino acid. In some embodiments, the mature polypeptide comprises an amino-terminal methionine, as shown in the amino acid sequence of SEQ ID NO: 5. Those skilled in the art will recognize that the sequence disclosed in the nucleic acid sequence of SEQ ID NO: 1 represents a single allele of human IL-21 and that allelic variation and alternative splicing are expected to occur.

[0073] The terms "IL-21" and "IL-21 polypeptide" include variants, fragments, isoforms, species homologs of human IL-21, and analogs having at least one common epitope with IL-21. Exemplary IL-21 polypeptides include the amino acid sequence of SEQ ID NOS: 2, 4, 5 and 6.

[0074] The present invention also provides isolated IL-21 polypeptide variants that have a substantially similar sequence identity to the amino acid sequence of SEQ ID NO:2, or its ortholog. The term "substantially similar sequence identity" is used herein to denote polypeptides comprising at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% sequence identity to the sequences shown in the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6, or their orthologs. The present invention further includes nucleic acid molecules that encode such polypeptides. Methods for determining percent identity are known to those skilled in the art.

[0075] As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology=# of identical positions/total # of positions x100), taking

into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[0076] The percent identity between two amino acid sequences can be determined using the algorithm of Meyers, E. et al. (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman et al. (*J. Mol. Biol.*, 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG® software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0077] Additionally or alternatively, the protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul et al., *J. Mol. Biol.*, 215:403-410 (1990). BLAST^{SM} protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST^{SM} can be utilized as described in Altschul et al., *Nucleic Acids Res.*, 25(17):3389-3402 (1997). When utilizing BLAST^{SM} and Gapped BLAST^{SM} programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. (See www.ncbi.nlm.nih.gov).

[0078] In general, when designing modifications to molecules or identifying specific fragments, determination of structure will be accompanied by evaluating activity of modified molecules. For extensive discussion of modifications to the IL-21 polynucleotide and polypeptide, see, U.S. Patent Nos. 6,307,024 and 6,686,178 which are incorporated herein by reference.

[0079] The present invention also includes administration of molecules having the functional activity of IL-21. Thus, administration of functional fragments and functionally modified polypeptides of IL-21 polypeptides and nucleic acid molecules encoding such

functional fragments and modified polypeptides are encompassed by the present invention. A "functional" IL-21 or fragment thereof as defined herein is characterized by its proliferative or differentiating activity, by its ability to induce or inhibit specialized cell functions, in particular for immune effector cells, such as NK cells, T cells, B cells and dendritic cells. Functional IL-21 also includes the ability to exhibit anticancer and antiviral effects *in vitro* or *in vivo*, or by its ability to bind specifically to an anti-IL-21 antibody or IL-21 receptor (either soluble or immobilized).

[0080] A variety of polypeptide fusions (and related multimeric proteins comprising one or more polypeptide fusions) can also be used. For example, an IL-21 polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patent Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-IL-21 polypeptide fusions can be expressed in genetically engineered cells (to produce a variety of multimeric IL-21 analogs). Auxiliary domains can be fused to IL-21 polypeptides to target them to specific cells, tissues, or macromolecules. For example, a IL-21 polypeptide or protein could be targeted to a predetermined cell type by fusing a IL-21 polypeptide to a ligand or monoclonal antibody that specifically binds to a receptor on the surface of that target cell. In this way, polypeptides and proteins can be targeted for therapeutic or diagnostic purposes. A IL-21 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., *Connective Tissue Research*, 34:1-9 (1996).

[0081] Regardless of the particular nucleotide sequence of a variant IL-21 polynucleotide, the polynucleotide encodes a polypeptide that is characterized by its proliferative or differentiating activity, its ability to induce or inhibit specialized cell functions, or by the ability to bind specifically to an anti-IL-21 antibody or IL-21 receptor. More specifically, variant IL-21 polynucleotides will encode polypeptides which exhibit at least 50%, and in certain embodiments, greater than 70%, 80%, 90% or 95%, of the activity of the polypeptide as shown in SEQ ID NO: 2.

[0082] For any IL-21 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the genetic code and methods known in the art.

[0083] The IL-21 polypeptides used in the present invention can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. In some embodiments, the exogenous DNA encodes the amino acid sequence of SEQ ID NO: 5, which encodes mature IL-21 polypeptide having a methionine at the amino-terminus which may be useful for initiation of protein synthesis in a microbial host. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY (1987). Expression constructs and methods for producing IL-21 are described in U.S. Patent No. 6,686,178 and PCT Publication No. WO 04/055168, incorporated herein by reference.

[0084] IL-21 conjugates used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C1-C10)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, bis-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A IL-21 conjugate can also comprise a mixture of such water-soluble polymers.

Anti-PD-1 Antibodies

[0085] PD-1 is an immunoinhibitory receptor belonging to the CD28 family (Freeman et al., *J. Exp. Med.*, 192:1027 (2000); Okazaki et al., *Curr. Opin. Immunol.*, 14:779 (2002)) and binds to two ligands, PD-L1 and PD-L2. PD-1 is induced on T-cells, B-cells and myeloid cells in-vitro (Agata et al., *Int. Immunol.*, 8:765 (1996)), but is

predominantly expressed on previously activated T-cells *in vivo* (Iwai et al., *Immunol. Lett.*, 83:215 (2002)).

[0086] The terms "Programmed Death 1", "Programmed Cell Death 1", "Protein PD-1", "PD-1", "PD1", "PDCD1", "hPD-1" and "hPD-I" are used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The complete PD-1 sequence can be found under GENBANK® Accession No. U64863.

[0087] Studies indicate that PD-1 plays a critical role in immune responses. Engagement of PD-1 by PD-L1 leads to inhibition of T cell proliferation and cytokine production such as IL-2 and IFN-gamma (Freeman et al., *J. Exp. Med.*, 192:1027 (2000). In addition, PD-1 deficient mice exhibit a breakdown of peripheral tolerance and develop systemic autoimmune disease (Nishimura et al., *Immunity*, 11:141-151 (1999); Nishimura et al., *Science*, 291:319-322 (2001)). Over-expression of PD-L1 has been observed in numerous human cancers, including melanomas and carcinomas of lung, ovary, colon, bladder, breast, cervix, liver, and head and neck, and glioblastoma (Dong et al., *Nat. Med.*, 8:793-800 (2002); Brown et al., *J. Immunol.*, 170:1257-1266 (2003); Strome et al., *Cancer Res.*, 63:6501 (2003); Wintterle et al., *Cancer Res.*, 63:7462-7467 (2003)), and PD-L1/PD-1 interaction has been suggested to play a pivotal role in the immune evasion of tumors from the host immune system (Blank et al., *Cancer Immunol. Immunother.*, 54(4):307-314 (2005)). Therefore, blockade of PD-L1/PD-1 interaction, *e.g.*, with an antibody which specifically binds PD-1, serves as one possible mechanism for enhancing anti-tumor immunity.

[0088] Exemplary anti-PD-1 antibodies and methods for their use are described by Goldberg et al., *Blood*, 110(1):186-192 (2007), Thompson et al., *Clin. Cancer Res.*, 13(6):1757-1761 (2007), and Korman et al., International Application No. PCT/JP2006/309606 (PCT Publication No. WO 2006/121168 A1), and U.S. Patent No. 8,008,449, each of which are expressly incorporated by reference herein.

[0089] The antibodies for use in the present invention include, but are not limited to, monoclonal antibodies, synthetic antibodies, polyclonal antibodies, multispecific antibodies (including bi-specific antibodies), human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv) (including bi-specific scFvs), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and epitope-

binding fragments of any of the above. In particular, antibodies for use in the present invention include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain a PD-1 binding site that immunospecifically binds to PD-1. The immunoglobulin molecules for use in the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Preferably, the antibodies for use in the invention are IgG, more preferably, IgG1.

[0090] The antibodies for use in the invention may be from any animal origin including birds and mammals (*e.g.*, human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken). Preferably, the antibodies are human or humanized monoclonal antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from mice or other animals that express antibodies from human genes.

[0091] The antibodies for use in the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may immunospecifically bind to different epitopes of a polypeptide or may immunospecifically bind to both a polypeptide as well a heterologous epitope, such as a heterologous polypeptide or solid support material. See, *e.g.*, PCT Publication Nos. WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt, et al., *J. Immunol.*, 147:60-69 (1991); U.S. Patent Nos. 4,474,893, 4,714,681, 4,925,648, 5,573,920, and 5,601,819; and Kostelny et al., *J. Immunol.*, 148:1547-1553 (1992).

[0092] The antibodies for use in the invention include derivatives of the antibodies. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding an antibody to be used with the methods for use in the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the derivatives include less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original molecule. In a preferred embodiment, the derivatives have conservative amino acid substitutions are made at one

or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

[0093] The antibodies for use in the present invention include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, synthesis in the presence of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0094] The present invention also provides antibodies for use in the invention that comprise a framework region known to those of skill in the art. In certain embodiments, one or more framework regions, preferably, all of the framework regions, of an antibody to be used in the compositions and methods for use in the invention are human. In certain other embodiments for use in the invention, the fragment region of an antibody for use in the invention is humanized. In certain embodiments, the antibody to be used with the methods for use in the invention is a synthetic antibody, a monoclonal antibody, an intrabody, a chimeric antibody, a human antibody, a humanized chimeric antibody, a

humanized antibody, a glycosylated antibody, a multispecific antibody, a human antibody, a single-chain antibody, or a bispecific antibody.

[0095] In certain embodiments, an antibody for use in the invention has a half-life in a subject, preferably a human, of about 12 hours or more, about 1 day or more, about 3 days or more, about 6 days or more, about 10 days or more, about 15 days or more, about 20 days or more, about 25 days or more, about 30 days or more, about 35 days or more, about 40 days or more, about 45 days or more, about 2 months or more, about 3 months or more, about 4 months or more, or about 5 months or more. Antibodies with increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor (see, *e.g.*, PCT Publication No. WO 97/34631 and U.S. Patent Application Serial No. 10/020,354, entitled "Molecules with Extended Half-Lives, Compositions and Uses Thereof", filed December 12, 2001, by Johnson et al.; and U.S. Publication Nos. 2005/003700 and 2005/0064514, which are incorporated herein by reference in their entireties). Such antibodies can be tested for binding activity to antigens as well as for *in vivo* efficacy using methods known to those skilled in the art, for example, by immunoassays described herein.

[0096] Further, antibodies with increased *in vivo* half-lives can be generated by attaching to the antibodies polymer molecules such as high molecular weight polyethyleneglycol (PEG). PEG can be attached to the antibodies with or without a multifunctional linker either through site-specific conjugation of the PEG to the amino-terminus or carboxyl-terminus of the antibodies or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation will be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by, *e.g.*, size exclusion or ion-exchange chromatography. PEG-derivatized antibodies can be tested for binding activity to antigens as well as for *in vivo* efficacy using methods known to those skilled in the art, for example, by immunoassays described herein.

[0097] In certain embodiments, an antibody for use in the present invention includes antigen-binding portions of an intact antibody that retain capacity to bind PD-1. Examples

include (i) a Fab fragment, a monovalent fragment consisting of the 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 consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., *Nature*, 341:544-546 (1989)), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). 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, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); See, *e.g.*, Bird et al., *Science*, 242:423-426 (1988); and Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883 (1988)). Such single chain antibodies are included by reference to the term "antibody."

[0098] In certain embodiments, an antibody for use in the present invention comprises a heavy chain variable region derived from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region derived from a particular germline light chain immunoglobulin gene.

[0099] For example, in a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L6 gene, wherein the antibody specifically binds PD-1, preferably human PD-1. In yet another preferred embodiment, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody:

(a) comprises a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene (which gene encodes the amino acid sequence set forth in SEQ ID NO: 15);

(b) comprises a light chain variable region that is the product of or derived from a human V_K L6 gene (which gene encodes the amino acid sequence set forth in SEQ ID NO: 16); and

(c) specifically binds to PD-1.

[00100] An example of an antibody having a heavy and light chain variable region derived from the human V_H 3-33 and V_K L6 immunoglobulin germline sequences, respectively, is 5C4.

[00101] As used herein, a human antibody comprises heavy or light chain variable regions that is "the product of" or "derived from" a particular germline sequence if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[00102] In yet another embodiment, an antibody of the invention comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention.

[00103] For example, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence of SEQ ID NO: 7;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence of SEQ ID NO: 8; and

the antibody exhibits one or more of the following properties:

(c) the antibody binds to human PD-1 with a K_D of $1x10^{-7}$ M or less;

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(e) the antibody increases T-cell proliferation in an MLR assay;

(f) the antibody increases interferon-gamma production in an MLR assay;

(g) the antibody increases IL-2 secretion in an MLR assay;

(h) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(i) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(j) the antibody stimulates antigen-specific memory responses;

(k) the antibody stimulates antibody responses; and/or

(l) the antibody inhibits tumor cell growth *in vivo*.

[00104] In other embodiments, the V_H and/or V_L amino acid sequences may be 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences set forth above. An antibody having V_H and V_L regions having high (*i.e.*, 80% or greater) homology to the V_H and V_L regions of the sequences set forth above, can be obtained by mutagenesis (*e.g.*, site-directed or PCR-mediated mutagenesis) of nucleic acid molecules encoding SEQ ID NOs: 7 and 8, followed by testing of the encoded altered antibody for retained function

(*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein.

[00105] As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positionsx100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[00106] The percent identity between two amino acid sequences can be determined using the algorithm of Meyers, E. et al. (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman et al. (*J. Mol. Biol.*, 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG® software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[00107] Additionally or alternatively, the protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul et al., *J. Mol. Biol.*, 215:403-410 (1990). BLAST^{SM} protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST^{SM} can be utilized as described in Altschul et al., *Nucleic Acids Res.*, 25(17):3389-3402 (1997). When utilizing BLAST^{SM} and Gapped BLAST^{SM} programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. (See www.ncbi.nlm.nih.gov).

[00108] In certain embodiments, an antibody of the invention comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences and a light chain variable

region comprising CDR1, CDR2 and CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the 5C4 antibody or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-PD-1 antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid of SEQ ID NO: 11, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NO: 14, and conservative modifications thereof; and

the antibody exhibits one or more of the following properties:

(c) the antibody binds to human PD-1 with a K_D of $1x10^{-7}$ M or less;

(d) the antibody does not substantially bind to human CD28, CTLA-4 or ICOS;

(e) the antibody increases T-cell proliferation in an MLR assay;

(f) the antibody increases interferon-gamma production in an MLR assay;

(g) the antibody increases IL-2 secretion in an MLR assay;

(h) the antibody binds to human PD-1 and cynomolgus monkey PD-1;

(i) the antibody inhibits the binding of PD-L1 and/or PD-L2 to PD-1;

(j) the antibody stimulates antigen-specific memory responses;

(k) the antibody stimulates antibody responses; and/or

(l) the antibody inhibits tumor cell growth *in vivo*.

[00109] In a preferred embodiment, the heavy chain variable region CDR2 sequence comprises an amino acid sequence of SEQ ID NO: 10, and conservative modifications thereof, and the light chain variable region CDR2 sequence comprises an amino acid sequence of SEQ ID NO: 13, and conservative modifications thereof. In another preferred embodiment, the heavy chain variable region CDR1 sequence comprises an amino acid sequence of SEQ ID NO: 9, and conservative modifications thereof; and the light chain

variable region CDR1 sequence comprises an amino acid sequence of SEQ ID NO: 12, and conservative modifications thereof.

[00110] As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the invention can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein.

[00111] In some embodiments, an anti-PD-1 antibody, or antigen-binding portion thereof of the invention comprises a heavy chain variable region comprising amino acids having the sequence set forth in SEQ ID NO: 7 and a light chain variable region comprising amino acids having the sequence set forth in SEQ ID NO: 8.

[00112] In other embodiments, an anti-PD-1 antibody, or antigen-biding portion thereof, of the invention comprises a heavy chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 9; a heavy chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 10; a heavy chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 11; a light chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 12; a light chain variable region CDR2 comprising

amino acids having the sequence set forth in SEQ ID NO: 13; and a light chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 14.

[00113] In some embodiments, an anti-PD-1 antibody of the present invention is an antibody selected from the group consisting of 17D8, 2D3, 4H1, 4A11, 7D3, 5F4 and 5C4. In other embodiments, an anti-PD-1 antibody of the present invention is an antibody that cross-competes with 17D8, 2D3, 4H1, 4A11, 7D3, 5F4 or 5C4 for binding to the same epitope of PD-1. Such cross-competing antibodies can be identified based on their ability to cross-compete with 17D8, 2D3, 4H1, 5F4, 4A11, 7D3 or 5C4 in standard PD-1 binding assays. For example, BIACORE® analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the antibodies of the current invention. The ability of a test antibody to inhibit the binding of, for example, 17D8, 2D3, 4H1, 5F4, 4A11, 7D3 or 5C4, to human PD-1, demonstrates that the test antibody can compete with 17D8, 2D3, 4H1, 5F4, 4A11, 7D3 or 5C4 for binding to human PD-1 and thus binds to the same epitope on human PD-1 as 17D8, 2D3, 4H1, 5F4, 4A11, 7D3 or 5C4. 17D8, 2D3, 4H1, 4A11, 7D3, 5F4 and 5C4, and methods for their use, are described in U.S. Patent No. 8,008,449, which is expressly incorporated by reference herein.

[00114] In some embodiments, an anti-PD-1 antibody of the present invention is 5C4. The 5C4 antibody is also known as MDX-1106, BMS-936558 and nivolumab.

Cancers

[00115] In some embodiments, the invention provides a method of treating cancer in a subject, comprising administering to the subject a therapeutically effective amount of an IL-21 polypeptide and a therapeutically effective amount of an anti-PD-1 antibody, or antigen-binding portion thereof. Preferably, the IL-21 polypeptide comprises at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or greater than 99% sequence identity to the amino acid sequence shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ NO: 5 or SEQ ID NO: 6. Preferably, the antibody is a human anti-PD-1 antibody (such as any of the human anti-human PD-1 antibodies described herein). Additionally or alternatively, the antibody may be a chimeric or humanized anti-PD-1 antibody. In some embodiments, the anti-PD-1 antibody comprises a heavy chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID

NO: 9; a heavy chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 10; a heavy chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 11; a light chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 12; a light chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 13; and a light chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 14. In other embodiments, the anti-PD-1 antibody is 5C4.

[00116] Preferred cancers whose growth may be inhibited using the combination therapy of IL-21 polypeptide and anti-PD-1 antibody include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer (*e.g.*, clear cell carcinoma), prostate cancer (*e.g.*, hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.*, non-small cell lung cancer). Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the antibodies of the invention.

[00117] Examples of other cancers that may be treated using the methods of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations

of said cancers. The present invention is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai et al., *Int. Immunol.*, 17:133-144 (2005)).

[00118] In some embodiments, the cancers whose growth may be inhibited using the combination therapy of IL-21 polypeptide and anti-PD-1 antibody are virally-associated cancers. Exemplary virally-associated cancers include, but are not limited to, cancers associated with Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma viruses (HPV), human T lymphotropic virus type 1 (HTLV-1), human T lymphotropic type 2 (HTLV-2) and human herpesvirus, such as human herpesvirus 8 (HHV-8). The cancers associated with particular viruses are known to those of ordinary skill in the art. For example, examples of EBV-associated cancers include, but are not limited to, lymphomas, nasopharyngeal cancer, gastric carcinoma, parotid carcinoma, breast carcinoma, and leiomyosarcoma. Examples of cancers associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) include, but are not limited to cancers of the liver. Examples of cancers associated with human papilloma viruses (HPV) include, but are not limited to, oropharangeal head and neck cancer, nasopharyngeal head and neck cancer, and cancers of the cervix, vulva, vagina, penis and anus. Examples of cancers associated with human T lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) include, but are not limited to, adult T-cell leukemia and hairy-cell leukemia, respectively. Examples of cancers associated with human herpesvirus 8 (HHV-8) include, but are not limited to, Kaposi sarcoma. In some embodiments, the virally-associated cancer is a cancer associated with HPV. In other embodiments, the virally-associated cancer is a cancer associated with HCV.

Pharmaceutical Compositions

[00119] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjugate, or bispecific

molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[00120] The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.*, Berge, S.M. et al., *J. Pharm. Sci.*, 66:1-19 (1977)). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N′-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[00121] A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00122] Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00123] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of

microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00124] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00125] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. 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. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[00126] 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 sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile

injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00127] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 percent to about ninety-nine percent of active ingredient, preferably from about 0.1 percent to about 70 percent, most preferably from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

[00128] Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate 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 subjects to be treated; each unit contains 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 (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00129] For administration of the anti-PD-1 antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-PD-1

antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

[00130] For administration of the IL-21 polypeptide, the dosage ranges from 1-150 μg/kg body weight. In some embodiments, the dose of IL-21 polypeptide is selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg body weight.

Combination Therapy

[00131] The present invention is based, in part, on the following experimental data. Preclinical studies have been conducted to assess the efficacy of mouse IL-21 (mIL-21) alone or in combination with mPD-1 mAb (a chimeric rat anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc) in 4 different syngeneic mouse tumor models: the solid tumor models MC38 (murine colon carcinoma) and EMT-6 (murine mammary carcinoma), and the solid tumor (SC) and lung metastasis (IV) models utilizing the B16 F10 mouse melanoma cell line. These mouse tumor models have been used in the field of oncology to predict results of cancer therapies in human clinical studies.

[00132] In the first of two MC38 studies (Study 1408-226), mIL-21 administered as monotherapy intraperitoneally (IP) at 200 μg/mouse (~10 mg/kg) 3 times on Days 7, 10, and 14, elicited modest antitumor activity (30% tumor growth inhibition [TGI] at Day 29). The %TGI was calculated as: [(mean tumor volume in IgG control group - mean tumor volume of other group, divided by mean tumor volume in IgG group) × 100]. mPD-1 mAb administered at 200 μg/mouse IP on Days 8, 12, and 15 also elicited modest antitumor activity (60% TGI at Day 29 and a single tumor-free mouse of the 10 treated). The combination of both agents resulted in synergistic antitumor activity, as evidenced by complete regressions observed in 7 of 10 mice and 99.9% median TGI (Figure 1). Synergy was demonstrated in this study, with the observation that 7 mice experienced complete regression on the combination whereas one mouse would have been expected to have experienced complete remission if the combination had an additive effect (chi-square, p=0.0455). The regressions in the combination treatment group were durable, as

there were no cases of tumor recrudescence after mice became tumor-free. Furthermore, a significant survival benefit was observed with the combination treatment, while there was only minimal benefit with the monotherapies when compared with the IgG control group (Figure 3). The combination treatments were also well tolerated; there were no noteworthy changes in body weights among any of the treated mice.

[00133] In the second MC38 study (Study 1106-248), 2 dose regimens of mIL-21 were evaluated: 50 μg × 6 and 200 μg × 3. The mean and median tumor growth graphs are shown in Figure 4. On Day 31, the TGI (median) was 25% in mice administered 200 μg mIL-21 while 60% TGI was observed in mice administered 200 μg PD-1 mAb. In addition, 2 of 10 PD-1 mAb treated mice were tumor-free on Day 31 (Figure 4). Similar trends were observed in the % mean TGI values on Day 21. Combination of both agents resulted in 100% TGI with 8 of 10 mice exhibiting complete regressions. Synergy was demonstrated at the 200 μg dose of mIL-21, with the observation that 8 mice experienced complete regression with 200 μg mIL-21 + mPD-1 mAb, whereas 2 mice would have been expected to have experienced complete remission if the contributions of 200 μg mIL-21 alone and mPD-1 mAb alone were additive (chi square, p=0.0339). Synergy was not demonstrated at the 50 μg dose of mIL-21; however, enhanced anti-tumor effects were observed for the combination of both agents compared to the administration of each alone. The regressions in the combination treatment group were durable as there were no cases of tumor recrudescence through the end of the study on Day 70. Furthermore, a survival benefit was observed (on Day 70) in both combination treatment groups while there was only 0% to 20% survival in the groups that received mIgG isotype control mAb or mIL 21 or mPD-1 mAb alone (Figure 6). Similar antitumor activity was observed in mIL-21-treated animals, regardless of treatment regimen (200 μg × 3 vs. 50 μg × 6). There were no significant changes in body weights or clinical signs (*e.g.*, lethargy, mobility, coat condition, etc.) among any of the treated mice.

[00134] In the EMT-6 mammary carcinoma model, mIL-21 was efficacious as monotherapy, administered at 50 μg/mouse 3 times weekly for 2 weeks (Study EMT-6 #39) with a TGI of 52% by Day 31. mIL-21 (50 μg/mouse) was administered 3 times a week for 2 weeks; mPD-1 mAb (10 mg/kg) was administered every 4 days for 3 doses. mPD-1 mAb at a dose of 10 mg/kg was ineffective in this model, resulting in TGI of 0% (Day 31). Combination treatment with mIL-21 and mPD-1 mAb did not lead to enhanced

antitumor responses compared to mIL-21 alone. There were no noteworthy changes in body weight in the experimental group treated with the combination treatment compared with the other groups.

[00135] The combination of mIL-21 and mPD-1 mAb was evaluated in 2 additional settings using the B16-F10 melanoma SC and IV models. Mice treated in Study TGM 1109 (SC model) with the combination of mIL-21 and mPD1 mAb had a lower median tumor volume than mice treated with phosphate-buffered saline (PBS), mIL-21, or mPD-1 mAb alone (Figure 14), though no animals were tumor-free in any group. Groups of mice treated with the combination of mIL-21 and mPD-1 mAb tended to be protected from reaching a specific tumor volume (450 mm^3) compared to mice treated with PBS or mIL 21 or mPD-1 mAb alone (Figure 16). A tumor volume of 450 mm^3 was chosen as this was the largest tumor volume that all mice on study achieved and thus, provided a consistent endpoint measurement. Synergy analysis was performed using a Cox regression model. Although the result was not statistically significant, the data were deemed to be suggestive of a trend for synergistic interaction, given the small group size, between mIL-21 and mPD-1 mAb ($p=0.1348$).

[00136] In the IV model (Study TGM 1108), mice treated with a combination of mIL-21 and mPD-1 mAb had a significantly lower average number of surface lung metastases ($p<0.05$) compared to PBS-treated mice by 1-way analysis of variance (ANOVA), as shown in Figure 12. Mice treated with either mIL-21 alone or mPD-1 mAb alone did not have significantly different mean numbers of metastases compared to PBS-treated mice. Synergy with respect to the average number of lung metastases was evaluated using a linear model with terms for treatment with mPD-1 mAb, mIL-21, and the interaction of mPD-1 mAb and mIL-21; no clear evidence of synergy was identified. However, despite the lack of synergy, there was an enhanced effect of combination treatment, compared to monotherapy with mIL-21 or mPD-1 mAb alone, on the reduction of lung metastases.

[00137] In certain embodiments, the combination of IL-21 polypeptide and anti-PD-1 antibody discussed herein may be administered concurrently as a single composition in a pharmaceutically acceptable carrier, or concurrently as separate compositions with IL-21 polypeptide in a pharmaceutically acceptable carrier and anti-PD-1 antibody in a pharmaceutically acceptable carrier. In another embodiment, the combination of IL-21 polypeptide and anti-PD-1 antibody can be administered sequentially. For example, an

IL-21 polypeptide and an anti-PD-1 antibody can be administered sequentially, such as IL-21 polypeptide being administered first and anti-PD-1 antibody second, or anti-PD-1 antibody being administered first and IL-21 polypeptide second. Furthermore, if more than one dose of the combination therapy is administered sequentially, the order of the sequential administration can be reversed or kept in the same order at each time point of administration, sequential administrations may be combined with concurrent administrations, or any combination thereof. For example, the first administration of a combination IL-21 polypeptide and anti-PD-1 antibody may be concurrent, the second administration may be sequential with IL-21 polypeptide first and anti-PD-1 antibody second, and the third administration may be sequential with anti-PD-1 antibody first and IL-21 polypeptide second, etc. Another representative dosing scheme may involve a first administration that is sequential with anti-PD-1 antibody first and IL-21 polypeptide second, and subsequent administrations may be concurrent.

[00138] In some embodiments, anti-PD-1 antibody is administered at a dose of 3 mg/kg every other week and IL-21 polypeptide is administered at a dose selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg weekly during weeks 1-4 of a 6-week cycle. In other embodiments, anti-PD-1 antibody is administered at a dose of 3 mg/kg every other week and IL-21 polypeptide is administered at a dose selected from the group consisting of 10, 30, 50, 75and 100 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. In some embodiments, the combination therapy is administered to treat clear cell renal cell carcinoma (ccRCC). In other embodiments, the combination is administered to treat non-small cell lung cancer (NSCLC). In some embodiments, the combination thereapy is administered to treat melanoma. In other embodiments, the combination thereapy is admnisteraed to treat prostate cancer. In some embodiments, the combination therapy is administered to treat breast cancer. In other embodiments, the combination thereapy is administeraeted to treat colon cancer. In some embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HPV. In other embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HCV.

[00139] In some embodiments, anti-PD-1 antibody is administered at a dose of 1 mg/kg every other week and IL-21 polypeptide is administered at a dose selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg weekly during weeks 1-4 of a 6-week

cycle. In other embodiments, anti-PD-1 antibody is administered at a dose of 1 mg/kg every other week and IL-21 polypeptide is administered at a dose selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. In some embodiments, the combination therapy is administered to treat clear cell renal cell carcinoma (ccRCC). In other embodiments, the combination is administered to treat non-small cell lung cancer (NSCLC). In some embodiments, the combination thereapy is administered to treat melanoma. In other embodiments, the combination thereapy is admnisteraed to treat prostate cancer. In some embodiments, the combination therapy is administered to treat breast cancer. In other embodiments, the combination thereapy is administeraeted to treat colon cancer. In some embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HPV. In other embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HCV.

[00140] In some embodiments, the anti-PD-1 antibody 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 polypeptide is administered at a dose of selected 10 μg/kg weekly during weeks 1-4 of a 6-week cycle. In other embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 is administered at a dose of selected 30 μg/kg weekly during weeks 1-4 of a 6-week cycle. In some embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 is administered at a dose of selected 50 μg/kg weekly during weeks 1-4 of a 6-week cycle. In other embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 is administered at a dose of selected 75 μg/kg weekly during weeks 1-4 of a 6-week cycle. In some embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 is administered at a dose of selected 100 μg/kg weekly during weeks 1-4 of a 6-week cycle. The afore mentioned dosages and administration schedules of the combination of 5C4 and rIL-21 may be administered to treat cancers. In some embodiments, the combination therapy is administered to treat clear cell renal cell carcinoma (ccRCC). In other embodiments, the combination is administered to treat non-

small cell lung cancer (NSCLC). In some embodiments, the combination thereapy is administered to treat melanoma. In other embodiments, the combination thereapy is admnisteraed to treat prostate cancer. In some embodiments, the combination therapy is administered to treat breast cancer. In other embodiments, the combination thereapy is administeraeted to treat colon cancer. In some embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HPV. In other embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HCV.

[00141] In some embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 is administered at a dose of selected 10 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. In other embodiments, anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and rIL-21 is administered at a dose of selected 30 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. In some embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and IL-21 polypeptide is administered at a dose of selected 50 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. In other embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and IL-21 polypeptide is administered at a dose of selected 75 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. In some embodiments, the anti-PD-1 antibody is 5C4 and the IL-21 polypeptide is rIL-21, and 5C4 is administered at a dose of 3 mg/kg every other week and IL-21 polypeptide is administered at a dose of selected 100 μg/kg 3 times per week during weeks 1 and 3 of a 6-week cycle. The afore mentioned dosages and administration schedules of the combination of 5C4 and rIL-21 may be administered to treat cancers. In some embodiments, the combination therapy is administered to treat clear cell renal cell carcinoma (ccRCC). In other embodiments, the combination is administered to treat non-small cell lung cancer (NSCLC). In some embodiments, the combination thereapy is administered to treat melanoma. In other embodiments, the combination thereapy is admnisteraed to treat prostate cancer. In some embodiments, the combination therapy is administered to treat breast cancer. In other embodiments, the combination thereapy is

administeraeted to treat colon cancer. In some embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HPV. In other embodiemtns, the combination is administered to treat a virally-associated cancer such as a cancer associated with HCV.

[00142] Optionally, combination therapy with IL-21 polypeptide and anti-PD-1 antibody or a binding fragment thereof can be further combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He et al., *J. Immunol.*, 173:4919-4928 (2004)). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

[00143] A combination of IL-21 polypeptide and anti-PD-1 antibody can be further combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., *ASCO Educational Book Spring*, 60-62 (2000); Logothetis, C., *ASCO Educational Book Spring*, 300-302 (2000); Khayat, D., *ASCO Educational Book Spring*, 414-428 (2000); Foon, K., *ASCO Educational Book Spring*, 730-738 (2000); see also Restifo et al., Chapter 61: "Cancer Vaccines", DeVita et al., eds., *Cancer: Principles and Practice of Oncology*, Fifth Edition, pp. 3023-3043 (1997)). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al., *Proc. Natl. Acad. Sci. USA*, 90:3539-3543 (1993)).

[00144] The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, *Immunity*, 10:281-287 (1999)). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. In certain embodiments, combination therapy with IL-21 polypeptide and anti-PD-1 antibody may be used in conjunction with a collection of

recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self-antigens and are, therefore, tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim et al., *Science*, 266:2011-2013 (1994)). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (*i.e.*, bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

[00145] Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with combination therapy with IL-21 polypeptide and anti-PD-1 antibody is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot et al., *Science*, 269:1585-1588 (1995); Tamura et al., *Science*, 278:117-120 (1997)).

[00146] Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle et al., *Nat. Med.*, 4:328-332 (1998)). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler et al., *Nat. Med.*, 6:332-336 (2000)). As a method of vaccination, DC immunization may be effectively further combined with combination therapy with IL-21 polypeptide and anti-PD-1 antibody to activate more potent anti-tumor responses.

[00147] Combination therapy with IL-21 polypeptide and anti-PD-1 antibody may also be further combined with standard cancer treatments. For example, combination therapy with IL-21 polypeptide and anti-PD-1 antibody may be effectively combined with chemotherapeutic regimes. In these instances, as is observed with the combination therapy with IL-21 polypeptide and anti-PD-1 antibody, it may be possible to reduce the

dose of other chemotherapeutic reagent administered with the combination of the instant disclosure (Mokyr et al., *Cancer Res.*, 58:5301-5304 (1998)). An example of such a combination is combination therapy with IL-21 polypeptide and anti-PD-1 antibody further in combination with decarbazine for the treatment of melanoma. Another example is combination therapy with IL-21 polypeptide and anti-PD-1 antibody further in combination with interleukin-2 (IL-2) for the treatment of melanoma. Other combination therapies include combination therapy with IL-21 polypeptide and anti-PD-1 antibody in combination with radiation, surgery, or hormone deprivation. Angiogenesis inhibitors may also be combined with a combination therapy with IL-21 polypeptide and anti-PD-1 antibody.

[00148] Combination therapy with IL-21 polypeptide and anti-PD-1 antibody can also be used in combination with bispecific antibodies that target Fc-alpha or Fc-gamma receptor-expressing effector cells to tumor cells (see, *e.g.*, U.S. Patent Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would be augmented by the use of combination therapy with IL-21 polypeptide and anti-PD-1 antibody. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

[00149] In another example, combination therapy with IL-21 polypeptide and anti-PD-1 antibody can be used in conjunction with anti-neoplastic antibodies, such as RITUXAN® (rituximab), HERCEPTIN® (trastuzumab), BEXXAR® (tositumomab), ZEVALIN® (ibritumomab), CAMPATH® (alemtuzumab), Lymphocide (eprtuzumab), AVASTIN® (bevacizumab), and TARCEVA® (erlotinib), and the like. In an exemplary embodiment, a treatment of a cancer tumor may include an anti-cancer antibody in combination with combination therapy with IL-21 polypeptide and anti-PD-1 antibody, concurrently or sequentially or any combination thereof, which may potentiate an anti-tumor immune responses by the host.

[00150] Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins, which are expressed by the tumors and which are immunosuppressive. These include, among others,

TGF-beta (Kehrl, J. et al., *J. Exp. Med.*, 163:1037-1050 (1986)), IL-10 (Howard, M. et al., *Immunol. Today*, 13:198-200 (1992)), and Fas ligand (Hahne, M. et al., *Science*, 274:1363-1365 (1996)). In another example, antibodies to each of these entities may be further combined with combination therapy with IL-21 polypeptide and anti-PD-1 antibody to counteract the effects of immunosuppressive agents and favor anti-tumor immune responses by the host.

[00151] Other antibodies that may be used to activate host immune responsiveness can be further used in combination with combination therapy with IL-21 polypeptide and anti-PD-1 antibody. These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. et al., *Nature*, 393:474-478 (1998)) and can be used in conjunction with combination therapy with IL-21 polypeptide and anti-PD-1 antibody. Activating antibodies to T cell costimulatory molecules, such as OX-40 (Weinberg, A. et al., *Immunol.*, 164:2160-2169 (2000)), 4-1BB (Melero, I. et al., *Nat. Med.*, 3:682-685 (1997)), and ICOS (Hutloff, A. et al., *Nature*, 397:262-266 (1999)) may also provide for increased levels of T cell activation.

[00152] In further embodiments, combination therapy with IL-21 polypeptide and anti-PD-1 antibody can be further combined with the use of any non-absorbable steroid. As used herein, a "non-absorbable steroid" is a glucocorticoid that exhibits extensive first pass metabolism such that, following metabolism in the liver, the bioavailability of the steroid is low, *i.e.*, less than about 20%. In one embodiment of the invention, the non-absorbable steroid is budesonide. Budesonide is a locally-acting glucocorticosteroid, which is extensively metabolized, primarily by the liver, following oral administration. ENTOCORT® EC (Astra-Zeneca) is a pH- and time-dependent oral formulation of budesonide developed to optimize drug delivery to the ileum and throughout the colon. ENTOCORT® EC is approved in the U.S. for the treatment of mild to moderate Crohn's disease involving the ileum and/or ascending colon. The usual oral dosage of ENTOCORT® EC for the treatment of Crohn's disease is 6 to 9 mg/day. ENTOCORT® EC is released in the intestines before being absorbed and retained in the gut mucosa. Once it passes through the gut mucosa target tissue, ENTOCORT® EC is extensively metabolized by the cytochrome P450 system in the liver to metabolites with negligible glucocorticoid activity. Therefore, the bioavailability is low (about 10%). The low

bioavailability of budesonide results in an improved therapeutic ratio compared to other glucocorticoids with less extensive first-pass metabolism. Budesonide results in fewer adverse effects, including less hypothalamic-pituitary suppression, than systemically-acting corticosteroids. However, chronic administration of ENTOCORT® EC can result in systemic glucocorticoid effects such as hypercorticism and adrenal suppression. See PDR 58, Suppl. Third Edition, 608-610 (2004).

[00153] In still further embodiments, combination therapy with IL-21 polypeptide and anti-PD-1 antibody in conjunction with a non-absorbable steroid can be further combined with a salicylate. Salicylates include 5-ASA agents such as, for example: sulfasalazine (AZULFIDINE®, Pharmacia & Upjohn); olsalazine (DIPENTUM®, Pharmacia & UpJohn); balsalazide (COLAZAL®, Salix Pharmaceuticals, Inc.); and mesalamine (ASACOL®, Procter & Gamble Pharmaceuticals; PENTASA®, Shire US; CANASA®, Axcan Scandipharm, Inc.; ROWASA®, Solvay).

[00154] In accordance with the methods of the present invention, a salicylate administered in combination with combination therapy with IL-21 polypeptide and anti-PD-1 antibody and further in combination with a non-absorbable steroid, can include any overlapping or sequential administration of the salicylate and the non-absorbable steroid for the purpose of decreasing the incidence of colitis induced by the immunostimulatory antibodies. Thus, for example, methods for reducing the incidence of colitis induced by combination therapy with IL-21 polypeptide and anti-PD-1 antibody encompass administering a salicylate and a non-absorbable steroid concurrently or sequentially (*e.g.*, a salicylate is administered 6 hours after a non-absorbable steroid), or any combination thereof. Further, according to the present invention, a salicylate and a non-absorbable steroid can be administered by the same route (*e.g.*, both are administered orally) or by different routes (*e.g.*, a salicylate is administered orally and a non-absorbable steroid is administered rectally), which may differ from the route(s) used to administer the combination therapy with IL-21 polypeptide and anti-PD-1 antibody.

[00155] The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Example 1

Non-clinical Evaluation of the Combination of Mouse IL-21 and mPD-1 mAb in Mouse Tumor Models

[00156] Combination therapy with immunomodulatory agents is emerging as an improved option for the treatment and management of cancer because of the potential induction of rapid and robust antitumor immune responses. Interleukin 21 (IL-21), a member of the common gamma chain (γ_c) cytokine family, is produced primarily by CD4+ T cells and natural killer T (NKT) cells and has multiple effects on the innate and adaptive immune systems. IL-21, administered as a single agent, resulted in objective responses in about 20% of patients with melanoma and renal cell carcinoma. Programmed death-1 (PD-1) blockade by monoclonal antibodies (mAbs) has been shown in nonclinical studies to prolong antigen-specific T cell responses. In clinical studies, early results suggest that a PD-1-blocking mAb has antitumor activity in multiple cancers. Based on their complementary mechanisms of action, combination of IL-21 and PD-1 blockade should produce additive or synergistic antitumor immune responses resulting in improved clinical activity. Nonclinical studies were conducted to evaluate the tolerability and the antitumor activity of recombinant mouse IL-21 (mIL-21) in combination with mPD-1 mAb in multiple syngeneic tumor models.

[00157] 5C4 is a human anti-human PD-1 antibody that blocks the binding of PD-1 to PD-L1 and PD-L2 expressed on T cells, APCs, and B cells (Keir, M.E. et al., *Annu. Rev. Immunol.*, 26:677-704 (2008)). Blocking the downregulation of the immune responses elicited by the interaction of these molecules provides benefit in animal models. In clinical trials, 5C4 administered at doses of 0.1 to 10 mg/kg has been shown to be generally well tolerated and has demonstrated efficacy in multiple tumor types (melanoma, renal cell, and lung cancer) (Brahmer, J.R. et al., *J. Clin. Oncol.*, 28(19):3167-3175 (Jul. 1, 2010)). As 5C4 does not cross-react with mouse PD-1, a mouse-specific anti-PD-1 mAb, 4H2, was generated for use in mouse models (Li, B. et al., *Clin. Cancer Res.*, 15:1507-1509 (2009)).

[00158] The efficacy of mIL-21 and mouse anti-PD-1 mAb alone or in combination in 4 different syngeneic mouse tumor models was evaluated, including the solid tumor

models MC38 (murine colon carcinoma) and EMT-6 (murine mammary carcinoma), and the solid tumor (subcutaneous [SC]) and lung metastasis (intravenous [IV]) models utilizing the B16-F10 mouse melanoma cell line. Enhanced antitumor activity of the combination treatment was observed in the MC38 and B16-F10 SC models, with evidence of synergy in the MC38 model, and in 1 of the 2 SC B16-F10 experiments conducted. No enhanced efficacy of combination treatment over single-agent treatment was observed in the EMT-6 model. In all studies, the combination therapy was well tolerated.

1.1: MC38 Study 1408-226

1.1.1: Materials and Methods

1.1.1.a: Animals

[00159] Twelve-week old female C57/BL6 mice (Charles River Laboratories, Hollister, CA) were used for these studies. Mice were housed in microisolator cages and administered autoclaved food and water ad libitum. The MC38 studies were conducted at the Bristol-Myers Squibb (BMS) site in Milpitas, CA (Biologics Discovery California).

1.1.1.b: Reagents

Recombinant Mouse IL-21 (mIL-21) (SEQ ID NO: 19)

[00160] Mouse IL-21 (SEQ ID NO: 19) was produced and purified by ZymoGenetics and certified to have < 0.5 EU/mg endotoxin level and > 90% purity. Stock solutions of mouse IL-21 were kept at -80 °C and dosing solutions were prepared daily in phosphate-buffered saline (PBS). SEQ ID NO: 19 is the amino acid sequence of mature mouse IL-21 (mouse IL-21 lacking a signal sequence) having a methionine on the amino-terminus.

PD-1-Specific Monoclonal Antibody (mPD-1 mAb)

[00161] The antibody, 4H2 (also referred to herein as mPD-1 mAb), is a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc. It was generated using well known laboratory techniques as described in detail in U.S. Patent No. 8,008,449. Briefly, to generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were

screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS.

[00162] mPD-1 mAb was produced and purified by BMS (< 0.1 EU/mL endotoxin level, > 95% purity and ~1% high molecular weight species) and formulated in PBS. Stock solutions were kept at 4 °C and dosing solutions were prepared in PBS. Of note, mouse IgG1 has similarly low effector function (FcR binding, complement fixation) as human IgG4, the isotype of the therapeutic anti-human PD-1 mAb (5C4, BMS-936558).

mIgG1 Control mAb

[00163] The mAb of unknown specificity (MOPC-21; catalog #BE0083) was produced and purified by BioXCell (West Lebanon, NH) and formulated in PBS at 2.58 mg/mL.

MC38 Cell Line

[00164] The MC38 cell line was provided by Dr. James Allison (Memorial Sloan-Kettering Cancer Center). Cells were grown in DME + 10% fetal bovine serum (FBS). Cells were seeded from a vial of frozen MC38 cells (Lot #CLO463-121510), quality controlled, and subsequently confirmed to be mycoplasma-free (Report 1012-0047). Subconfluent cells were harvested on the day of implantation and were ~86% viable.

1.1.1.c: Study Designs and Tumor Growth Assessment

[00165] Two million MC38 tumor cells were injected SC into C57BL/6 mice; 8 days later, tumor volumes were determined, followed by randomization and treatments. The initial tumor volumes ranged from 53.9-56.2 $mm^3/2$ (*i.e.*, length [L] × width [W] × height/2). The treatments are shown in Table 1.

1.1.1.d: Tumor Growth Assessment

[00166] Percent mean or median tumor growth inhibition (% mean or median TGI) values were calculated using the following formula: [(mean or median control tumor size - mean or median treated tumor growth) divided by mean or median control tumor growth] × 100. Calculations for the mean were determined only when 100% of mice

were alive on study (*i.e.*, if even one mouse was removed from the study, mean tumor volumes were no longer determined); calculations for the median were determined on the last day in which at least 60% of mice were alive on study. Percent tumor-free (TF) was calculated based on the percent of mice without tumors at the end of the study.

1.1.1.e: Statistical Analysis

[00167] For each of the combination groups, synergy was evaluated by comparing the number of complete remissions that would be expected if the effects seen in the PD-1 alone and IL-21 alone groups were additive, to the number of complete remissions that were actually observed. This was evaluated using a Chi-Square test with one degree of freedom.

1.1.2: Results

[00168] The initial tumor volumes ranged from 53.9-56.2 $mm^3/2$. The mean and median tumor growth graphs are shown in Figure 1, while the individual mouse data are shown in Figure 2 and listed in Chart 1. On Day 29, mIL-21 administered at 200 µg/mouse 3 times (Days 8, 12, and 15) resulted in 30% median TGI while mPD-1 mAb administered at 200 µg/mouse resulted in 60% median TGI and a single tumor-free mouse (Table 2). Combination of both agents resulted in synergistic antitumor activity, as evidenced by complete regressions observed in 7 of 10 mice and 99.9% median TGI (Figure 1, Figure 2, Table 2). Synergy was demonstrated in this study, with the observation that 7 mice experienced complete regression on the combination compared to the single mouse that would be expected to have experienced complete remission if the combination had an additive effect (Chi-Square, p=0.0455). While the mean TGI values were somewhat lower reflecting the earlier day of analysis (Day 19 vs. Day 29), a potent combinatorial effect was observed (Table 2). The regressions in the combination treatment group were durable, as there were no cases of tumor recrudescence for the remainder of the study. Furthermore, a survival benefit was observed in the combination treatment group (70% of mice were alive on Day 75), while there were no survivors in the mIgG control or mIL-21 groups, and only a single survivor in the mPD-1 mAb treated group (Figure 3). There were no significant changes in body weights or clinical signs (*e.g.*, lethargy, mobility, coat condition, etc.) among any of the treated mice (Chart 2).

1.2: MC38 Study 1106-248

1.2.1: Materials and Methods

1.2.1.a: Animals

[00169] Twelve-week old female C57/BL6 mice (Charles River Laboratories, Hollister, CA) were used for these studies. Mice were housed in microisolator cages and administered autoclaved food and water ad libitum. The MC38 studies were conducted at the Bristol-Myers Squibb (BMS) site in Milpitas, CA (Biologics Discovery California).

1.2.1.b: Reagents

Recombinant Mouse IL-21 (mIL-21) (SEQ ID NO: 19)

[00170] Mouse IL-21 was produced and purified by ZymoGenetics and certified to have < 0.5 EU/mg endotoxin level and > 90% purity. Stock solutions of mouse IL-21 were kept at -80 °C and dosing solutions were prepared daily in phosphate-buffered saline (PBS).

PD-1-Specific Monoclonal Antibody (mPD-1 mAb)

[00171] The antibody, 4H2 (also referred to herein as mPD-1 mAb), is a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc. It was generated using well known laboratory techniques as described in detail in U.S. Patent No. 8,008,449. Briefly, to generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS.

[00172] mPD-1 mAb was produced and purified by BMS (< 0.1 EU/mL endotoxin level, > 95% purity and ~1% high molecular weight species) and formulated in PBS. Stock solutions were kept at 4 °C and dosing solutions were prepared in PBS. Of note, mouse IgG1 has similarly low effector function (FcR binding, complement fixation) as human IgG4, the isotype of the therapeutic anti-human PD-1 mAb (5C4, BMS-936558).

mIgG1 Control mAb

[00173] The mAb of unknown specificity (MOPC-21; catalog #BE0083) was produced and purified by BioXCell (West Lebanon, NH) and formulated in PBS at 2.58 mg/mL.

MC38 Cell Line

[00174] The MC38 cell line was provided by Dr. James Allison (Memorial Sloan-Kettering Cancer Center). Cells were grown in DME + 10% fetal bovine serum (FBS). Cells were seeded from a vial of frozen MC38 cells (Lot #CLO463-121510), quality controlled, and subsequently confirmed to be mycoplasma-free (Report 1012-0047). Subconfluent cells were harvested on the day of implantation and were ~86% viable.

1.2.1.c: Study Designs and Tumor Growth Assessment

[00175] Two million MC38 tumor cells were injected SC into C57BL/6 mice; 7 days later, tumor volumes were determined, followed by randomization and treatments. The initial tumor volumes ranged from 56.5-58.2 $mm^3/2$. The treatments are shown in Table 3.

1.2.1.d: Tumor Growth Assessment

[00176] Percent mean or median tumor growth inhibition (% mean or median TGI) values were calculated using the following formula: [(mean or median control tumor size - mean or median treated tumor growth) divided by mean or median control tumor growth] × 100. Calculations for the mean were determined only when 100% of mice were alive on study (*i.e.*, if even one mouse was removed from the study, mean tumor volumes were no longer determined); calculations for the median were determined on the last day in which at least 60% of mice were alive on study. Percent tumor-free (TF) was calculated based on the percent of mice without tumors at the end of the study.

1.2.1.e: Statistical Analysis

[00177] For each of the combination groups, synergy was evaluated by comparing the number of complete remissions that would be expected if the effects seen in the PD-1 alone and IL-21 alone groups were additive, to the number of complete remissions that

were actually observed. This was evaluated using a Chi-Square test with one degree of freedom.

1.2.2: Results

[00178] The mean and median tumor growth graphs are shown in Figure 4, while the individual mouse data are shown in Figure 5 and listed in Chart 3. On Day 31, the TGI (median) was 25% in mice administered 200 μg mIL-21 while 60% TGI was observed in mice administered 200 μg mPD-1 mAb. In addition, 2 of 10 mPD-1 mAb-treated mice were tumor-free on Day 31 (Figure 4, Figure 5, Table 4). Similar trends were observed in the % mean TGI values on Day 21. Combination of both agents resulted in 100% TGI with 8 of 10 mice exhibiting complete regressions. Synergy was demonstrated at the mIL-21 dose of 200 μg, with the observation that 8 mice experienced complete regression with 200 μg mIL-21 + mPD-1 mAb compared to the 2 mice that would be expected to have experienced complete remission if the contributions of 200 μg of mIL-21 alone and mPD-1 mAb alone were additive (Chi-Square, p=0.0339). While enhanced anti-tumor effects were observed for the combination of both agents compared to the administration of each alone, synergy was not demonstrated at the 50 μg dose of mIL-21. The regressions in the combination treatment group were durable as there were no cases of tumor recrudescence through the end of the study on Day 70. Furthermore, a survival benefit was observed (on Day 70) in both combination treatment groups while there was only 0% to 20% survival in the groups that received mIgG isotype control mAb or mIL-21 or mPD-1 mAb alone (Figure 6). Similar antitumor activity was observed in mIL-21-treated animals, regardless of treatment regimen (200 μg × 3, vs. 50 μg × 6). There were no significant changes in body weights or clinical signs (*e.g.*, lethargy, mobility, coat condition, etc.) among any of the treated mice (Chart 4).

1.3: EMT-6 Study #39

1.3.1. Materials and Methods

1.3.1.a: Animals

[00179] Nine-to-11 week old female Balb/c mice (Harlan Laboratories, Frederick, MD) were housed in sterilized micro-isolator cages and provided sterile food and water ad libitum. This study was conducted at the BMS site in Lawrenceville, NJ.

1.3.1.b: Reagents

Recombinant Mouse IL-21 (mIL-21) (SEQ ID NO: 19)

[00180] Mouse IL-21 was produced and purified by ZymoGenetics and certified to have < 0.5 EU/mg endotoxin level and > 90% purity. Stock solutions of mouse IL-21 were kept at -80 °C and dosing solutions were prepared daily in phosphate-buffered saline (PBS).

PD-1-Specific Monoclonal Antibody (mPD-1 mAb)

[00181] The antibody, 4H2 (also referred to herein as mPD-1 mAb), is a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc. It was generated using well known laboratory techniques as described in detail in U.S. Patent No. 8,008,449. Briefly, to generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS.

[00182] mPD-1 mAb was produced and purified by BMS (< 0.1 EU/mL endotoxin level, > 95% purity and ~1% high molecular weight species) and formulated in PBS. Stock solutions were kept at 4 °C and dosing solutions were prepared in PBS. Of note, mouse IgG1 has similarly low effector function (FcR binding, complement fixation) as human IgG4, the isotype of the therapeutic anti-human PD-1 mAb (5C4, BMS-936558).

EMT-6 Cell Line

[00183] EMT-6 mouse mammary carcinoma cells were obtained from Dr. Dietmar Siemann (University of Florida). EMT-6 cells were cultured in DMEM + GLUTAMAX® media (Gibco, Cat #10566), supplemented with 10% FBS (Summit Biotechnology, Cat #FP-200-05). Cells were dissociated from tissue culture flasks with 0.25% Trypsin-EDTA (Gibco, Cat #25200). Cell viability was > 90%.

1.3.1.c: Study Design and Tumor Growth Assessment

[00184] Balb/c mice were injected SC with 1.5 × 106 EMT-6 cells in 0.2 mL Hank's Balanced Salt Solution (HBSS). Seven days post tumor cell implantation, mice were randomized into groups of 8 animals with a mean tumor volume of ~100 mm^3. Recombinant mouse IL-21 (50μg, IP) and PD-1-specific monoclonal antibody (10 mg/kg, IP) were administered as described in Table 5. Tumors were measured with calipers 2-dimensionally twice weekly and tumor volume was calculated as L × (W2/2), with L being the longer of the 2 measurements. Body weights were recorded biweekly, and any mouse with >20% body weight loss was removed from the study.

1.3.2: Results

[00185] As shown in Figure 7 and Figure 8, mIL-21 as monotherapy, administered at 50 μg/mouse 3 times weekly for 2 weeks was efficacious in the EMT-6 model with a median TGI of 52% on Day 31. In contrast, mPD-1 mAb at a dose of 10 mg/kg, administered every 4 days for 3 doses, was ineffective in this model, resulting in TGI of 0% on Day 31 (Table 6). Combination treatment with mIL-21 and mPD-1 mAb (TGI = 28.7%) did not enhance antitumor responses as compared to mIL-21 alone. There were no significant changes in body weight or clinical signs (*e.g.*, lethargy, mobility, coat condition, etc) in the combination treatment group compared with the other groups (see Chart 5).

1.4 TGM Study 1104 (SC B16-F10 Model)

1.4.1: Materials and Methods

1.4.1.a: Animals

[00186] Female C57BL/6 mice (Harlan Sprague-Dawley, Livermore, CA) of 8.5 weeks old were used for this study. Mice were housed in microisolator cages and administered autoclaved food and water ad libitum. This study was conducted at the BMS site in Seattle, WA (ZymoGenetics).

1.4.1.b: Reagents

Recombinant Mouse IL-21 (mIL-21) (SEQ ID NO: 19)

[00187] Mouse IL-21 was produced and purified by ZymoGenetics and certified to have < 0.5 EU/mg endotoxin level and > 90% purity. Stock solutions of mouse IL-21 were kept at -80 °C and dosing solutions were prepared daily in phosphate-buffered saline (PBS).

PD-1-Specific Monoclonal Antibody (mPD-1 mAb)

[00188] The antibody, 4H2 (also referred to herein as mPD-1 mAb), is a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc. It was generated using well known laboratory techniques as described in detail in U.S. Patent No. 8,008,449. Briefly, to generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS.

[00189] mPD-1 mAb was produced and purified by BMS (< 0.1 EU/mL endotoxin level, > 95% purity and ~1% high molecular weight species) and formulated in PBS. Stock solutions were kept at 4 °C and dosing solutions were prepared in PBS. Of note, mouse IgG1 has similarly low effector function (FcR binding, complement fixation) as human IgG4, the isotype of the therapeutic anti-human PD-1 mAb (5C4, BMS-936558).

PBS Vehicle

[00190] PBS (HyClone Laboratories, Cat #SH 30256.01, Logan, Utah) was used as vehicle control and to prepare mIL-21 and anti-mPD-1 mAb dosing solutions.

B16-F10 Cell Line

[00191] The B16-F10 mouse melanoma cells were originally obtained from ATCC (Manassas, VA). B16-F10 cells were cultured in RPMI 1640 media (HyClone Laboratories, Cat #SH30096.01), supplemented with 10% FBS (HyClone Laboratories, Cat #SH30071.02), 2 mM L-glutamine (Gibco Life Technologies, Grand Island, NY, Cat #25030), and 1 mM sodium pyruvate (Gibco Life Technologies, Cat #11360). Cells were

dissociated from tissue culture flasks with TrypLE Express cell dissociation solution (Gibco, Cat #12604). Cell viability was assessed prior to implantation via trypan blue exclusion and shown to be > 95% viable.

1.4.1.c: Study Design and Tumor Growth Assessment

[00192] C57BL/6 mice were injected SC in the hind-flank region (just proximal to the hip bone) with 1×10^5 B16-F10 cells in 0.05 mL HBSS (HyClone Laboratories, Cat #SH3026801). The day of tumor implantation was considered Study Day 1. On Day 6, mice were assigned to treatment groups as outlined in Table 7. Tumors were then measured 2-dimensionally with calipers at least 4 times per week and tumor volume was calculated as L*(W2/2), with L being the longer of the two measurements. Mice were terminated when the measured tumor volume neared 1500 mm^3 or when the tumors began to ulcerate.

1.4.1d: Statistical Analysis

[00193] Significant differences ($p < 0.05$) between groups for mean tumor growth over time were analyzed by 2-way analysis of variance (ANOVA), using treatment and time as variables, followed by Fisher's test. Significant differences in the time (in days) until the tumor volume in mice reached 300 mm^3 were analyzed using survival proportions followed by log-rank test for trend and presented as a Kaplan-Meier plot. To evaluate synergy, a Cox regression model including terms for treatment with mIL-21, mPD-1 mAb, and the interaction between mIL-21 and mPD-1 mAb was fit to the time (in days) until the tumor volume in the mice reached 300 mm^3.

1.4.2: Results

[00194] In this model, SC delivery of mouse B16-F10 melanoma cells results in the development of solid SC tumors which is cell number-dependent. Delivery of 1×10^5 cells per mouse typically results in a tumor burden that is consistent and allows significant reductions to be observed when mice are treated with an efficacious treatment. Administering too many cells can result in either overly aggressive tumor growth that is difficult to inhibit or, conversely, too few cells results in inconsistently low tumor burden.

[00195] As shown in Figure 9, mice treated with either mIL-21 alone or in combination with anti-mPD1 mAb had lower median tumor volumes than mice treated with PBS or mPD-1 mAb alone. When calculating median tumor volumes, only groups that had ≥ 70% mice alive were included. When individual animal tumor growth was evaluated (Figure 10 and Chart 6), treatment with mIL-21 alone, or with the combination of mIL-21 and mPD-1 mAb, was able to noticeably delay tumor growth in more mice as compared to treated with PBS or mPD-1 mAb alone. There were no tumor-free mice in any of the groups at Day 35. When analyzed using survival proportions, groups of mice treated with either mIL-21 alone or a combination of mIL-21 and mPD-1 mAb tended to be more protected from reaching a specific tumor volume (300 mm^3) than mice treated with PBS or mPD-1 mAb alone, though the differences between groups were not statistically significant (Figure 11). A tumor volume of 300 mm^3 was chosen since this was the largest tumor volume that all mice on study achieved and thus, provided a consistent endpoint measurement. There was a higher than normal amount of tumor ulcerations in this study which resulted in needing to terminate a large number of mice in each of the groups before their tumors reached the maximum allowed volume of 1500 mm^3. While enhanced anti-tumor effects were observed for the administration of mIL-21 and the combination of both agents compared to the administration of mPD-1 mAb alone, synergy was not demonstrated using a Cox regression model. There were no significant changes in body weights among any of the treated mice (Chart 7).

1.5 TGM Study 1108 (IV B16-F10 Model)

1.5.1: Materials and Methods

1.5.1.a: Animals

[00196] Female C57BL/6 mice (Harlan Sprague-Dawley, Livermore, CA) at 8 weeks of age were used for this study. Mice were housed in microisolator cages and administered autoclaved food and water ad libitum. This study was conducted at the BMS site in Seattle, WA (ZymoGenetics).

1.5.1.b: Reagents

Recombinant Mouse IL-21 (mIL-21) (SEQ ID NO: 19)

[00197] Mouse IL-21 was produced and purified by ZymoGenetics and certified to have < 0.5 EU/mg endotoxin level and > 90% purity. Stock solutions of mouse IL-21 were kept at -80 °C and dosing solutions were prepared daily in phosphate-buffered saline (PBS).

PD-1-Specific Monoclonal Antibody (mPD-1 mAb)

[00198] The antibody, 4H2 (also referred to herein as mPD-1 mAb), is a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc. It was generated using well known laboratory techniques as described in detail in U.S. Patent No. 8,008,449. Briefly, to generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS.

[00199] mPD-1 mAb was produced and purified by BMS (< 0.1 EU/mL endotoxin level, > 95% purity and ~1% high molecular weight species) and formulated in PBS. Stock solutions were kept at 4 °C and dosing solutions were prepared in PBS. Of note, mouse IgG1 has similarly low effector function (FcR binding, complement fixation) as human IgG4, the isotype of the therapeutic anti-human PD-1 mAb (5C4, BMS-936558).

PBS Vehicle

[00200] PBS (HyClone Laboratories, Cat #SH 30256.01, Logan, Utah) was used as vehicle control and to prepare mIL-21 and mPD-1 mAb dosing solutions.

B16-F10 Cell Line

[00201] The B16-F10 mouse melanoma cells were originally obtained from ATCC (Manassas, VA). B16-F10 cells were cultured in RPMI 1640 media (HyClone Laboratories, Cat #SH30096.01), supplemented with 10% FBS (HyClone Laboratories, Cat #SH30071.02), 2 mM L-glutamine (Gibco Life Technologies, Grand Island, NY, Cat #25030), 1 mM sodium pyruvate (Gibco Life Technologies, Cat #11360). Cells were

dissociated from tissue culture flasks with TrypLE™ Express cell dissociation solution (Gibco, Cat #12604). Cell viability was assessed prior to implantation via trypan blue exclusion and shown to be > 95% viable.

1.5.1.c: Study Design and Tumor Growth Assessment

[00202] C57BL/6 mice were injected IV via the tail vein with 1×10^5 B16-F10 cells in 0.1 mL HBSS (HyClone Laboratories, Cat #SH3026801). The day of tumor implantation was considered Study Day 1. On Day 5, mice were assigned to treatment groups as outlined in Table 8. Body weights were obtained prior to B16-F10 implantation (Day -2) and approximately 3 to 4 times per week thereafter; to be consistent, body weights were taken between approximately 8 am and 10 am and the time of day was always recorded. On Day 20 post tumor cell implant, mice were anesthetized with isoflurane and euthanized by cervical dislocation. Lungs were harvested, inflated with PBS, and the number of surface metastases was enumerated.

1.5.1.d: Statistical Analysis

[00203] Significant differences ($p < 0.05$) in the mean number of lung surface metastases between groups were analyzed using 1-way ANOVA followed by Tukey's multiple comparison test. To evaluate synergy, a linear regression model including terms for treatment with mIL-21, mPD-1 mAb, and the interaction between mIL-21 and mPD-1 mAb was fit to the number of lung metastases.

1.5.2: Results

[00204] As shown in Figure 12, the group of mice treated with a combination of mIL-21 and mPD-1 mAb had a significantly lower average number of surface lung metastases compared to PBS-treated mice (1-way ANOVA, $p < 0.05$). The average number of lung metastases in mice treated with mIL-21 or mPD-1 mAb alone was not significantly different from that of the PBS-treated mice ($p > 0.05$). Synergy with respect to the average number of lung metastases was evaluated using a linear model with terms for treatment with mPD-1 mAb, mIL-21, and the interaction of mPD-1 mAb and mIL-21; no clear evidence of synergy was identified. However, despite the lack of synergy, there was an enhanced effect of combination treatment, compared to monotherapy with mIL-21 or

mPD-1 alone, on the reduction of lung metastases. Lung metastases counts for individual mice are presented in Chart 8.

[00205] All mice in the experiment appeared to tolerate the treatments well as evidenced by their healthy appearance, no signs of morbidity, and the fact that all mice survived until the end of the study period (Day 20 post-cell implant). In addition, all groups of mice gained weight over the course of the study, an additional sign that the treatments were well tolerated. Although the group of mice treated with the combination of mIL-21 and mPD-1 mAb tended to gain less weight than the other groups, the differences were not statistically different (Figure 13, Chart 9).

1.6 TGM Study 1109 (SC B16-F10 Model)

1.6.1: Materials and Methods

1.6.1.a: Animals

[00206] Female C57BL/6 mice (Harlan Sprague-Dawley, Livermore, CA) at 8.5 weeks of age were used for this study. Mice were housed in microisolator cages and administered autoclaved food and water ad libitum. This study was conducted at the BMS site in Seattle, WA (ZymoGenetics).

1.6.1.b: Reagents

Recombinant Mouse IL-21 (mIL-21) (SEQ ID NO: 19)

[00207] Mouse IL-21 was produced and purified by ZymoGenetics and certified to have < 0.5 EU/mg endotoxin level and > 90% purity. Stock solutions of mouse IL-21 were kept at -80 °C and dosing solutions were prepared daily in phosphate-buffered saline (PBS).

PD-1-Specific Monoclonal Antibody (mPD-1 mAb)

[00208] The antibody, 4H2 (also referred to herein as mPD-1 mAb), is a chimeric rat-mouse anti-mouse-PD-1 antibody containing rat anti-mPD-1 Fabs linked to a mouse IgG1 Fc. It was generated using well known laboratory techniques as described in detail in U.S. Patent No. 8,008,449. Briefly, to generate the rat anti-mouse PD-1 antibody, rats were immunized with mouse cells transfected to express a recombinant mouse PD-1 fusion protein (R&D Systems Catalog No. 1021-PD) and monoclonal antibodies were

screened for binding to mouse PD-1 antigen by ELISA assay. The rat anti-PD-1 antibody V regions were then recombinantly linked to a murine IgG1 constant region using standard molecular biology techniques and rescreened for binding to mouse PD-1 by ELISA and FACS.

[00209] mPD-1 mAb was produced and purified by BMS (< 0.1 EU/mL endotoxin level, > 95% purity and ~1% high molecular weight species) and formulated in PBS. Stock solutions were kept at 4 °C and dosing solutions were prepared in PBS. Of note, mouse IgG1 has similarly low effector function (FcR binding, complement fixation) as human IgG4, the isotype of the therapeutic anti-human PD-1 mAb (5C4, BMS-936558).

PBS Vehicle

[00210] PBS (HyClone Laboratories, Cat #SH 30256.01, Logan, Utah) was used as vehicle control and to prepare mIL-21 and mPD-1 mAb dosing solutions.

B16-F10 Cell Line

[00211] The B16-F10 mouse melanoma cells were originally obtained from ATCC (Manassas, VA). B16-F10 cells were cultured in RPMI 1640 media (HyClone Laboratories, Cat #SH30096.01), supplemented with 10% FBS (HyClone Laboratories, Cat #SH30071.02), 2 mM L-glutamine (Gibco Life Technologies, Grand Island, NY, Cat #25030), and 1 mM sodium pyruvate (Gibco Life Technologies, Cat #11360). Cells were dissociated from tissue culture flasks with TrypLE Express cell dissociation solution (Gibco, Cat #12604). Cell viability was assessed prior to implantation via trypan blue exclusion and shown to be > 95% viable.

1.6.1.c: Study Design

[00212] C57BL/6 mice were injected SC in the hind-flank region (just proximal to the hip bone) with 1×10^5 B16-F10 cells in 0.05 mL HBSS (HyClone Laboratories, Cat #SH3026801). The day of tumor implantation was considered Study Day 1. Six days post-implant, mice were randomized into groups of 10 animals each and treated as outlined in Table 9. Tumors were then measured 2-dimensionally with calipers at least 4 times per week and tumor volume was calculated as L×(W2/2), with L being the longer of

the 2 measurements. Mice were terminated when the measured tumor volume neared 1500 mm^3 or when the tumors began to ulcerate.

1.6.1.d: Statistical Analysis

[00213] Significant differences ($p < 0.05$) between groups for mean tumor growth over time were analyzed using 2-way ANOVA using treatment and time as variables, followed by Fisher's test. Significant differences in the time (in days) until the tumor volume in mice reached 450 mm^3 were analyzed using survival proportions followed by log-rank test for trend and presented as a Kaplan-Meier plot. To evaluate synergy, a Cox regression model including terms for treatment with mIL-21, mPD-1 mAb, and the interaction between mIL-21 and mPD-1 mAb was fit to the time (in days) until the tumor volume in the mice reached 450 mm^3.

1.6.2: Results

[00214] As shown in Figure 14, mice treated with the combination of mIL-21 and mPD1 mAb had a lower median tumor volume than mice treated with PBS, mIL-21, or mPD-1 mAb alone. Additionally, a trend of delayed tumor growth was observed in mice treated with the combination of mIL-21 and mPD-1 mAb as compared to mice treated with PBS or mPD-1 mAb alone (Figure 15 and Chart 10). There were no tumor-free mice in any of the groups at Day 40. When analyzed using survival proportions, groups of mice treated with the combination of mIL-21 and mPD-1 mAb tended to be protected from reaching a specific tumor volume (450 mm^3) compared to mice treated with PBS or mIL-21 or mPD-1 mAb alone (Figure 16). A tumor volume of 450 mm^3 was chosen since this was the largest tumor volume that all mice on study achieved and thus, provided a consistent endpoint measurement. Synergy analysis was performed using a Cox regression model. Although the result was not statistically significant, the data were deemed to be suggestive of a trend for synergistic interaction, given the small group size, between mIL-21 and mPD-1 mAb ($p=0.1348$).

[00215] Although the differences in survival proportions between groups were not statistically significant, the *p* value was 0.08 when analyzed by log-rank analysis for trend. Given the small group size ($n = 10$), this suggests a trend towards the combination

therapy group of mice surviving longer than the groups of mice treated with either of the treatments alone (Figure 16).

[00216] There were no significant changes in body weights among any of the treated mice (Chart 11).

1.7: Conclusions from Example 1

[00217] In a series of studies using 3 tumor types (MC38, EMT-6, and B16-F10), both agents given concurrently elicited enhanced efficacy in 2 of the 3 models evaluated (MC38 and B16-F10) compared with the activity observed with each agent alone (summarized in Table 10). mIL-21 was evaluated at dose levels ranging from 50 μg to 200 μg per mouse following different dosing schedules, and mPD-1 mAb was dosed at 200 to 300 μg per mouse every 3 to 4 days, for 1 or 3 cycles (Table 10). Synergistic activity was observed in the MC38 colon carcinoma model. In the MC38 tumor model, mIL-21 at the highest dose tested (200 μg per mouse) and mPD-1 mAb at 200 μg per mouse, showed little single-agent efficacy; however, concurrent therapy produced prolonged antitumor effects.

[00218] In addition to evaluating antitumor efficacy, mortality, body weight, and clinical signs of toxicity were monitored in each study. No increases in mortality, body weight changes, or clinical signs of toxicity were observed in mice administered up to 200 μg/mouse (approximately 10 mg/kg) mIL-21 and 300 μg/mouse (approximately 15 mg/kg) anti-mouse PD-1 mAb in combination, as compared to either agent alone or to the control groups.

[00219] In the EMT-6 model, mIL-21 induced delayed tumor growth, while therapy with mPD-1 mAb was ineffective (Figure 7 and Figure 8). Combination treatment with mIL-21 and mPD-1 mAb did not enhance antitumor responses compared to mIL-21 alone. In clinical studies, it has been reported that expression of PD-L1 in tumors correlates with response to PD-1 blockade (Brahmer, J.R. et al., *J. Clin. Oncol.*, 28(19):3167-3175 (Jul. 1, 2010)). It is possible that lack of PD-L1 in mouse tumors may be associated with unresponsiveness to mPD-1 mAb, but the studies presented here did not address this question.

[00220] In the B16-F10 murine melanoma models tested, enhanced antitumor effects were observed with mIL-21 and mPD-1 mAb combination treatment. The beneficial

effects of combination treatment were observed in both the IV metastasis model (TGM 1108) and the solid tumor SC model (TGM 1104 and TGM 1109), and these models performed well within experimental and historical expectations with regard to the number of lung metastases (TGM 1108) and the expected solid tumor growth and incidence of early necrotic tumors (TGM 1109) in the PBS-treated groups. The B16-F10 murine melanoma models are difficult models in which to demonstrate efficacy of antitumor therapeutics, as the tumors are aggressive and poorly immunogenic. In addition, demonstrating antitumor efficacy is more difficult when treatments are started after the establishment of tumors, as was done in the 3 studies described herein.

[00221] Mouse IL-21 has previously been shown to have antitumor activity in the metastatic and solid tumor B16-F10 models, but only when dosed at a greater frequency (*i.e.*, daily injections as compared to 3 times weekly, as was done in the studies described herein) and/or when treatment was initiated prior to tumor establishment (Søndergaard, H., *Cancer Immunol. Immunother.*, 56(9):1417-1428 (Sep 2007). The fact that the combination of mIL-21 and mPD-1 mAb was able to significantly reduce the number of lung surface metastases in TGM 1108 using the dosing regimens described (Table 8), and was also able to reduce the tumor burden in TGM 1109, illustrates the benefits of combination treatment with these therapeutics.

[00222] Taken together, these data demonstrate that combination mIL-21 and mPD-1 mAb therapy is well tolerated in mice and provides synergistic anti-tumor activity and/or greater antitumor efficacy than single-agent monotherapy in most of the models tested. Efficient and productive adaptive immune responses to tumors require the orchestration of various signaling pathways. Pharmacological interventions that modulate T cell responses via blockade of PD-1 or signaling through IL-21 have demonstrated preclinical and clinical activity. The complementary mechanisms of action of anti-PD-1 antibody and IL-21 polypeptide provide a rational for the enhanced antitumor effects of combination therapy with anti-PD-1 antibody and IL-21 polypeptide.

Example 2

Clinical Evaluation of the Combination of Recombinant IL-21 and Anti-PD-1 Antibody (5C4)

[00223] This is a Phase 1, open-label study of rIL-21 administered in combination with 5C4 to subjects with advanced or metastatic solid tumors refractory to or relapsed from at least one prior therapy. Recombinant human IL-21 (also referred to herein as rIL-21) (SEQ ID NO: 5), is supplied as a sterile 1 mg/mL solution to be administered as an intravenous (IV) push over 1 to 2 minutes. 5C4 is available as a sterile 10 mg/mL formulation to be administered as an IV infusion over 60 minutes. In Part 1, rIL-21 is administered at doses of 10, 30, 50, 75 or 100 μg/kg on 1 of 2 schedules: (1) weekly during Weeks 1 through 4 of a 6-week cycle (Arm A) or (2) 3 times per week during Weeks 1 and 3 of a 6-week cycle (Arm B). In both arms, 5C4 is administered at a dose of 3 mg/kg once every 2 weeks during the 6-week cycle (*i.e.*, during Weeks 1, 3, and 5). In Part 2, cohort expansion is carried out at the dose selected in Part 1 for both treatment schedules. In both parts of the study, treatment can continue for up to 2 years with the potential for 1 additional year of therapy for subjects who become eligible for retreatment during the follow-up period.

Study Design

[00224] rIL-21 is administered in combination with 5C4 to subjects with advanced or metastatic solid tumors unresponsive (*i.e.*, not achieving a complete response [CR] or partial response [PR]) to at least 1 prior therapy. The study is conducted in 2 parts (Part 1: Dose Escalation and Part 2: Cohort Expansion). The first part of the study consists of dose escalation of rIL-21 administered in combination with 5C4. The study uses a 3 + 3 design for dose escalation. Part 2 of the study begins enrollment after the MTD (or maximum administered dose [MAD] if no MTD is determined) for each dosing regimen has been determined in Part 1.

[00225] Subjects with CR, PR, or stable disease (SD) continue to receive treatment until the first occurrence of at least one of the following: (1) achievement of a confirmed CR; (2) clinical deterioration suggesting that no further benefit from treatment is likely; (3) meeting other pre-specified criteria for discontinuation of treatment; (4) other intolerability to therapy; or (5) administration of 2 years of therapy. Subjects with confirmed CR receive 2 additional cycles, then stop treatment and enter follow-up. Subjects with progressive disease (PD) are permitted to continue treatment as long as the subject is receiving clinical benefit, as assessed by the Investigator, and tolerating

treatment; however, subjects must discontinue treatment upon the next documented event of PD.

[00226] A schematic of rIL-21 and 5C4 administration in each of the 2 arms of the study is provided in Figure 17.

Part 1 - Dose Escalation

[00227] The dose levels of rIL-21 and 5C4 to be administered for each dose cohort are provided in Table 11. Dose escalation is carried out in 2 arms; treatment in both arms occurs in 6-week cycles. Arm A consists of weekly administration of rIL-21 during Weeks 1 through 4 of a 6-week cycle. In Arm B, rIL-21 is administered 3 times per week during Weeks 1 and 3 of a 6-week cycle. In both arms 5C4 is administered once every 2 weeks (*i.e.*, during Weeks 1, 3, and 5). Each 6-week cycle of treatment consists of 3 doses of 5C4 administered 2 weeks apart given in combination with rIL-21 administered on the assigned dose schedule. In both arms 5C4 is administered as a single agent during Week 5. If the first cohort of either arm is determined to exceed the MTD, the subsequent cohort in that is treated with 10 μg/kg rIL-21 in combination with 1 mg/kg 5C4.

Part 2 - Cohort Expansion

[00228] Cohort expansion is carried out in subjects with clear cell renal cell carcinoma (ccRCC) or non-small cell lung cancer (NSCLC) to further establish safety and obtain preliminary estimates of efficacy at the dose combination recommended for each arm in Part 1. During Part 2, approximately 50 subjects with ccRCC and 50 subjects with NSCLC are randomized in a 1:1 ratio to the dose selected for each arm in Part 1. Separate randomization schedules are generated for NSCLC and ccRCC subjects; randomization of NSCLC subjects is stratified by disease histology (squamous vs. non- squamous). At least 12 NSCLC subjects with squamous histology are treated in each arm.

Study Population

[00229] Male and female subjects greater than or equal to 18 years of age with histologic or cytologic confirmation of locally advanced, non-resectable, or metastatic solid tumors meeting all eligibility criteria are eligible to participate in the dose escalation

portion of the study. Subjects carrying a diagnosis of RCC (with a clear cell component) or NSCLC are enrolled in the cohort expansion portion of the study.

Study Assessments and Endpoints

Safety Outcome Measures

[00230] Safety, as measured by the rate of adverse events (AEs) and serious adverse events (SAEs), is the primary endpoint and is assessed during treatment and for 100 days of follow-up. All subjects who receive at least 1 dose of rIL-21 or 5C4 are evaluated for safety. Assessments are based on AE reports and the results of vital sign measurements, electrocardiograms (ECGs), physical examinations, radiology exams, and clinical laboratory tests. Adverse events are categorized using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA); both AEs and laboratory tests are graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.

Efficacy Measures

[00231] Efficacy is assessed as a secondary objective using the following secondary endpoints, all of which are evaluated according to both RECIST 1.1 and irRECIST 1.1 criteria from the start of treatment through the last follow-up assessment: (i) best overall response (BOR), (ii) objective response rate (ORR; *i.e.*, CR + PR), (iii) duration of response (DOR) and (iv) progression-free survival rate (PFSR) at landmark timepoints (*e.g.*, 12, 24, 48, and 96 weeks).

Example 3

Clinical Evaluation of the Combination of Recombinant IL-21 and Anti-PD-1 Antibody (5C4) in Virally-Associated Tumors

[00232] Immunotherapy agents are emerging as impressive treatment option for cancers associated with virus. For example, the PD-1:PD-L1 pathway may play a role in both persistence of HPV infection (through expression of PD-L1 in the tonsillar crypt epithelium - the site of initial infection) as well as resistance to immune elimination during malignant progression (Lyford-Pike et al., *Cancer Res*, 73:1733-1741 (2013)). The study in this Example 3 relates to two patients who were patients in the on-going

Phase I clinical study described in Example 2. Two cancer patients known to be positive for human papilloma virus were treated with rIL-21 administered in combination with 5C4. The data described below demonstrate a benefit associated with combination therapy with rIL-21 and 5C4 in patients with virally-associated cancer.

[00233] The two patients, Patient 3-002 and Patient 2-005, were patients in the study described in Example 2. Patient 3-002 had tonsil cancer and Patient 2-005 had penile cancer. Both patients received 10 mcg/kg rIL-21 and 3 mg/kg 5C4. Both patients were administered 10 mcg/kg rIL-21 weekly during weeks 1 through 4 of a 6-week cycle and 3 mg/kg 5C4 every other week. The results are summarized in Table 12.

[00234] Patient 3-002 had progressive disease during therapy but experienced a reduction in tumor after 5 cycles. Patient 2-005 showed a remarkable reduction in tumor and was considered a partial responder. Collectively these data demonstrate a benefit associated with combination therapy with rIL-21 and 5C4 in patients with virally-associated cancer.

Table 1

Study design for MC38 Study 1408-226

Group	N	Treatment 1		Treatment 2	
		Treatment Dose	Schedule Route	Treatment Dose	Schedule Route
1	10	mIgG 400 µg	d8, d12, d15 IP	PBS (N/A)	d7, d10, d14 IP
2	10	mIgG 200 µg	d8, d12, d15 IP	mIL-21 200 µg	d7, d10, d14 IP
3	10	mPD-1 mAb 200 µg	d8, d12, d15 IP	mIgG 200 µg	d7, d10, d14 IP
4	10	mPD-1 mAb 200 µg	d8, d12, d15 IP	mIL-21 200 µg	d7, d10, d14 IP

Table 2

Percent Mean and Median Tumor Growth Inhibition and Tumor-Free Mice

(MC38 Study 1408-226)

Group #	1	2	3	4
Treatment	mIgG	mIgG + mIL-21	mPD-1 mAb	mPD-1 mAb + mIL-21
% mean TGI @ d19	0	0	5.3	74
% median TGI @ d29	0	30	60	99.9
% tumor-free mice	0	0	10	70

Table 3

Study Design for MC38 Study 1106-248

Group	N	Treatment 1		Treatment 2	
		Treatment Dose	Schedule, Route	Treatment Dose	Schedule, Route
1	10	mIgG 200 µg	d7, d10, d14 IP	PBS (N/A)	d7, d10, d14 IP
2	10	mIgG 200 µg	d7, d10, d14 IP	mIL-21 200 µg	d7, d10, d14 IP
3	10	mIgG 200 µg	d7, d10, d14 IP	mIgG 50 µg	d7, d10, d13, d15, d17, d20 IP
4	10	mPD-1 mAb 200 µg	d7, d10, d14 IP	PBS (N/A)	d7, d10, d14 IP
5	10	mPD-1 mAb 200 µg	d7, d10, d14 IP	mIL-21 200 µg	d7, d10, d14 IP
6	10	mPD-1 mAb 200 µg	d7, d10, d14 IP	mIL-21 50 µg	d7, d10, d13, d15, d17, d20 IP

Table 4

Percent Mean and Median Tumor Growth Inhibition and Percent Tumor-Free Mice (MC38 Study 1106-248)

Group #	1	2	3	4	5	6
Treatment	mIgG	mIgG + mIL-21 (200 µg)	mIgG + mIL-21 (50 µg)	mPD-1 mAb	mPD-1 mAb + mIL-21 (200 µg)	mPD-1 mAb + mIL-21 (50 µg)
% mean TGI @ d21	0	34	50	64	93	95
% median TGI @ d31	0	25	7	60	100	100
% tumor-free mice	0	0	20	20	80	80

Table 5

Study Design for EMT-6 Study #39

Group #	Treatment 1			Treatment 2		
	Compound	Dose	Schedule	Compound	Dose	Schedule
1	No Treatment					
3	mPD-1 mAb	10 mg/kg	q4D×3			
4	mIL-21	50µg	M, W, F[a] × 2			
6	mPD-1 mAb	10 mg/kg	q4D×3	mIL-21	50µg	M, W, F × 2

[a] M,W,F = Monday, Wednesday, Friday

Table 6

Antitumor Activity of mIL-21 in Combination with mPD-1 mAb in the EMT-6 Mammary Carcinoma Tumor Model (EMT-6 Study #39)

Group #	1	2	3	4
Treatment	Control (untreated)	mPD-1 mAb[a] (10 mg/kg)	mIL-21 (50 µg)	mIL-21+mPD-1 mAb (50 µg + 10 mg/kg)
% TGI[b]	-	0	52	29
T-C (days)[c]	-	0	8.3	5.0

[a] mPD-1 mAb treatments were given on Days 7, 11, and 15; mIL-21 was administered on Days 7, 9, 11, 14, 16, and 18

[b] %TGI = mean tumor growth inhibition calculated 2 weeks post final PD-1 mAb treatment (Day x post implantation) using the formula $(100 - [(T_t/T_o) / (C_t/C_o)]) / 100 - (C_t/C_o)$ where T_t = mean tumor size of treated animals on Day x; T_o = mean initial tumor size of treated animals; C_t = mean tumor size of control animals on Day x; C_o = mean initial tumor size of control animals

[c] T-C = time for treated groups to reach tumor target size - time for control group to reach tumor target size (target size = 1000 mm^3)

Table 7

Study Design for TGM Study 1104

Group	N	Treatment 1		Treatment 2	
		Treatment, Dose	Schedule, Route	Treatment, Dose	Schedule, Route
1	10	PBS 100 µL	d6, d8, d9, d10, d12, d13, d15, d17 IP	None (N/A)	(N/A) (N/A)
2	10	mIL-21 75 µg	d6, d8, d10, d13, d15, d17 IP	None (N/A)	(N/A) (N/A)
3	10	None (N/A)	(N/A) (N/A)	mPD-1 mAb 300 µg	d6, d9, d12, d15 IP
4	10	mIL-21 75 µg	d6, d8, d10, d13, d15, d17 IP	mPD-1 mAb 300 µg	d6, d9, d12, d15 IP

Table 8

Study Design for TGM Study 1108

Group	N	Treatment 1		Treatment 2	
		Treatment, Dose	Schedule, Route	Treatment, Dose	Schedule, Route
1	10	PBS 100 µL	d5, d7, d8, d9, d11, d12, d14, d16 IP	None (N/A)	(N/A) (N/A)
2	10	mIL-21 75 µg	d5, d7, d9, d12, d14, d16 IP	None (N/A)	(N/A) (N/A)
3	10	None (N/A)	(N/A) (N/A)	mPD-1 mAb 300 µg	d5, d8, d11, d14 IP
4	10	mIL-21 75 µg	d5, d7, d9, d12, d14, d16 IP	mPD-1 mAb 300 µg	d5, d8, d11, d14 IP

Table 9

Study Design for TGM Study 1109

Group	N	Treatment 1		Treatment 2	
		Treatment, Dose	Schedule, Route	Treatment, Dose	Schedule, Route
1	10	PBS 100 µL	d6, d8, d9, d10, d12, d13, d15, d17 IP	None (N/A)	(N/A) (N/A)
2	10	mIL-21 75 µg	d6, d8, d10, d13, d15, d17 IP	None (N/A)	(N/A) (N/A)
3	10	None (N/A)	(N/A) (N/A)	mPD-1 mAb 300 µg	d6, d9, d12, d15 IP
4	10	mIL-21 75 µg	d6, d8, d10, d13, d15, d17 IP	mPD-1 mAb 300 µg	d6, d9, d12, d15 IP

Table 10

Summary of Nonclinical Tumor Model Studies Evaluating Combination Treatment of mIL-21 and mPD-1 mAb[a]

Study ID	Model	mIL-21			mPD-1 mAb		
		Dose	# Doses	Frequency	Dose	# Doses	Frequency
1408-226	MC38	200 µg	3	q3-4d	200 µg	3	q3-4d
1106-248	MC38	200 µg or 50 µg	3	q3-4d (200) or 6 doses (50)	200 µg	3	q3-4d
EMT-6 #39	EMT-6	50 µg	6	MWF (x2)	200 µg (~10 mpk)	3	q4dx3
TGM 1104	B16-F10 (SC)	75 µg	6	MWF (x2)	300 µg (~15 mpk)	4	q3d
TGM 1108	B16-F10 (IV)	75 µg	6	MWF (x2)	300 µg (~15 mpk)	4	q3d
TGM 1109	B16-F10 (SC)	75 µg	6	MWF (x2)	300 µg (~15 mpk)	4	q3d

[a] All mIL-21 and mPD-1 mAb treatments were administered IP

Table 11

Dose Escalation Schedule for rIL-21 and Anti-PD-1 Antibody, 5C4, (Arm A and Arm B)

Dose Cohort	rIL-21 (IV; µg/kg)	anti-PD-1 antibody (IV; mg/kg)
1	10	3
2	30	3
3	50	3
4	100*	3

* Further dose escalation of rIL-21 may occur in consultation with the Sponsor/Medical Monitor and Investigators, but will not exceed 50% of the previous highest tolerated dose.

Table 12

Summary of Results for Clinical Evaluation of the Combination of Recombinant IL-21 and Anti-PD-1 Antibody (5C4) in Virally-Associated Tumors

Patient No.	Type of CA	HPV +? (Y or N)	Cohort	Response
3-002	Penile cancer	Positive	Weekly/10mcg	PD
2-005	tonsil	Positive	Weekly/10mcg	PR

[1] Pt 3-002: PD after 4 Cycles. Came off after 5 cycles, but had some good tumor reduction (-26% from BL, -15% from nadir) after 5 cycles

[2] Pt 2-00: SD initially, PR after 4 Cycles.

Chart 1

MC38 Study 1408-226 Individual Tumor Measurements (mm^3)

		RUL	TS	TS	TS	TS	RUL	RUL	TS	TS	FD
Group 1	Treatment	3/29	4/12	4/19	4/5	3/29	3/29	3/22	4/15	4/1	3/29
Date	mIgG	249523	249536	249542	249575	249597	249605	249607	249628	249633	249650
3/4	8	45.3	45.6	32.7	33.4	72.5	72.9	126.4	10.3	58.4	57.3
3/8	12	147.3	72.9	58.8	152.5	248.1	281.2	147.5	21.1	95.3	169.4
3/11	15	186.5	158.8	63.6	283.1	291.7	393.1	325.9	25.5	191.0	279.6
3/15	19	380.1	165.0	111.5	442.2	621.6	448.8	280.8	67.3	244.9	321.6
3/22	26	752.5	582.4	321.4	1191.7	1563.0	1140.8	280.2	308.0	1332.9	1003.0
3/25	29	1137.2	1067.3	590.6	1384.9	2463.5	1292.1	280.2	596.9	1902.5	1314.0
3/29	33		1354.4	794.6	1873.8				1006.9	2631.6	
4/1	36		1483.1	1024.4	2162.9				1176.7		
4/5	40		2030.9	1110.4					1856.4		
4/12	47			1955.3					2451.3		
4/15	50			2589.5							
4/19	54										
4/22	57										
4/26	61										

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS		RUL	TS	TS	TS	TS	TS	TS	FD
Group 2	Treatment	4/5		3/29	5/3	4/19	4/15	4/19	4/15	4/5	4/5
Date	PD-1	249518	249559	249567	249568	249579	249580	249602	249606	249621	249634
3/4	8	45.7	72.0	126.0	34.4	57.1	78.7	58.5	45.2	32.4	12.1
3/8	12	407.0	255.9	424.7	113.4	71.5	199.1	79.0	81.7	230.6	159.2
3/11	15	526.5	383.8	613.3	110.4	73.5	206.4	41.4	180.7	410.0	155.5
3/15	19	441.3	293.5	834.6	16.0	109.7	186.8	12.6	304.9	473.0	248.9
3/22	26	687.9	79.1	1553.9	0.0	414.0	233.0	18.7	640.1	1093.3	676.3
3/25	29	1273.9	20.3	1889.2	0.0	943.3	430.8	31.1	1314.9	1402.9	753.9
3/29	33	1682	13	1889.2	0.0	1137.7	796.9	47.7	1765.9	1827.9	1370.8
4/1	36	2137.2	6.4	1889.2	0.0	1290.2	1157.5	139.3	1608.6	2653.6	1951.1
4/5	40	2137.2	3.2	1889.2	72.8	1537.8	1820.6	252	1731.3	2653.6	1951.1
4/12	47	2137.2	0	1889.2	99.3	1616.2	2755.1	631.5	3179.3	2653.6	1951.1
4/15	50		0		365.7	2647.9		916.8			
4/19	54		0		670.1			1298.3			
4/22	57		0		959.2			1805.3			
4/26	61		0		1572.3			2143.8			
4/29	64		0		2273.4						
5/3	68		0								
5/6	71		0								
5/10	75		0								

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS	TS	TS	TS	TS	RUL	TS	TS	BWL	TS
Group 3	Treatment	3/29	4/26	3/29	4/19	4/22	4/12	4/19	4/12	3/25	4/12
Date	IL-21 + IgG	249513	249519	249549	249555	249571	249572	249620	249623	249636	249643
3/4	8	20.1	25.3	68.1	80.3	54.8	42.7	49.1	40.2	96.5	62.2
3/8	12	261.6	47.5	169.1	190.1	143.9	116.8	142.2	116.6	245.9	125
3/11	15	493	35.4	331.7	139.8	232.5	165.1	204.2	199.6	294.8	168
3/15	19	1002.1	6	524.4	71.3	156.5	156	240.5	177.9	477.2	335.2
3/22	26	1308.5	26.2	1129.9	186.7	170.7	185.3	247.6	471.3	517.3	790.7
3/25	29	2173.6	48.9	2183.2	337.3	219.8	291.6	353	837.1	517.3	1115.9
3/29	33	2173.6	133.7	2183.2	558.7	339.5	441.2	610.8	1138.1	517.3	1756.5
4/1	36	2173.6	247.9	2183.2	1069.5	500.4	567.9	1021.8	1888.5	517.3	1649.3
4/5	40	2173.6	328.4	2183.2	1363.7	599.0	710	1241.1	2758.4	517.3	2830.5
4/12	47		720.7		1995.4	1128.2		1666.4			
4/15	50		1198.4		2904.9	1785.0		2544.4			
4/19	54		1643.0			2138.6					
4/22	57		2146.7								
4/26	61										

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS		TS		TS					
Group 4	Treatment	4/15		4/15		4/12					
Date	IL-21 + PD-1	249533	249550	249574	249583	249592	249600	249604	249614	249653	249654
3/4	8	42.6	53.6	49.2	21.9	67.5	62.9	95.2	80.4	25.2	40.5
3/8	12	133.5	12.6	167.9	18.3	226.8	173.7	254.5	127.1	12.6	61.5
3/11	15	232.2	4.9	182	0	185.3	150.4	241.5	85.7	0	32.6
3/15	19	367.3	0	183.8	0	169.1	26.7	20.9	18.2	0	3.8
3/22	26	584.4	0	522.2	0	379.6	4.9	17.7	6.8	0	1.3
3/25	29	780.8	0	656.2	0	632.6	0	6.5	2.4	0	0
3/29	33	1079.9	0	896.3	0	1141.8	0	5.7	0	0	0
4/1	36	1104	0	1186.2	0	1600.0	0	7.4	0	0	0
4/5	40	1387.2	0	1556.5	0	2070.2	0	5.0	0	0	0
4/12	47	2262.4	0	2009.4	0	2070.2	0	8.8	0	0	0
4/15	50	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
4/19	54	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
4/22	57	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
4/26	61	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
4/29	64	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
5/3	68	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
5/6	71	2262.4	0	2009.4	0	2070.2	0	0	0	0	0
5/10	75	2262.4	0	2009.4	0	2070.2	0	0	0	0	0

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

Chart 2

MC38 Study 1408-226 Individual Mouse Weights (grams)

		RUL	TS	TS	TS	TS	RUL	RUL	TS	TS	FD
Group 1	Treatment	3/29	4/12	4/19	4/5	3/29	3/29	3/22	4/15	4/1	3/29
date	mIgG	249523	249536	249542	249575	249597	249605	249607	249628	249633	249650
3/4	8	22.7	24.8	22.9	22.5	22.5	21.4	22.1	23.4	23.4	23.4
3/8	12	22.9	24.9	23.3	21.7	22.6	21.6	23.0	23.7	22.7	23.2
3/11	15	24.0	25.2	24.2	23.1	24.1	22.5	22.8	24.4	23.0	23.2
3/15	19	24.4	25.3	24.7	22.7	24.1	23.2	23.8	24.6	23.4	23.5
3/22	26	22.0	25.0	24.0	23.5	24.9	23.2	20.8	24.7	23.8	20.1
3/25	29	22.0	25.7	24.7	23.7	25.0	23.0		24.6	24.8	19.1
3/29	33	22	26	24.6	24.4		23		24.7	23.6	
4/1	36		25.2	25.4	23				25.4		
4/5	40		21.1	26.2					26.1		
4/12	47			28.8					26.8		
4/15	50			30							
4/19	54										
4/22	57										
4/26	61										
4/29	64										
5/3	68										
5/6	71										
5/10	75										
5/13	78										

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS		RUL	TS	TS	TS	TS	TS	TS	FD
Group 2	Treatment	4/5		3/29	5/3	4/19	4/15	4/29	4/15	4/5	4/5
date	PD-1	249518	249559	249567	249568	249579	249580	249602	249606	249621	249634
3/4	8	20.8	26.6	24.2	22.4	23.6	21.2	23.6	22.6	24.1	23.2
3/8	12	20.9	25.5	24.3	22.5	23.7	20.8	23.9	22.4	24.7	22.9
3/11	15	21.7	26.3	24.9	22.8	24.9	21.6	24.9	23.0	25.5	23.5
3/15	19	20.3	25.8	25.3	23.5	25.5	22.3	25.2	23.2	26.3	24.1
3/22	26	21.8	24.2	25.2	23.4	24.2	21.5	24.0	23.0	26.3	23.0
3/25	29	21.6	24.7	25.0	23.7	24.8	22.3	23.5	24.1	26.7	23.6
3/29	33	21.3	24.4	25	23.5	24.7	22.2	23.4	24.8	27.1	23.5
4/1	36	20.9	23.8		23.9	25.3	22.8	23.8	25.2	28.6	24.5
4/5	40		23.7		23.9	25.2	23.6	24.4	25.9		
4/12	47		23.6		24.4	23.6	27.1	24.6	28.4		
4/15	50		24.5		24.1	23.6		25.3			
4/19	54		24.4		24.1			25.9			
4/22	57		24.4		24.3			26.7			
4/26	61		24.5		25.9			27.4			
4/29	64		24.4		28.2						
5/3	68		24.4								
5/6	71		24								
5/10	75		25.1								
5/13	78		24.9								

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS	TS	TS	TS	TS	RUL	TS	TS	BWL	TS
Group 3	Treatment	3/29	4/26	3/29	4/19	4/22	4/12	4/19	4/12	3/25	4/12
date	IL-21 + IgG	249513	249519	249549	249555	249571	249572	249620	249623	249636	249643
3/4	8	24.2	22.5	23.1	22.6	22.1	24.1	24.1	22.3	25.0	24.1
3/8	12	24.2	23.7	22.5	22.6	22.2	23.5	23.5	21.9	25.3	24.9
3/11	15	24.9	23.4	23	22.9	22.3	23.5	23.9	23.4	25.6	24
3/15	19	24.9	23.1	23.7	22.8	22.7	24	24.4	23.4	26.4	25.2
3/22	26	26.5	22.7	24.3	22.7	21.9	23.2	24.8	22.7	19.1	25
3/25	29	26.4	23	25.6	23.2	22.7	24	25.6	23.5		26.2
3/29	33		22.8		23.3	22.1	23.4	24.5	23.6		26.9
4/1	36		23		23.9	23	22.3	25.1	25		28
4/5	40		23.6		24.6	23.5	21.7	25.9	26.8		29.7
4/12	47		23.8		26.4	24	21.7	26.5			
4/15	50		24		28.1	24.3		27.3			
4/19	54		25			25.7					
4/22	57		26								
4/26	61										
4/29	64										
5/3	68										
5/6	71										
5/10	75										
5/13	78										

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS		TS		TS					
Group 4	Treatment	4/15		4/14		4/12					
date	IL-21 + PD-1	249533	249550	249574	249583	249592	249600	249604	249614	249653	249654
3/4	8	23.9	24.2	22.3	20.5	21.9	24.8	23.5	20.6	24.3	22.6
3/8	12	23.2	24.1	22.7	21	22.2	25	24.6	21.1	25	22.7
3/11	15	23	25.2	23	20.8	23	25.7	25.3	21.5	25	22.8
3/15	19	23.5	26.2	23.2	21.6	23.1	25.8	25.1	21.7	24.2	23.3
3/22	26	23.5	24.9	23.1	21.6	22.8	24.8	23.4	21.1	24.2	22.9
3/25	29	24	24.5	24.2	21.6	23	25.2	24.5	21.3	24.5	23.2
3/29	33	24.7	24.2	24.6	21.4	23.4	24.9	24.7	21	24.3	22.7
4/1	36	25.5	24.6	24.6	21.9	24.5	24.7	25.1	20.9	24.8	23
4/5	40	25.8	24.9	25.6	21	26.4	25	24.3	21.5	24.4	23.1
4/12	47	28	24.7	27	21.8		25.1	25.1	21.6	24.6	23.6
4/15	50		24.8		21.6		25.2	25.3	21.6	24.1	22.6
4/19	54		26.2		21.9		25.8	24.8	21.8	25	22.7
4/22	57		26.6		22.3		26	25.1	21.8	24.2	22.7
4/26	61		26.6		22.5		25.9	26.7	22.3	24.3	22.5
4/29	64		26.8		22.3		26.1	27	22.7	24.8	22.6
5/3	68		26.8		21.9		26.5	25.2	22.5	24.3	22.7
5/6	71		26.8		21.6		26.1	25.7	22.2	24.6	23
5/10	75		26.8		21.7		26.4	26	22.5	24.8	23.1
5/13	78		27.4		22		27	25.5	22	25.2	23.7

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

Chart 3

MC38 Study 1106-248 Individual Tumor Measurements (mm^3)

		TS	TS	TS	TS	TS	TS	TS	FD	FD	TS
Group 1	treatment	7/1	7/1	7/12	7/19	7/12	7/19	7/1	7/1	7/12	7/15
date	mIgG	259833	259862	259870	259872	259893	259912	259935	259943	259944	259946
6/14	7	129.2	76.5	61.1	32.2	19.8	47.7	76.4	59.0	33.2	47.1
6/17	10	332.6	255.4	172.6	76.6	44.6	104.2	244.6	167.4	164.6	203.0
6/21	14	778.0	760.0	458.4	80.5	71.6	302.2	1344.1	238.4	297.3	147.6
6/24	17	1397.6	1310.7	866.0	233.7	119.8	622.7	1667.2	435.2	537.1	296.9
6/28	21	2016.1	2117.3	1323.5	393.1	177.3	682.7	2358.0	937.2	1098.9	318.3
7/1	24	2016.1	2117.3	1452.4	649.2	590.3	823.4	2358.0	937.2	1157.1	509.5
7/5	28	2016.1	2117.3	1987.4	1123.9	917.6	1411.1	2358.0	937.2	1719.5	1098.4
7/8	31	2016.1	2117.3	2475.9	1364.7	1063.4	1695.3	2358.0	937.2	1666.3	1335.1
7/12	35				1548.0		1797.2				2069.3
7/15	38				2339.3		1197.1				
7/19	42										
7/22	45										
7/26	49										
7/29	52										
8/2	56										
8/5	59										
8/16	70										

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		FD	FD	FD	TS	TS	TS	TS	TS	TS	RUL
Group 2	treatment	7/15	7/12	7/5	7/19	7/22	7/1	7/15	7/29	7/26	7/12
date	mIgG + IL-21 (200ug)	259839	259846	259849	259850	259864	259865	259871	259875	259903	259922
6/14	7	47.0	75.9	57.9	47.9	61.5	126.5	31.9	19.9	77.5	34.1
6/17	10	163.6	344.9	252.1	142.0	201.3	369.2	120.2	37.3	142.8	83.0
6/21	14	185.8	419.4	477.4	189.9	179.4	998.9	366.0	52.8	160.9	246.0
6/24	17	288.1	607.9	889.6	337.9	221.8	1474.2	646.0	64.2	143.3	177.8
6/28	21	505.2	998.0	1121.7	495.0	374.3	2264.4	931.7	123.5	240.9	473.8
7/1	24	648.6	912.6	1391.4	501.0	425.4	2264.4	1174.0	187.8	206.3	762.1
7/5	28	1049.0	1589.3	1391.4	1002.9	824.8	2264.4	1430.1	428.1	524.8	1407.3
7/8	31	1385.5	1914.4	1391.4	1146.4	1102.0	2264.4	1773.1	341.0	634.2	1124.9
7/12	35	1708.4	1914.4	1391.4	1756.5	1614.2	2264.4	2754.2	604.9	1089.6	1124.9
7/15	38				2496.2	1973.6			869.7	1477.0	
7/19	42					2363.8			1113.4	1673.7	
7/22	45								1407.2	2221.1	
7/26	49								2074.4		
7/29	52										
8/2	56										
8/5	59										
8/16	70										

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS	TS	TS	TS	FD		FD		TS	TS
Group 3	treatment	7/15	7/19	8/2	7/12	7/12		7/26		7/5	7/12
date	mIgG + IL-21 (50ug)	259841	259873	259887	259890	259910	259911	259919	259928	259938	259945
6/14	7	74.7	44.1	30.8	48.9	57.7	35.3	22.4	114.2	61.7	77.9
6/17	10	192.8	63.9	123.0	258.6	97.1	135.0	212.5	156.3	229.0	187.9
6/21	14	201.3	157.1	153.8	550.1	243.2	156.6	117.9	321.2	593.0	379.6
6/24	17	442.9	327.7	123.5	739.4	368.1	104.6	79.0	204.3	1109.1	629.0
6/28	21	550.2	399.2	197.4	1272.5	487.5	14.9	95.1	106.8	1569.9	1001.6
7/1	24	774.7	611.6	269.8	1604.8	715.9	6.1	157.3	38.6	2191.1	1161.8
7/5	28	1413.2	1158.6	393.4	1970.7	1011.6	0.0	148.3	33.9	2191.1	1572.2
7/8	31	1895.8	1686.2	507.7	2561.6	1446.2	0.0	210.0	19.4	2191.1	2017.7
7/12	35	2213.1	1934.2	674.9	2561.6	1446.2	0.0	505.1	7.7	2191.1	2017.7
7/15	38		2765.4	814.7			0.0	618.1	0.0		
7/19	42			1141.9			0.0	1272.9	0.0		
7/22	45			1198.6			0.0	1520.7	0.0		
7/26	49			1531.2			0.0		0.0		
7/29	52			2203.9			0.0		0.0		
8/2	56						0.0		0.0		
8/5	59						0.0		0.0		
8/16	70						0.0		0.0		

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

			TS		TS	TS	TS	TS	TS	TS	TS
Group 4	treatment		7/8		7/15	7/26	7/15	8/2	7/26	7/29	7/8
date	PD-1	259847	259857	259861	259878	259885	259894	259896	259909	259929	259948
6/14	7	84.2	57.4	37.2	23.3	63.0	74.5	48.9	30.5	44.0	112.2
6/17	10	193.0	145.7	43.4	77.5	93.1	118.7	147.5	77.5	90.1	193.6
6/21	14	117.9	607.1	0.0	178.9	151.7	318.0	61.6	150.5	128.1	368.5
6/24	17	29.2	786.6	0.0	299.4	176.9	354.6	152.4	112.3	79.4	530.6
6/28	21	8.4	1270.9	0.0	502.9	348.5	552.3	206.8	115.4	170.5	984.4
7/1	24	0.0	1666.4	0.0	884.9	335.0	787.0	344.8	152.5	286.8	1307.8
7/5	28	0.0	2402.5	0.0	1621.7	666.6	1131.7	532.9	354.5	498.3	2057.8
7/8	31	0.0	2402.5	0.0	1583.5	682.5	1302.9	649.6	541.8	640.2	2057.8
7/12	35	0.0	2402.5	0.0	2024.7	1191.2	2196.5	864.5	984.4	1271.4	2057.8
7/15	38	0.0	2402.5	0.0	2024.7	1495.3	2196.5	1027.6	1273.4	1287.2	2057.8
7/19	42	0.0	2402.5	0.0	2024.7	1850.1	2196.5	1054.3	1621.0	1530.4	2057.8
7/22	45	0.0	2402.5	0.0	2024.7	2200.2	2196.5	1639.9	2932.9	1836.1	2057.8
7/26	49	0.0		0.0				1792.6		2585.9	
7/29	52	0.0		0.0				2600.4			
8/2	56	0.0		0.0							
8/5	59	0.0		0.0							
8/16	70	0.0		0.0							

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

		TS			TS						
Group 5	treatment	7/15			7/19						
date	PD-1 + IL-21 (200ug)	259867	259884	259889	259892	259908	259914	259915	259916	259923	259930
6/14	7	30.0	73.2	23.3	43.9	48.9	37.2	84.2	106.5	63.5	56.8
6/17	10	99.3	112.4	36.9	124.9	202.5	47.3	53.5	155.3	93.4	53.9
6/21	14	210.9	20.8	36.9	306.6	60.6	9.9	107.6	158.7	114.8	0.0
6/24	17	300.1	3.7	8.6	303.9	35.1	0.0	19.3	38.1	99.1	0.0
6/28	21	361.0	0.0	0.0	379.6	0.0	0.0	3.8	0.0	23.7	0.0
7/1	24	482.9	0.0	0.0	466.1	0.0	0.0	0.0	0.0	17.6	0.0
7/5	28	965.5	0.0	0.0	716.4	0.0	0.0	0.0	0.0	6.1	0.0
7/8	31	1700.8	0.0	0.0	1070.8	0.0	0.0	0.0	0.0	2.0	0.0
7/12	35	2347.2	0.0	0.0	1821.9	0.0	0.0	0.0	0.0	0.0	0.0
7/15	38	2347.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
7/19	42	2347.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
7/22	45	2347.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
7/26	49	2347.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
7/29	52	2348.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
8/2	56	2349.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
8/5	59	2349.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0
8/16	70	2349.2	0.0	0.0	2243.0	0.0	0.0	0.0	0.0	0.0	0.0

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

				TS	TS						
Group 6	treatment			7/29	7/22						
date	PD-1 + IL-21 (50ug)	259831	259835	259837	259840	259844	259921	259931	259932	259937	259949
6/14	7	37.3	28.3	63.6	103.2	43.5	56.8	37.3	24.2	72.3	86.2
6/17	10	76.7	34.4	126.1	183.5	88.2	97.6	52.7	143.5	230.0	233.2
6/21	14	13.7	10.2	199.2	332.3	28.4	75.1	21.8	225.7	111.5	304.0
6/24	17	0.0	3.2	274.0	252.7	18.9	48.7	9.9	121.1	31.0	129.9
6/28	21	0.0	0.0	237.3	275.9	0.0	11.5	0.0	27.6	7.8	20.2
7/1	24	0.0	0.0	221.1	307.9	0.0	5.9	0.0	0.0	0.0	7.9
7/5	28	0.0	0.0	463.2	596.9	0.0	5.0	0.0	0.0	0.0	0.0
7/8	31	0.0	0.0	703.0	1081.4	0.0	2.7	0.0	0.0	0.0	0.0
7/12	35	0.0	0.0	1044.8	1248.3	0.0	0.0	0.0	0.0	0.0	0.0
7/15	38	0.0	0.0	1249.2	1735.7	0.0	0.0	0.0	0.0	0.0	0.0
7/19	42	0.0	0.0	1793.8	2320.8	0.0	0.0	0.0	0.0	0.0	0.0
7/22	45	0.0	0.0	1935.9	2320.8	0.0	0.0	0.0	0.0	0.0	0.0
7/26	49	0.0	0.0	2497.0	2320.8	0.0	0.0	0.0	0.0	0.0	0.0
7/29	52	0.0	0.0	2497.0	2320.8	0.0	0.0	0.0	0.0	0.0	0.0
8/2	56	0.0	0.0	2497.0	2320.8	0.0	0.0	0.0	0.0	0.0	0.0
8/5	59	0.0	0.0	2497.0	2320.8	0.0	0.0	0.0	0.0	0.0	0.0
8/16	70	0.0	0.0	2497.0	2320.8	0.0	0.0	0.0	0.0	0.0	0.0

BWL = body weight loss; FD = found dead; RUL = ulcerated; TS = tumor sacrifice

Chart 4

Individual Mouse Weights (grams) for MC38 Study 1106-248

Group 1	treatment										
date	IgG	259833	259862	259870	259872	259893	259912	259935	259943	259944	259946
6/14	7	23	25.5	23.4	23.7	23	24.6	29.3	22.9	21.5	21.6
6/17	10	22.6	25.5	23.2	24.1	23.5	25.1	28.8	23.5	21.7	21.4
6/21	14	22.9	25.7	23.8	24	23.5	25.1	29	23	22.3	20.9
6/24	17	23.6	26	23.9	23	23.9	23.8	27.3	22.4	22.4	21.4
6/28	21	25.4	26.8	24.7	23.9	24.7	25	28.7	22.8	23.2	22.3
7/1	24			25.1	23.6	24.3	25.4			23.5	22.9
7/5	28			25.9	23.6	25.2	26			22.3	23.7
7/8	31			27.3	22.2	23.1	26.9			19	24
7/12	35				22.4		22.9				25.9
7/15	38				20.8		19.1				
7/19	42										
7/22	45										
7/26	49										
7/29	52										
8/2	56										
8/5	59										
8/16	70										

Group 2	treatment										
date	IgG + IL-21 (200)	259839	259846	259849	259850	259864	259865	259871	259875	259903	259922
6/14	7	24.6	23.9	22.9	23.1	24.7	23.7	21.9	26	24	22.6
6/17	10	24	23.8	22.7	23.2	24.7	23.5	22.1	24.1	23.4	22.7
6/21	14	24.7	22.9	22.5	23.5	25	24.4	22.3	25.2	24.2	22.8
6/24	17	24.3	23.1	22.9	22.9	25	24.8	22.4	24.7	24.2	22.8
6/28	21	24.9	24.4	23.5	24	25.9	27.4	23.2	25.2	24.3	23.8
7/1	24	24.9	23.8	23.7	24.3	25.9		23.5	24.6	24.7	23.3
7/5	28	25.2	24.6		24.7	26.3		24.3	25.2	23.9	23.2
7/8	31	25.8	23.4		25	27.3		25.5	24.5	24.9	23.2
7/12	35	23.6			26	27.6		27.5	25.5	25.3	18.6
7/15	38				26.8	27.8			25.6	25	
7/19	42					25			26.1	21.8	
7/22	45								26.8	24	
7/26	49								26.6		
7/29	52										
8/2	56										
8/5	59										
8/16	70										

Group 3	treatment										
date	mIgG + IL-21 (50ug)	259841	259873	259887	259890	259910	259911	259919	259928	259938	259945
6/14	7	24.6	26.3	22.4	22.7	22.4	24.2	20	21.9	24.7	23.5
6/17	10	24.4	25.1	22.1	23	21.4	24	23.4	21.9	24.7	22.9
6/21	14	24	24.6	21.5	23.2	21.8	23.6	20	21	25	23.9
6/24	17	24.1	25	21.9	23.6	21.6	24	19.7	21.1	24.8	22.1
6/28	21	24.5	25.7	22	23.3	22.4	24.6	19.9	21.6	26	24.1
7/1	24	24.8	25.6	22.6	23.2	22.7	24.6	20.1	21.2	26.3	23.9
7/5	28	25.4	26.1	22.4	21.5	22.6	25.3	20.5	21.3		24.4
7/8	31	26.1	26.8	22.8	22.7	23.2	25.7	21	21.5		25.7
7/12	35	28.5	27.8	23.2			26.7	21.2	21.8		
7/15	38		28.5	23.4			25.1	20.8	21.3		
7/19	42			24.2			25.4	22.1	22.4		
7/22	45			24.5			25.6	23.2	22.6		
7/26	49			25.1			25.3		22.8		
7/29	52			26.7			26		22.7		
8/2	56						25.6		22.6		
8/5	59						26.3		22.8		
8/16	70						27.4		23.6		

Group 4	treatment										
date	PD-1	259847	259857	259861	259878	259885	259894	259896	259909	259929	259948
6/14	7	24	25.7	23.1	22.9	22.5	27.7	22.6	22.8	23.2	23.2
6/17	10	24.9	24.6	22.2	23.4	22.4	25.8	22.6	22.9	23	23.4
6/21	14	24.9	26.4	23.1	23.3	22.5	25.8	22.8	23.3	22.5	23.4
6/24	17	24.1	26.4	23	23.6	21.9	26	22.8	22.6	22.7	23.7
6/28	21	24.8	26.2	23.6	24.3	21.9	26.2	23.4	23.7	22.9	24.7
7/1	24	25.2	26.9	24	24	21.9	26.2	24	23.8	22.9	26
7/5	28	25.5	28.4	24.3	24.6	22.6	26.9	23.9	24.8	23.3	27.3
7/8	31	25.8		24.4	25.2	23.1	24.2	24.7	25	23.5	
7/12	35	24.9		24.3	27.1	23.7	24.3	25.5	25.1	24.3	
7/15	38	25.5		23.1		23.7		24.6	25.5	24.8	
7/19	42	26.9		24.2		25		25.6	26.3	26.3	
7/22	45	27.8		24		25.4		26.7	27.6	26.9	
7/26	49	27.9		24.3				27.7		28	
7/29	52	28.2		24.2				27.9			
8/2	56	28.7		25							
8/5	59	28.8		25.7							
8/16	70	31.2		27							

Group 5	treatment										
date	PD-1 + IL-21 (200)	259867	259884	259889	259892	259908	259914	259915	259916	259923	259930
6/14	7	24.3	23.7	22.8	22.1	24.8	25.1	23.3	27.4	22.7	22.9
6/17	10	24.3	23.8	23.1	21.8	24.6	25.4	20.5	26.5	22.9	22.3
6/21	14	24.2	23.8	22.9	22.4	25.5	24.3	22.9	25.7	23.6	22.9
6/24	17	24.4	23.1	23.2	22.7	25.6	24.7	23.6	26	23.5	22.6
6/28	21	24.9	24.9	23.1	23.4	25.9	25.2	23.7	26.2	23.7	23.3
7/1	24	25.1	23.6	23.5	23.6	25.8	25.6	24.3	26.4	24.2	23.3
7/5	28	25.4	24.7	23.7	24.6	26.9	25.7	23.8	26	24.3	24.3
7/8	31	26.4	24.7	23.3	24.9	26.7	24.9	24.3	26.2	24.2	24.8
7/12	35	28.8	24.8	23.7	26.7	27.6	25.3	24.2	26.6	25.3	23
7/15	38		24.9	23.6	27.6	27.3	25.1	25	27	24.2	23.5
7/19	42		25.5	23.9		26.5	26.1	24.4	26.3	25.2	24.3
7/22	45		24.9	24.2		26.6	25.2	24.5	27.4	24.9	24.3
7/26	49		25.2	23.6		27.9	25.1	25	26.9	24.8	24.8
7/29	52		26.3	24		29.1	25.5	24.8	26.7	24.6	25.5
8/2	56		26.9	24.5		30.4	26.3	24.8		24.8	25.4
8/5	59		25.6	27.4		31	26.5	24.1	28.2	25.8	25.8
8/16	70		25.8	23.9		30.7	27.9	24.9	29.9	26.3	25.7

Group 6	treatment										
date	PD-1 + IL-21 (50ug)	259831	259835	259837	259840	259844	259921	259931	259932	259937	259949
6/14	7	21.8	22.7	23.1	21.8	24.8	22.9	22.3	26.4	22.4	24.6
6/17	10	20.8	21.8	21.7	21.2	24.2	22.1	22.8	26.8	21.5	24.7
6/21	14	20.8	22.6	22.3	21.1	22.6	22.5	22.2	25.5	20.8	24.5
6/24	17	20.7	22.1	23.5	22.1	22.9	22.6	22.2	25.7	21.3	25.6
6/28	21	20.3	23.0	24.0	21.8	24.2	21.7	23.0	26.0	22.9	25.5
7/1	24	20.2	22.5	24.3	22.2	23.9	22.2	22.7	26.0	21.5	26.1
7/5	28	21.3	22.5	24.0	22.3	24.1	22.6	23.0	26.7	21.9	25.8
7/8	31	21.4	22.6	24.2	23.5	24.6	22.4	23.0	27.3	21.5	25.9
7/12	35	22.1	22.8	24.3	23.6	25.5	23.2	22.8	28.3	21.5	25.2
7/15	38	20.9	22.7	24.8	24.8	26.3	22.7	22.2	28.3	21.9	25.3
7/19	42	23.3	24.0	26.5	27.3	27.2	22.8	23.6	29.2	22.6	25.8
7/22	45	24.6	23.4	24.1		26.9	23.0	23.2	29.4	22.2	25.7
7/26	49	23.7	23.3	25.5		26.7	23.7	23.6	29.9	22.5	26.9
7/29	52	24.5	24.0			26.8	23.2	23.9	30.4	22.9	27.1
8/2	56	25.1	24.3			26.8	23.4	24.0	30.6	23.1	27.0
8/5	59	24.6	24.5			27.0	23.1	26.4	30.7	22.8	27.8
8/16	70	24.2	25.5			28.1	23.9	24.0	31.5	23.2	27.7

Chart 5

EMT-6 Study #39 Group Body Weights (grams)

		Bodyweight Data											
		06/20/11 (day 7)	06/23/11 (day 10)	06/26/11 (day 13)	06/30/11 (day 17)	07/05/11 (day 22)	07/08/11 (day 25)	07/11/11 (day 28)	07/14/11 (day 31)	07/18/11 (day 35)	07/21/11 (day 38)	07/25/11 (day 42)	07/28/11 (day 45)
1: Control	Group Total	159.0	163.0	164.0	166.0	171.0	173.0	175.0	173.0	174.0	174.0		
	mean	19.9	20.4	20.5	20.8	21.4	21.6	21.9	21.6	21.8	21.8		
	n	8	8	8	8	8	8	8	8	8	8		
3: BMS-968593; 10 mg/kg; Q4DX3	Group Total	161.0	166.0	169.0	170.0	171.0	175.0	176.0	175.0	175.0	174.0		
	mean	20.1	20.8	21.1	21.3	21.4	21.9	22.0	21.9	21.9	21.8		
	n	8	8	8	8	8	8	8	8	8	8		
4: BMS-986014; 50 ug/mouse; M,W,F (x2)	Group Total	165.0	168.0	171.0	170.0	177.0	177.0	180.0	179.0	184.0	184.0	180.0	179.0
	mean	20.6	21.0	21.4	21.3	22.1	22.1	22.5	22.4	23.0	23.0	22.5	22.4
	n	8	8	8	8	8	8	8	8	8	8	8	8
6: BMS-968593; 10 mg/kg; Q4DX3 + BMS-986014; 50 ug/mouse; M,W,F (x2)	Group Total	158.0	163.0	165.0	169.0	170.0	172.0	171.0	171.0	174.0	174.0	167.0	
	mean	19.8	20.4	20.6	21.1	21.3	21.5	21.4	21.4	21.8	21.8	20.9	
	n	8	8	8	8	8	8	8	8	8	8	8	

Note: individual body weights were not collected in this study.

Chart 6

TGM 1104 Individual Tumor Measurements (mm^3)

Group 1	PBS	8/12	8/4	8/9	8/12	8/9	8/11	8/5	8/8	8/9	8/16
date	day	5878	5879	5880	5881	5882	5883	5884	5885	5887	5945
7/26	6	5	22	27	11	16	16	30	3	27	11
7/27	7	13	33	42	12	14	13	30	8	33	20
7/29	9	23	78	46	9	27	28	61	23	38	32
7/30	10	12	138	52	7	32	38	54	29	26	24
8/1	12	16	280	119	18	48	75	166	108	109	26
8/3	14	59	687	186	32	135	162	811	211	237	40
8/5	16	137	687	562	74	282	500	799	423	500	106
8/6	17	150	687	531	83	531	402	799	677	437	96
8/8	19	341	687	929	161	1000	760	799	1302	1114	194
8/9	20	466		1270	233	1344	759			1372	316
8/10	21	562			207		888				273
8/11	22	641			325		1124				435
8/12	23	849			389						519
8/15	26										846
8/16	27										1259

Group 2	mIL-21	8/24	8/10	8/17	8/15	8/12	8/10	8/8	8/7	8/9	8/11
date	day	5888	5889	5892	5944	5947	5893	5894	5897	5943	5949
7/26	6	15	15	19	7	16	29	24	20	20	10
7/27	7	11	18	16	8	19	33	43	25	33	11
7/29	9	15	31	14	18	39	30	35	50	38	10
7/30	10	9	33	18	6	33	36	50	61	34	12
8/1	12	15	91	28	10	38	64	146	137	119	17
8/3	14	18	140	27	32	66	119	169	341	164	68
8/5	16	32	289	63	58	154	161	360	499	352	150
8/6	17	14	268	52	119	196	208	504	608	474	187
8/8	19	25	584	190	363	414	744	865	608	953	381
8/9	20	21	712	207	426	590	946	865	608	953	509
8/10	21	20	712	268	581	514	1124				645
8/11	22	23		333	635	849					832
8/12	23	29		329	607	907					
8/15	26	119		534	924						
8/16	27	166		715							
8/17	28	133		1064							
8/18	29	358									
8/19	30	312									
8/21	32	542									
8/22	33	634									
8/23	34	777									

Group 3	mPD-1 mAb	8/7	8/11	8/14	8/8	8/6	8/6	8/6	8/5	8/6	8/6
date	day	5899	5900	5901	5902	5952	5903	5904	5905	5906	5907
7/26	6	13	8	20	8	19	13	18	12	21	18
7/27	7	65	40	56	21	60	23	27	24	22	19
7/29	9	38	28	34	24	30	47	34	79	54	29
7/30	10	51	22	26	27	47	60	49	68	66	30
8/1	12	84	47	25	55	143	249	135	228	195	100
8/3	14	241	135	49	133	246	257	224	552	429	158
8/5	16	516	268	77	276	428	581	403	638	629	424
8/6	17	719	332	132	485	645	982	574	638	745	509
8/8	19	1260	567	344	1130						
8/9	20		796	377							
8/10	21		981	493							
8/11	22		1666	515							
8/12	23			504							

Group 4	mIL-21 + mPD-1 mAb	8/12	8/9	8/7	8/23	8/12	8/11	8/16	8/6	8/6	8/6
date	day	5918	5919	5920	5921	5922	5923	5924	5926	5927	5953
7/26	6	20	5	13	26	5	12	7	16	18	6
7/27	7	31	11	27	19	13	18	14	18	25	11
7/29	9	25	18	25	14	23	20	20	34	54	18
7/30	10	30	42	18	15	12	25	27	34	72	19
8/1	12	42	61	26	20	16	32	24	111	157	60
8/3	14	74	127	59	18	59	63	51	198	299	131
8/5	16	121	125	118	21	137	108	63	443	652	256
8/6	17	167	444	128	18	150	131	66	590	694	288
8/8	19	*NM*		259	52	341	337	146			
8/9	20	*NM*			61	466	420	256			
8/10	21	502			63	562	424	269			
8/11	22	750			101	641	827	376			
8/12	23	750			107	828		415			
8/15	26				231			1030			
8/16	27				257			1327			
8/17	28				350						
8/18	29				640						
8/19	30				727						
8/21	32				1029						
	NM = no measurement										

Chart 7

TGM 1104 Individual Mouse Weights (grams)

Group 1	PBS	8/12	8/4	8/9	8/12	8/9	8/11	8/5	8/8	8/9	8/16
date	day	5878	5879	5880	5881	5882	5883	5884	5885	5887	5945
7/19	-2	20.1	18.7	19.5	21.2	20.4	19.9	18.5	22.0	20.2	19.5
7/26	6	20.5	18.8	19.6	21.7	20.8	20.0	18.9	21.7	20.4	20.2
7/28	8	20.7	18.8	19.2	21.6	20.4	18.9	18.4	21.4	20.6	19.7
7/30	10	21.0	19.4	19.7	21.7	21.1	20.0	18.8	22.1	20.8	20.4
8/2	13	22.0	19.4	20.0	21.7	21.5	20.5	19.0	22.2	20.9	21.0
8/4	15	21.7	17.6	20.2	21.7	21.8	20.2	19.5	21.8	21.6	20.6
8/6	17	22.4		20.2	22.5	22.7	20.9		22.0	22.1	20.8
8/9	20	22.4		20.9	22.9	22.5	21.4			23.3	21.5
8/10	21	22.7			22.7		21.4				21.5
8/11	22	22.6			22.6		21.4				20.7
8/12	23	21.8			22.6						20.7

Group 2	mIL-21	8/24	8/10	8/17	8/15	8/12	8/10	8/8	8/7	8/9	8/11
date	day	5888	5889	5892	5944	5947	5893	5894	5897	5943	5949
7/19	-2	20.2	19.6	19.2	18.0	19.4	20.3	19.9	19.8	20.4	19.3
7/26	6	20.2	20.2	20.2	18.6	19.7	20.4	20.6	20.1	20.8	19.4
7/28	8	20.2	20.7	19.7	18.4	19.6	19.8	21.0	20.1	20.7	19.3
7/30	10	20.5	20.5	19.8	18.8	20.4	20.4	21.5	20.5	21.2	19.5
8/2	13	20.8	20.9	20.4	18.9	20.2	20.4	21.6	20.7	22.1	19.7
8/4	15	20.7	20.8	19.9	19.2	20.6	20.9	21.7	20.8	21.4	20.0
8/6	17	20.6	21.3	20.1	19.0	20.3	20.8	21.8	21.3	22.1	20.7
8/9	20	21.3	21.6	20.6	19.4	21.4	21.5			22.3	20.9
8/10	21	21.4	21.9	20.7	19.1	21.1	21.4				21.2
8/11	22	21.3		20.4	19.4	20.9					21.2
8/12	23	21.1		20.5	19.4	21.2					

Group 3	mPD-1 mAb	8/7	8/11	8/14	8/8	8/6	8/6	8/6	8/5	8/6	8/6
date	day	5899	5900	5901	5902	5952	5903	5904	5905	5906	5907
7/19	-2	17.8	20.8	19.5	19.1	22.1	17.7	18.6	18.0	19.5	20.3
7/26	6	18.0	20.7	19.8	19.4	22.2	18.1	19.0	18.0	20.4	20.4
7/28	8	18.0	20.1	20.2	19.7	22.3	17.5	19.0	18.8	20.2	21.2
7/30	10	18.3	20.4	21.0	20.0	22.9	17.7	18.7	18.5	21.2	21.4
8/2	13	19.1	20.5	21.1	20.2	23.0	18.0	19.2	18.5	21.1	21.2
8/4	15	19.1	21.1	20.6	20.2	23.3	18.3	19.2	18.1	21.1	21.5
8/6	17	20.0	21.2	21.5	20.4	23.2	18.7	19.6		21.8	21.5
8/9	20		22.5	20.0							
8/10	21		22.3	22.0							
8/11	22		22.7	21.6							
8/12	23			21.8							

Group 4	mIL-21 + mPD-1 mAb	8/12	8/9	8/7	8/23	8/12	8/11	8/16	8/6	8/6	8/6
date	day	5918	5919	5920	5921	5922	5923	5924	5926	5927	5953
7/19	-2	19.4	20.0	19.2	20.5	20.4	17.5	19.9	18.3	18.9	21.0
7/26	6	19.9	20.8	19.8	21.3	21.1	17.7	20.0	18.2	19.0	21.1
7/28	8	19.4	20.3	19.5	20.9	20.7	18.0	20.7	18.3	19.6	21.1
7/30	10	19.9	20.9	19.7	21.4	21.0	18.2	20.4	18.7	19.6	21.4
8/2	13	20.7	20.5	20.3	21.9	22.0	18.2	21.4	19.2	19.5	21.4
8/4	15	20.7	21.4	20.1	21.1	21.7	18.4	21.1	19.3	19.4	21.8
8/6	17	20.2	21.9	20.4	21.6	22.4	18.5	21.0	19.6	20.0	21.6
8/9	20	no weight			22.5	22.4	19.6	21.9			
8/10	21	21.3			22.4	22.7	19.5	21.8			
8/11	22	21.1			21.8	22.6	20.0	21.7			
8/12	23	21.4			21.9	21.8		21.7			

Chart 8

TGM 1108 Individual Lung Metastasis Counts at Termination (Day 20)

Group 1	**PBS**	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	**day**	6321	6383	6323	6324	6325	6352	6353	6354	6355	6386	6387	6388	6389	6390
9/26	20	75	58	45	55	63	52	63	56	48	79	54	71	86	63

Group 2	**mIL-21**	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	**day**	6326	6327	6328	6329	6330	6371	6372	6385	6374	6375
9/26	20	67	40	76	39	30	18	60	59	72	70

Group 3	**mPD-1 mAb**	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	**day**	6381	3402	3403	3404	6351	6366	6367	6368	6369	6370
9/26	20	35	64	53	25	55	17	67	37	72	54

Group 4	**mIL-21 + mPD-1 mAb**	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	**day**	6331	6332	6333	6334	6335	6356	6357	6358	6359	6360
9/26	20	10	88	34	39	27	42	46	45	12	46

Chart 9

TGM 1108 Individual Mouse Weights (grams)

Group 1	**PBS**	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	**day**	6321	6383	6323	6324	6325	6352	6353	6354	6355	6386	6387	6388	6389	6390
9/5	-2	20.5	19.2	19.8	20.3	20.4	20.2	20.5	19.1	18.7	19.4	18.5	19.7	19.2	19.4
9/11	5	21.4	18.0	20.0	20.7	21.0	20.7	21.6	19.6	18.8	19.9	19.7	19.9	20.1	20.2
9/13	7	21.8	18.2	20.0	20.8	21.1	20.4	21.3	20.0	19.3	20.5	19.5	19.6	20.0	19.7
9/16	10	21.5	18.3	20.4	21.0	21.5	21.1	21.9	20.0	19.7	20.2	19.8	19.6	20.3	20.0
9/20	14	21.7	18.7	20.7	21.5	22.1	21.8	22.4	20.0	20.1	21.6	20.3	19.8	20.5	20.6
9/22	16	21.8	18.6	21.2	21.4	21.7	21.0	21.9	20.0	20.3	20.9	20.0	20.0	20.1	20.6
9/24	18	21.8	19.0	20.3	21.7	21.7	21.4	22.1	19.9	20.2	21.2	19.7	19.4	20.1	20.4
9/26	20	21.8	19.4	21.1	21.8	21.8	21.2	22.4	20.1	20.5	21.4	19.2	20.0	20.4	20.8

Group 2	mIL-21	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	day	6326	6327	6328	6329	6330	6371	6372	6385	6374	6375
9/5	-2	20.3	19.3	18.4	19.6	20.7	19.3	19.2	19.2	18.4	19.8
9/11	5	20.3	19.6	18.8	19.7	20.9	20.2	19.8	19.8	19.5	20.3
9/13	7	20.6	20.4	19.2	20.1	21.5	20.6	19.6	20.3	19.1	20.6
9/16	10	21.2	19.8	19.2	20.0	21.3	20.4	19.9	20.6	19.3	20.6
9/20	14	21.2	20.4	19.6	20.2	22.5	20.9	20.3	20.9	19.4	20.9
9/22	16	21.2	20.8	19.4	20.1	21.9	20.9	20.0	21.0	19.7	20.8
9/24	18	20.8	20.5	19.5	20.1	22.3	20.9	20.0	20.9	19.1	20.5
9/26	20	21.2	20.3	19.1	20.3	22.0	21.2	20.2	21.3	18.9	20.8

Group 3	mPD-1 mAb	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	day	6381	3402	3403	3404	6351	6366	6367	6368	6369	6370
9/5	-2	19.8	18.6	20.7	18.8	19.5	20.2	20.0	20.9	19.2	19.1
9/11	5	20.4	19.5	20.9	19.5	19.4	20.9	20.3	21.5	20.3	20.0
9/13	7	20.2	19.7	22.1	19.4	20.2	21.0	20.3	21.6	19.9	20.7
9/16	10	20.4	19.6	22.2	19.3	20.3	21.0	20.9	21.6	20.0	20.5
9/20	14	20.0	21.0	22.2	20.1	20.8	22.0	20.7	22.4	19.8	20.6
9/22	16	20.5	21.0	22.2	19.9	21.4	21.1	20.6	22.5	19.9	20.9
9/24	18	20.1	20.2	22.2	20.0	21.2	21.6	20.4	22.2	18.9	20.5
9/26	20	20.6	20.7	22.3	20.6	22.0	21.8	20.0	22.5	19.5	20.2

Group 4	mIL-21 + mPD-1 mAb	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26	9/26
date	day	6331	6332	6333	6334	6335	6356	6357	6358	6359	6360
9/5	-2	19.8	18.6	19.4	18.3	20.2	20.2	17.5	20.9	19.5	20.4
9/11	5	20.5	18.7	20.2	18.8	20.4	20.1	18.1	21.9	19.4	20.9
9/13	7	21.0	19.2	19.8	19.1	20.5	20.3	18.7	21.7	19.7	20.7
9/16	10	21.3	18.8	19.9	18.6	21.0	20.4	18.4	21.8	19.7	20.9
9/20	14	20.8	19.3	20.0	18.7	20.9	21.1	18.8	22.5	19.9	21.4
9/22	16	20.8	19.0	19.7	19.0	20.9	21.1	18.8	22.2	20.4	21.7
9/24	18	20.8	19.2	19.5	19.2	21.0	20.6	18.9	21.5	20.0	21.3
9/26	20	20.9	19.2	19.6	19.6	21.0	20.5	18.8	22.5	20.5	21.7

Chart 10

TGM 1109 Individual Tumor Measurements (mm^3)

Group 1	**PBS**	10/4	9/29	9/30	9/30	9/30	10/5	10/9	10/5	10/4	10/5
date	**day**	6515	6516	6526	3518	6519	6529	6521	6522	6523	6524
9/19	6	35	11	21	45	43	19	25	31	23	34
9/21	8	50	32	40	53	70	29	16	52	40	32
9/22	9	52	48	80	52	77	60	35	67	81	49
9/23	10	66	92	83	99	82	56	27	*NM*	60	31
9/25	11	176	159	228	296	176	74	26	*NM*	188	63
9/26	12	242	327	389	416	174	78	43	*NM*	871	96
9/28	14	536	413	664	826	414	195	52	211	515	82
9/29	15	422	449	807	605	598	345	68	462	519	119
9/30	16	640		1470	1409	1746	376	116	618	703	233
10/2	18	835					436	164	602	721	303
10/3	19	1315					734	205	1131	976	571
10/4	20	1493					977	374	1298	1789	773
10/5	21						1684	497	2086		1555
10/6	22							681			
10/7	23							625			
10/8	24							790			
10/9	25							1098			
		NM = no measurement									

Group 2	mIL-21	10/3	10/3	10/2	10/3	9/30	10/6	9/30	10/7	9/29	10/6
date	day	6530	6525	6532	6533	6534	6535	6528	6537	6538	6539
9/19	6	19	39	19	22	44	21	53	23	55	19
9/21	8	19	44	22	36	23	38	50	29	54	23
9/22	9	50	59	50	74	38	61	89	51	94	60
9/23	10	50	40	47	83	55	36	93	53	95	*NM*
9/25	11	203	144	100	108	123	63	192	42	148	*NM*
9/26	12	148	194	107	171	842	86	571	44	244	*NM*
9/28	14	257	378	236	222	166	201	413	64	351	101
9/29	15	433	484	269	428	342	260	685	86	596	166
9/30	16	658	826	376	838	1089	361	1150	144		273
10/2	18	1102	1152	497	1076		672		234		484
10/3	19	1548	1483		1728		860		415		1006
10/4	20						982		543		668
10/5	21						1467		635		891
10/6	22						1902		1012		1711
10/7	23								1322		
		NM = no measurement									

Group 3	mPD-1 mAb	9/30	10/2	9/30	10/3	10/2	10/7	10/4	10/5	10/4	10/2
date	day	6540	6541	6542	6543	6527	6545	6546	6511	6512	6549
9/19	6	46	36	31	17	36	8	30	14	20	41
9/21	8	40	56	39	38	43	14	56	31	52	50
9/22	9	55	58	55	63	53	15	74	46	46	75
9/23	10	53	84	52	57	53	14	40	27	39	73
9/25	11	85	169	164	119	200	38	98	44	68	266
9/26	12	101	273	187	205	215	34	139	59	108	289
9/28	14	247	377	352	305	490	83	256	197	242	569
9/29	15	403	405	1331	787	605	228	458	228	460	725
9/30	16	613	686	1194	583	1045	318	606	471	697	1242
10/2	18		1399		886	1315	299	943	540	897	1466
10/3	19				1620		541	1436	931	1406	
10/4	20						663	1761	1087	1825	
10/5	21						827		1767		
10/6	22						1185				
10/7	23						1315				
		NM = no measurement									

Group 4	mIL-21 + mPD-1 mAb	9/29	10/6	10/11	10/7	10/4	10/11	10/9	9/30	10/4	10/10
date	day	6504	6501	6562	6563	6500	6565	6497	6567	6499	6569
9/19	6	70	20	8	18	23	9	20	18	23	21
9/21	8	69	32	15	28	46	15	33	44	69	31
9/22	9	140	44	12	30	34	19	43	47	48	52
9/23	10	110	46	14	19	27	13	27	50	49	36
9/25	11	98	43	13	39	75	107	26	131	72	*NM*
9/26	12	137	83	14	56	160	11	22	133	142	*NM*
9/28	14	695	121	13	79	216	13	47	244	320	*NM*
9/29	15	706	254	33	136	355	34	102	382	413	74
9/30	16		416	33	241	622	20	*NM*	444	514	80
10/2	18		515	23	465	741	31	258		805	209
10/3	19		652	39	539	1104	35	509		1259	308
10/4	20		991	50	713	1477	50	560		1319	395
10/5	21		1193	40	842		103	761			413
10/6	22		1394	57	1037		131	1235			808
10/7	23			32	1234		206	881			528
	NM = no measurement										

Group 4	mIL-21 + mPD-1 mAb	9/29	10/6	10/11	10/7	10/4	10/11	10/9	9/30	10/4	10/10
date	day	6504	6501	6562	6563	6500	6565	6497	6567	6499	6569
10/8	25			79			378	1392			764
10/9	26			83			721				1609
10/10	27			133			838				
10/11	28			176			940				
10/12	29			182			1007				
10/13	30			256			1594				
10/14	31			441							
10/15	32			465							
10/16	33			571							
10/17	34			693							
10/18	35			776							
10/19	36			784							
10/20	37			1149							
10/21	38			1323							
10/22	39			1411							
	NM = no measurement										

Chart 11

TGM 1109 Individual Mouse Weights (grams)

Group 1	PBS	10/4	9/29	9/30	9/30	9/30	10/5	10/9	10/5	10/4	10/5
date	day	6515	6516	6526	3518	6519	6529	6521	6522	6523	6524
9/12	-2	19.3	19.6	20.25	19.4	19.25	21.05	20.65	18.8	18.85	18.8
9/19	6	20.1	20.6	20.5	20.7	20.1	21.2	20.1	18.9	19.1	18.5
9/21	8	19.8	20	19.8	19.2	19.7	21.4	20.2	18.9	19.2	18.7
9/22	9	20.2	19.7	19.8	19.6	19.6	20.5	19.7	18.5	18.8	17.7
9/23	10	19.7	19.5	19.8	19.3	19.2	20.5	19.4	18.8	18.6	17.5
9/25	11	20.3	20.4	20.4	20.2	19.8	21.1	20.2	19.5	19.5	18.2
9/26	12	20.1	19.7	20.4	19.5	19.9	21.1	19.3	19.1	19.2	18.2
9/28	14	20.5	18.3	21.1	20.3	19.9	20.3	19.9	19.6	19.7	18.6
9/29	15	20.4	17.3	21.3	21.9	19.6	21.4	19.7	19.7	19.9	17.8
9/30	16	20.2		22.5	21.8	20	21.3	20	19.6	19.7	17.7
10/2	18	21.4					21.9	20	19.3	20.5	17.9
10/3	19	21.9					22	20.2	19.6	21.9	18.6
10/4	20	22					22.5	20.5	19.9	22.1	18.9
10/5	21						22.8	20.5	20.4		19.2
10/6	22							20.7			
10/7	23							20.5			
10/8	24							20.9			
10/9	25							21.2			

Group 2	mIL-21	10/3	10/3	10/2	10/3	9/30	10/6	9/30	10/7	9/29	10/6
date	day	6530	6525	6532	6533	6534	6535	6528	6537	6538	6539
9/12	-2	19.0	19.3	19.3	20.8	18.2	20.2	19.9	19.3	21.4	19.7
9/19	6	19.3	20	20.8	20.1	19.2	20.7	20.2	19.3	20.5	20.3
9/21	8	19.2	19.5	20.6	20.7	18.9	21.1	20.1	18.9	20.8	19.9
9/22	9	19.1	19.3	20.3	20.4	18.8	20.9	19.6	19.1	20.7	21.1
9/23	10	18.9	19.3	20.5	20.1	18.7	20.9	19.7	18.9	21.0	19.9
9/25	11	19.9	19.9	20.5	20.3	19.4	21.1	19.8	19.3	21.0	20.6
9/26	12	19.7	19.7	20.1	20.6	19.7	21.0	20.2	19.4	20.9	20.2
9/28	14	19.7	20.0	18.6	20.7	19.2	20.9	20.9	19.2	20.4	20.9
9/29	15	19.9	20.2	18.6	20.7	19.4	21.0	21.5	19.0	20.0	20.6
9/30	16	19.8	20.6	18.0	21.3	19.0	21.4	21.7	19.1		20.2
10/2	18	20.7	20.9	18.6	21.7		21.3		18.9		20.3
10/3	19	21.6	21.5		21.8		22.3		19.1		20.7
10/4	20						22.2		19.4		20.7
10/5	21						22.5		19.6		21.2
10/6	22						23.4		20.1		21.5
10/7	23								20.8		

Group 3	mPD-1 mAb	9/30	10/2	9/30	10/3	10/2	10/7	10/4	10/5	10/4	10/2
date	day	6540	6541	6542	6543	6527	6545	6546	6511	6512	6549
9/12	-2	21.7	19.8	19.2	20.5	18.5	17.8	20.5	19.4	19.5	22.1
9/19	6	22.4	20.2	19.2	20.6	18.8	18.1	20.6	19.8	19.2	22.9
9/21	8	22.7	20.2	19.4	19.5	18.9	18.5	21.0	19.6	19.2	22.9
9/22	9	22.7	20.2	19.2	18.0	18.5	18.8	20.7	19.4	19.1	22.7
9/23	10	25.7	19.9	18.9	18.4	18.4	18.6	20.5	19.6	18.8	22.4
9/25	11	24.8	20.5	19.3	18.9	19.2	18.6	21.2	19.7	19.2	23.0
9/26	12	24.2	20.7	19.5	18.9	18.8	19.2	21.0	19.9	19.2	22.6
9/28	14	24.4	20.7	19.9	19.2	19.1	19.2	21.4	19.9	20.0	21.0
9/29	15	25.3	20.8	19.7	19.4	19.3	19.3	21.4	19.8	20.0	23.7
9/30	16	26.5	21.0	20.3	19.3	19.2	18.9	21.8	20.0	19.9	23.9
10/2	18		21.3		19.4	20.2	18.8	20.3	19.8	19.3	23.3
10/3	19				20.5		19.5	22.0	20.6	20.5	
10/4	20						19.7	21.7	22.0	19.9	
10/5	21						20.2		22.8		
10/6	22						20.4				
10/7	23						20.2				

Group 4	mIL-21 + mPD-1 mAb	9/29	10/6	10/11	10/7	10/4	10/11	10/9	9/30	10/4	10/10
date	day	6504	6501	6562	6563	6500	6565	6497	6567	6499	6569
9/12	-2	18.3	19.8	21.6	20.4	19.9	19.7	18.6	17.8	20.2	17.9
9/19	6	18.7	20.4	22.0	21.4	20.3	20.2	19.0	18.5	20.6	18.2
9/21	8	18.8	20.4	22.1	20.1	19.9	20.0	19.4	18.8	20.6	18.4
9/22	9	19.0	20.0	21.7	21.1	20.4	19.4	19.3	18.2	20.3	18.6
9/23	10	19.2	19.8	21.8	21.1	20.3	19.6	19.4	18.6	20.4	18.7
9/25	11	19.2	20.1	22.4	21.1	20.3	20.0	19.1	18.8	20.4	18.4
9/26	12	19.5	19.9	22.6	22.0	20.6	20.3	19.4	18.8	21.0	18.7
9/28	14	18.3	20.0	22.1	21.4	20.7	20.4	19.5	19.0	21.3	19.1
9/29	15	16.5	20.2	22.4	21.4	20.7	20.2	18.9	18.9	21.1	18.8
9/30	16		20.1	22.6	21.7	20.7	20.3	no weight	16.9	20.5	18.7
10/2	18		19.9	22.0	21.3	20.8	19.8	19.2		21.1	17.9
10/3	19		21.2	22.8	22.2	22.0	20.9	19.5		22.2	18.8
10/4	20		21.1	22.9	22.8	22.3	21.2	19.5		22.6	18.7
10/5	21		22.8	23.1	22.7		21.4	19.9			19.2
10/6	22		22.2	22.5	22.2		20.7	21.0			19.0
10/7	23			22.1	23.3		21.0	20.8			19.4
10/8	24			22.1			21.2	21.2			19.4
10/9	25			22.9			21.8	21.5			19.6
10/10	26			23.2			22.5				20.2
10/11	27			23.3			22.3				

CLAIMS

1. A method for treating cancer in a subject, comprising (a) administering an IL-21 polypeptide to the subject and (b) administering an anti-PD-1 antibody or antigen-binding portion thereof to the subject.

2. The method of claim 1, wherein the IL-21 polypeptide and the anti-PD-1 antibody or antigen-binding portion thereof are administered sequentially.

3. The method of claim 1, wherein the IL-21 polypeptide and the anti-PD-1 antibody or antigen-binding portion thereof are administered concurrently.

4. The method of claim 2 or 3, wherein the IL-21 polypeptide is administered before the anti-PD-1 antibody or antigen-binding portion thereof.

5. The method of claim 2 or 3, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered before the IL-21 polypeptide.

6. The method of claim 2, wherein the IL-21 polypeptide and the anti-PD-1 antibody or antigen-binding portion thereof are admixed as a single composition and administered concurrently.

7. The method of claim 2 or 3, comprising administering to a subject (a) a composition comprising an IL-21 polypeptide and a pharmaceutically acceptable carrier and (b) a composition comprising an anti-PD-1 antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier.

8. The method of claim 1, wherein the cancer is selected from the group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer, a virally-associated cancer and lung cancer.

9. The method of any one of claims 1-8, wherein the subject is a human.

10. The method of any one of claims 1-8, wherein the anti-PD-1 antibody or antigen-binding portion thereof is a human antibody.

11. The method of claim 10, wherein the anti-PD-1 human antibody or antigen-binding portion thereof is a monoclonal antibody.

12. The method of any of claims 1-8, wherein the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain variable region comprising amino acids having the sequence set forth in SEQ ID NO: 7 and a light chain variable region comprising amino acids having the sequence set forth in SEQ ID NO: 8.

13. The method of any of claims 1-8, wherein the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 9; a heavy chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 10; a heavy chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 11; a light chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 12; a light chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO: 13; and a light chain variable region CDR3 comprising amino acids having the sequence set forth in SEQ ID NO: 14.

14. The method of any of claims 1-8, wherein the anti-PD-1 antibody is 5C4.

15. The method of any one of claims 1-8, wherein the IL-21 polypeptide has 95% sequence identity with the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

16. The method of any one of claims 1-8, wherein the IL-21 polypeptide comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

17. The method of any one of claims 1-8, wherein the IL-21 polypeptide comprises the amino acid sequence of SEQ ID NO: 5.

18. A method for treating cancer in a subject, comprising (a) administering an Il-21 polypeptide comprising the amino acid sequence of SEQ ID NO: 5 and (b) administering 5C4 to the subject.

19. The method of claim 18, wherein the dose of 5C4 is 3 mg/kg.

20. The method of claim 18, wherein the dose of 5C4 is 1 mg/kg.

21. The method of claim 19 wherein the 5C4 is administered every other week.

22. The method of claim 21, wherein the dose of the IL-21 polypeptide is selected from the group consisting of 10, 30, 50, 75 and 100 μg/kg.

23. The method of claim 22, wherein the IL-21 polypeptide is administered weekly during weeks 1-4 of a 6-week cycle.

24. The method of claim 22, wherein the IL-21 polypeptide is administered 3 times per week during weeks 1 and 3 of a 6-week cycle.

25. The method of claim 23 or 24, wherein the administration of the IL-21 polypeptide and the 5C4 is for up to 2 years.

FIG. 1

Mean and Median Antitumor Activity of mIL-21 and mPD-1 mAb Alone or in Combination (MC38 Study 1408-226)

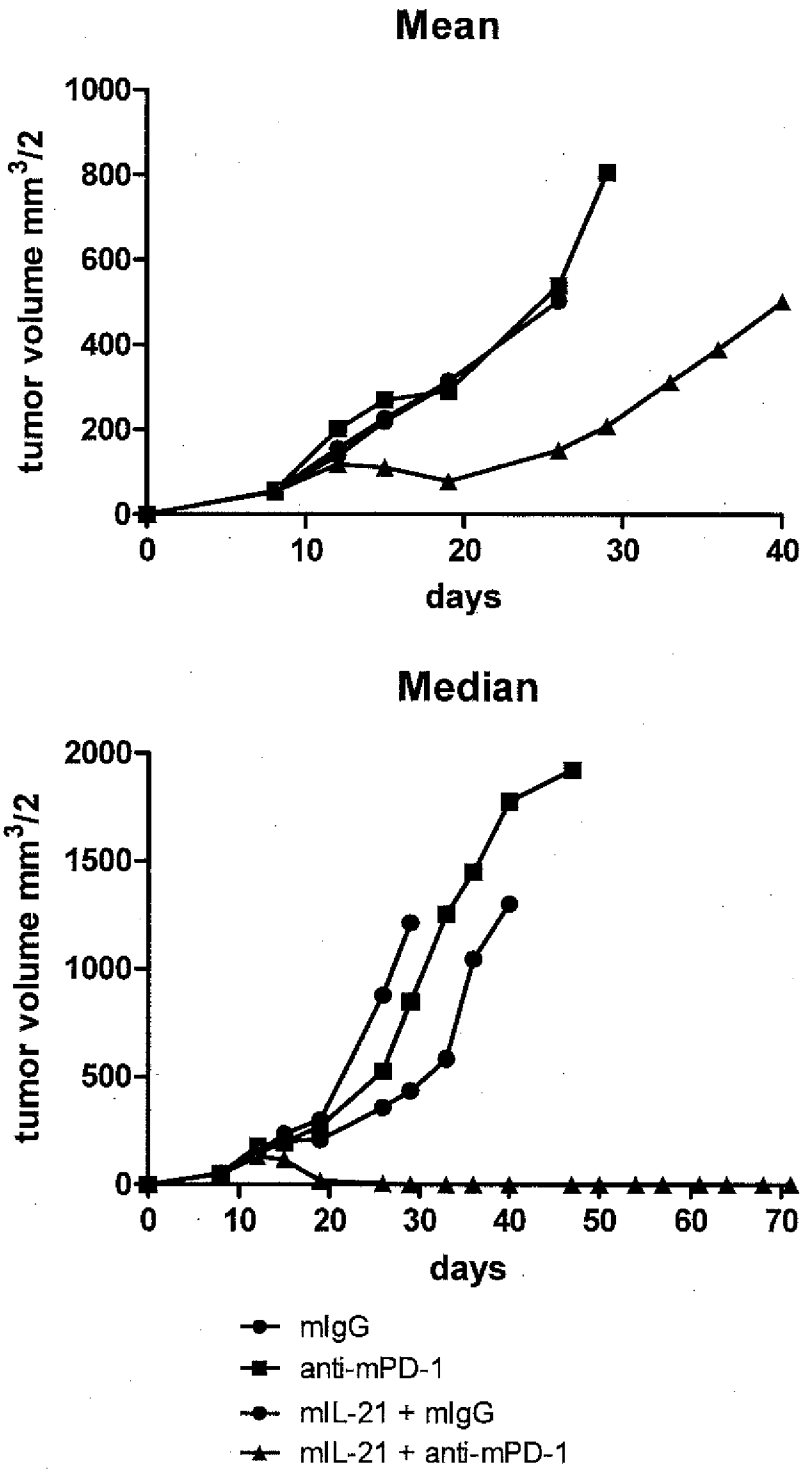
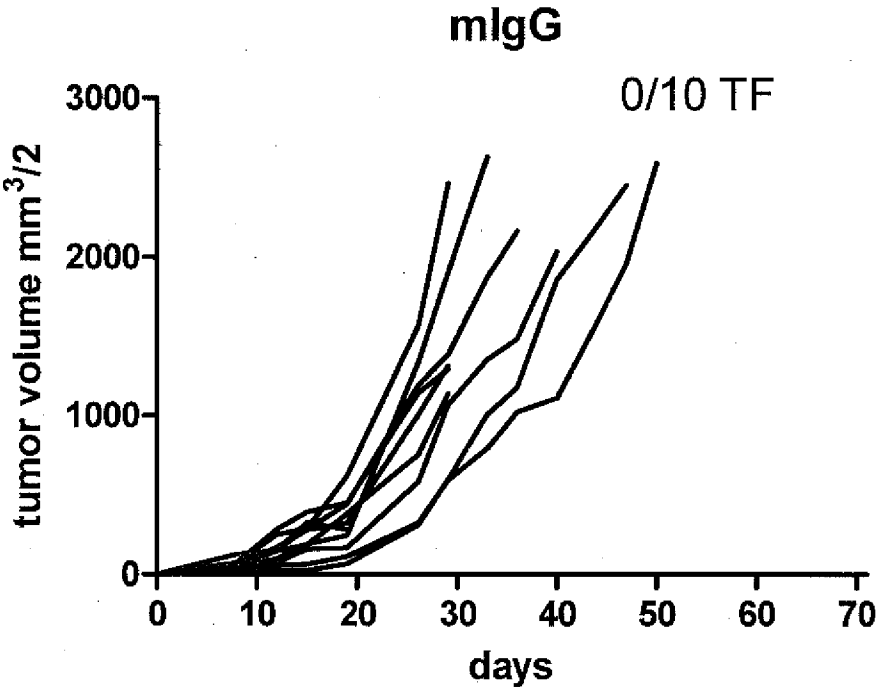


FIG. 2

Individual Mouse Tumor Volume Data (MC38 Study 1408-226)



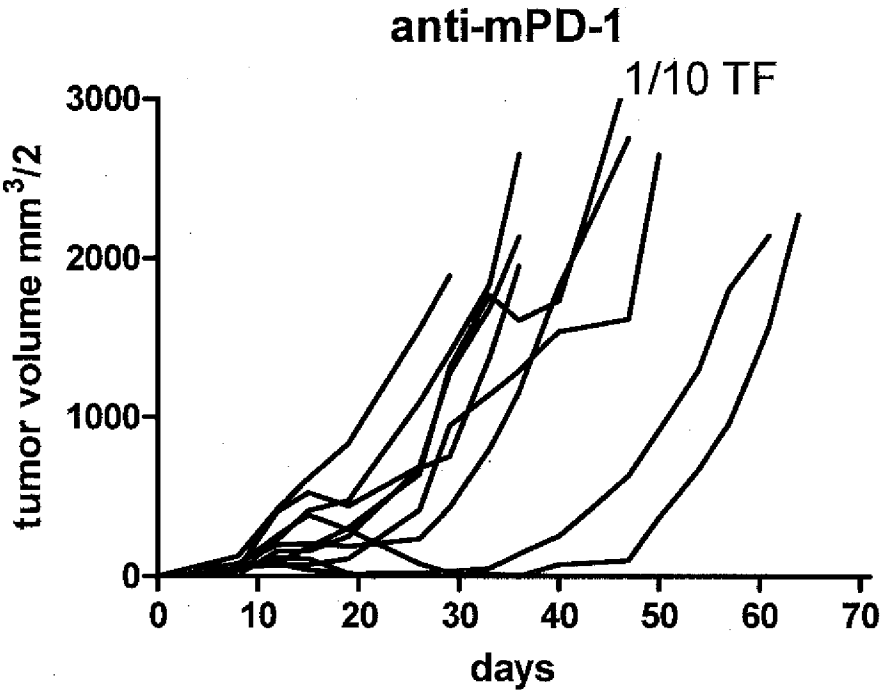


FIG. 2 (continued)

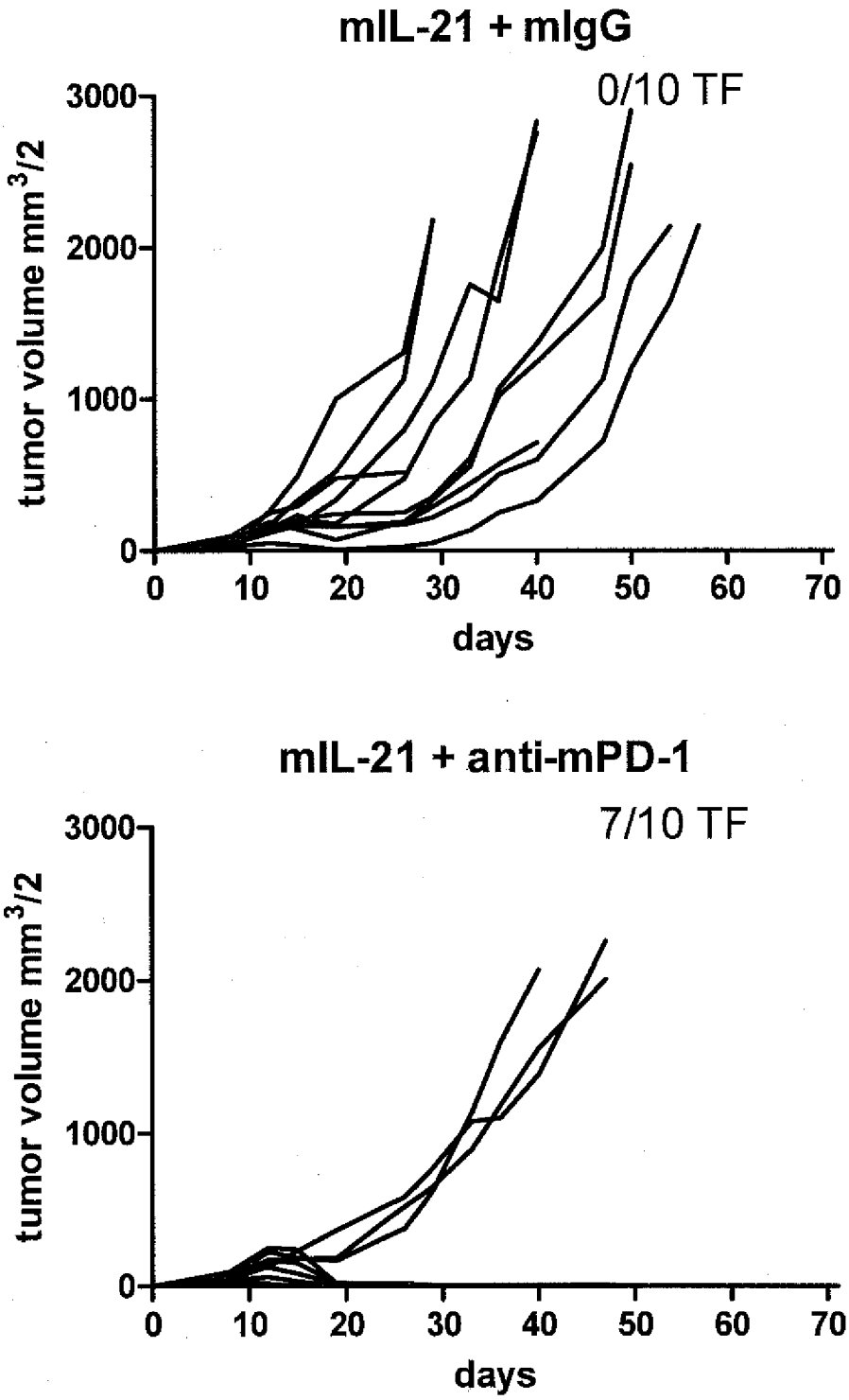


FIG. 3

Survival of Mice Treated with mIL-21 and mPD-1 mAb Alone or in Combination
(MC38 Study 1408-226)

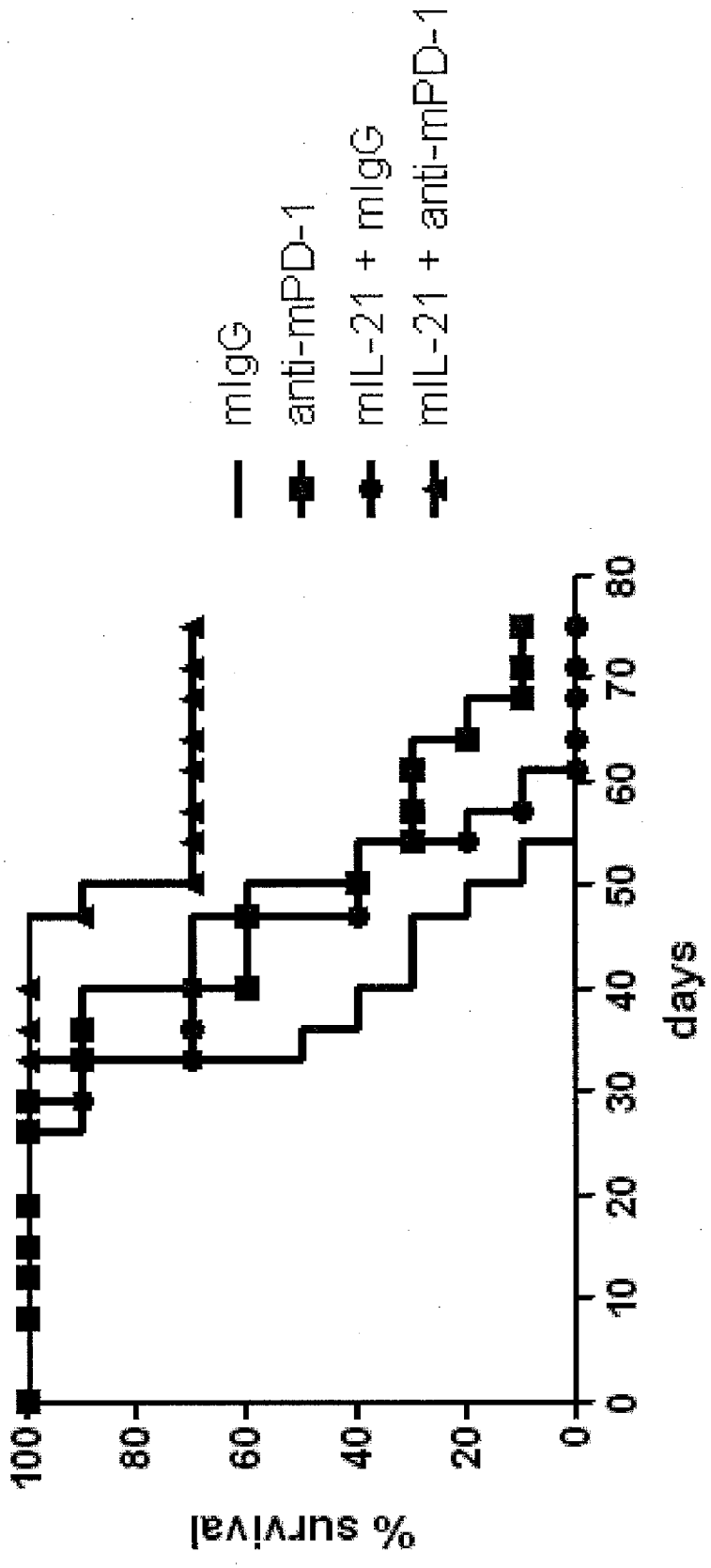


FIG. 4

Mean and median tumor activity of mIL-21 and mPD-1 mAb Alone or in Combination (MC38 Study 1106-248)

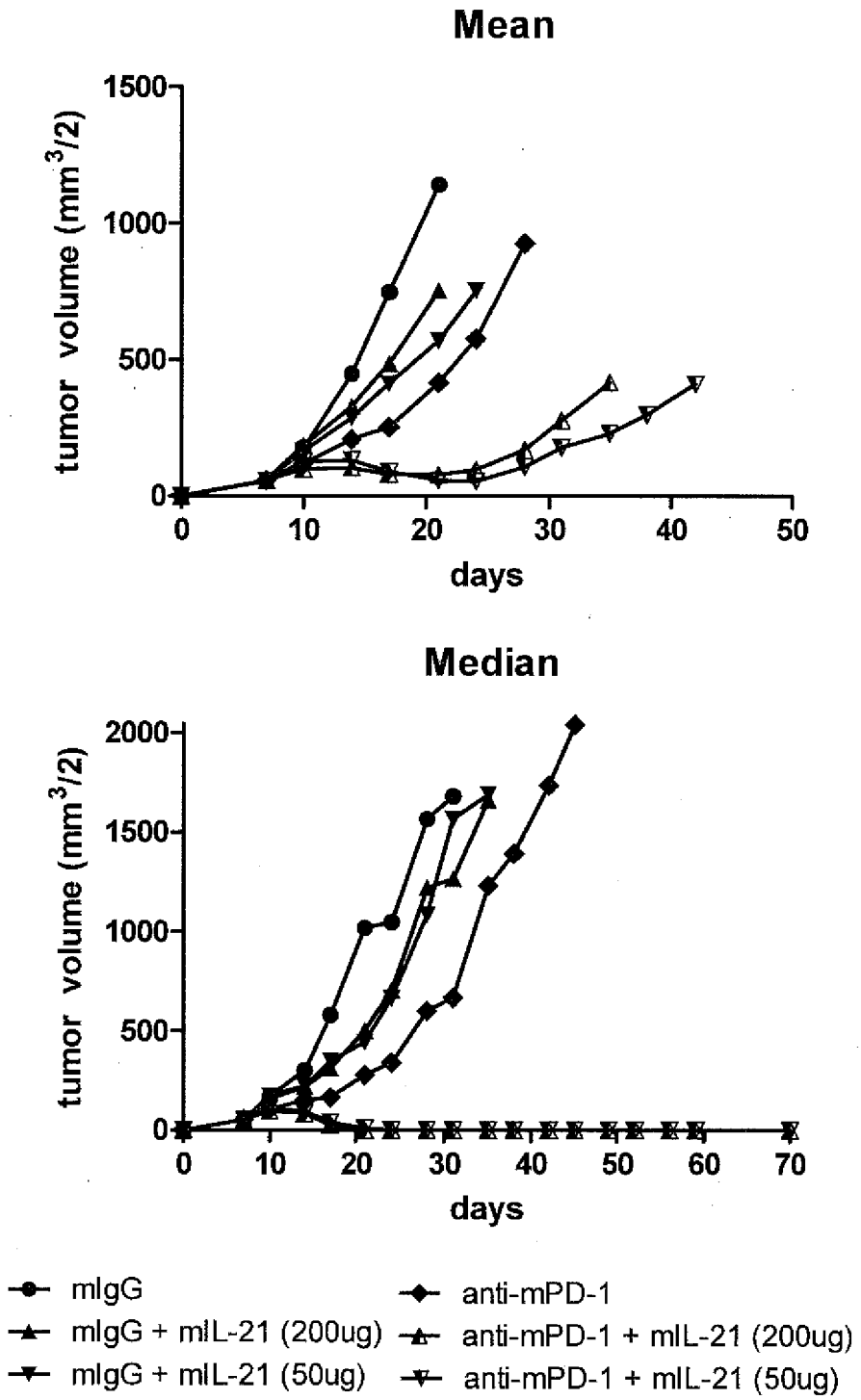


FIG. 5

Individual Mouse Tumor Volume Data (MC38 Study 1106-248)

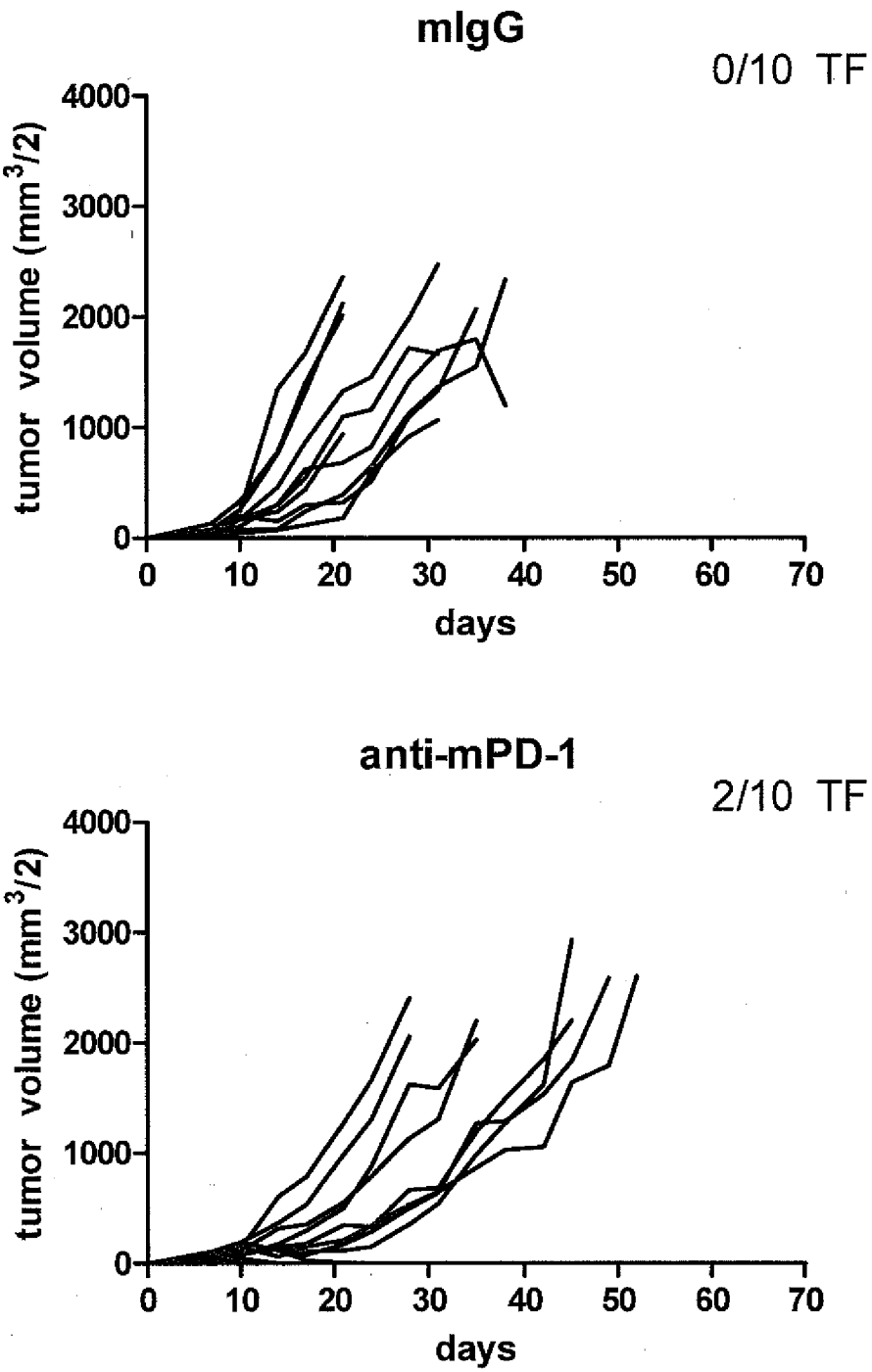


FIG. 5 (continued)

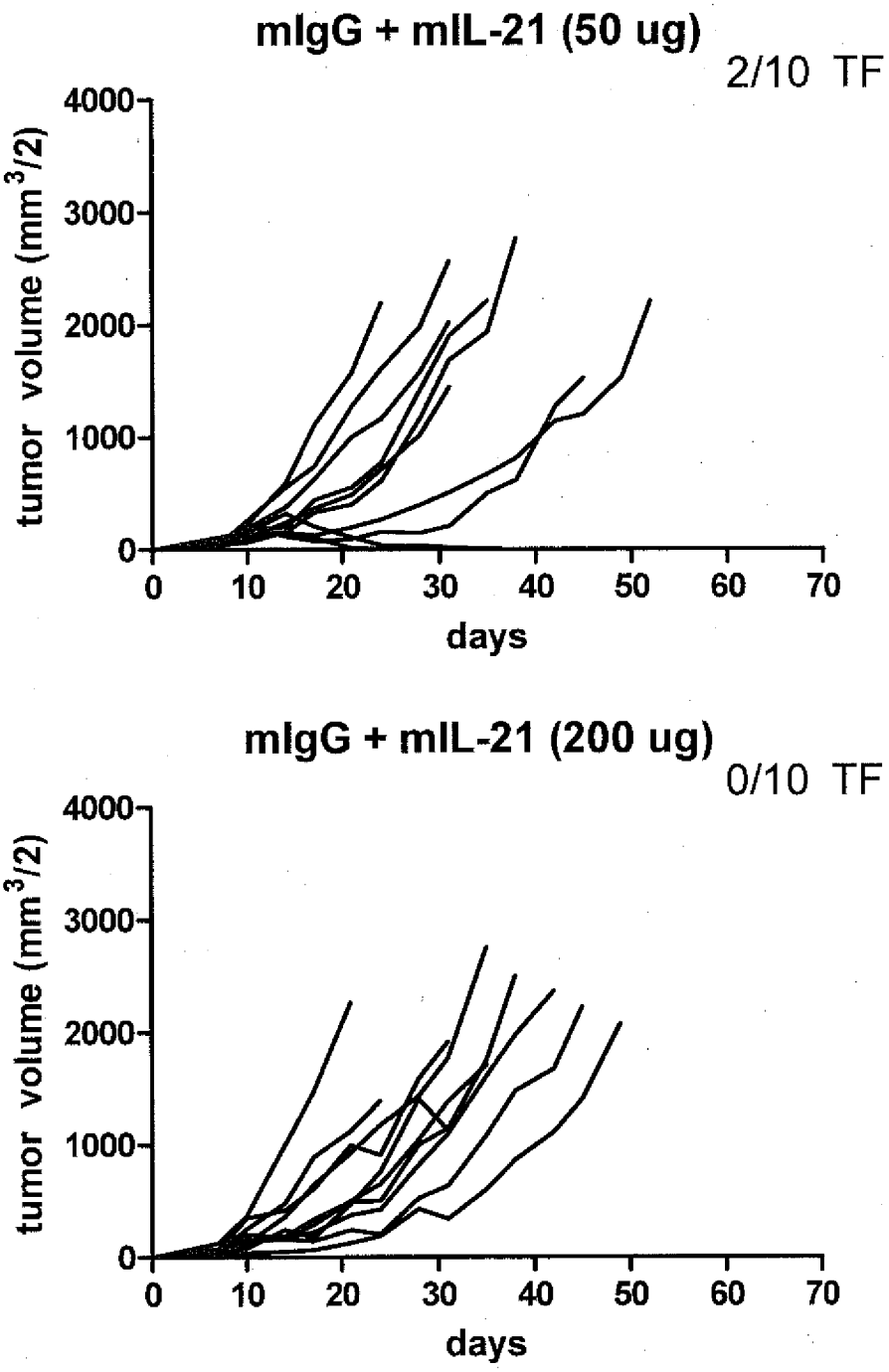


FIG. 5 (continued)

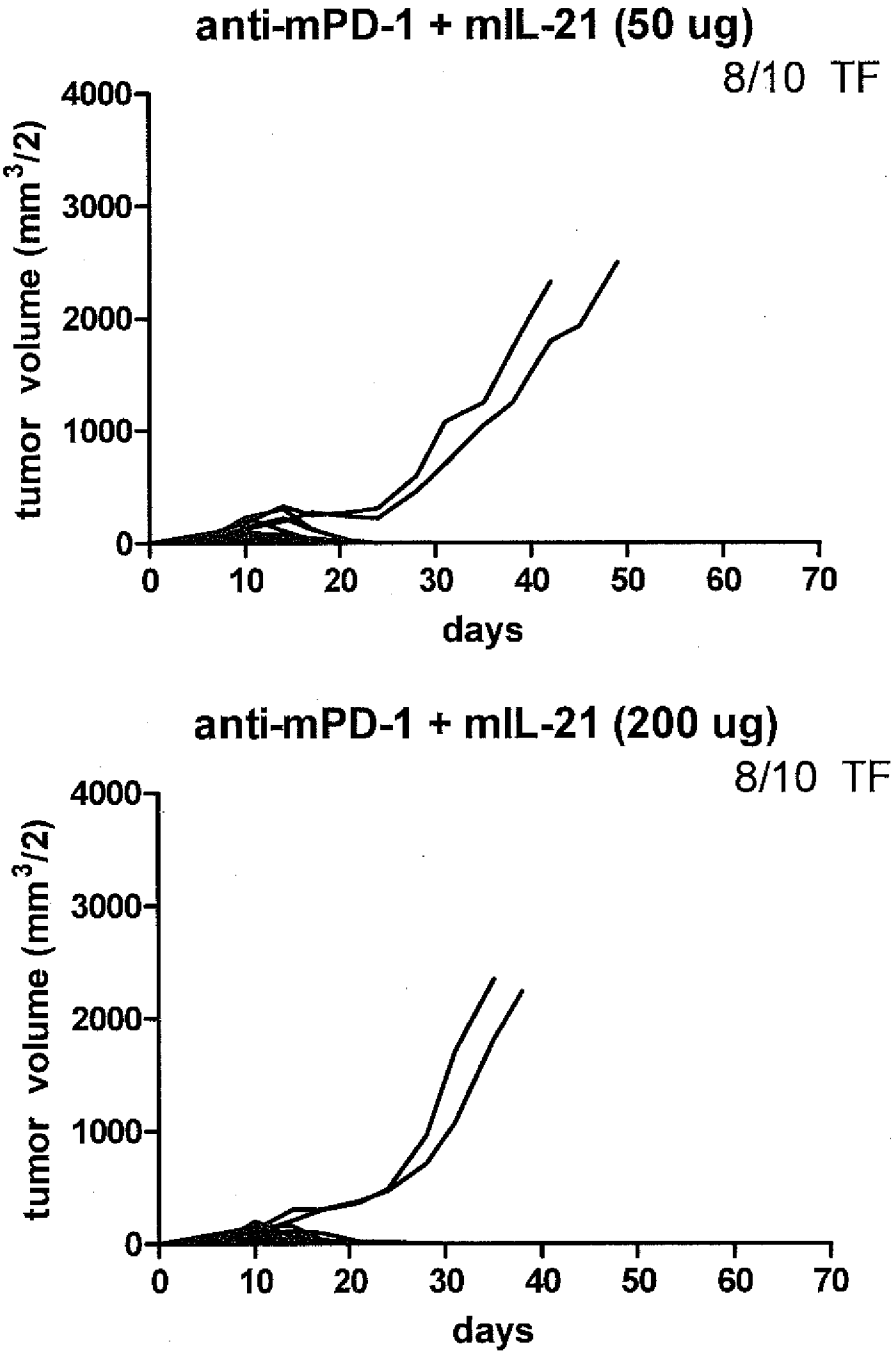


FIG. 6

Survival of Mice Treated with mIL-21 and mPD-1 mAb Alone or in Combination in the MC38 Tumor Model (MC38 Study 1106-248)

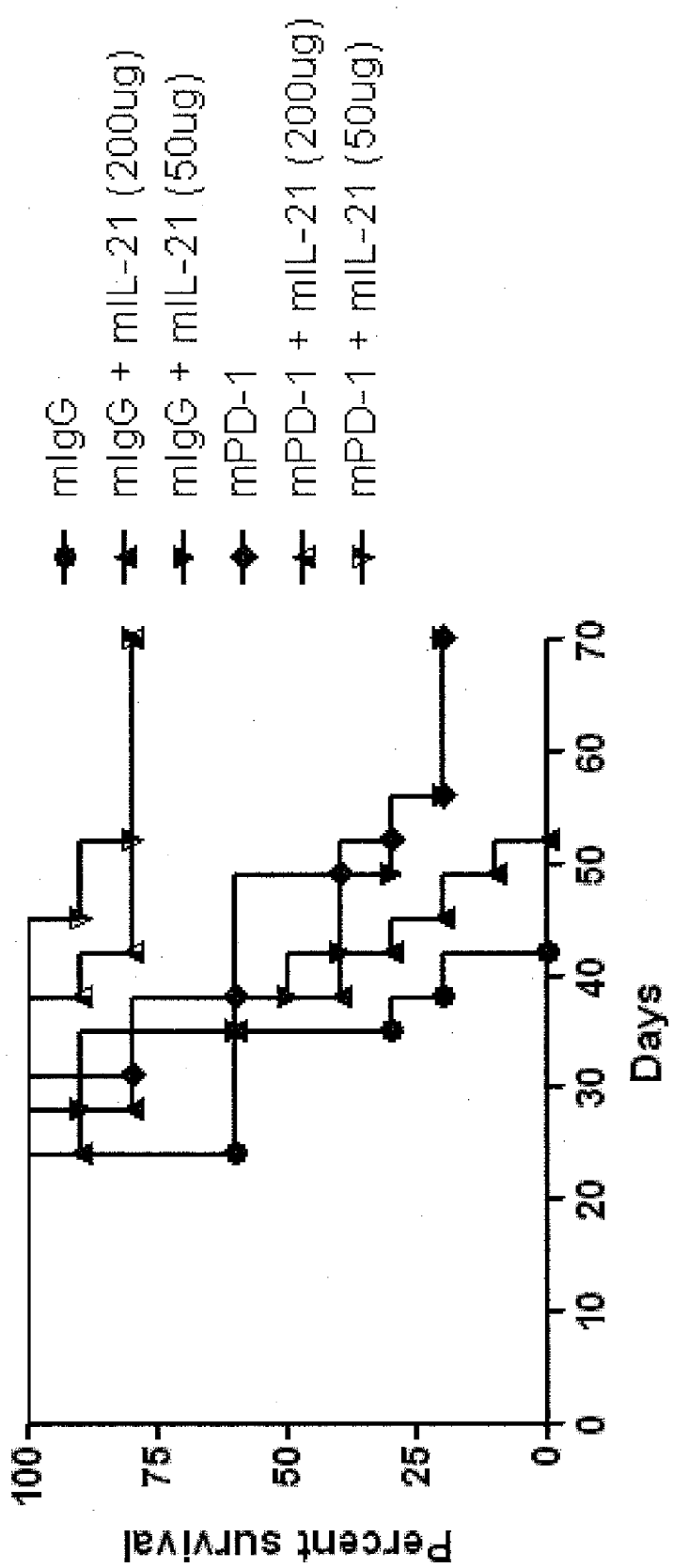


FIG. 7

Median Tumor Volume in Mice Treated with mIL-21 and mPD-1 mAb, Alone or in Combination, in the EMT-6 Established Tumor Model (EMT-6 Study #39)

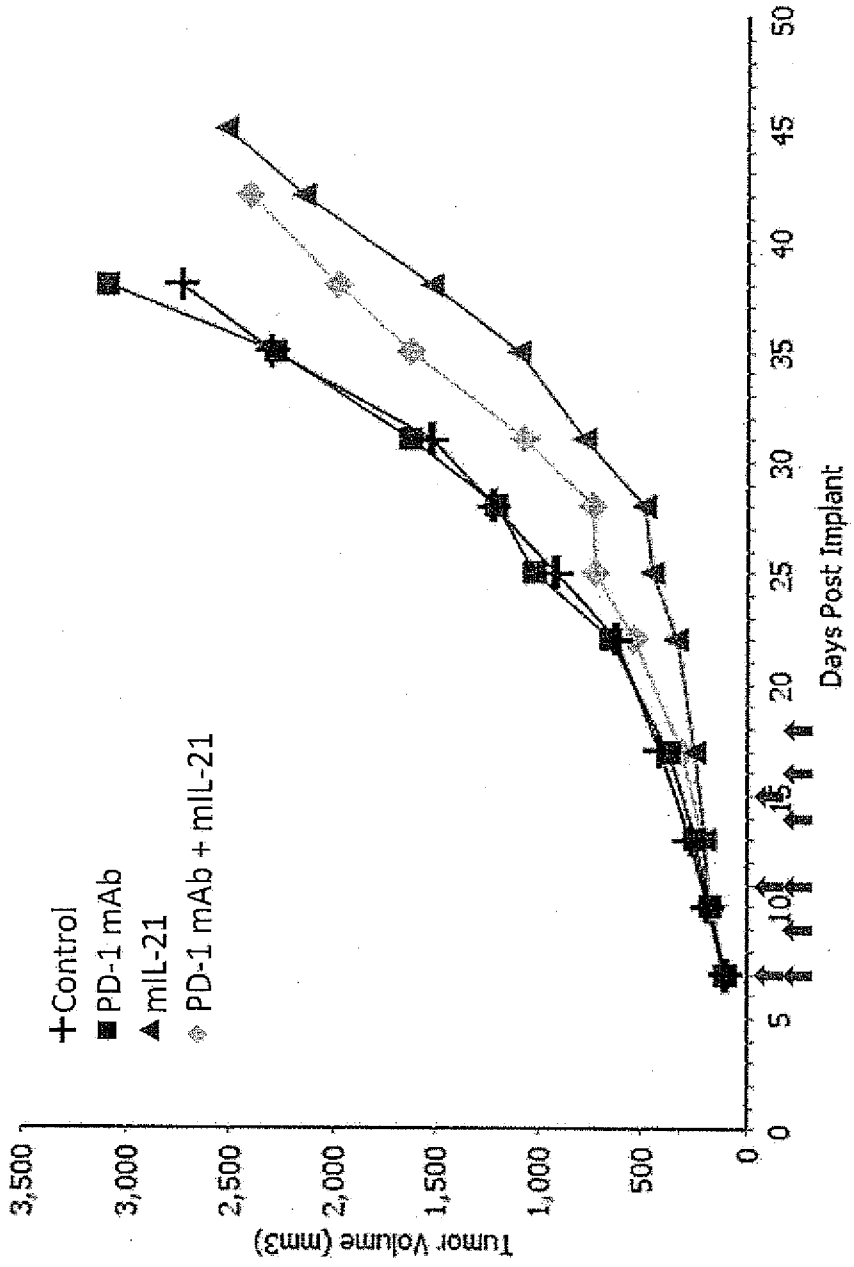


FIG. 8

Tumor Volumes Measured in Individual Mice (EMT-6 Study #39)

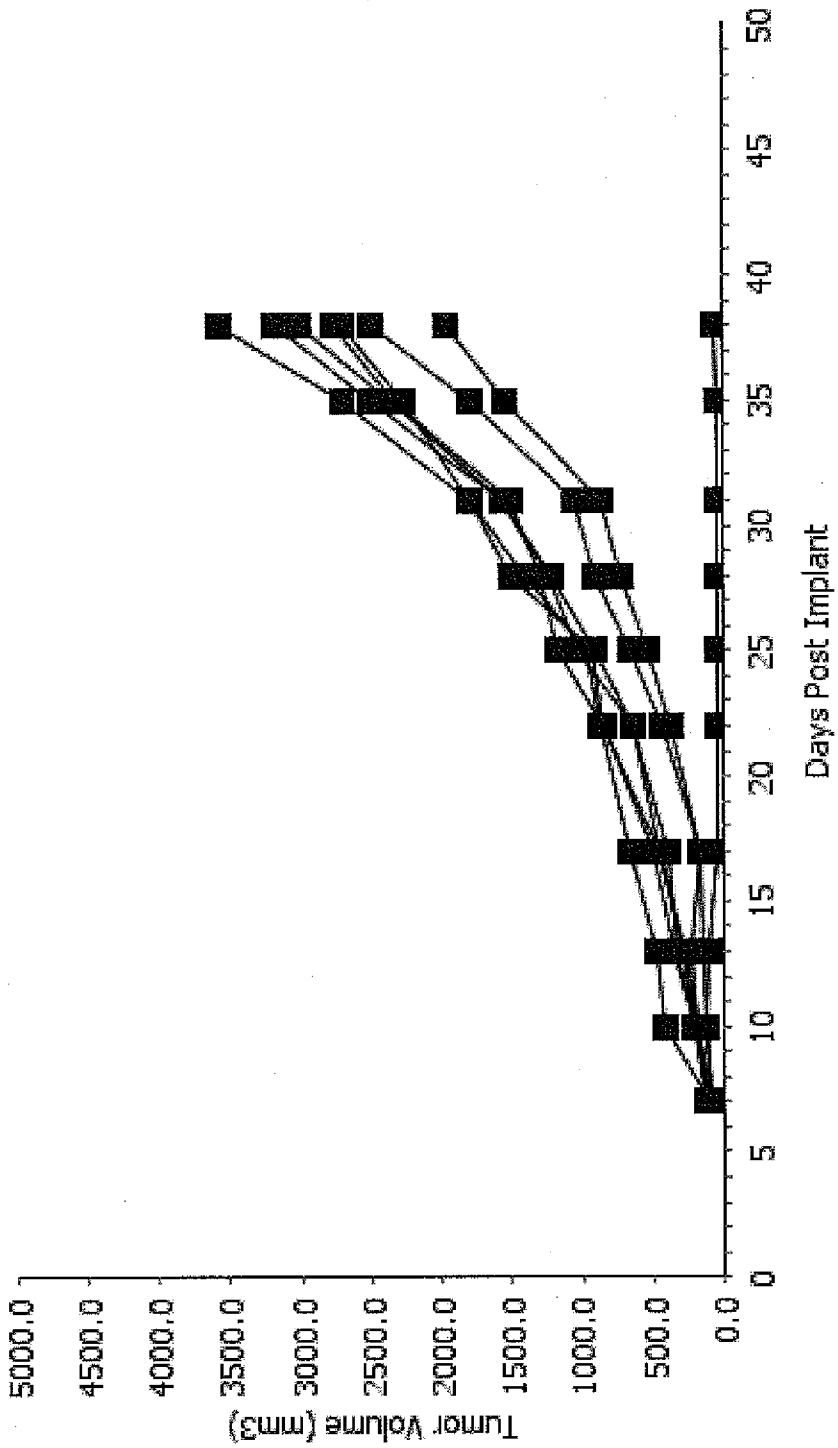


FIG. 8 (continued)

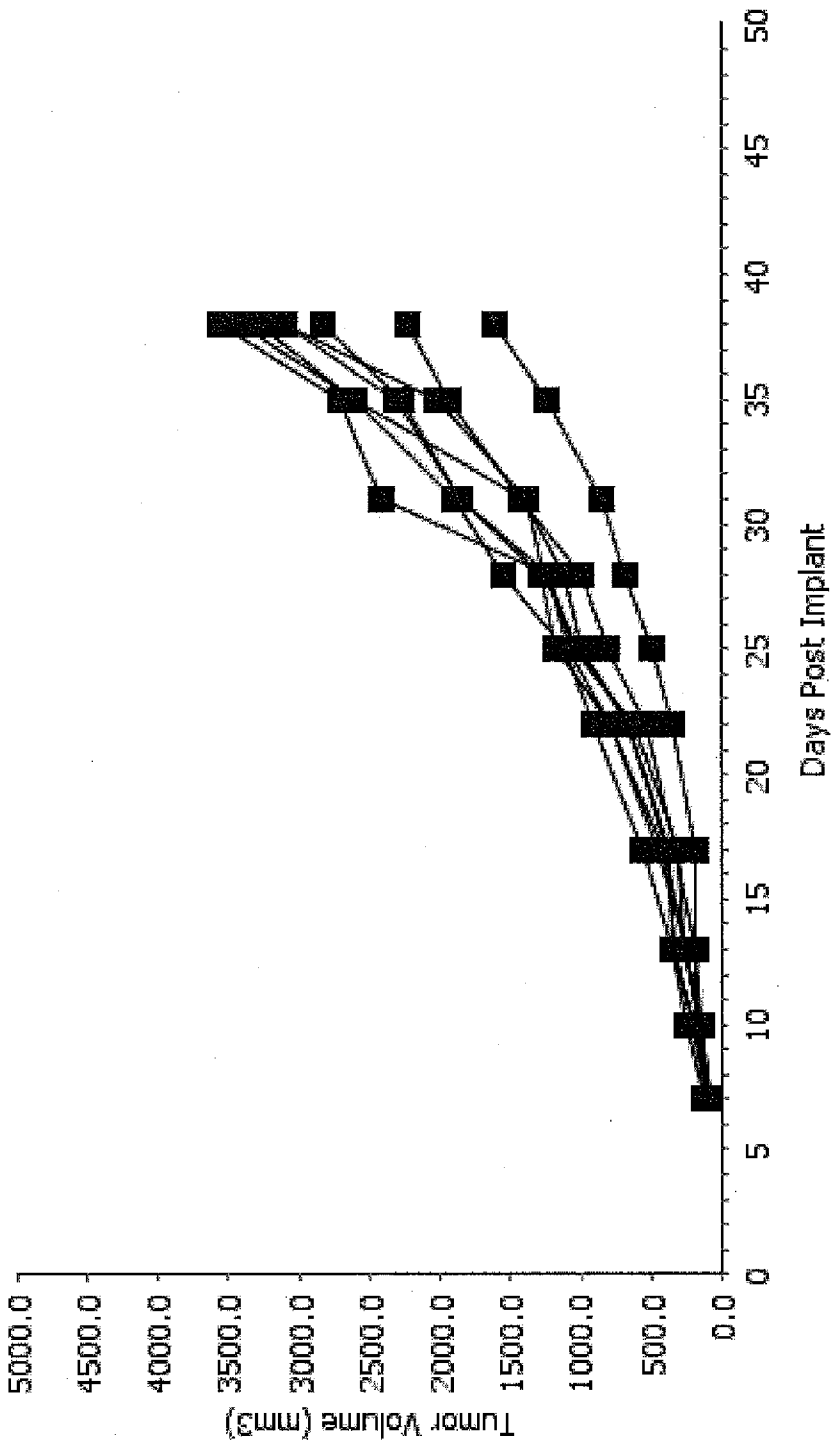


FIG. 8 (continued)

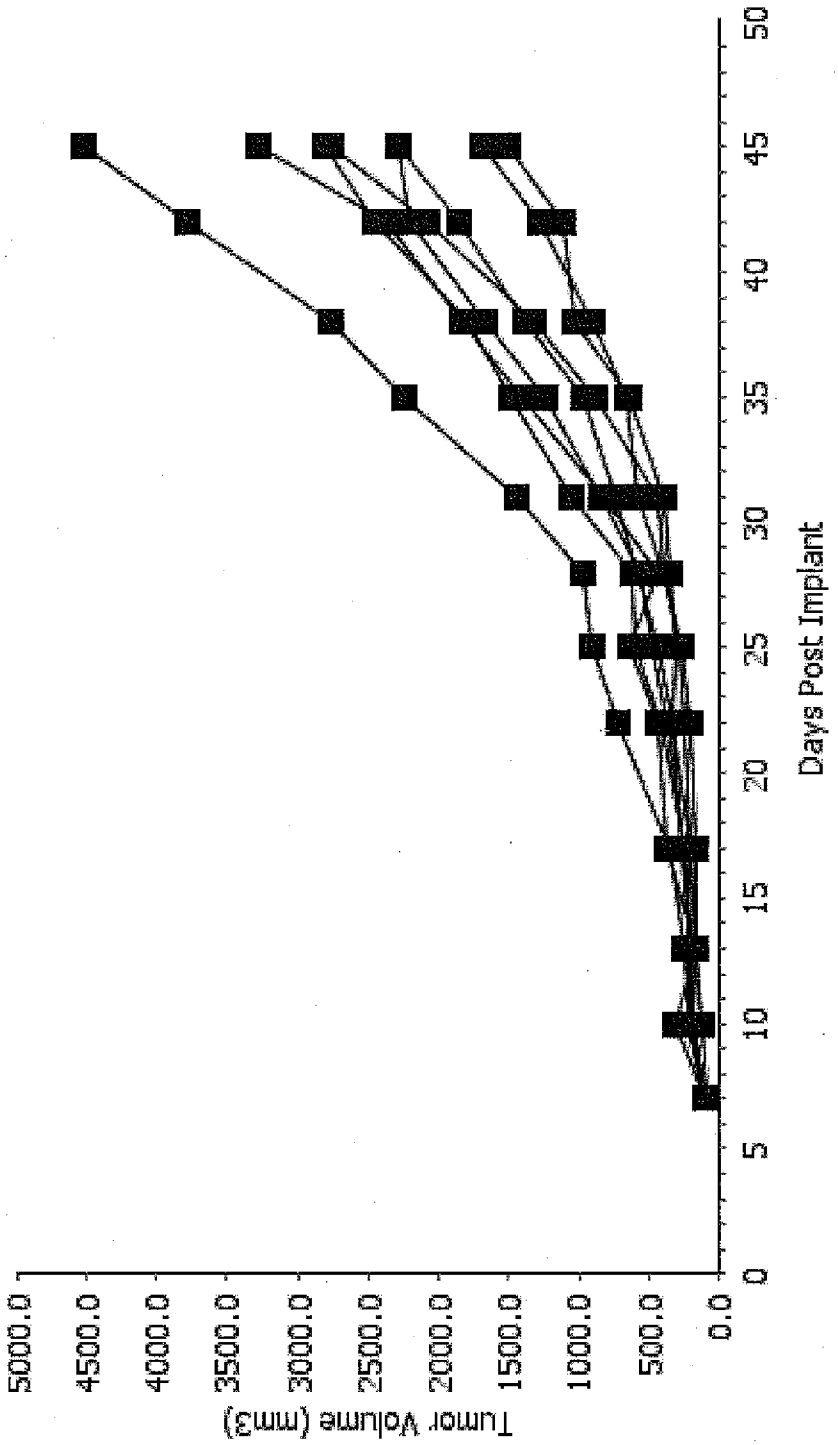


FIG. 8 (continued)

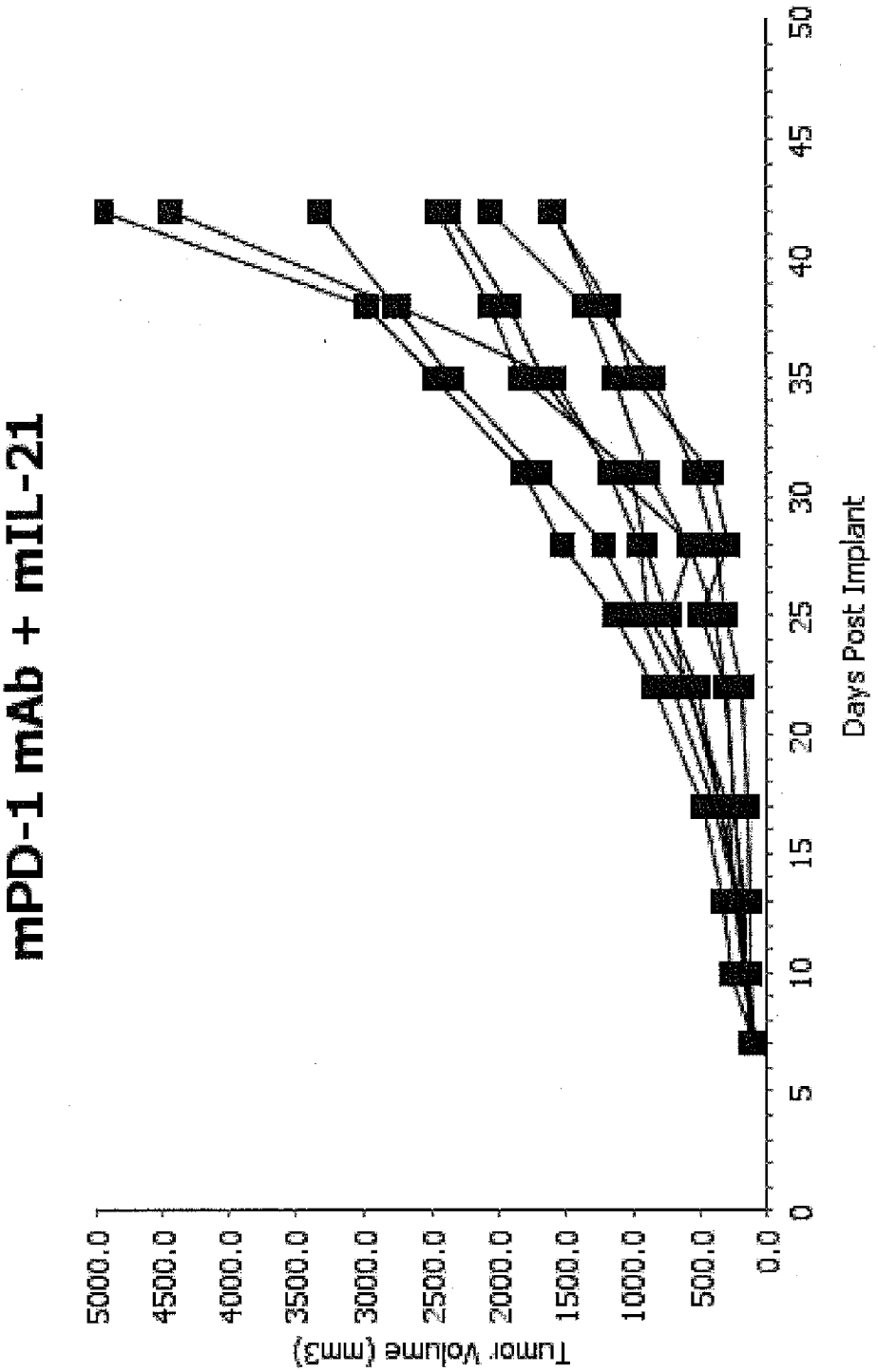


FIG. 9

Median Tumor Volume in Mice Treated with PBS, mIL-21, or mPD-1 mAb, Alone or in Combination, in the SC B16-F10 Mouse Model (TGM Study 1104)

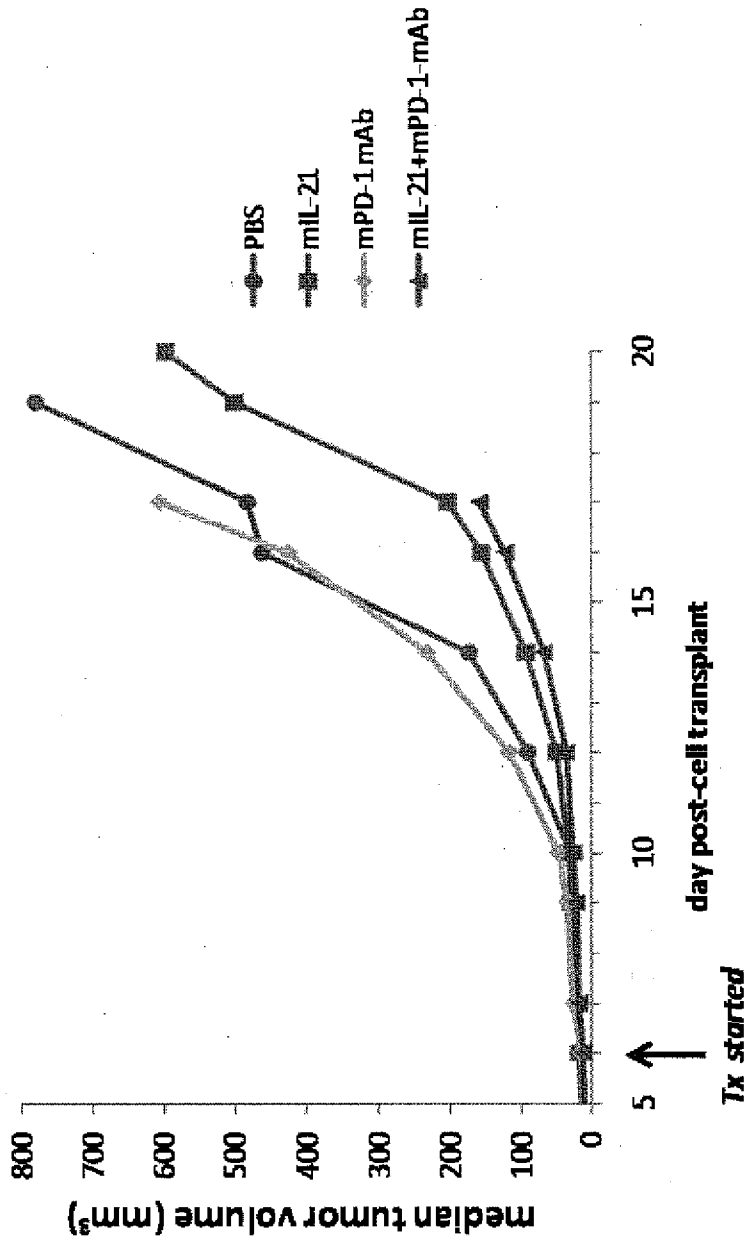


FIG. 10

Tumor Volume in Individual Mice Treated with PBS, mIL-21 or mPD-1 mAb, Alone or in Combination, in the SC B16-F10 Mouse Model

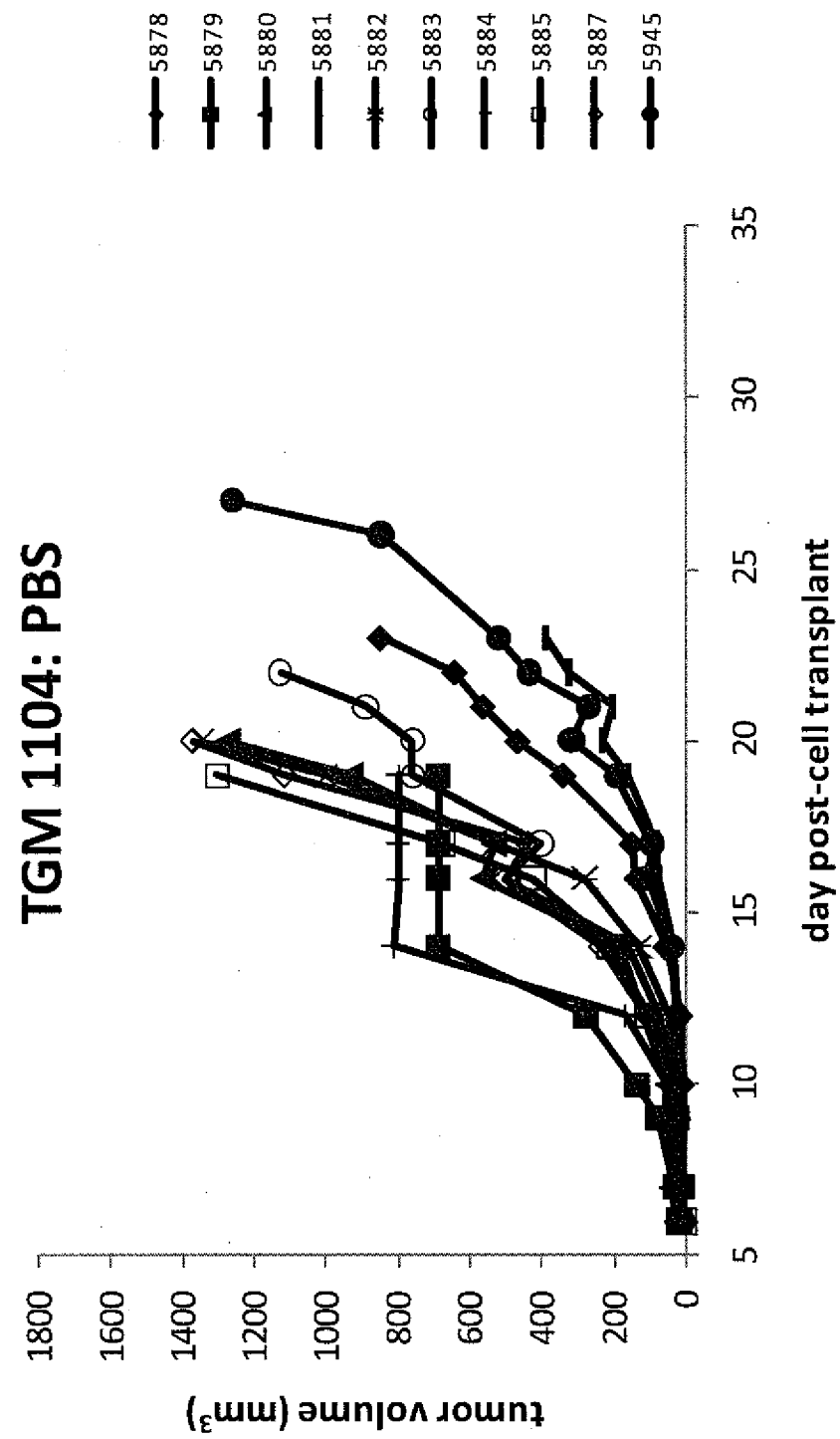


FIG. 10 (continued)

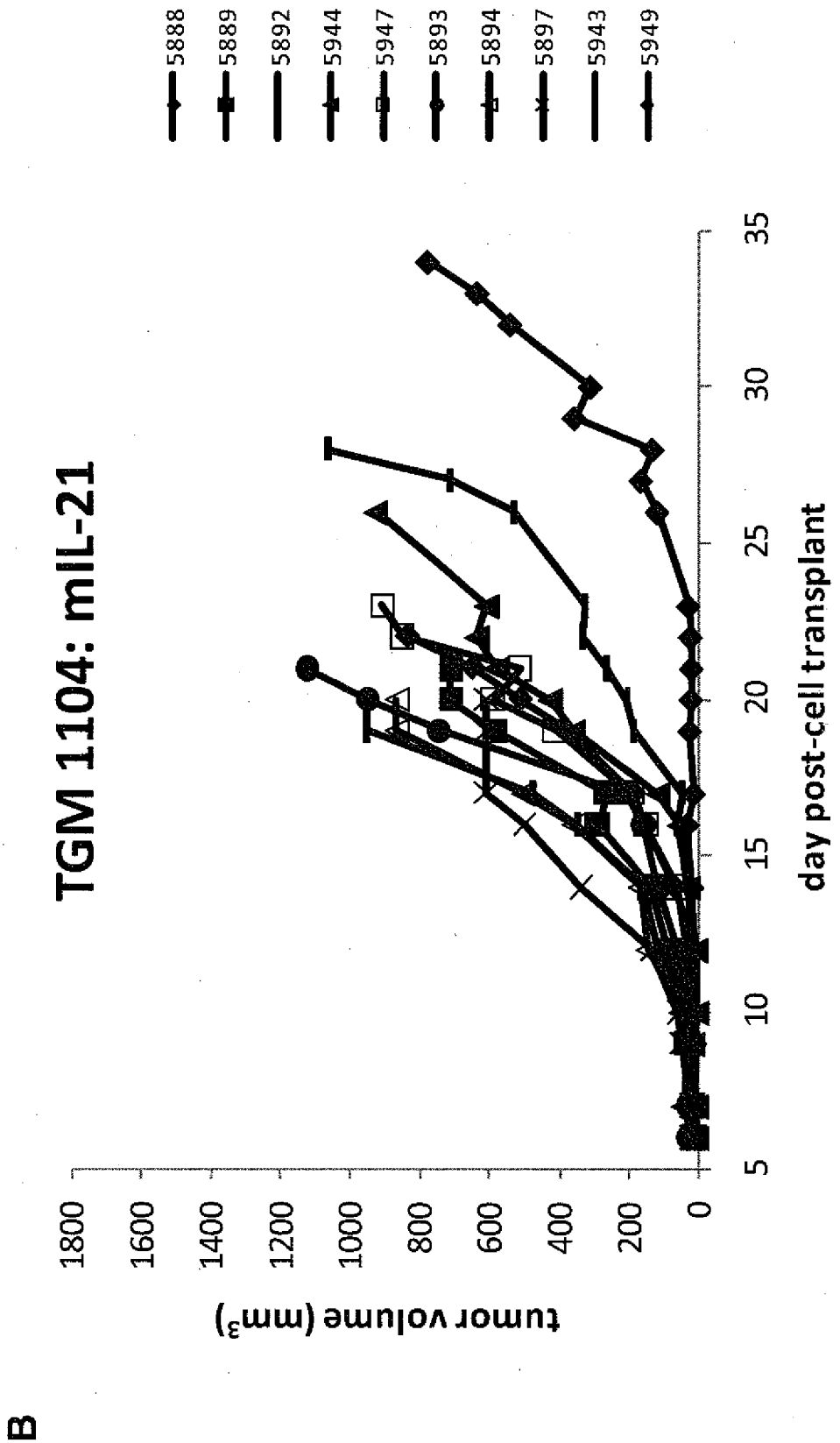


FIG. 10 (continued)

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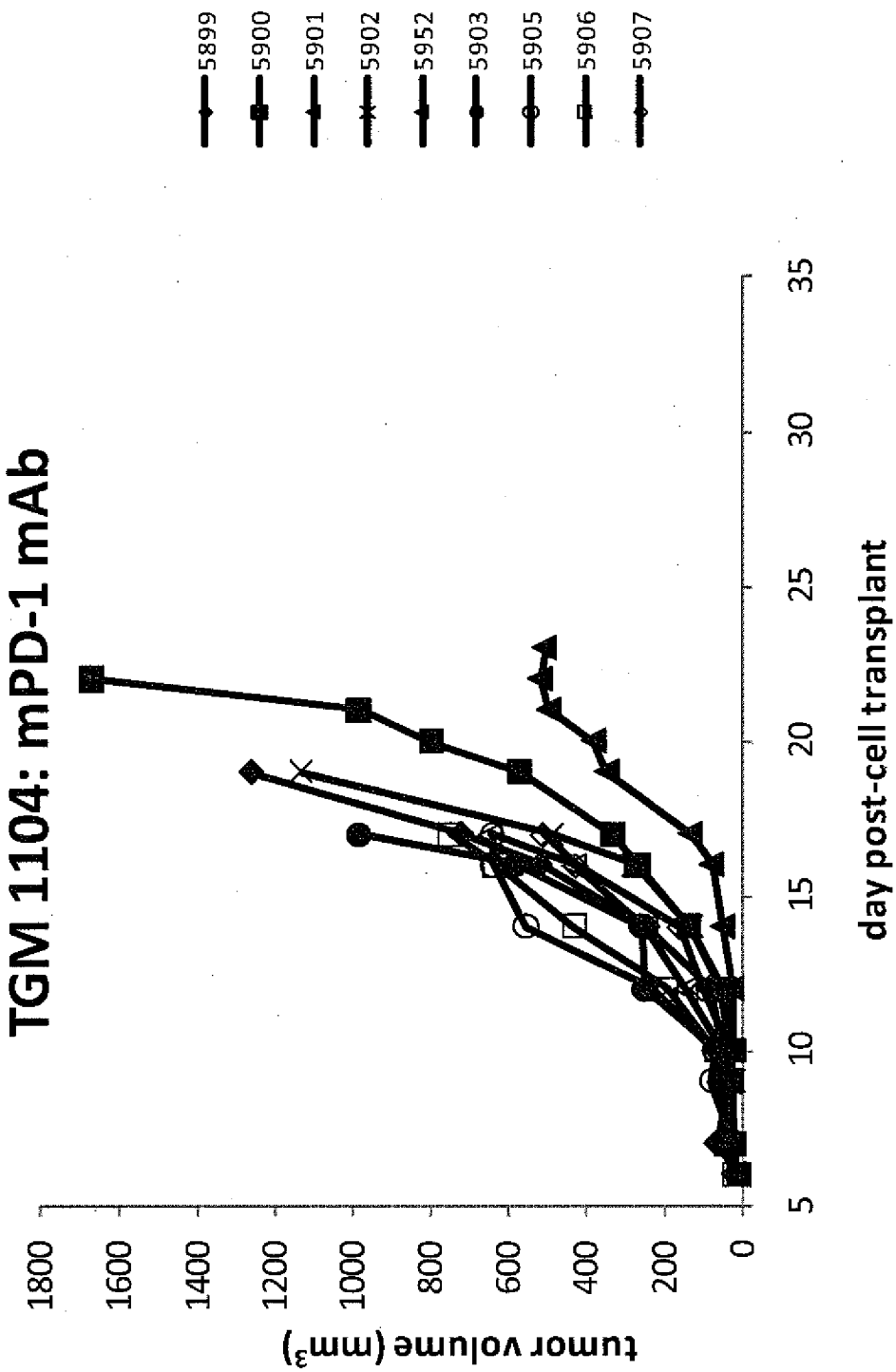

FIG. 10 (continued)

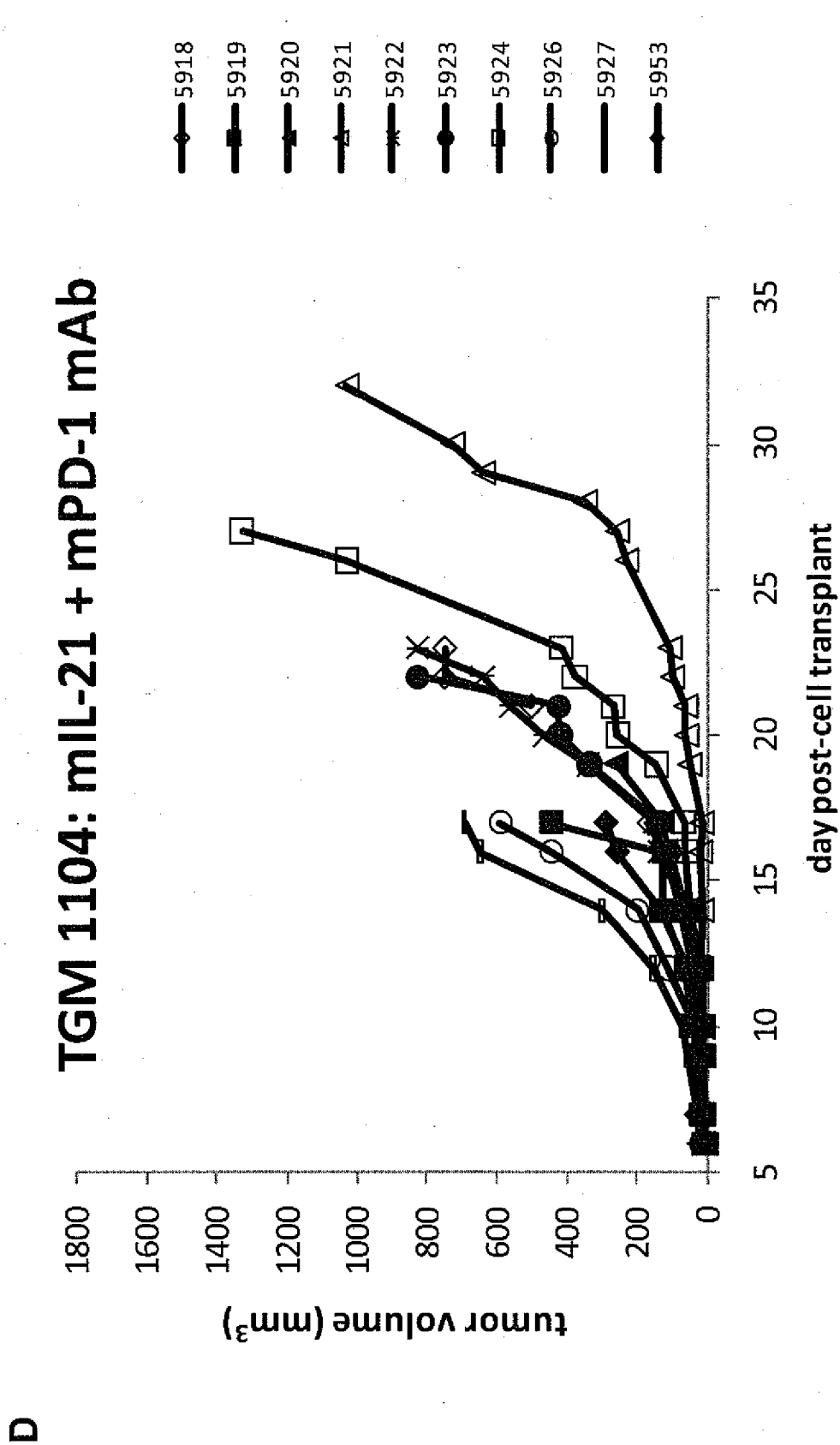

FIG. 11

Survival Analysis for Mice Treated with PBS, mIL-21, or mPD-1 mAb, Alone or in Combination, in the SC B16-F10 Mouse Model

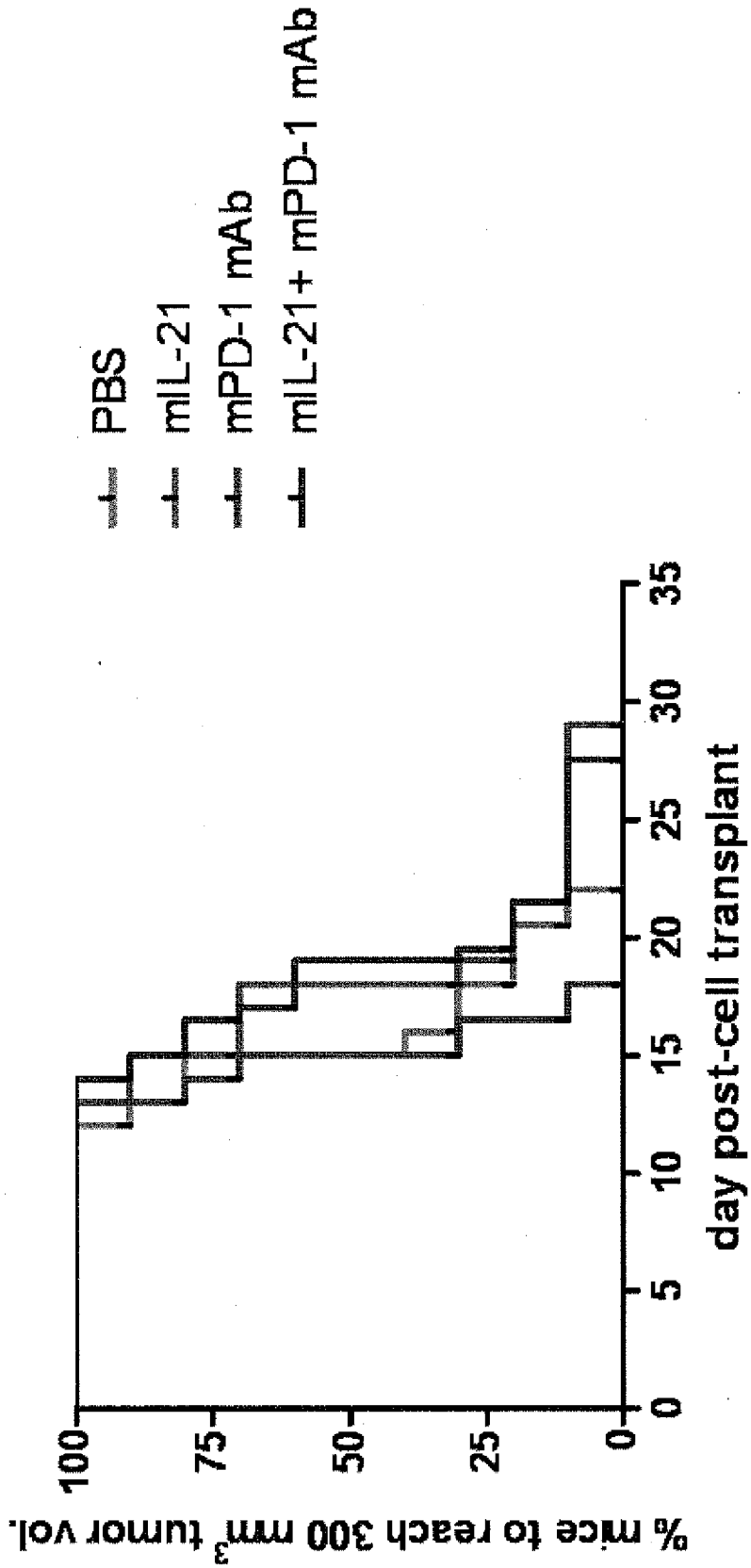

FIG. 12

Number of Lung Metastases in Mice Treated with PBS, mIL-21 or mPD-1 mAb, Alone or in Combination, in the IV B16-F10 Mouse Model (TGM 1108) (mean + SD)

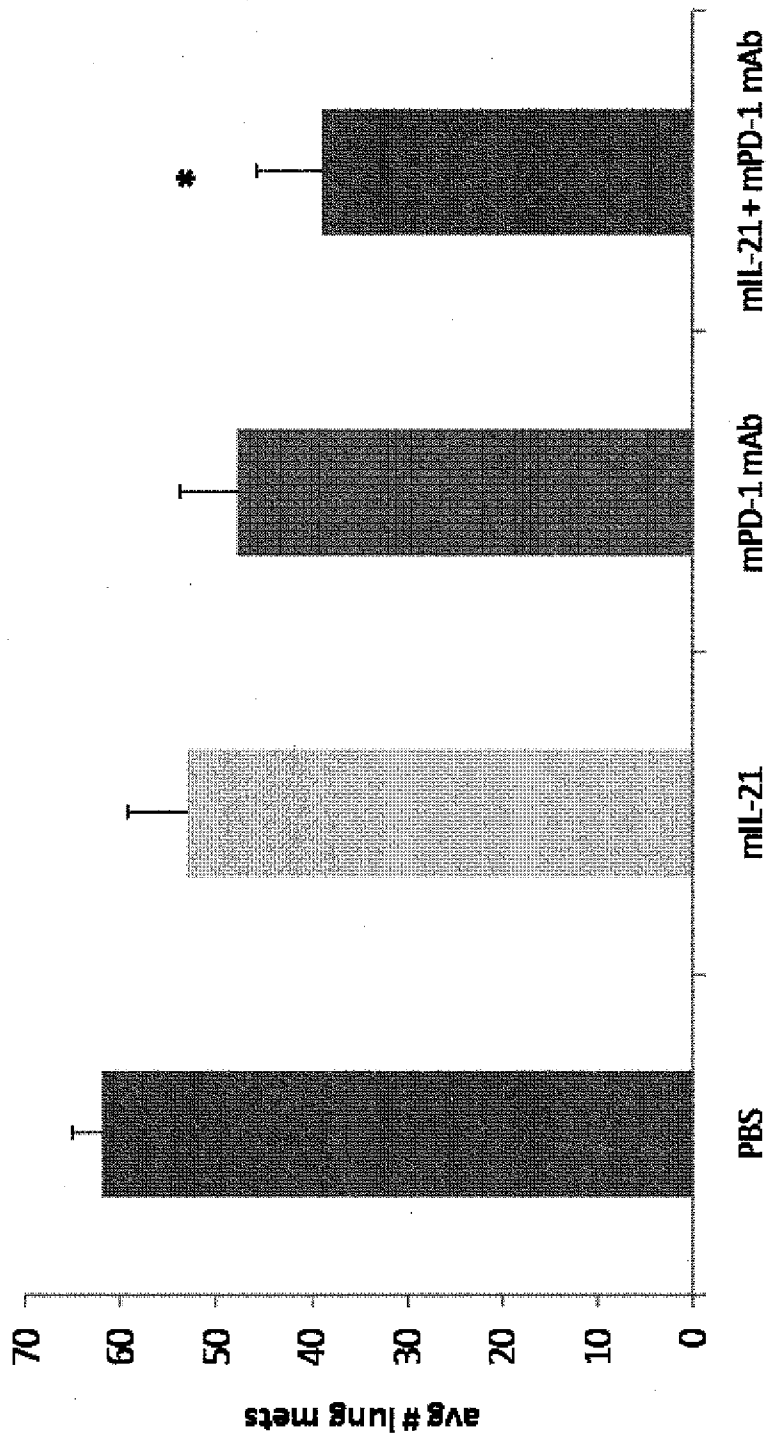

FIG. 13

Change in Body Weight (BW) in Mice Treated with PBS, mIL-21 or mPD-1 mAb, Alone or in Combination, in the IV B16-F10 Mouse Model (TGM 1108) (mean ± SD)

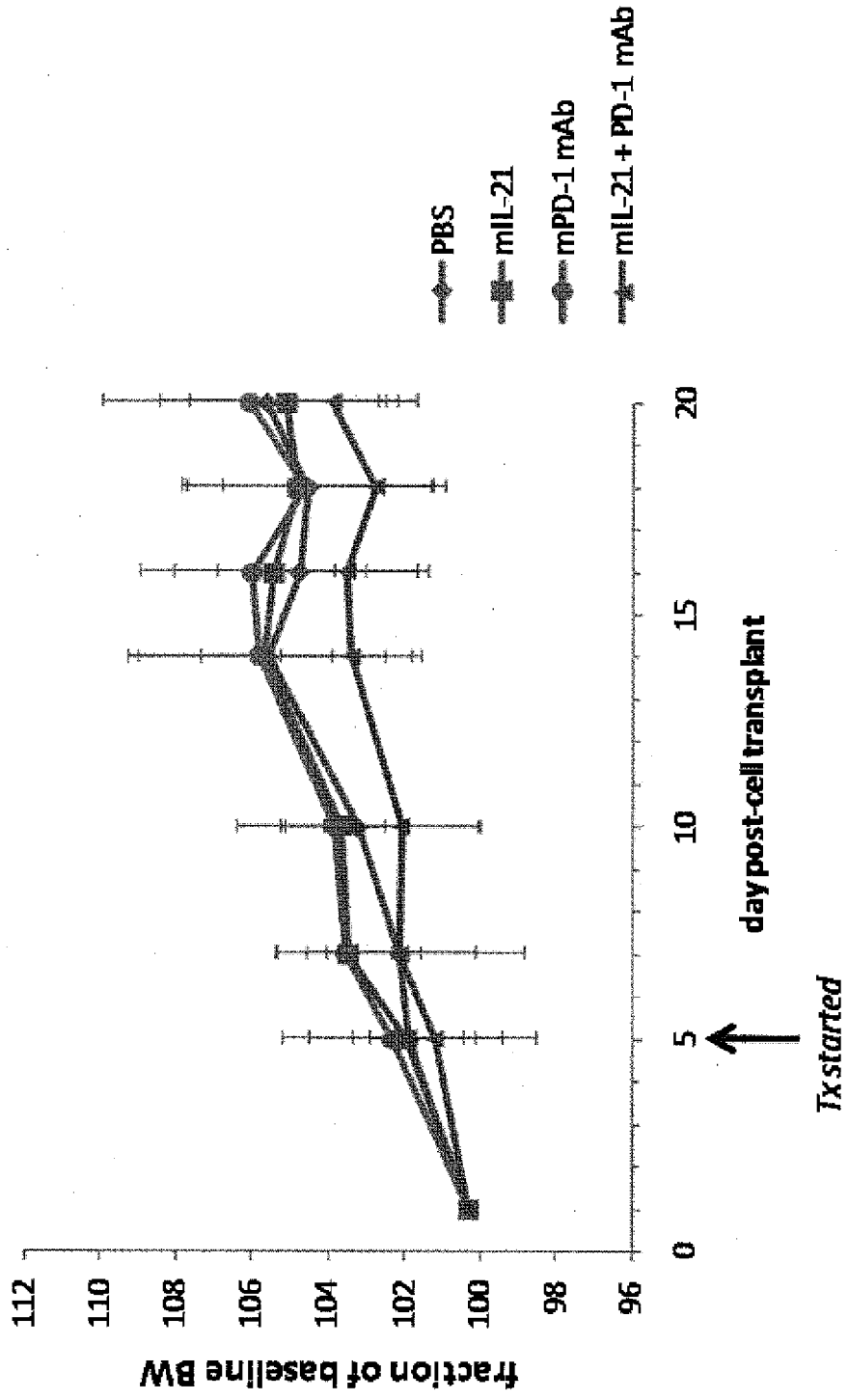

FIG. 14

Median Tumor Volume in Mice Treated with PBS, mIL-21, or mPD-1 mAb, Alone or in Combination, in the SC B16-F10 Mouse Model (TGM 1109)

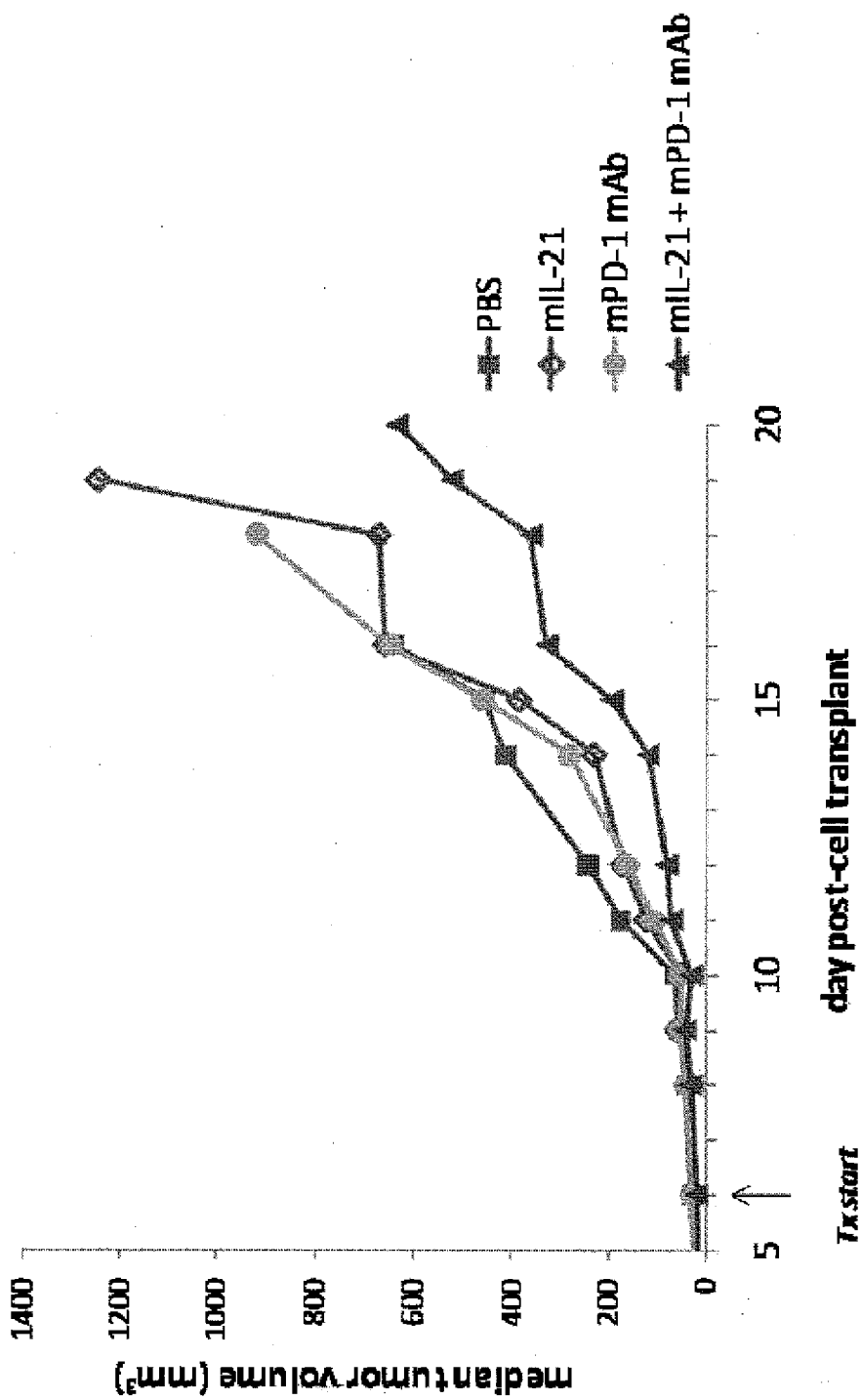

FIG. 15

Tumor Volume in Individual Mice Treated with PBS, mIL-21, or mPD-1 mAb, Alone or in Combination, in the SC B16-F10 Mouse Model

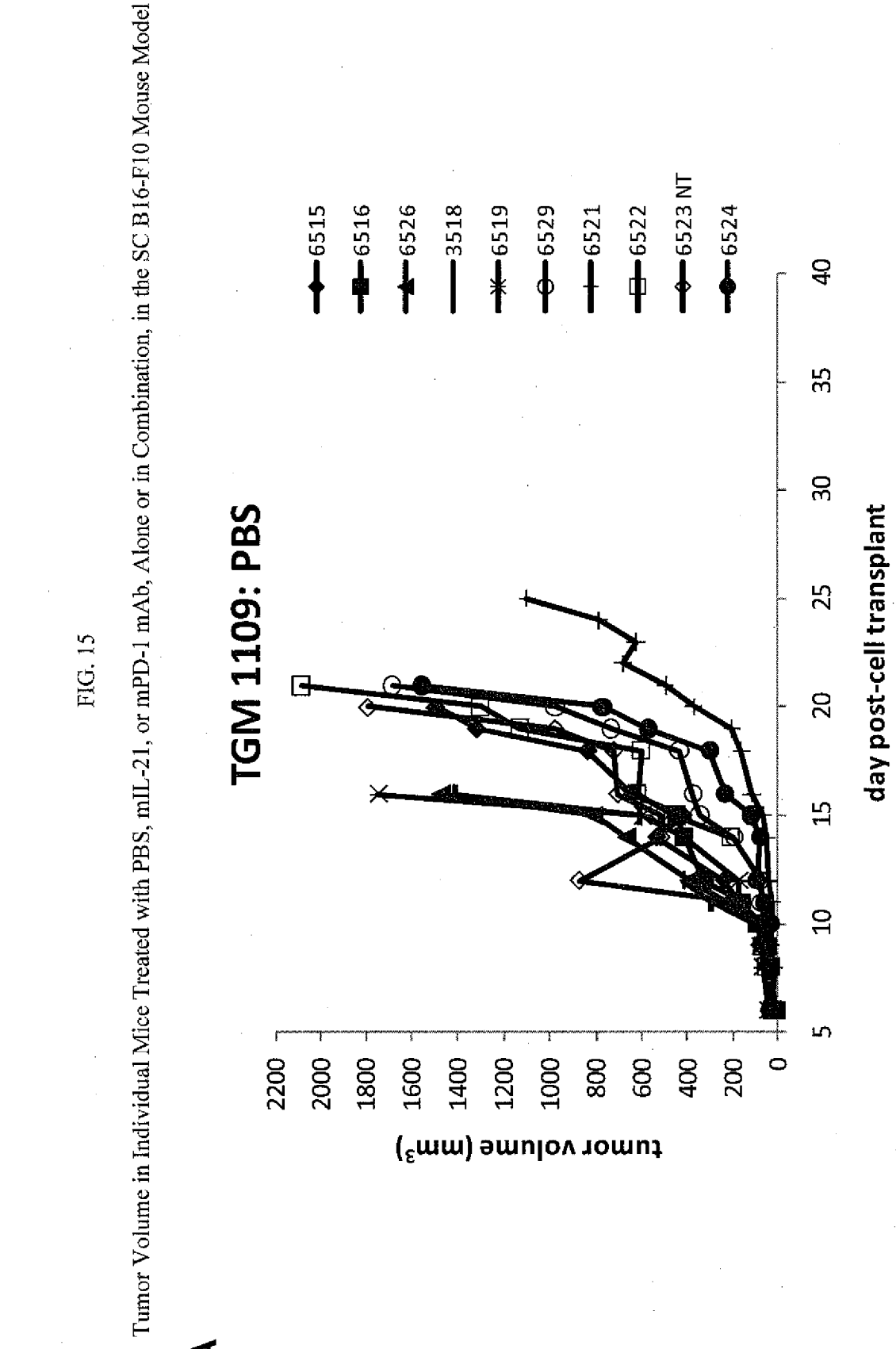

FIG. 15 (continued)

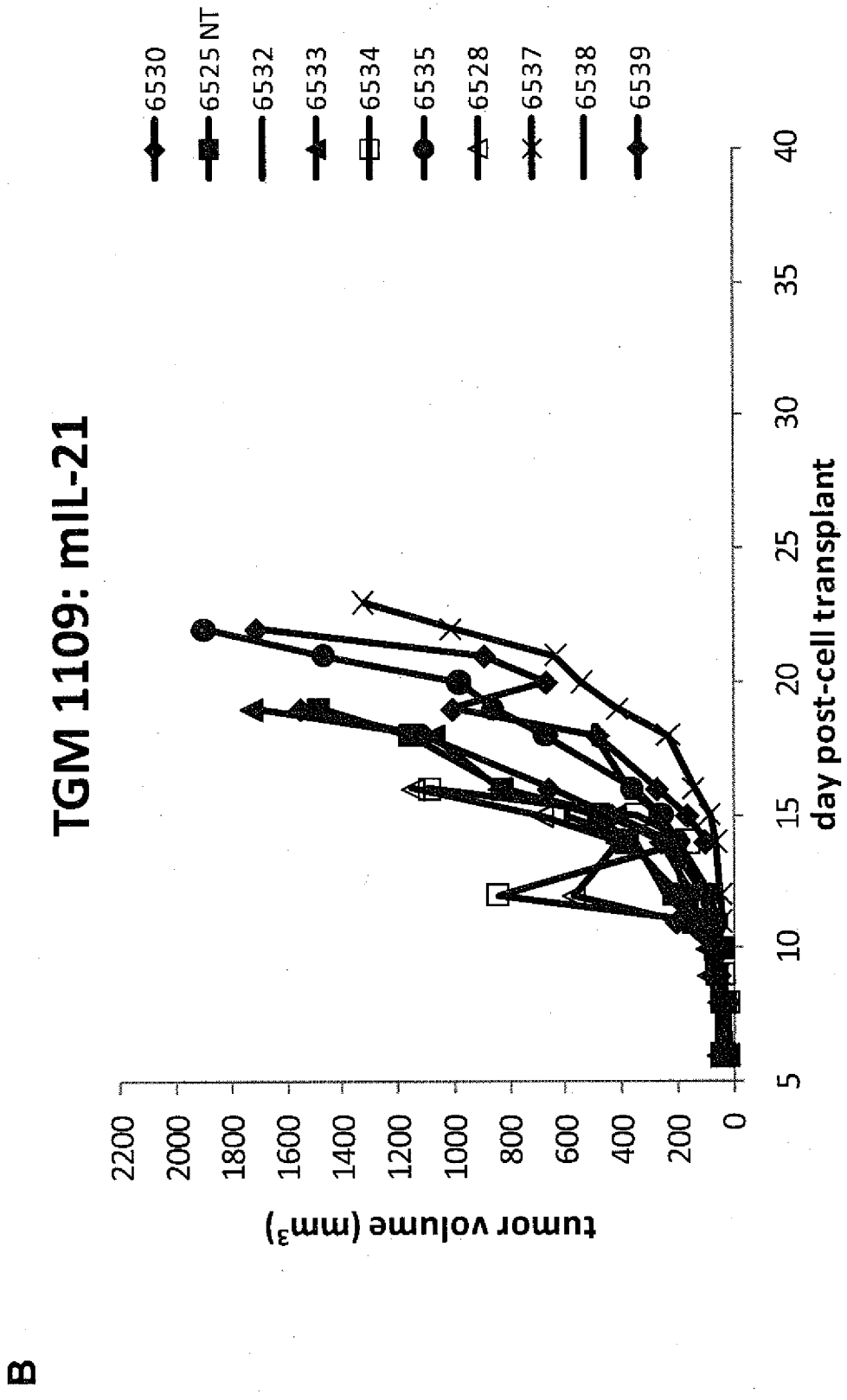

FIG. 15 (continued)

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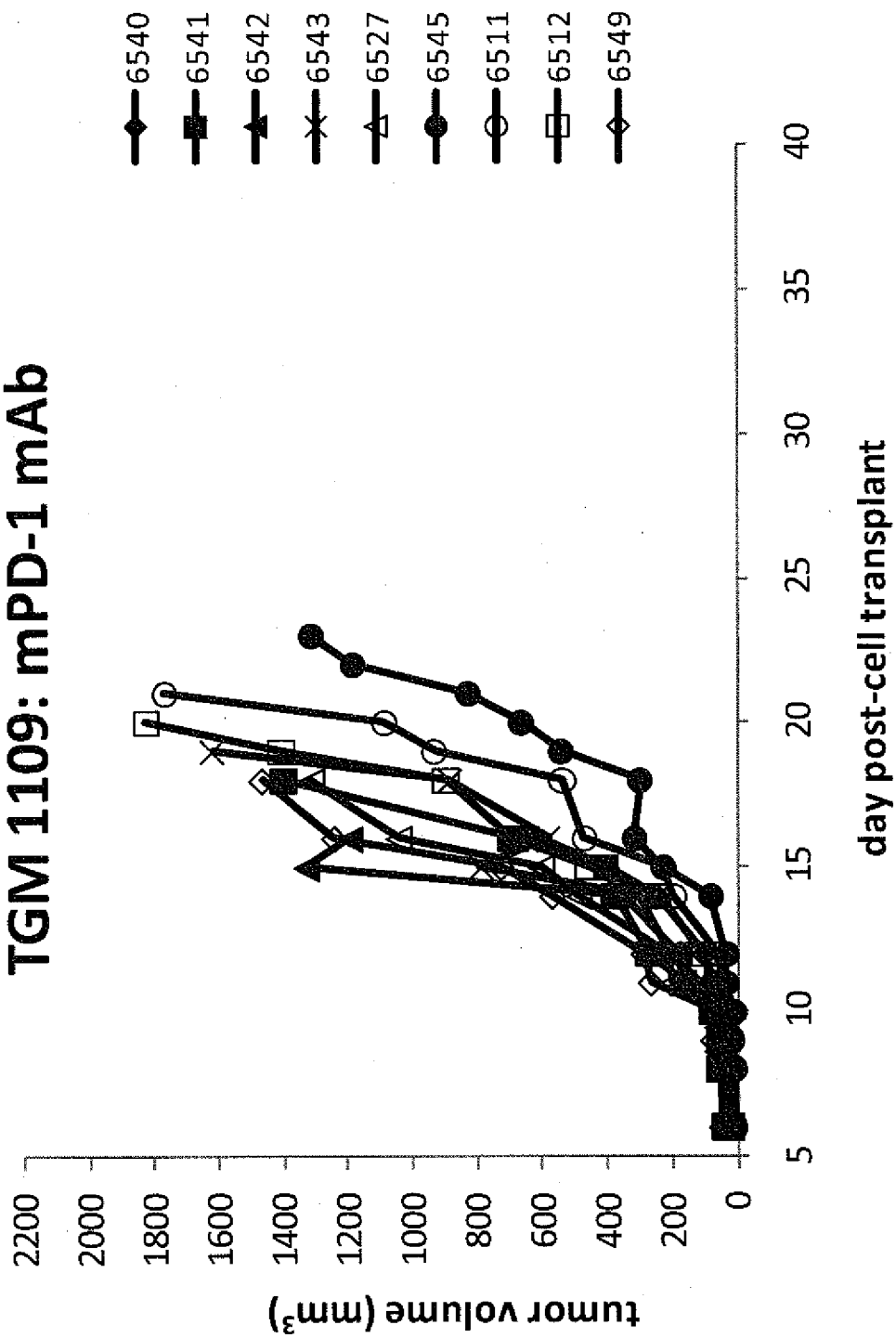


FIG. 15 (continued)

D

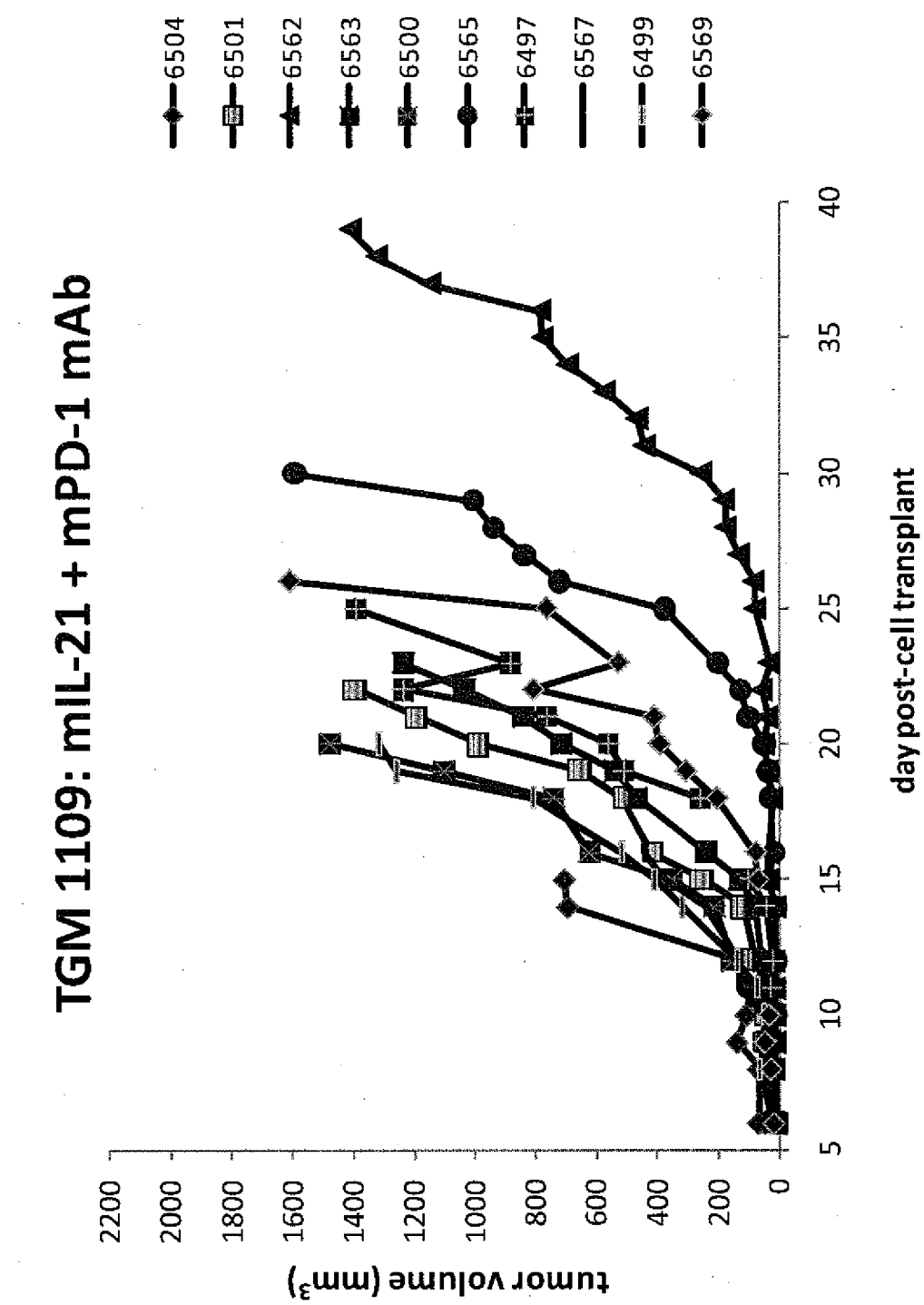
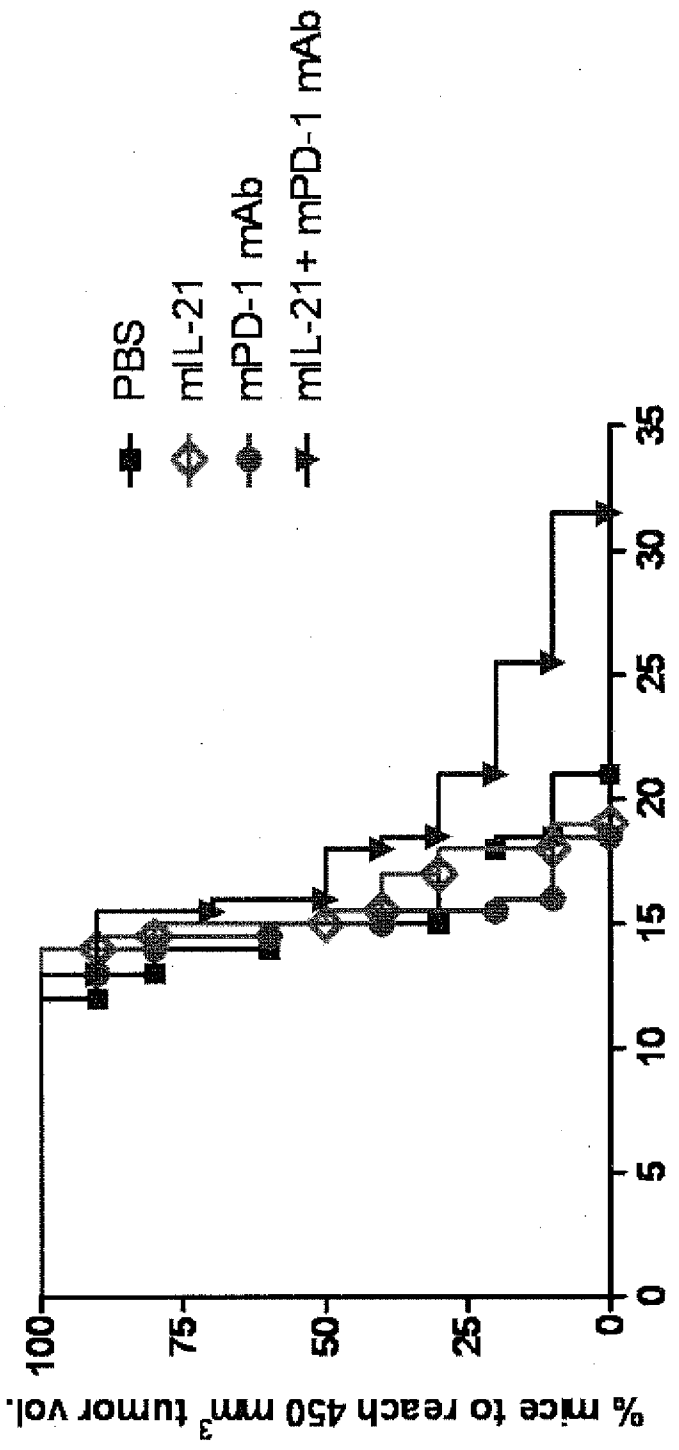


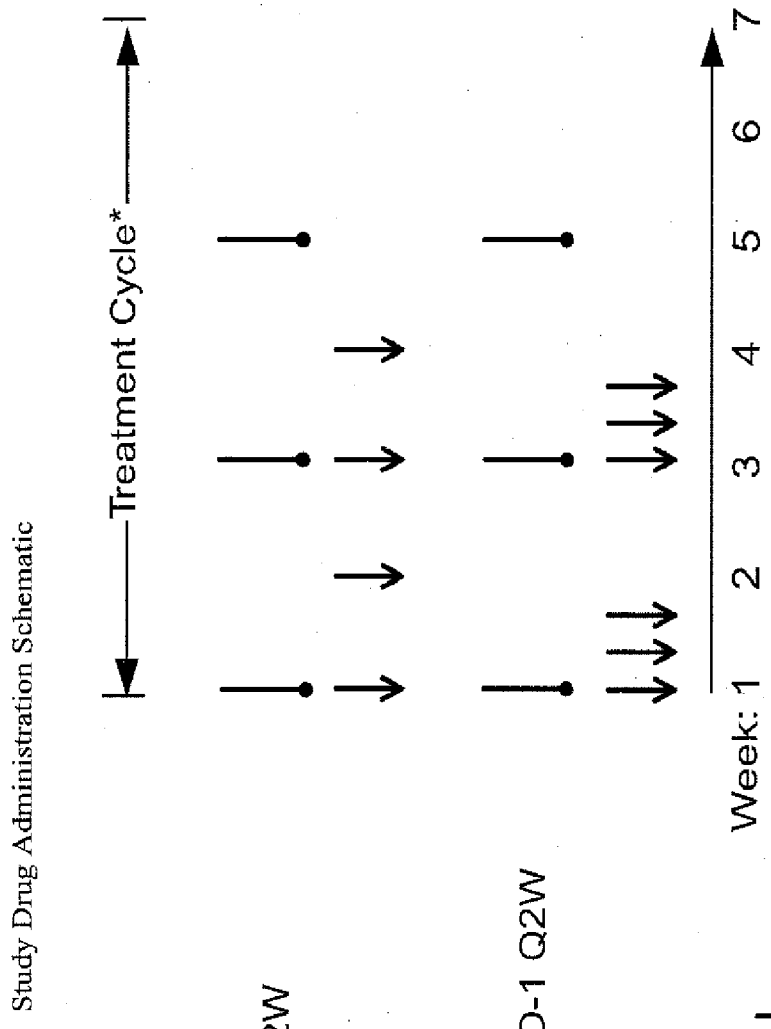
FIG. 16

Survival Analysis for Mice Treated with PBS, mIL-21, or mPD-1 mAb, Alone or in Combination, in the SC B16-F10 Mouse Model



p = 0.08 for comparison of survival curves using logrank test for trend

FIG. 17



Note: Treatment of subjects in the first dose cohort of Arm B will not begin until it has been determined that the first dose cohort of Arm A does not exceed the MTD. Subsequent dosing cohorts for Arm A and Arm B will be enrolled in parallel.
* Treatment cycles may be repeated for up to 2 years.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/039814

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 C07K16/28 A61K38/20 A61K39/395
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 428 216 A2 (ZYMOGENETICS INC [US]) 14 March 2012 (2012-03-14) abstract page 7,; claim 1	1-25
Y	WO 03/103589 A2 (ZYMOGENETICS INC [US]; NELSON ANDREW J [US]; HUGHES STEVEN D [US]; HOL) 18 December 2003 (2003-12-18) the whole document	1-25
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2013	01/08/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sirim, Pinar

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/039814

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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