



(51) International Patent Classification:

A61K 31/437 (2006.01) C12N 7/01 (2006.01)
A61K 35/763 (2015.01) A61P 35/00 (2006.01)
A61K 39/395 (2006.01)

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

(21) International Application Number:

PCT/US2017/056878

Published:

— with international search report (Art. 21(3))

(22) International Filing Date:

17 October 2017 (17.10.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/410,206 19 October 2016 (19.10.2016) US

(71) Applicant: THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

[US/US]; 412 Low Memorial Library., 535 W. 116th Street, New York, New York 10027 (US).

(72) Inventors: SAENGER, Yvonne; 215 West 91st Street #42, New York, New York 10025 (US). GARTRELL, Robyn; 601 West 164th Street, Apt. 55, New York, New York 10032 (US).

(74) Agent: WATT, Stuart L.; AMGEN Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: COMBINATION OF BRAF INHIBITOR, TALIMOGENE LAHERPAREPVEC, AND IMMUNE CHECKPOINT INHIBITOR FOR USE IN THE TREATMENT CANCER (MELANOMA)

(57) Abstract: Provided herein is a new therapeutic regimen comprising a BRAF inhibitor, talimogene laherparepvec, and an immune checkpoint inhibitor. In certain embodiments, the combination therapy disclosed herein can be used to treat solid malignant tumors.



WO 2018/075447 A1

COMBINATION OF BRAF INHIBITOR, TALIMOGENE LAHERPAREPVEC, AND IMMUNE CHECKPOINT INHIBITOR FOR
USE IN THE TREATMENT CANCER (MELANOMA)**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit under 35 U.S.C. 119(e) of United States provisional patent application number 62/410,206, filed October 19, 2016, which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present disclosure relates to a combination therapy for the treatment of solid malignant tumors, particularly to combinations of talimogene laherparepvec, a BRAF inhibitor, and an immune checkpoint inhibitor, wherein the combination therapy shows enhanced anti-tumor effect.

BACKGROUND

[0003] Cancer remains one of the most deadly threats to human health. Worldwide, the number of melanoma cases is increasing at a rate that is faster than that of any other human cancer (Roberts et al, Br. J. Dermatol (2002) 146:7-17). Melanoma accounts for less than 2 percent of skin cancer cases but causes a majority of skin cancer deaths. The clinical outcome of patients with melanoma is highly dependent on the stage at presentation. Early-stage melanoma can often be completely removed via surgery of the affected area. But once it has metastasized, it is much harder to treat. In most cases, it is not possible to completely eliminate or cure the cancer once it has metastasized. Depending upon where and how large the metastases are, treatment may involve chemotherapy, surgery, gene therapy, immunotherapy, radiation therapy, and combinations of these.

[0004] Talimogene laherparepvec is an oncolytic virus developed for the treatment of advanced solid tumors. Talimogene laherparepvec was approved by the United States Food and Drug Administration (FDA) in October 2015 and by the European Commission in December 2015 for the treatment of metastatic melanoma. Talimogene laherparepvec is derived from a strain of herpes simplex virus type 1 (HSV-1) and has been genetically engineered to attenuate neurotropic and infectious properties while increasing selectivity for malignant rather than healthy cells. The virus has also been modified to produce granulocyte-

macrophage colony-stimulating factor (GM-CSF), an inflammatory cytokine which is a chemo-attractant for various immune cells.

[0005] Despite recent success in the development of new therapies for melanoma, the vast majority of patients eventually succumb to their disease; hence, there remains a significant unmet need for improvement of outcomes in solid malignant tumor patient populations, including more specifically the melanoma patient population.

SUMMARY OF THE INVENTION

[0006] In certain embodiments, the present invention relates to a method for treating a tumor (e.g., a solid malignant tumor) or cancer in a subject comprising: administering to the subject a therapeutically effective amount of a BRAF inhibitor, an oncolytic virus, and an immune checkpoint inhibitor.

[0007] In some embodiments, the oncolytic virus is an HSV-1-based oncolytic virus. In specific embodiments, the oncolytic virus is talimogene laherparepvec.

[0008] In further embodiments, the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049, and combinations thereof. In certain embodiments, the immune checkpoint inhibitor is a PD-1 antagonist. In specific embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody.

[0009] In certain embodiments, the administration of the immune checkpoint inhibitor is continued until it induces tumor regression, remission, or eradication.

[00010] In some embodiments, the administration of each BRAF inhibitor, oncolytic virus, and the immune checkpoint inhibitor is continued until it induces tumor regression, remission, or eradication.

[00011] In certain embodiments, the BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof. In a specific embodiment, the BRAF inhibitor is vemurafenib.

[00012] In some embodiments, the tumor (e.g., solid malignant tumor) or cancer is a BRAF mutant tumor or cancer. In some embodiments, the BRAF mutant tumor or cancer is melanoma, thyroid cancer, brain cancer, lung cancer, gastric cancer, or colon cancer. In certain embodiments, the BRAF mutant tumor (e.g., solid malignant tumor) or cancer is melanoma.

[00013] In certain embodiments, the present invention relates to method for treating melanoma (e.g., BRAF mutant melanoma) in a subject comprising: administering to the subject a therapeutically effective amount of BRAF inhibitor, an oncolytic virus, and an immune checkpoint inhibitor.

[00014] In some embodiments, the oncolytic virus is an HSV-1-based oncolytic virus. In specific embodiments, the oncolytic virus is talimogene laherparepvec.

[00015] In further embodiments, the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049, and combinations thereof. In some embodiments, the immune checkpoint inhibitor is a PD-1 antagonist. In specific embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody.

[00016] In some aspects, administration of the checkpoint inhibitor is continued until it induces melanoma tumor regression, remission, or eradication. In further aspects, the administration of each BRAF inhibitor, oncolytic virus, and checkpoint inhibitor is continued until it induces melanoma tumor regression, remission, or eradication.

[00017] In some embodiments, the BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.

[00018] In certain embodiments, the present disclosure relates to a method of reducing CD4+FOXP3+ cellular population within a tumor (e.g., solid malignant tumor), the method comprising delivering to the tumor an effective amount of BRAF inhibitor, an oncolytic virus, and a checkpoint inhibitor.

[00019] In certain embodiments, the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049 and combinations thereof. In some embodiments, the immune checkpoint inhibitor is a PD-1 antagonist. In specific embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody.

[00020] In some embodiments, the BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.

[00021] In certain embodiments, the present invention provides a method of stimulating an immune response in a subject (e.g., a subject with a BRAF mutant tumor such

as, e.g., melanoma) comprising: administering to the subject a therapeutically effective amount of a BRAF inhibitor, an oncolytic virus, and a checkpoint inhibitor.

[00022] In some embodiments, the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049, and combinations thereof. In further embodiments, the immune checkpoint inhibitor is a PD-1 antagonist. In specific embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody.

[00023] In some embodiments, administration of each BRAF inhibitor, oncolytic virus, and checkpoint inhibitor is continued until stimulation of the immune response is achieved.

[00024] In certain embodiments, BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[00025] Figure 1 is a timeline and scheme for the administration of treatments to mice of the desired genotype (i.e., mice which are heterozygous BRAF (BRAF^{V600E/+}), homozygous for floxed PTEN (PTEN^{flox/flox}) and *Tyr::CreERT2*). As illustrated, mice with the desired genotype were raised until they were 6-8 weeks old, then underwent tumor induction, were treated 4-6 weeks post tumor induction, and were sacrificed at the end of the study.

[00026] Figure 2 is a graph of tumor volume (y-axis) vs days of treatment measured in six distinct treatment groups: (1) group 1 received control chow, isotype control 2A3, and PBS (phosphate buffered saline) (line 1); (2) group 2 received BRAF inhibitor PLX 4720 containing chow (BRAFi chow), isotype control 2A3, and PBS (line 2); (3) group 3 received BRAFi chow, anti-PD-1 antibody, and PBS (line 3); (4) group 4 received BRAFi chow, isotype control 2A3, and OncoVex^{mGM-CSF} (line 4); (5) group 5 received BRAFi chow, anti-PD-1 antibody, and OncoVex^{mGM-CSF} (line 5); and (6) group 6 received control chow, anti-PD-1 antibody, and OncoVex^{mGM-CSF} (6).

[00027] Figure 3 is a Kaplan-Meier survival curve of tumor-bearing animals treated with the following: (1) control chow, isotype control 2A3, and PBS (also referred to herein as Control Group; line 1); (2) BRAF inhibitor PLX 4720 containing chow (BRAFi chow), isotype control 2A3, and of PBS (also referred to herein as BRAFi only; line 2); (3) BRAFi chow, anti-PD-1 antibody, and PBS (also referred to herein as BRAFi + α -PD1; line 3); (4) BRAFi chow, isotype control 2A3, and OncoVex^{mGM-CSF} (also referred to as herein as BRAFi

+ OncoVex^{mGM-CSF}; line 4); (5) BRAFi chow, anti-PD-1 antibody, and OncoVex^{mGM-CSF} (also referred to herein as BRAFi + α -PD1 + OncoVex^{mGM-CSF}; line 5); and (6) control chow, anti-PD-1 antibody, and OncoVex^{mGM-CSF} (also referred to herein as α -PD1 + OncoVex^{mGM-CSF}; line 6).

[00028] Figure 4 expands upon the data in Figures 2 and 3 and represents tumor growth curves, survival curves, and ulceration rates between treatment groups. Figure 4A is the mean tumor volume comparison of all mice up through 33 days. Figure 4B is the mean tumor volume comparison of mice in groups receiving BRAFi up through 57 days. A 2-way ANOVA analysis was performed comparing group 2 (BRAFi only) to other BRAFi treatment groups and found significant tumor growth inhibition in group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0141$). Figure 4C is the survival comparison of mice in each treatment group using the log-rank (Mantel-Cox) test. Findings show prolonged survival for group 2 (BRAFi only) ($p<0.0001$), group 3 (BRAFi + α -PD1) ($p=0.0002$), group 4 (BRAFi + OncoVex^{mGM-CSF}) ($p<0.0001$), and group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p<0.0001$) in comparison to group 1 (control) mice. Figure 4D shows comparison of survival in treatment groups receiving BRAFi using the log-rank (Mantel-Cox) test. A trend toward significance in group 4 (BRAFi + OncoVex^{mGM-CSF}) ($p=0.1218$) and group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p=0.1561$) when comparing to group 2 (BRAFi only) mice was observed. Figure 4E shows that tumor ulceration lead to stoppage of intratumoral injections. The number of mice receiving each treatment was evaluated using two-tailed fisher's exact test. The number of mice ulcerated was higher for those receiving OncoVex^{mGM-CSF} vs. not receiving OncoVex^{mGM-CSF} ($p=0.0003$), while comparison of BRAFi and anti-PD1 treatment was not significant. Figure 4F also shows the size of tumors at timepoints when intratumoral injections were stopped due to ulceration in each group was evaluated. The size of tumor was significantly smaller in group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0214$) and group 6 (α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0074$) when compared to group 1 (Control Group) mice ($ns>0.05$, $*\leq 0.05$, $**\leq 0.01$, $***\leq 0.001$, $****<0.0001$).

[00029] Figure 5 is a scatter plot showing significantly decreased percent of CD4+Foxp3+ cells following the treatment of tumor-bearing animals with (1) control chow, isotype control 2A3, and PBS (Control); (2) BRAF inhibitor PLX 4720 containing chow (BRAFi chow), isotype control 2A3, and PBS (BRAFi Only); (3) BRAFi chow, anti-PD-1 antibody, and PBS (BRAFi + anti-PD1); (4) BRAFi chow, isotype control 2A3, and OncoVex^{mGM-CSF} (BRAFi + OncoVex^{mGM-CSF}); (5) BRAFi chow, anti-PD-1 antibody, and

OncoVex^{mGM-CSF} (BRAFi + anti-PD1 + OncoVex^{mGM-CSF}); and (6) control chow, anti-PD-1 antibody, and OncoVex^{mGM-CSF} (anti-PD1 + OncoVex^{mGM-CSF}).

[00030] Figure 6 expands upon the data in Figure 5 and is the flow cytometry evaluation of intratumoral T Cell Infiltration. Figure 6A is the percent of CD8+/CD3+ cytotoxic T lymphocytes (CTLs) in all 6 treatment groups. CTLs were found to be increased in group 3 (BRAFi + α -PD1) ($p=0.0002$) ($p=0.0476$), group 4 (BRAFi + OncoVex^{mGM-CSF}) ($p=0.0004$), group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0001$), and group 6 (α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0001$) when compared to group 1 (control). Figure 6B shows that CTL infiltration was highest in groups receiving OncoVex^{mGM-CSF} when compared to groups not receiving OncoVex^{mGM-CSF} ($p<0.0001$). Figure 6C shows the percent of CD4+FOXP3+/CD4+ regulatory T- cells (Tregs) in all 6 treatment groups. Infiltration of Tregs is decreased in group 4 (BRAFi + OncoVex^{mGM-CSF}) ($p=0.0082$), group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p<0.0001$), and group 6 (α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0031$) when compared to group 1 (control). Figure 6D shows that Treg infiltration is lowest in groups receiving OncoVex^{mGM-CSF} when compared to groups not receiving OncoVex^{mGM-CSF} ($p<0.0001$). (ns>0.05, * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.001, **** $\leq$ 0.0001).

DETAILED DESCRIPTION

[00031] In one aspect, the present disclosure provides a method of treating a tumor (e.g., a solid malignant tumor) or cancer, the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor ("BRAFi"), an oncolytic virus, and an immune checkpoint inhibitor. In one embodiment, the oncolytic virus is derived from a herpes simplex virus. In another aspect, the present disclosure provides a method of treating a tumor (e.g., a solid malignant tumor) or cancer, the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor, an HSV-1 based oncolytic virus (e.g., talimogene laherparepvec), and an immune checkpoint inhibitor.

[00032] The present disclosure also provides a method of treating melanoma (e.g., BRAF mutant melanoma), the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor, an oncolytic virus, and an immune checkpoint inhibitor. In one embodiment, the oncolytic virus is derived from a herpes simplex virus. Additionally, the present disclosure provides a method of treating melanoma (e.g., BRAF mutant melanoma), the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor, an HSV-1 based oncolytic virus (e.g., talimogene laherparepvec), and an immune checkpoint inhibitor.

[00033] The present disclosure provides a combination therapy for treating various types of tumors (e.g., solid malignant tumors) including BRAF mutant tumors. Particularly, the present disclosure provides compositions and methods combining an effective amount of BRAF inhibitor, an oncolytic virus, and an immune checkpoint inhibitor. In one embodiment, the oncolytic virus is derived from a herpes simplex virus. The present disclosure also provides compositions and methods combining an effective amount of BRAF inhibitor, an HSV-1 based oncolytic virus (e.g., talimogene laherparepvec), and a checkpoint inhibitor. In some embodiments, the combination therapy demonstrates significant enhancement of the anti-tumor effect compared to either agent alone. In particular embodiments, the enhancement of the anti-tumor effect is synergistic.

[00034] In addition, the present disclosure provides pharmaceutical compositions comprising an effective amount of BRAF inhibitor, an oncolytic virus, and a checkpoint inhibitor. In one embodiment, the oncolytic virus is derived from a herpes simplex virus. The present disclosure also provides pharmaceutical compositions comprising an effective amount of BRAF inhibitor, an HSV-1 based oncolytic virus (e.g., talimogene laherparepvec), and a checkpoint inhibitor.

[00035] The present disclosure is based in part on the unexpected discovery that combination of BRAF inhibitor, a herpes simplex virus based oncolytic virus (e.g., talimogene laherparepvec), and an immune checkpoint inhibitor (e.g., a PD-1 inhibitor) shows a synergistic inhibiting effect on formation and progression of cancer. This phenomenon was observed in a spontaneous *in vivo* model of melanoma (Examples 2 and 3). Thus, it was shown that the combination of the present disclosure possesses beneficial therapeutic properties, e.g. synergistic interaction, and a strong *in-vivo* anti-tumor response, which can be used as a treatment. These characteristics render it particularly useful for the treatment of cancers and tumors (e.g., solid malignant tumors) including BRAF mutant cancers and tumors.

[00036] Thus, in particular embodiments, the present disclosure provides a method of treating tumors (e.g., solid malignant tumors) or cancers, including BRAF mutant tumors or cancers, the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor, an oncolytic virus, and a PD-1 inhibitor. For example, the present disclosure provides a method of treating tumors (e.g., solid malignant tumors), including BRAF mutant tumors, the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor, a herpes simplex virus based oncolytic virus (e.g., talimogene laherparepvec), and a PD-1 inhibitor.

[00037] In one embodiment, the PD-1 inhibitor is an anti PD-1 antibody. In a specific embodiment, the anti-PD-1 antibody is pembrolizumab (MK-3475). In another embodiment, the anti-PD-1 antibody is nivolumab (MDX 1106/BMS 936558).

[00038] In one embodiment, the BRAF inhibitor is PLX4720. While not being bound by theory, it is expected that the benefit of the methods described herein will be most pronounced for BRAF mutant tumors. Thus, in certain embodiments, the tumors will be screened prior to treatment for BRAF mutations and tumors positive for BRAF mutations will be treated as described herein.

[00039] Accordingly, the present disclosure provides a method of treating tumors (e.g., solid malignant tumors), including BRAF mutant tumors, the method comprising administering to a patient, a therapeutically effective amount of BRAF inhibitor PLX4720, talimogene laherparepvec, and an anti-PD-1 antibody. In a particular embodiment, the solid malignant tumor is melanoma (e.g., BRAF mutant melanoma).

DEFINITIONS

[00040] As used herein, the term “immune checkpoint inhibitor” refers to molecules that completely or partially reduce, inhibit, interfere with or modulate one or more checkpoint proteins. Checkpoint proteins regulate T-cell activation or function. Numerous checkpoint proteins are known, such as, Programmed Death 1 (PD-1) with its ligands PD-L1 and PD-L2, Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and its ligands CD80 and CD86, B7-H1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\gamma\delta$, and memory CD8⁺ ($\alpha\beta$) T cells), OX-40, CD137, CD40, CD 160 (also referred to as BY55), CGEN-15049, CHK 1 and CHK2 kinases, A2aR and various B-7 family ligands (Pardoll, Nature Reviews Cancer 12: 252-264, 2012). These proteins are responsible for co-stimulatory or inhibitory interactions of T-cell responses. Immune checkpoint proteins regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. Examples of immune checkpoint inhibitors include antibodies or molecules/agents derived from antibodies.

[00041] As used herein, the terms “patient” or “subject” are used interchangeably and mean a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Preferably, the patient is a human.

[0001] The term “melanoma” is used in the broadest sense and refers to all stages and all forms of cancer arising from melanocytes. The methods of the present invention can be used to treat several different stages of cancer (e.g., melanoma). Most staging systems

include information relating to whether the cancer has spread to nearby lymph nodes, where the tumor is located in the body, the cell type, whether the cancer has spread to a different part of the body, the size of the tumor, and the grade of tumor (i.e., the level of cell abnormality the likelihood of the tumor to grow and spread). For example, Stage 0 refers to the presence of abnormal cells that have not spread to nearby tissue – i.e., cells that may become a cancer. Stage I, Stage II, and Stage III cancer refer to the presence of cancer. The higher the Stage, the larger the cancer tumor and the more it has spread into nearby tissues. Stage IV cancer is cancer that has spread to distant parts of the body. In a particular example, the stage of melanoma is Stage IIIc to IV melanoma.

[00042] Melanoma is typically a malignant tumor associated with skin cancer.

[00043] As used herein, the terms “reduce or inhibit” is meant the ability to cause an overall decrease of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. Reduce or inhibit can refer to the symptoms of the disorder being treated, the presence or size of metastases, or the size of the primary tumor.

[00044] As used herein, the phrases “treating cancer” and “treatment of cancer” and “treatment of tumors” mean to decrease, reduce, or inhibit the replication of cancer cells; decrease, reduce or inhibit the spread (formation of metastases) of cancer; decrease tumor size; decrease the number of tumors (i.e. reduce tumor burden); lessen or reduce the number of cancerous cells in the body; prevent recurrence of cancer after surgical removal or other anti-cancer therapies; or ameliorate or alleviate the symptoms of the disease caused by the cancer.

[00045] As used herein, the term “synergistic” refers to an interaction of a BRAF inhibitor and an oncolytic virus (e.g., talimogene laherparepvec) with an immune checkpoint inhibitor wherein the observed effect (e.g., reduction of tumor volume) in the presence of the combination of compounds together is higher than the sum of the individual effects of each compound administered separately. In one embodiment, the observed combined effect of the compounds is significantly higher than the sum of the individual effects.

[00046] As used herein, the term “BRAF inhibitor” refers to a compound or agent that inhibits, decreases, lowers, or reduces at least one activity of any of the isoforms or mutants of BRAF kinase. Examples of BRAF inhibitors include, but are not limited to, GSK2118436 (dabrafenib), PLX4720, and PLX4032.

[00047] As used herein, the term “anti PD-1 antibody” refers to an antibody that inhibits PD-1 activity by, e.g., inhibiting the interaction between PD-1 and its ligands (e.g., PD-L1 and PD-L2) and, thus, can enhance T-cell responses and can mediate antitumor

activity. The term "inhibits the activity" relates to a decrease (or reduction) in lymphocyte proliferation or activity that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more. PD-1- mediated activity can be determined quantitatively using T cell proliferation assays known in the art.

[00048] As used herein, the term "effective amount" refers to the quantity of a component that is sufficient to treat a subject without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention, i.e. a therapeutically effective amount. The specific effective amount will vary with such factors as the particular condition being treated, the physical condition of the patient, the type of mammal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or its derivatives.

[00049] The term "CD4+Foxp3+ T regulatory cells (Tregs)" used herein refers to regulatory T cells expressing a Foxp3 protein. Foxp3 is expressed by CD4+CD25+ Tregs, and gain-of-function, overexpression and analysis of Foxp3-deficient Scurfy (sf) mice show Foxp3 is essential to the development and maintenance of murine Tregs.

[00050] The combination therapy described in the present disclosure can be used for the treatment of various types of solid malignant tumors (e.g., BRAF mutant tumors). Non-limiting examples of preferred types of cancers/tumors (including BRAF mutant tumors) for treatment include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), pancreatic adenocarcinoma, breast cancer, colon cancer, lung cancer (e.g. non-small cell lung cancer), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies, and more generally solid tumors. Additionally, the present disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the combinations described herein. In specific embodiments, the combination therapy described in the present disclosure can be used for the treatment of BRAF mutant tumors. Such BRAF mutant tumors include, e.g., melanoma, thyroid cancer, brain cancer, lung cancer, gastric cancer, and colon cancer. In a particular embodiment, the BRAF mutant tumor is melanoma. In other embodiments, the combination therapy described in the present disclosure can be used for the treatment of phosphatase and tensin homolog (PTEN) negative tumors.

Uses of the Combination Therapy

[00051] The combination therapy described in the present disclosure is particularly advantageous because the anti-tumor effect of the combination is enhanced compared to the effect of each compound alone, or the effects of a combination of any two compounds selected from BRAF inhibitor, oncolytic virus (e.g., talimogene laherparepvec), and a PD-1 inhibitor. Moreover, it is anticipated that the dosage of each agent in a combination therapy can be reduced as compared to monotherapy with each agent, while still achieving an overall anti-tumor effect. In addition, due to the synergistic effect, the total amount of compounds administered to a patient can advantageously be reduced, which may result in decreased side effects.

[00052] Enthusiasm relating to the potential of combinations of BRAF inhibitors with checkpoint inhibitors (e.g., ipilimumab) has been historically muted in light of the prevailing viewpoint that the two agents were too toxic for administration in combination to patients. The present invention demonstrates however that BRAF inhibitors can be safely administered in combination with some checkpoint inhibitors, such as anti-PD-1 compounds (e.g., anti-PD-1 antibodies).

[00053] The antibody or antigen binding fragments of the anti-PD-1 or immune modulating antibody, in combination with an oncolytic virus (e.g., talimogene laherparepvec) and a BRAF inhibitor can be used to increase, enhance, stimulate or up-regulate an immune response. The combination treatments described herein are particularly suitable for treating subjects having a disorder that can be treated by increasing the T-cell mediated immune response. Preferred subjects include human patients in need of enhancement of an immune response.

[00054] T regulatory cells (Tregs) play an important role in the induction and maintenance of immunologic tolerance (Malek and Castro, *Immunity*. 2010 Aug 27; 33(2): 153–165). In many human cancers and mouse models of tumor growth, the frequency of Treg and their suppressor functions are increased as compared to those reported for healthy subjects. Moreover, there is considerable experimental and clinical evidence suggesting Tregs are engaged in suppression of antitumor immune responses and thus contribute to tumor escape from the host immune system (Droese et al. *BMC Cancer*. 2012; 12:134).

[00055] Two main types of regulatory T cells have been identified--natural and induced (or adaptive)-and both play significant roles in tuning down effector immune responses. The expression of surface markers such as CD25 on the cell surface and intracellular forkhead transcription factor (FOXP3) has been used to differentiate between

these two Treg subsets by flow cytometry. Adaptive CD4(+)Foxp3(+) regulatory T (iTregs) expressing the Foxp3 have been shown to suppress T cell-mediated host immune responses against self- and nonself-antigens. In several different types of cancer, increased numbers of CD4+FOXP3+ Tregs have been associated with poor prognosis. For example, high CD4+Foxp3+ Tregs infiltration is significantly associated with shorter overall survival (OS) in the majority of solid tumors including cervical, renal, melanoma, and breast cancer (Shang et al. *Sci Rep.* 2015 Oct 14;5:15179).

[00056] The combination therapy described in the present disclosure is additionally suitable for reducing the CD4⁺FOXP3⁺ cellular population within tumors (e.g., solid malignant tumors), including BRAF mutant tumors, , such as in a method comprising administering a therapeutically effective amount of BRAF inhibitor, an oncolytic virus (e.g., talimogene laherparepvec), and an immune checkpoint inhibitor. Accordingly, the combination therapy of the present disclosure can be used for the treatment of a Foxp3+ T regulatory cell (Treg) related disease. The Foxp3+ Treg related disease may be a cancer or a tumor. As disclosed herein, the percentage of CD4+ Foxp3+ Tregs was significantly lower in cells receiving anti-PD-1 therapy, OncoVex^{mGM-CSF} and BRAF inhibition, compared with those that received only OncoVex^{mGM-CSF} and BRAF inhibition combined therapy or anti-PD-1 and OncoVex^{mGM-CSF} combination (Example 3). OncoVex^{mGM-CSF} is an HSV-1 modified in the same manner as talimogene laherparepvec except that it contains the gene encoding murine GM-CSF instead of human GM-CSF.

[00057] The administration of the immune checkpoint inhibitor is continued until it induces tumor regression or eradication. Furthermore, the duration of each component of the combined treatment disclosed here will continue until it induces tumor regression or eradication.

[00058] Furthermore, the present disclosure relates to a pharmaceutical composition comprising a therapeutically effective amount of (i) an oncolytic virus (e.g., an HSV-1 such as talimogene laherparepvec), (ii) a BRAF inhibitor or a pharmaceutically acceptable salt thereof, and (iii) a PD-1 inhibitor, or a pharmaceutically acceptable salt thereof. Alternatively, the present disclosure relates to a pharmaceutical composition comprising a therapeutically effective amount of (i) an oncolytic virus (e.g., an HSV-1 such as talimogene laherparepvec), and (ii) a BRAF inhibitor or a pharmaceutically acceptable salt thereof. In another aspect, the present disclosure encompasses a pharmaceutical composition comprising a therapeutically effective amount of (i) an oncolytic virus (e.g., an HSV-1 such as talimogene laherparepvec), and (ii) a PD-1 inhibitor, or a pharmaceutically acceptable salt

thereof. In further aspect, the present disclosure encompasses a pharmaceutical composition comprising a therapeutically effective amount of (i) a BRAF inhibitor or a pharmaceutically acceptable salt thereof, and (ii) a PD-1 inhibitor, or a pharmaceutically acceptable salt thereof. Alternatively, a pharmaceutical composition of the present disclosure may comprise (i) an oncolytic virus (e.g., an HSV-1 such as talimogene laherparepvec), or (ii) a BRAF inhibitor or a pharmaceutically acceptable salt thereof, or (iii) a PD-1 inhibitor, or a pharmaceutically acceptable salt thereof. In certain embodiments, a PD-1 inhibitor is an anti-PD-1 antibody. In further embodiments, anti-PD-1 antibody is nivolumab or pembrolizumab. In one embodiment, a BRAF inhibitor is PLX4720.

[00059] The various components of the combination therapy described in the present disclosure may be administered concurrently with, or separately from, other components in a treatment regimen. Additionally, the combination therapy described herein further comprises administering a chemotherapeutic agent, targeted therapy or radiation to the subject either prior to, simultaneously with, or after treatment with the combination therapy. In an additional aspect, the tumor may be resected prior to the administration of the BRAF inhibitor, an oncolytic virus (e.g., talimogene laherparepvec), and a PD-1 inhibitor.

[00060] The components of the combination therapy described herein can be administered simultaneously, separately or sequentially.

Oncolytic Viruses

[00061] Advances in cancer vaccines and immunotherapy have generated a considerable interest in viral therapy such that “oncolytic viruses” may be exploited to potentiate an anti-tumor immune response by exposing the immune system to tumor antigens.

[00062] Pre-clinical and clinical studies evaluating the potential of several oncolytic viruses are ongoing. Thus, in one aspect, the oncolytic virus of the current invention is CAVATAK™ (a coxsackievirus A21 (CVA21)), HF-10 (spontaneously mutated HSV-1), ONCOS-102 (engineered human serotype 5 adenovirus), enadenotucirev (A11/Ad3 chimeric Group B adenovirus), genetically engineered strains of vesicular stomatitis virus, REOLYSIN® (human reovirus), pexastimogene devacirepvec (vaccinia poxvirus), NDV-GM-CSF (newcastle disease virus encoding GM-CSF), BV-2711 (HSV-1-based virus), PSI-001, VCN-01 (adenovirus expressing PH20 hyaluronidase), OrienX-010 (HSV-1 expressing GM-CSF), or Ad-RTS hIL-12 (adenovirus vector engineered to express human IL-12).

[00063] In some embodiments, the oncolytic virus is a herpes simplex virus. In other embodiments, the oncolytic virus is a herpes simplex virus type 1 (HSV-1). In yet other

embodiments, the HSV-1 lacks functional ICP34.5 genes, lacks a functional ICP47 gene and comprises a gene encoding human GM-CSF. The ICP34.5 gene is located in the terminal repeats of the long region of HSV and therefore there are two copies per genome. Previous studies have shown that functional deletion of the neurovirulence gene, ICP34.5, renders the virus avirulent. The ICP47 gene is located in the unique short region of HSV. The ICP47 gene product interacts with the transporter associated with antigen processing (TAP1 and TAP2) and blocks antigen processing via MHC class I molecules. Deletion of the ICP47 gene allows greater antigen processing within infected cells and is intended to result in a concomitant increase in the immune response to infected cells. GM-CSF is a cytokine known to be involved in the stimulation of immune responses. Using homologous recombination with plasmid DNA, heterologous genes, such as that encoding human GM-CSF, can be inserted into the HSV viral genome, and viral genes, such as ICP34.5 and ICP47, can be functionally deleted. In one embodiment, the herpes simplex virus is talimogene laherparepvec.

[00064] Talimogene laherparepvec is derived from HSV-1, an enveloped, double-stranded DNA virus which replicates in the nuclei of infected cells. HSV-1 induces cell lysis resulting in the spread of viral progeny to nearby cells (Roizman et al. Lippincott Williams & Wilkins: Philadelphia. p. 1823–1897 (2013). Talimogene laherparepvec, HSV-1 [strain JS1] ICP34.5-/ICP47-/hGM-CSF, (previously known as OncoVex^{GM-CSF}), is an intratumorally delivered oncolytic immunotherapy comprising an immune-enhanced HSV-1 that selectively replicates in solid tumors. (Lui et al., Gene Therapy, 10:292-303, 2003; US Patent No. 7,223,593 and US Patent No. 7,537,924). The HSV-1 was derived from Strain JS1 as deposited at the European collection of cell cultures (ECAAC) under accession number 01010209. In talimogene laherparepvec, the HSV-1 viral genes encoding ICP34.5 have been functionally deleted. Functional deletion of ICP34.5, which acts as a virulence factor during HSV infection, limits replication in non-dividing cells and renders the virus non-pathogenic. The safety of ICP34.5-functionally deleted HSV has been shown in multiple clinical studies (MacKie et al, Lancet 357: 525-526, 2001; Markert et al, Gene Ther 7: 867-874, 2000; Rampling et al, Gene Ther 7:859-866, 2000; Hunter et al, J Virol Aug; 73(8): 6319- 6326, 1999). In addition, ICP47 (which blocks viral antigen presentation to major histocompatibility complex class I and II molecules) has been functionally deleted from talimogene laherparepvec. Functional deletion of ICP47 also leads to earlier expression of US11, a gene that promotes virus growth in tumor cells without decreasing tumor selectivity. The coding sequence for human GM-CSF, a cytokine involved in the stimulation of immune

responses, has been inserted into the viral genome of talimogene laherparepvec. The insertion of the gene encoding human GM-CSF is such that it replaces nearly all of the ICP34.5 gene, ensuring that any potential recombination event between talimogene laherparepvec and wild-type virus could only result in a disabled, non-pathogenic virus and could not result in the generation of wild-type virus carrying the gene for human GM-CSF. The HSV thymidine kinase (TK) gene remains intact in talimogene laherparepvec, which renders the virus sensitive to anti-viral agents such as acyclovir. Therefore, acyclovir can be used to block talimogene laherparepvec replication, if necessary.

[00065] Talimogene laherparepvec produces a direct oncolytic effect by replication of the virus in the tumor, and induction of an anti-tumor immune response enhanced by the local expression of GM-CSF. In the context of melanoma, and other disseminated diseases, this dual activity is beneficial as a therapeutic treatment. The intended clinical effects include the destruction of injected tumors, the destruction of local, locoregional, and distant uninjected tumors, a reduction in the development of new metastases, a reduction in the rate of overall progression and of the relapse rate following the treatment of initially present disease, and prolonged overall survival.

[00066] Talimogene laherparepvec has been tested for efficacy in a variety of *in vitro* (cell line) and *in vivo* murine tumor models and has been shown to eradicate tumors or substantially inhibit their growth at doses comparable to those used in clinical studies. Nonclinical evaluation has also confirmed that GM-CSF enhances the immune response generated, enhancing both injected and uninjected tumor responses, and that increased surface levels of MHC class I molecules result from the deletion of ICP47. Talimogene laherparepvec has been injected into normal and tumor-bearing mice to assess its safety. In general, the virus has been well tolerated, and doses up to 1×10^8 PFU/dose have given no indication of any safety concerns. (See, for example, Liu et al., *Gene Ther* 10: 292-303, 2003).

[00067] Clinical studies have been or are being conducted in several advanced tumor types (advanced solid tumors, melanoma, squamous cell cancer of the head and neck, breast cancer, and pancreatic cancer), with over 400 subjects treated with talimogene laherparepvec (see, for example, Harrington et al., *J Clin Oncol.* 27(15a):abstract 6018, 2009; Kaufman et al., *Ann Surg Oncol.* 17: 718-730, 2010; Kaufman and Bines, *Future Oncol.* 6(6): 941-949, 2010). Indeed, talimogene laherparepvec was approved by the U.S. FDA in October 2015 and by the European Commission in December 2015 for the treatment of metastatic melanoma

Inhibition of BRAF Signaling

[00068] BRAF gene mutations have been identified in 60% to 70% of malignant melanomas, 83% of anaplastic thyroid carcinoma, 35% to 69% of papillary thyroid carcinoma, 4% to 16% of colon cancer, 63% of low-grade ovarian carcinoma, 15% of Barrett's esophageal carcinoma, 4% of acute myeloid leukemia, 3-4.8% of head and neck squamous cell carcinoma, 2%-3% of non-small-cell lung cancer, 2% of gastric carcinoma, 2% of non-Hodgkin's lymphoma and has been reported in glioma, sarcoma, breast cancer, cholangiocarcinoma, and liver cancer. The combination of oncolytic virus (e.g., talimogene laherparepvec), BRAF inhibitor, and PD-1 inhibitor combinations of the present invention is particularly useful in treating tumors comprising BRAF mutations, such as those discussed above.

[00069] Thus, in one embodiment, the type of solid malignant tumor to be treated with the combination therapy of the present disclosure includes, but is not limited to, melanoma. In one embodiment, the melanoma comprises a BRAF gene mutation. In other embodiments, additional tumors to be treated with the combination therapy of the present disclosure include, but are not limited to, tumors comprising BRAF gene mutations, including anaplastic thyroid carcinoma, papillary thyroid carcinoma, colon cancer, ovarian carcinoma, Barrett's esophageal carcinoma, head and neck squamous cell carcinoma, non-small-cell lung cancer, gastric carcinoma, non-Hodgkin's lymphoma, glioma, sarcoma, breast cancer, cholangiocarcinoma, and liver cancer.

[00070] Most mutations in BRAF that have been found in human cancers are point mutations that occur in the kinase domain and are clustered in exons 11 and 15 of the gene which contains several regulatory phosphorylation sites (S446, S447, D448, D449, T599, and S602). (Beeram, et al., *Journal of Clinical Oncology* (2005), 23(27):6771-6790). The most prevalent mutation is the T1799A transversion mutation which accounts for more than 80% of mutations in the BRAF gene and results in a V600E mutation in B-Raf protein. This mutation is thought to mimic phosphorylation in the activation segment of B-Raf since it inserts a negatively charged residue near two activating phosphorylation sites, T599 and S602, and thus results in constitutively active B-Raf protein in a Ras independent manner. (Xing, M., *Endocrine-Related Cancer* (2005), 12:245-262).

[00071] Additional mutations of the BRAF gene in human cancer, include but are not limited to, ARG461ILE, ILE462SER, GLY463GLU, and LYS600GLU (Rajagopalan, H., et al. (Letter) *Nature* 418: 934, 2002), GLY465VAL and LEU596ARG (Naoki, K., et al., *Cancer Res.* 62: 7001-7003, 2002), and GLY468ARG, GLY468ALA and ASP593GLY (Lee,

J. W., et al., Brit. J Cancer 89: 1958-1960, 2003), the references of which are each incorporated by reference in their entirety.

[00072] In one embodiment, the oncogenic BRAF to be inhibited in the combination therapy of the present disclosure has a mutation selected from the group consisting of VAL600GLU (also named as VAL599GLU) (Davies et al. Nature. Jun. 27, 2002; 417(6892):949-54), ARG461ILE, ILE462SER, GLY463GLU, and LYS600GLU (Rajagopalan, H., et al. (Letter) Nature 418: 934, 2002), GLY465VAL and LEU596ARG (Naoki, K., et al., Cancer Res. 62: 7001-7003, 2002), and GLY468ARG, GLY468ALA and ASP593GLY (Lee, J. W., et al., Brit. J Cancer 89: 1958-1960, 2003).

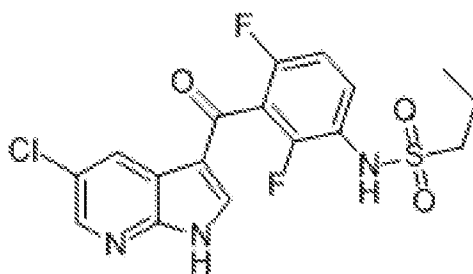
[00073] Multiple BRAF inhibitors are well known to those of skill in the art. For example, BRAF has been a target of small-molecule therapies to treat cancer (See e.g. Halilovic E, and Solit D B, Curr Opin Pharmacol 2008; 8:419-26; and Michaloglou et al., Oncogene 2008; 27:877-95).

[00074] In one embodiment of the present disclosure, the inhibitor of oncogenic BRAF is selected from the group consisting of: PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.

[00075] In another embodiment, the inhibitor of oncogenic BRAF is selected from the group consisting of Sorafenib (Bayer), RAF265 (Novartis), XL281 (BMS-908662, Bristol-Myers Squibb; Exelixis), AZ628 (Montagut et al (2008) Cancer Res 68:4853-61), GSK2118436 (dabrafenib), GDC-0879 (Selleck Chemicals LLC, Houston, Tex.), PLX4032 (Vemurafenib, Plexxikon, Berkeley, Calif., USA), and PLX4720 (Difluorophenyl-sulfonamide, Plexxikon Inc., Berkeley, Calif., USA).

[00076] In yet further embodiments, the inhibitor of oncogenic BRAF is PLX4720 (Plexxikon Inc., Berkeley, Calif., USA) (Tsai et al. Proc Natl Acad Sci USA 2008; 105:3041-3046).

[00077] The chemical structure of PLX-4720 is shown below:



Immune Checkpoint Inhibitors

[00078] Immune checkpoints regulate T cell function in the immune system. T cells play a central role in cell-mediated immunity. Checkpoint proteins interact with specific ligands which send a signal to the T cell and essentially turn off or inhibit T cell function. Cancer cells take advantage of this system by driving high levels of expression of checkpoint proteins on their surface which results in control of the T cells expressing checkpoint proteins on the surface of T cells that enter the tumor microenvironment, thus suppressing the anticancer immune response. As such, inhibition of checkpoint proteins results in complete or partial restoration of T cell function and an immune response to the cancer cells. Examples of checkpoint proteins include, but are not limited to CTLA-4, PD-L1, PD-L2, PD-1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\gamma\delta$, and memory CD8⁺ ($\alpha\beta$) T cells), CD 160 (also referred to as BY55), CGEN-15049, CHK 1 and CHK2 kinases, A2aR and various B-7 family ligands.

[00079] Checkpoint inhibitors include any agent that blocks or inhibits in a statistically significant manner, the inhibitory pathways of the immune system. Such inhibitors may include small molecule inhibitors or may include antibodies, or antigen binding fragments thereof, that bind to and block or inhibit immune checkpoint receptors or antibodies that bind to and block or inhibit immune checkpoint receptor ligands. Illustrative checkpoint molecules that may be targeted for blocking or inhibition include, but are not limited to, CTLA-4, PD-L1, PD-L2, PD-1, B7-H3, B7-H4, BTLA, HVEM, GAL9, LAG3, TIM3, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\gamma\delta$, and memory CD8⁺ ($\alpha\beta$) T cells), CD160 (also referred to as BY55), CGEN-15049, CHK 1 and CHK2 kinases, A2aR and various B-7 family ligands. B7 family ligands include, but are not limited to, B7-1, B7-2, B7-DC, B7-H1, B7-H2, B7-H3, B7-H4, B7-H5, B7-H6 and B7-H7. Checkpoint inhibitors include antibodies, or antigen binding fragments thereof, other binding proteins, biologic therapeutics or small molecules, that bind to and block or inhibit the activity of one or more of CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160 and CGEN- 15049. Illustrative immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking antibody), anti-OX40, PD-L1 monoclonal Antibody (Anti-B7-H1; MEDI4736), MK-3475 (PD-1 blocker), Nivolumab (anti-PD1 antibody), CT- 011 (anti-PD1 antibody), BY55 monoclonal antibody, AMP224 (anti-PDL1 antibody), BMS-936559 (anti-PDL1 antibody), MPLDL3280A (anti-PDL1 antibody), MSB0010718C (anti-PDL1 antibody) and Yervoy/ipilimumab (anti-CTLA-4 checkpoint inhibitor). Checkpoint

protein ligands include, but are not limited to PD-L1, PD-L2, B7-H3, B7-H4, CD28, CD86 and TIM-3.

[00080] Programmed cell death protein 1 (PD-1) is a 288 amino acid cell surface protein molecule expressed on T cells and pro-B cells and plays a role in their fate/differentiation. PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family. PD-1 plays a role in tumor-specific escape from immune surveillance. PD-1 is up-regulated in melanoma infiltrating T lymphocytes (TILs) (Dotti (2009) Blood 114 (8): 1457-58). Tumors have been found to express the PD-1 ligand (PDL-1 and PDL-2) which, when combined with the up-regulation of PD-1 in CTLs, may be a contributory factor in the loss in T cell functionality and the inability of CTLs to mediate an effective anti-tumor response.

[00081] Clinical trials in melanoma have shown robust anti-tumor responses with anti-PD-1 blockade. Significant benefit with PD-1 inhibition in cases of advanced melanoma, ovarian cancer, non-small-cell lung, prostate, renal-cell, and colorectal cancer have also been described. Studies in murine models have applied this evidence to glioma therapy. Anti-PD-1 blockade adjuvant to radiation promoted cytotoxic T cell population and an associated long-term survival benefit in mice with glioma tumor.

[00082] Accordingly, the type of tumor (e.g., solid malignant tumor) to be treated in the combination therapy of the present disclosure includes melanoma, ovarian cancer, non-small-cell lung carcinoma, prostate carcinoma, renal-cell carcinoma, and colorectal carcinoma.

[00083] One aspect of the present disclosure provides checkpoint inhibitors which are antibodies that can act as inhibitors of PD-1, thereby modulating immune responses regulated by PD-1. In one embodiment, the anti-PD-1 antibodies can be antigen-binding fragments. Anti-PD-1 antibodies disclosed herein are able to bind to human PD-1 and agonize the activity of PD-1, thereby inhibiting the function of immune cells expressing PD-1. Examples of PD-1 and PD-L1 blockers are described in US Patent Nos. 7,488,802; 7,943,743; 8,008,449; 8,168,757; 8,217,149, and PCT Published Patent Application Nos: WO03042402, WO2008156712, WO2010089411, WO2010036959, WO2011066342, WO2011159877, WO2011082400, and WO2011161699, each of which are incorporated herein by reference.

[00084] There are several PD-1 inhibitors currently being tested in clinical trials. CT-011 is a humanized IgG1 monoclonal antibody against PD-1. A phase II clinical trial in subjects with diffuse large B-cell lymphoma (DLBCL) who have undergone autologous stem cell transplantation was recently completed. Preliminary results demonstrated that 70% of subjects were progression-free at the end of the follow-up period, compared with 47% in the

control group, and 82% of subjects were alive, compared with 62% in the control group. This trial determined that CT-011 not only blocks PD-1 function, but it also augments the activity of natural killer cells, thus intensifying the antitumor immune response.

[00085] BMS 936558 is a fully human IgG4 monoclonal antibody targeting PD-1. In a phase I trial, biweekly administration of BMS-936558 in subjects with advanced, treatment-refractory malignancies showed durable partial or complete regressions. The most significant response rate was observed in subjects with melanoma (28%) and renal cell carcinoma (27%), but substantial clinical activity was also observed in subjects with non-small cell lung cancer (NSCLC), and some responses persisted for more than a year.

[00086] BMS 936559 is a fully human IgG4 monoclonal antibody that targets the PD-1 ligand PD-L1. Phase I results showed that biweekly administration of this drug led to durable responses, especially in subjects with melanoma. Objective response rates ranged from 6% to 17% depending on the cancer type in subjects with advanced-stage NSCLC, melanoma, RCC, or ovarian cancer, with some subjects experiencing responses lasting a year or longer.

[00087] MK 3475 is a humanized IgG4 anti-PD-1 monoclonal antibody in Phase III study alone or in combination with chemotherapy versus chemotherapy alone as first-line therapy for advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma. MK 3475 is currently undergoing numerous global Phase III clinical trials.

[00088] MPDL 3280A (atezolizumab) is a monoclonal antibody, which also targets PD-L1. MPDL 3280A received Breakthrough Therapy Designation from the U.S. Food and Drug Administration (FDA) for the treatment of people whose NSCLC expresses PD-L1 and who progressed during or after standard treatments.

[00089] AMP 224 is a fusion protein of the extracellular domain of the second PD-1 ligand, PD-L2, and IgG1, which has the potential to block the PD-L2/PD-1 interaction. AMP-224 is currently undergoing phase I testing as monotherapy in subjects with advanced cancer.

[00090] Medi 4736 is an anti-PD-L1 antibody that has demonstrated an acceptable safety profile and durable clinical activity in this dose-escalation study. Expansion in multiple cancers and development of MEDI4736 as monotherapy and in combination is ongoing.

[00091] Thus, in certain embodiments, the PD-1 blockers include anti-PD-1 antibodies and similar binding proteins such as nivolumab (MDX 1106, BMS 936558, ONO 4538), a fully human IgG4 antibody that binds to and blocks the activation of PD-1 by its ligands PD-L1 and PD-L2; pembrolizumab/lambrolizumab (MK-3475 or SCH 900475), a humanized monoclonal IgG4 antibody against PD-1; CT-011 a humanized antibody that binds PD-1; AMP-224 is a fusion protein of B7-DC; an antibody Fc portion; BMS-936559 (MDX- 1105-

01) for PD-L1 (B7-H1) blockade. Other immune-checkpoint inhibitors include lymphocyte activation gene-3 (LAG-3) inhibitors, such as IMP321, a soluble Ig fusion protein (Brignone et al., 2007, J. Immunol. 179:4202-4211). Other immune-checkpoint inhibitors include B7 inhibitors, such as B7-H3 and B7-H4 inhibitors. In particular, the anti-B7-H3 antibody MGA271 (Loo et al., 2012, Clin. Cancer Res. July 15 (18) 3834). Also included are TIM3 (T-cell immunoglobulin domain and mucin domain 3) inhibitors (Fourcade et al., 2010, J. Exp. Med. 207:2175-86 and Sakuishi et al., 2010, J. Exp. Med. 207:2187-94).

Pharmaceutical Compositions and Administration

[00092] The oncolytic virus (e.g., talimogene laherparepvec), a BRAF inhibitor, and an immune checkpoint inhibitor, as disclosed herein can be administered to a patient by various routes including, for example, orally or parenterally, such as intravenously, intramuscularly, subcutaneously, intraorbitally, intracapsularly, intraperitoneally, intrarectally, intracisternally, intratumorally, intravasally, intradermally or by passive or facilitated absorption through the skin using, for example, a skin patch or transdermal iontophoresis, respectively. The oncolytic virus (e.g., talimogene laherparepvec), a BRAF inhibitor, and an immune checkpoint inhibitor can also be administered to the site of a pathologic condition, for example, intravenously or intra-arterially into a blood vessel supplying a tumor. In one embodiment, the oncolytic virus (e.g., talimogene laherparepvec) is injected into the tumor (i.e., via intratumoral injection). In one embodiment, the BRAF inhibitor is administered orally. And, in one embodiment, the anti-PD-1 compound (e.g., antibody) is administered systemically (e.g., intravenously).

[00093] Thus, as discussed herein, the pharmaceutical formulations of the present invention may contain one, two, or all three of: an oncolytic virus (e.g., talimogene laherparepvec), a BRAF inhibitor, and an anti-PD-1 compound. Such pharmaceutical compositions can be used in the methods described here as appropriate. For example, in some embodiments of the methods described herein, the oncolytic virus (e.g., talimogene laherparepvec) is injected into the tumor, the BRAF inhibitor is administered orally, and the anti-PD-1 compound (e.g., antibody) is administered intravenously. As would be appreciated by those skilled in the field, the order of administration can be varied as appropriate.

[00094] The total amount of The oncolytic virus (e.g., talimogene laherparepvec), BRAF inhibitor, and immune checkpoint inhibitor can be administered to a subject as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be

administered using a fractionated treatment protocol, in which multiple doses are administered over a prolonged period of time.

[00095] Determining the dosage and duration of treatment according to any aspect of the present disclosure is well within the skills of a professional in the art. Those skilled in the field are readily able to monitor patients to determine whether treatment should be started, continued, discontinued or resumed at any given time. For example, dosages of the compounds are suitably determined depending on the individual cases taking symptoms, age and sex of the subject and the like into consideration. The amount of the compound to be incorporated into the pharmaceutical composition of the present disclosure varies with dosage route, solubility of the compound, administration route, administration scheme and the like. An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient and the method, route and dose of administration. The clinician using parameters known in the art makes determination of the appropriate dose. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved. Suitable dosages can be determined by further taking into account relevant disclosure in the known art.

[00096] Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high antibody concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. The antibody molecules can be administered by a variety of methods known in

the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Pharmaceutical Compositions and Administration of Talimogene Laherparepvec

[00097] Currently, talimogene laherparepvec is administered via intralesional injection. Clinical studies have demonstrated that talimogene laherparepvec can be injected directly into cutaneous, subcutaneous or nodal lesions that are visible, palpable, or can be injected with ultrasound-guidance. Talimogene laherparepvec is provided in 1 mL single-use vials in fixed dosing concentrations: 10^6 pfu/mL for initial dosing and 10^8 pfu/mL for subsequent dosing (Reske, et al. *J Immunol*, 2008. 180(11): p. 7525-36). The volume that is injected may vary depending on the tumor type. For example, talimogene laherparepvec is administered by intratumoral injection into injectable cutaneous, subcutaneous, and nodal tumors at a dose of up to 4.0 ml of 10^6 plaque forming unit/mL (PFU/mL) at day 1 of week 1 followed by a dose of up to 4.0 ml of 10^8 PFU/mL at day 1 of week 4, and every 2 weeks ($\pm$ 3 days) thereafter. The recommended volume of talimogene laherparepvec to be injected into the tumor(s) is dependent on the size of the tumor(s) and should be determined according to the injection volume guideline in Table 1 (and as shown in patent application PCT/US2013/057542).

Table 1. Talimogene Laherparepvec Injection Volume Guidelines Based on Tumor Size

Tumor Size (longest dimension)	Maximum Injection Volume
≥ 5.0 cm	4.0 ml
>2.5 cm to 5.0 cm	2.0 ml
>1.5 cm to 2.5 cm	1.0 ml

>0.5 cm to 1.5 cm	0.5 ml
≤0.5 cm	0.1 ml

All reasonably injectable lesions should be injected with the maximum dosing volume available on an individual dosing occasion. On each treatment day, prioritization of injections is recommended as follows: any new injectable tumor that has appeared since the last injection; by tumor size, beginning with the largest tumor; any previously uninjectable tumor(s) that is now injectable.

Pharmaceutical Compositions and Administration of BRAF inhibitor

[00098] In certain embodiments, a BRAF inhibitor is administered in an amount of about 0.1 mg/day to about 1200 mg/day, about 1 mg/day to about 100 mg/day, about 10 mg/day to about 1200 mg/day, about 10 mg/day to about 100 mg/day, about 100 mg/day to about 1200 mg/day, about 400 mg/day to about 1200 mg/day, about 600 mg/day to about 1200 mg/day, about 400 mg/day to about 800 mg/day or about 600 mg/day to about 800 mg/day.

[00099] In other embodiments, total daily dose of a BRAF inhibitor is selected from about 5 mg, about 10 mg, about 20 mg, about 25 mg, about 30mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, about 1500 mg, about 1550 mg, about 1600 mg, about 1650 mg, about 1700 mg, about 1750 mg, about 1800 mg, about 1850 mg, about 1900 mg, about 1950 mg, about 2000

mg, about 2050 mg, about 2100 mg, about 2150 mg, about 2200 mg, about 2250 mg, about 2300 mg, about 2350 mg, about 2400 mg, about 2450 mg, about 2500 mg, about 2550 mg, about 2600 mg, about 2650 mg, about 2700 mg, about 2750 mg, about 2800 mg, about 2850 mg, about 2900 mg, about 2950 mg, or about 3000 mg.

Pharmaceutical Compositions and Administration of Immune Checkpoint Inhibitor

[000100] The immune checkpoint inhibitor is administered in the form of a composition comprising one or more additional components such as a physiologically acceptable carrier, excipient or diluent. The compositions may comprise one or more substances selected from the group consisting of a buffer, an antioxidant such as ascorbic acid, a low molecular weight polypeptide (such as those having fewer than 10 amino acids), a protein, an amino acid, a carbohydrate such as glucose, sucrose or dextrans, a chelating agent such as EDTA, glutathione, a stabilizer, and an excipient. Neutral buffered saline or saline mixed with specific serum albumin are examples of appropriate diluents. In accordance with appropriate industry standards, preservatives such as benzyl alcohol may also be added. The composition may be formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Suitable components are nontoxic to recipients at the dosages and concentrations employed.

[000101] In certain embodiments, the checkpoint inhibitor is administered in 0.01mg/kg, 0.05mg/kg, 0.1mg/kg, 0.2mg/kg, 0.3mg/kg, 0.5mg/kg, 0.7mg/kg, 1mg/kg, 2mg/kg, 3mg/kg, 4mg/kg, 5mg/kg, 6mg/kg, 7mg/kg, 8mg/kg, 9mg/kg, 10mg/kg, or any combination thereof doses. In certain embodiments the checkpoint inhibitor is administered once a week, twice a week, three times a week, once every two weeks, or once every month. In certain embodiments, the checkpoint inhibitor is administered as a single dose, in two doses, in three doses, in four doses, in five doses, or in 6 or more doses.

[000102] In certain embodiments, the anti-PD-1 antibody is administered by injection (e.g., subcutaneously or intravenously) at a dose of about 1 to 30 mg/kg, e.g., about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. The dosing schedule can vary from e.g., once a week to once every 2, 3, or 4 weeks. In one embodiment, the anti-PD-1 antibody is administered at a dose from about 10 to 20 mg/kg every other week.

[000103] In one embodiment, the anti-PD-1 antibody molecule, e.g., nivolumab, is administered intravenously at a dose from about 1 mg/kg to 3 mg/kg, e.g., about 1 mg/kg, 2 mg/kg or 3 mg/kg, every two weeks. In one embodiment, the anti-PD-1 antibody molecule, e.g., nivolumab, is administered intravenously at a dose of about 2 mg/kg at 3-week intervals.

In one embodiment, nivolumab is administered in an amount from about 1 mg/kg to 5 mg/kg, e.g., 3 mg/kg, and may be administered over a period of 60 minutes, ca. once a week to once every 2, 3 or 4 weeks.

EXAMPLES

MATERIALS AND METHODS

BRAF^{F-V600E/+}; PTEN^{F/-}; Tyr::CreERT2 Mouse Model

[000104] All animal experiments were performed in accordance with institutional and national guidelines and were approved by the Institutional Animal Care and Use Committee at Columbia University. Mice were purchased from Jackson Laboratories (<https://www.jax.org/strain/013590>) and bred to desired genotype. The inducible mouse melanoma model was obtained by crossing the *C57BL/6J Tyr::CreERT2* (homo- or heterozygotic), *C57BL/6J PTEN^{F/-}* (homozygotic), and mixed-background *BRAF^{F-V600E/+}* (heterozygotic) mouse strains. Mice were genotyped at 7 days of age. Mice heterozygous BRAF (*BRAF^{F-V600E/+}*), homozygous for floxed PTEN (*PTEN^{F/-}*) and *Tyr::CreERT2* were kept for study. Homozygous and wild type mice were kept for breeding.

Tumor Induction

[000105] Tumors were induced on the skin of the melanoma model mice as described previously (Dankort et al. Nat. Genet. 2009 May;41(5):544-52). In brief, mice were anesthetized using isoflurane (Henry Schein Animal Health, Dublin, OH) and then 2 aliquots of 5 μ L of 10mg/mL 4-hydroxytamoxifen (4-TM) $\geq$ 70% Z isomer (remainder primarily E-isomer) (Sigma-Aldrich, St. Louis, MO) dissolved in pure ETOH (Fisher Scientific, Fair Lawn, NJ) was topically applied to the shaven right flank of 6 to 8-week-old mice for three consecutive days. Tumor outgrowth was monitored twice weekly, from 4 weeks following tumor induction.

Animal Treatment

[000106] Tumors developed 4-5 weeks post induction and mice with tumors between 5-60 mm³ of volume (calculated by $(L \times W \times H \times \pi)/6$) (or >5mm in diameter) were randomized into 6 possible treatment groups to treat 10 mice/group (Figures 5 and 6). Mice assigned to OncoVex^{mGM-CSF} treatment groups were transferred to an Animal Biosafety Level (ASBL) 2 facility. Treatments were administered through mouse chow, intraperitoneal

injection, and intratumoral injection. Control chow (Research Diets, New Brunswick, NJ) or BRAFi chow (Research Diets, New Brunswick, NJ) containing the drug PLX 4720 (Selleck Chemicals, Houston, TX), was given at start of study and continuously replenished. Intraperitoneal injections of 100 μ L of Isotype Control 2A3 (Bio X Cell, West Lebanon, NH) or anti-PD-1 of (Bio X Cell, West Lebanon, NH) diluted to 1:1 ratio with PBS (MP Biomedicals, LLC, Solon, OH) were given three days a week, every other week. Intratumoral injections of 100 μ L of PBS or 50 μ L of 1×10^7 pfu of OncoVex^{mGM-CSF} (Amgen, Thousand Oaks, CA) diluted with 50 μ L additional PBS were given biweekly. Each tumor reaching volume requirement were injected. Tumors were measured biweekly. Weight was measured once a week. Treatment was stopped in mice when there was suspected toxicity, the tumor appeared necrotic, the mouse appeared debilitated, or weight loss > 5%. End of study was determined by tumor volume ≥ 3000 mm³, $\geq 25\%$ of the tumor ulcerated, or the mouse had $\geq 20\%$ weight loss or other signs of debilitation.

[000107] A general scheme describing the use of these mice in these Examples is outlined in Figure 1.

Immune Monitoring

[000108] For analysis of tumor-infiltrating leukocytes, mice bearing tumors ≥ 3000 mm³ (newly scored dead on survival curve) were sacrificed by CO₂ inhalation, confirmed with neck dislocation, and tumors, spleen and lymph nodes were dissected. The tumor mass was homogenized, digested with 0.4 mg/mL collagenase (Sigma-Aldrich, St. Louis, MA), incubated at 37 °C for 20 minutes, further homogenized with a Gentle MACS Dissociator (Miltenyi Biotec, Inc., San Francisco, CA) and then strained through 40- μ m nylon filters (Fisher Scientific, Pittsburgh, PA). Spleen was cut and cells collected per protocol using RBC Cell lysis and straining. Lymph node cells were collected per protocol by cutting and straining. Cells were incubated with antibodies for 15 minutes on ice, fixed with fixation/permeabilization for 30 minutes, then incubated with the intracellular antibody FoxP3 for 30 minutes. Cells were then acquired with the BD Fortessa Flow Cytometer within two weeks of fixation and analyzed with FlowJo V10 (FlowJo, LLC, Ashland, OR). Fluorochrome conjugated antibodies specific for mouse CD45 (clone 30-F11) (eBioscience Inc., San Diego, CA), CD3 (17A2) (Biolegend, San Diego, CA), CD4 (clone RM4-5) (BD Biosciences, San Jose, CA), CD8 (clone 53-6.7) (Biolegend, San Diego, CA), PD-1 (clone J43) (BD Biosciences, San Jose, CA), NK1.1 (clone PK136) (eBioscience Inc., San Diego,

CA), FOXP3 (clone NRRF-20) (eBiosciences Inc., San Diego, CA), and fixable viability dye (eFlour 506) (eBiosciences Inc., San Diego, CA) were used for staining.

Statistical analysis

[000109] Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA). Survival analysis was performed using the log-rank (Mantel–Cox) test with Bonferroni correction. Significance was defined at a P value of <0.05, using a nonparametric Mann–Whitney t test. Bonferroni correction was used for multigroup comparisons. P-value was calculated as compared to control group. Multiplex IHC cell density and nearest neighbor analysis will be performed using Inform Software (Perkin Elmer, Waltham, MA).

GENERAL METHODS

[000110] Standard methods in molecular biology are described Sambrook, Fritsch and Maniatis (1982 & 1989 2nd Edition, 2001 3rd Edition) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA). Standard methods also appear in Ausbel, *et al.* (2001) *Current Protocols in Molecular Biology, Vols.1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

[000111] Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described (Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 1*, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described (see, *e.g.*, Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 2*, John Wiley and Sons, Inc., New York; Ausubel, *et al.* (2001) *Current Protocols in Molecular Biology, Vol. 3*, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J., pp. 384-391). Production, purification, and fragmentation of polyclonal and monoclonal antibodies are described (Coligan, *et al.* (2001) *Current Protocols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*). Standard techniques for characterizing ligand/receptor interactions are available (see, *e.g.*, Coligan, *et al.* (2001) *Current Protocols in Immunology*, Vol. 4, John Wiley, Inc., New York).

EXAMPLE 1

Generation of the $BRAF^{F-V600}$; $PTEN^{F-/-}$; $Tyr::CreERT2$ Mouse Model

[000112] One of the most common genetic alterations in human melanoma is a point mutation in the *BRAF* proto-oncogene. In addition to *BRAF* mutation, the progression of malignant melanoma is also driven by loss of expression of tumor suppression genes, such as phosphatase and tensin homolog (*PTEN*). In order to build a model that reflects both initiation and progression phases of melanoma, Dankort et al. have generated a conditional mouse model of $BRAF^{V600}$ induced, *PTEN* deficient metastatic human melanoma, which allows for the controlled timing and location of melanoma initiation (Nat. Genet. 2009 May;41(5):544-52). As most studies related to immunotherapy research have been conducted using C57BL/6J mice, the $BRAF^{F-V600}$; $PTEN^{F-/-}$; $Tyr::CreERT2$ inducible melanoma model was crossed on a C57BL/6J genetic background. Previous studies have demonstrated that C57BL/6J $Tyr::CreER$; $PTEN^{F-/-}$; $BRAF^{(F-V600E/+)}$ melanoma model can be used as a standard model in which targeted therapy combinations can be tested in a high-throughput manner (Hooijkaas et al. Am J Pathol. 2012 Sep;181(3):785-94).

[000113] Following generation of $BRAF^{F-V600}$; $PTEN^{F-/-}$; $Tyr::CreERT2$ animals, tumor formation was induced as described previously (Dankort et al. Nat. Genet. 2009 May;41(5):544-52).

EXAMPLE 2

*Combined Therapy using OncoVex^{mGM-CSF}, *BRAF* inhibition, and Immune Checkpoint Blockade Leads to Prevention/Establishment of Tumor Induction*

[000114] Melanomas are resistant to both radiotherapy and chemotherapy. Previous studies have demonstrated tumor reduction when melanomas (developed using a spontaneous model described herein) were treated with combination therapy using OncoVex^{mGM-CSF} and a *BRAF* inhibitor. In order to improve response to therapy, the effects of immune checkpoint blockade on tumor formation when combined with administration of OncoVex^{mGM-CSF} and *BRAF* inhibitor were tested.

[000115] $BRAF^{F-V600}$; $PTEN^{F-/-}$; $Tyr::CreERT2$ animals were divided into 6 groups, where tumor formation was induced using 4-HT. 4-5 weeks following tumor induction, each

of the 6 groups received a different treatment: (1) group 1 received control chow, intraperitoneal injection of isotype control 2A3, and intratumoral injection of PBS; (2) group 2 received BRAF inhibitor PLX 4720 containing chow (BRAFi chow), intraperitoneal injection of isotype control 2A3, and intratumoral injection of PBS; (3) group 3 received BRAFi chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of PBS; (4) group 4 received BRAFi chow, intraperitoneal injection of isotype control 2A3, and intratumoral injection of OncoVex^{mGM-CSF}; (5) group 5 received BRAFi chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of OncoVex^{mGM-CSF}; and (6) group 6 received control chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of OncoVex^{mGM-CSF}. Tumor volume was measured bi-weekly.

[000116] As shown in Figure 2, addition of immune checkpoint inhibitor (such as anti-PD-1 antibody) to OncoVex^{mGM-CSF} and BRAF inhibition combination therapy prevents/inhibits tumor formation significantly when compared to all other treatment groups. Animals treated with tamoxifen (4HT) for 35 days while receiving triple combination therapy (BRAFi chow, anti-PD-1 antibody, and OncoVex^{mGM-CSF}; group 5) did not develop tumors, while the control group established tumors (group 1) exceeding 1700 mm³ in size. Incorporating anti-PD-1 antibody into the treatment regimen consisting of OncoVex^{mGM-CSF} and BRAF inhibition significantly improved a therapeutic effect of combined OncoVex^{mGM-CSF} and BRAF inhibition therapy, where OncoVex^{mGM-CSF} and BRAF inhibition combination therapy resulted in tumors. These results indicate that inclusion of an immune checkpoint inhibitor into OncoVex^{mGM-CSF} and BRAF inhibition combination therapy substantially improves *in vivo* treatment outcome.

[000117] These results were further confirmed in Figures 4A and 4B. Here, tumor volume was calculated for each mouse and the mean of the tumor volumes of the mice in each group was plotted on a tumor growth curve up to 33 days (Figure 4A) and mean tumor volumes of the mice in groups receiving BRAFi chow is plotted on a tumor growth curve up to 57 days (Figure 4B). Group 5 mice (BRAFi chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of OncoVex^{mGM-CSF}) had significantly smaller tumor volume than group 2 mice (BRAFi alone) ($p=0.0141$) (Figure 4B). Mice were sacrificed at end of study which was determined by tumor volume of 3000 mm³ or greater, or greater than 2.6cm in a single dimension, more than 25% of the tumor was ulcerated, the mouse had >20% weight loss, or other signs of debilitation.

EXAMPLE 3

Combined Therapy using OncoVex^{mGM-CSF}, BRAF inhibition, and Immune Checkpoint Blockade Leads to Tumor Regression

[000118] The inclusion of an immune checkpoint inhibitor was tested in combination with OncoVex^{mGM-CSF} and BRAF inhibition therapy to determine whether this triple therapy can stimulate regression or slow down a progression of preexisting tumors in mice. To do so, the BRAF^{F-V600}; PTEN^{F-/-}; Tyr::CreERT2 animals were divided into 6 groups, where tumor formation was induced using 4-HT. Four-five weeks following tumor induction, when the animals had measurable melanoma tumors, each of the 6 groups received a different treatment: (1) group 1 received control chow, intraperitoneal injection of isotype control 2A3, and intratumoral injection of PBS; (2) group 2 received BRAF inhibitor PLX 4720 containing chow (BRAFi chow), intraperitoneal injection of isotype control 2A3, and intratumoral injection of PBS; (3) group 3 received BRAFi chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of PBS; (4) group 4 received BRAFi chow, intraperitoneal injection of isotype control 2A3, and intratumoral injection of OncoVex^{mGM-CSF}; (5) group 5 received BRAFi chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of OncoVex^{mGM-CSF}; and (6) group 6 received control chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of OncoVex^{mGM-CSF}. Tumor volume was measured bi-weekly.

[000119] Animals were monitored and mouse survival was plotted using Kaplan-Meier survival curve (Figure 3). As shown in Figure 3, animals in the control group succumbed to disease as early as 40 days following the start of treatment (Line 1), while animals receiving both OncoVex^{mGM-CSF} and BRAF inhibitor PLX 4720 remained alive for up to 130 days (Line 4). There was a statistically significant difference when PD-1 inhibition was included into the treatment regimen. As shown in Figure 3, significant improvement in survival, was observed when animals received BRAFi chow, intraperitoneal injection of anti-PD-1 antibody, and intratumoral injection of OncoVex^{mGM-CSF}, as manifested by the fact that a number of animals survived past 130 days (Line 5).

[000120] These results were further confirmed in Figures 4C and 4D. Survival of mice in each treatment group was plotted on a KM curve. Survival of mice in group 2 (BRAFi alone) ($p < 0.0001$), group 3 (BRAFi + α -PD1) ($p = 0.0002$), group 4 (BRAFi + OncoVex^{mGM-CSF}) ($p < 0.0001$), and group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p < 0.0001$) was significantly longer when compared to group 1 (control) (Figure 4C). Survival of mice in groups receiving BRAFi was also plotted using a KM curve and shows a trend towards significance in group 4 ($p = 0.1218$) and group 5 ($p = 0.1561$) when compared to group 2

(BRAFi alone) (Figure 4D). When more than 10% of the tumor was ulcerated, intratumoral injections were stopped. When comparing the number of mice whose treatment was stopped due to ulceration, findings showed a higher number of these mice were in groups receiving OncoVex^{mGM-CSF} while BRAFi and anti-PD1 did not affect ulceration to the same extent (Figure 4E). The size of the tumors of the mice in each group when intratumoral injections were stopped was also plotted and found that the tumor size of mice in group 5 ($p=0.0214$) and group 6 ($p=0.0074$) were significantly smaller than the tumor size of group 1 (control) mice (Figure 4F).

[000121] These experiments demonstrate that incorporation of an immune checkpoint inhibitor into OncoVex^{mGM-CSF} and BRAF inhibition combination therapy substantially improves survival of melanoma inflicted animals, which serve as a well-accepted model for human disease.

EXAMPLE 4

Combined Therapy using OncoVex^{mGM-CSF}, BRAF inhibition, and Immune Checkpoint Blockade Results in Decreased CD4+Foxp3+ Tregs Tumor Infiltration

[000122] The effect of combined OncoVex^{mGM-CSF}, BRAF inhibition, and immune checkpoint blockade therapy on intratumoral CD4+Foxp3+ Tregs infiltration is also analyzed herein. As shown in Figure 5, the percentage of CD4+ Foxp3+ Tregs was significantly lower in cells receiving anti-PD-1 therapy in addition to OncoVex^{mGM-CSF} and BRAF inhibition, compared with those that received only OncoVex^{mGM-CSF} and BRAF inhibition combined therapy.

[000123] These results were further confirmed in Figure 6. Flow cytometry was performed on tumor, spleen and lymph nodes of each mouse for analysis of tumor-infiltrating lymphocytes (TILs). Cells were stained with CD45, CD3, CD4, CD8, PD1, NK1.1, FOXP3, and fixable viability dye. The percent of CD8+/CD3+ (Cytotoxic T lymphocytes (CTLs)) was significantly higher in group 3 (BRAFi + α -PD1) ($p=0.0476$), group 4 (BRAFi + OncoVex^{mGM-CSF}) ($p=0.0004$), group 5 (BRAFi + α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0001$), and group 6 (α -PD1 + OncoVex^{mGM-CSF}) ($p=0.0001$) when compared to group 1 (control) (Figure 6A). CTL infiltration was highest in groups receiving OncoVex^{mGM-CSF} when compared to groups not receiving OncoVex^{mGM-CSF} ($p<0.0001$) (Figure 6B). The percent of CD4+FOXP3+/CD4+ (T-regulatory cells (Tregs)) is decreased in groups 4 ($p=0.0082$), 5 ($p<0.0001$), and 6 ($p=0.0031$) when compared to group 1 (control). (Figure 6C). Treg infiltration is lowest in groups receiving OncoVex^{mGM-CSF} when compared to groups not

receiving OncoVex^{mGM-CSF} ($p < 0.0001$) (Figure 6D). No trends were found in the spleen and lymph nodes.

EXAMPLE 5

Analysis of Tumor Samples Exposed to Combined Therapy Including OncoVex^{mGM-CSF}, BRAF inhibition, and Immune Checkpoint Blockade

[000124] Tumor tissue was zinc fixed and paraffin embedded at end of study to perform quantitative multiplex immunofluorescence (qmIF) using VectraTM (Perkin Elmer), a quantitative pathology workstation, allowing for evaluation of density and spatial relationships of immune cells. Tumor tissue from 36 mice was stained for qmIF (6 mice from each treatment group) for CD3, CD4, CD8, FOXP3, PDL1, F4/80, and DAPI. Multispectral images of these slides are obtained and are analyzed using inFormTM software (Perkin Elmer).

[000125] 6 mice are enrolled in each of the same 6 treatments groups (total of 36 mice) and tumors are analyzed from sacrificed mice at 30 days of treatment. Tumor tissues from these mice are formalin-fixed paraffin-embedded (FFPE) for NanoString analysis, to profile immune-related genes, as well as confirm infection of OncoVex^{mGM-CSF}, using the Mouse Immunology Panel and spiking in early, immediate early, and late HSV genes. 13 mice have completed this portion of the study. 12 mice are currently enrolled and the additional 11 mice are being bred and induced.

[000126] In other experiments, multiplex immunohistochemistry analyses are performed on slides cut from paraffin embedded blocks with dissected tumors from treatment mice. The slides are stained with antibodies for CD3, CD8, HSV, BRAF, FoxP3, and CD4. This panel is optimized and final antibodies are determined once of optimization is complete.

REFERENCES

1. Beeram et al. Raf: A Strategic Target for Therapeutic Development Against Cancer. Journal of Clinical Oncology (2005), 23(27):6771-6790).
2. Brignone et al. A soluble form of lymphocyte activation gene-3 (IMP321) induces activation of a large range of human effector cytotoxic cells. J Immunol. 2007 Sep 15;179(6):4202-11.
3. Dankort et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet. 2009 May;41(5):544-52.
4. Davies et al. Mutations of the *BRAF* gene in human cancer. Nature. 2002 Jun 27;417(6892):949-54.
5. Dotti, G. Blocking PD-1 in cancer immunotherapy. Blood. 2009 Aug 20;114(8):1457-8.
6. Drew M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012 Mar 22;12(4):252-64.
7. Droeser et al. Differential pattern and prognostic significance of CD4+, FOXP3+ and IL-17+ tumor infiltrating lymphocytes in ductal and lobular breast cancers. BMC Cancer. 2012; 12:134.
8. Fourcade et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med. 2010 Sep 27;207(10):2175-86.
9. Halilovic and Solit. Therapeutic strategies for inhibiting oncogenic BRAF signaling. Curr Opin Pharmacol. 2008 Aug;8(4):419-26.
10. Harrington et al., Phase I/II dose escalation study of OncoVexGM-CSF and chemoradiotherapy (CRT) in untreated stage III/IV squamous cell cancer of the head and neck (SCCHN) J Clin Oncol. 27(15a):abstract 6018, 2009.
11. Hooijkaas et al., Targeting *BRAFV600E* in an Inducible Murine Model of Melanoma. Am J Pathol. 2012 Sep;181(3):785-94).
12. Hunter et al. Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation of intracerebral injection in nonhuman primates. J Virol. 1999 Aug;73(8):6319-26.
13. Kaufman and Bines, OPTIM trial: a Phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma, Future Oncol. 2010 June 6(6): 941-949.

14. Kaufman et al. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. Ann Surg Oncol. 2010 Mar;17(3):718-30.
15. Lee et al. BRAF mutations in non-Hodgkin's lymphoma. Br J Cancer. 2003 Nov 17;89(10):1958-60.
16. Liu et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther. 2003 Feb;10(4):292-303.
17. Loo et al. Development of an Fc-enhanced anti-B7-H3 monoclonal antibody with potent antitumor activity. Clin Cancer Res. 2012 Jul 15;18(14):3834-45.
18. MacKie et al. Intralesional injection of herpes simplex virus 1716 in metastatic melanoma. Lancet. 2001 Feb 17;357(9255):525-6.
19. Malek and Castro. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity. 2010 Aug 27;33(2):153-65.
20. Markert et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther. 2000 May;7(10):867-74.
21. Michaloglou et al. BRAF(E600) in benign and malignant human tumours. Oncogene. 2008 Feb 7;27(7):877-95.
22. Montagut et al. Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res. 2008 Jun 15;68(12):4853-61.
23. Naoki et al. Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer Res. 2002 Dec 1;62(23):7001-3.
24. Rajagopalan et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature. 2002 Aug 29;418(6901):934.
25. Rampling et al. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. Gene Therapy 2000; 7: 859-866.
26. Roberts et al. U.K. guidelines for the management of cutaneous melanoma. Br J Dermatol. 2002 Jan;146(1):7-17.
27. Roizman et al. Lippincott Williams & Wilkins: Philadelphia. 2013. p. 1823–1897.
28. Sakuishi et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med. 2010 Sep 27;207(10):2187-94.
29. Shang et al. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep. 2015 Oct 14;5:15179.

30. Tsai et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci U S A. 2008 Feb 26;105(8):3041-6.
31. Xing et al. BRAF mutation in thyroid cancer. Endocr Relat Cancer. 2005 Jun;12(2):245-62.

* * *

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The entire disclosure of each of the patent documents, including certificates of correction, patent application documents, scientific articles, governmental reports, websites, and other references referred to herein is incorporated by reference herein in its entirety for all purposes. In case of a conflict in terminology, the present specification controls.

The invention can be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are to be considered in all respects illustrative rather than limiting on the invention described herein. Further, it

should be understood that the order of steps or order for performing certain actions is immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present specification will control.

WHAT IS CLAIMED IS:

1. A method for treating a solid malignant tumor in a subject comprising: administering to the subject a therapeutically effective amount of BRAF inhibitor, an oncolytic virus, and an immune checkpoint inhibitor.
2. The method of claim 1, wherein the oncolytic virus is an HSV-1-based oncolytic virus.
3. The method of claim 1, wherein the oncolytic virus is talimogene laherparepvec.
4. The method of claim 1, wherein the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049, and combinations thereof.
5. The method of claim 1, wherein the immune checkpoint inhibitor is a PD-1 antagonist.
6. The method of claim 5, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.
7. The method of claim 1, wherein the administration of the immune checkpoint inhibitor is continued until it induces tumor regression, remission, or eradication.
8. The method of claim 1, wherein the administration of each BRAF inhibitor, oncolytic virus, and the immune checkpoint inhibitor is continued until it induces tumor regression, remission, or eradication.
9. The method of claim 1, wherein the BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.
10. The method of claim 1, wherein the solid malignant tumor is melanoma.
11. A method for treating melanoma in a subject comprising: administering to the subject a therapeutically effective amount of a BRAF inhibitor, an oncolytic virus, and an immune checkpoint inhibitor.
12. The method of claim 11, wherein the oncolytic virus is an HSV-1-based oncolytic virus.
13. The method of claim 11, wherein the oncolytic virus is talimogene laherparepvec.

14. The method of claim 11, wherein the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049, and combinations thereof.
15. The method of claim 11, wherein the immune checkpoint inhibitor is a PD-1 antagonist.
16. The method of claim 11, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.
17. The method of claim 11, wherein the administration of the checkpoint inhibitor is continued until it induces melanoma tumor regression, remission, or eradication.
18. The method of claim 11, wherein the administration of each BRAF inhibitor, oncolytic virus, and checkpoint inhibitor is continued until it induces melanoma tumor regression, remission, or eradication.
19. The method of claim 11, wherein the BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.
20. A method of reducing CD4+FOXP3+ cellular population within a solid malignant tumor, the method comprising delivering to the tumor an effective amount of a BRAF inhibitor, an oncolytic virus, and a checkpoint inhibitor.
21. The method of claim 20, wherein the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049 and combinations thereof.
22. The method of claim 20, wherein the immune checkpoint inhibitor is a PD-1 antagonist.
23. The method of claim 20, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.
24. The method of claim 20, wherein BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.
25. A method of stimulating an immune response in a subject comprising: administering to the subject a therapeutically effective amount of a BRAF inhibitor, an oncolytic virus, and a checkpoint inhibitor.

26. The method of claim 25, wherein the immune checkpoint inhibitor is selected from a small molecule, protein, peptide, antibody or antigen binding fragment thereof, directed against CTLA-4, PD-L1, PD-L2, PD-1, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD 160, CGEN- 15049, and combinations thereof.
27. The method of claim 25, wherein the immune checkpoint inhibitor is a PD-1 antagonist.
28. The method of claim 25, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.
29. The method of claim 25, wherein the administration of each BRAF inhibitor, oncolytic virus, and checkpoint inhibitor is continued until stimulation of the immune response is achieved.
30. The method of claim 25, wherein the BRAF inhibitor is selected from the group consisting of PLX4720, Sorafenib, RAF265, XL281, AZ628, GSK2118436 (dabrafenib), GDC-0879, and PLX4032 (vemurafenib), or a derivative thereof.

Figure 1

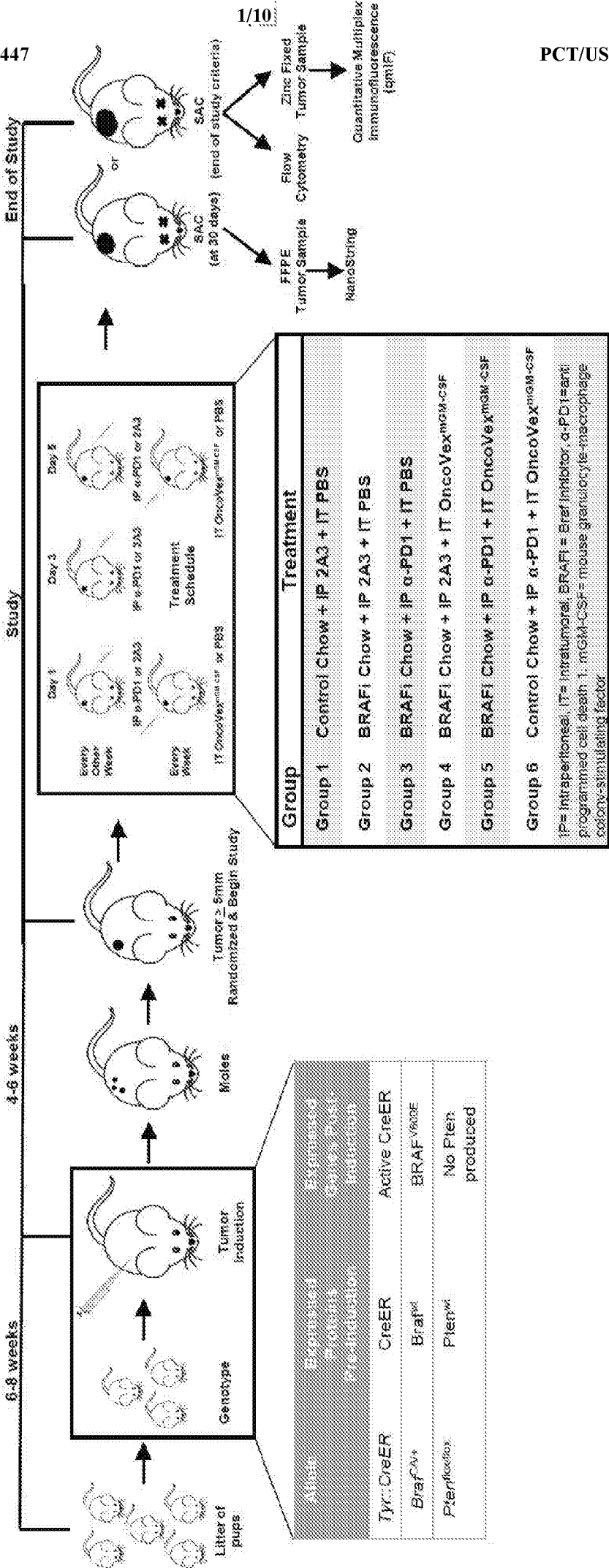


Figure 4A

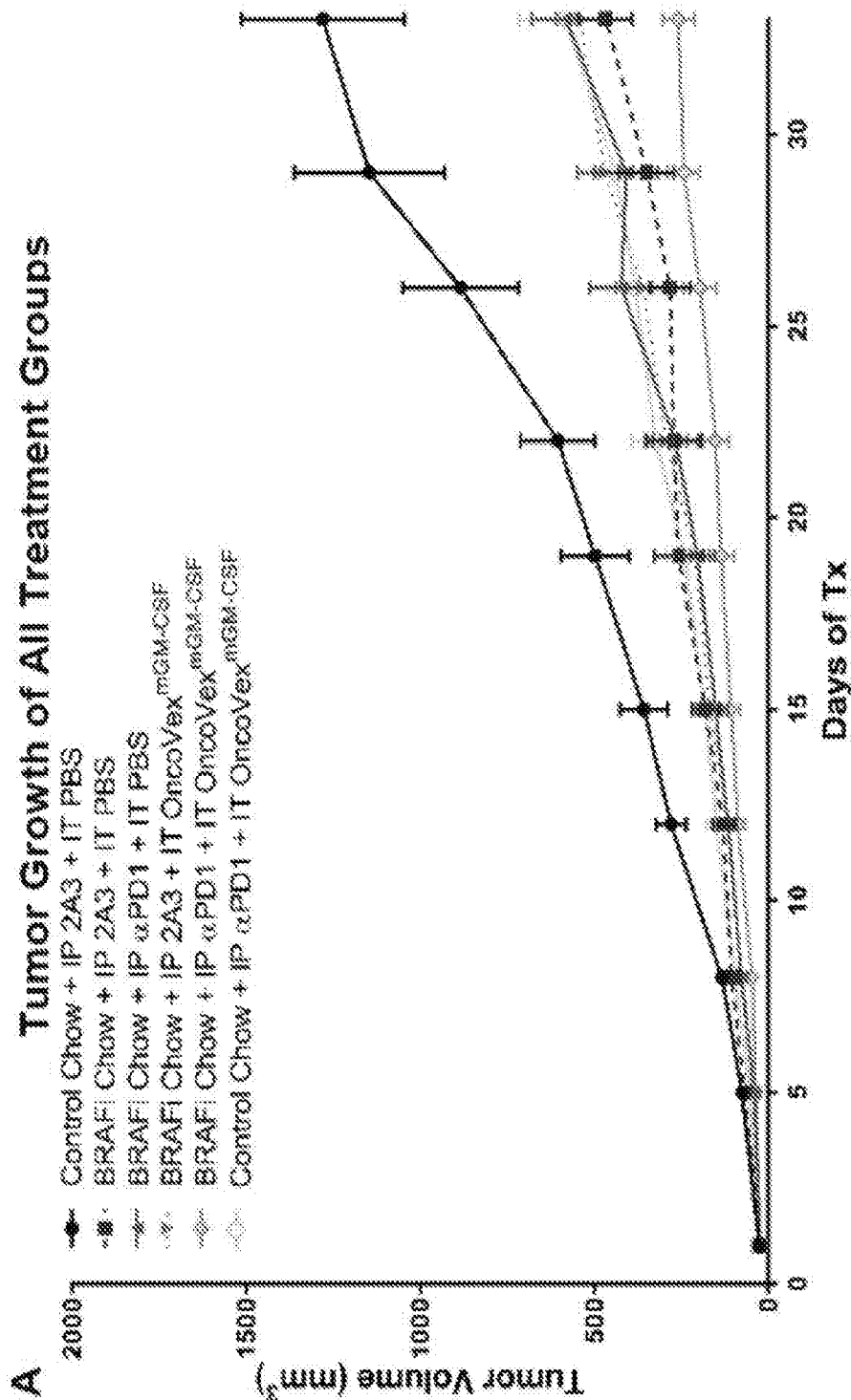


Figure 4B

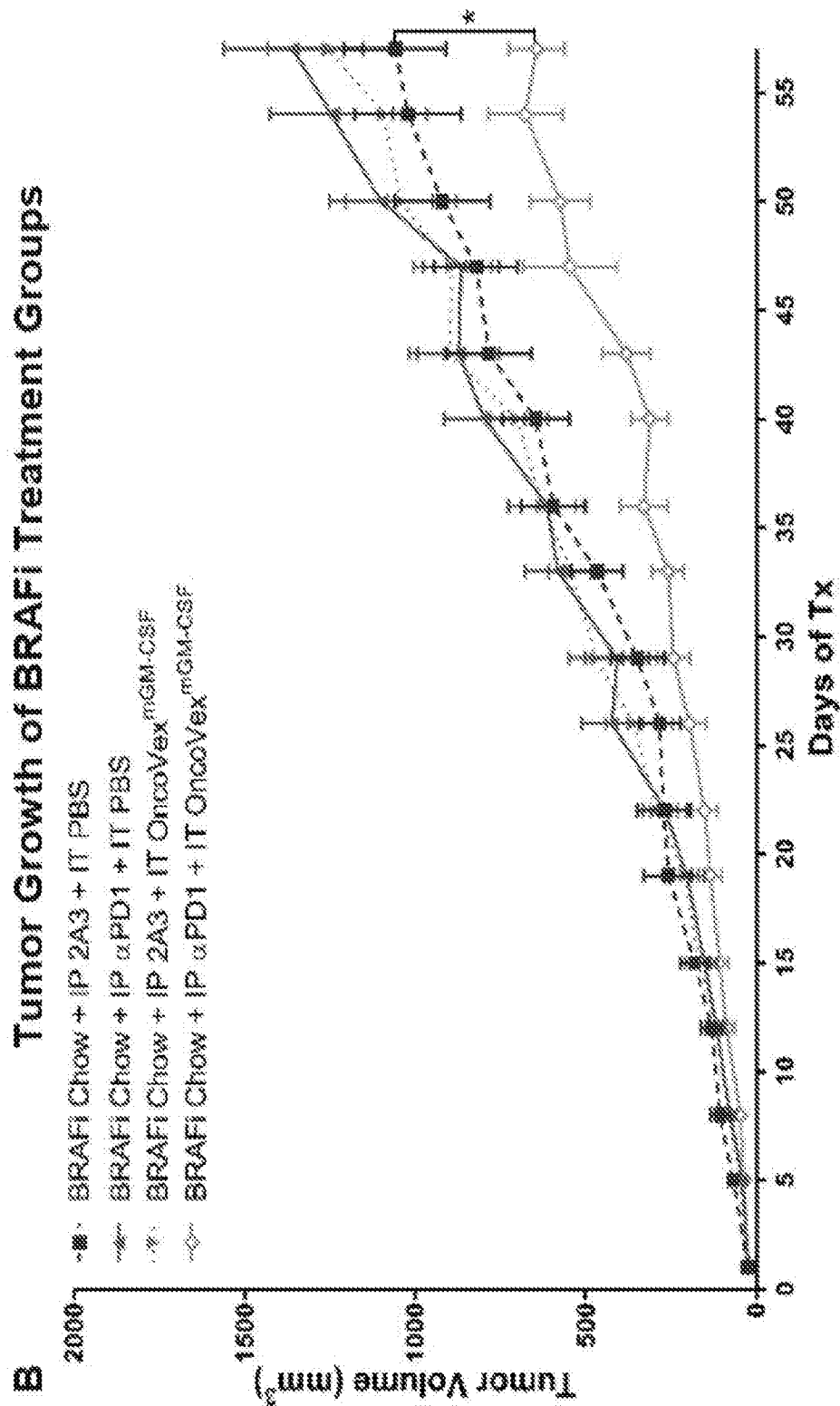
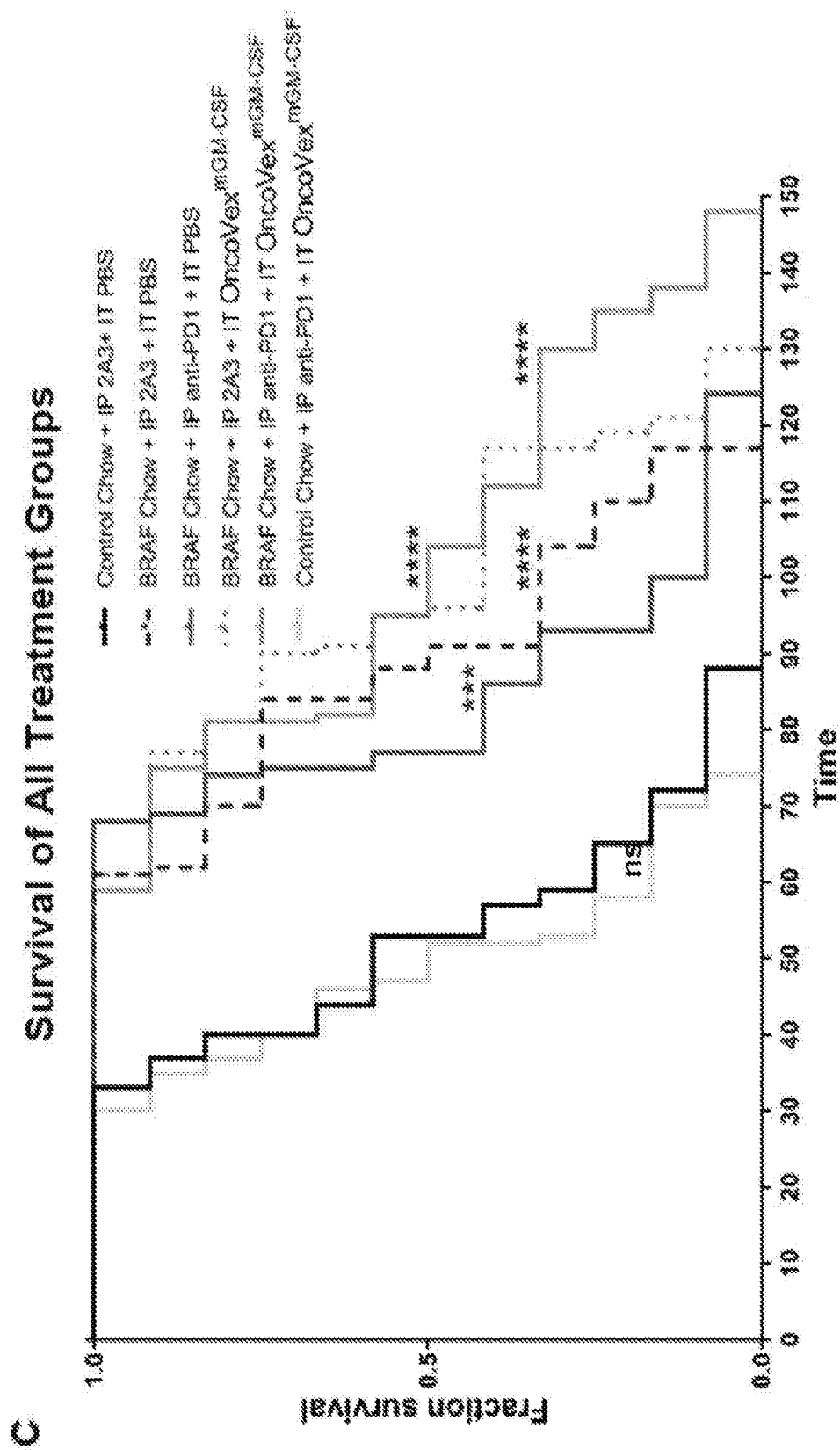


Figure 4C



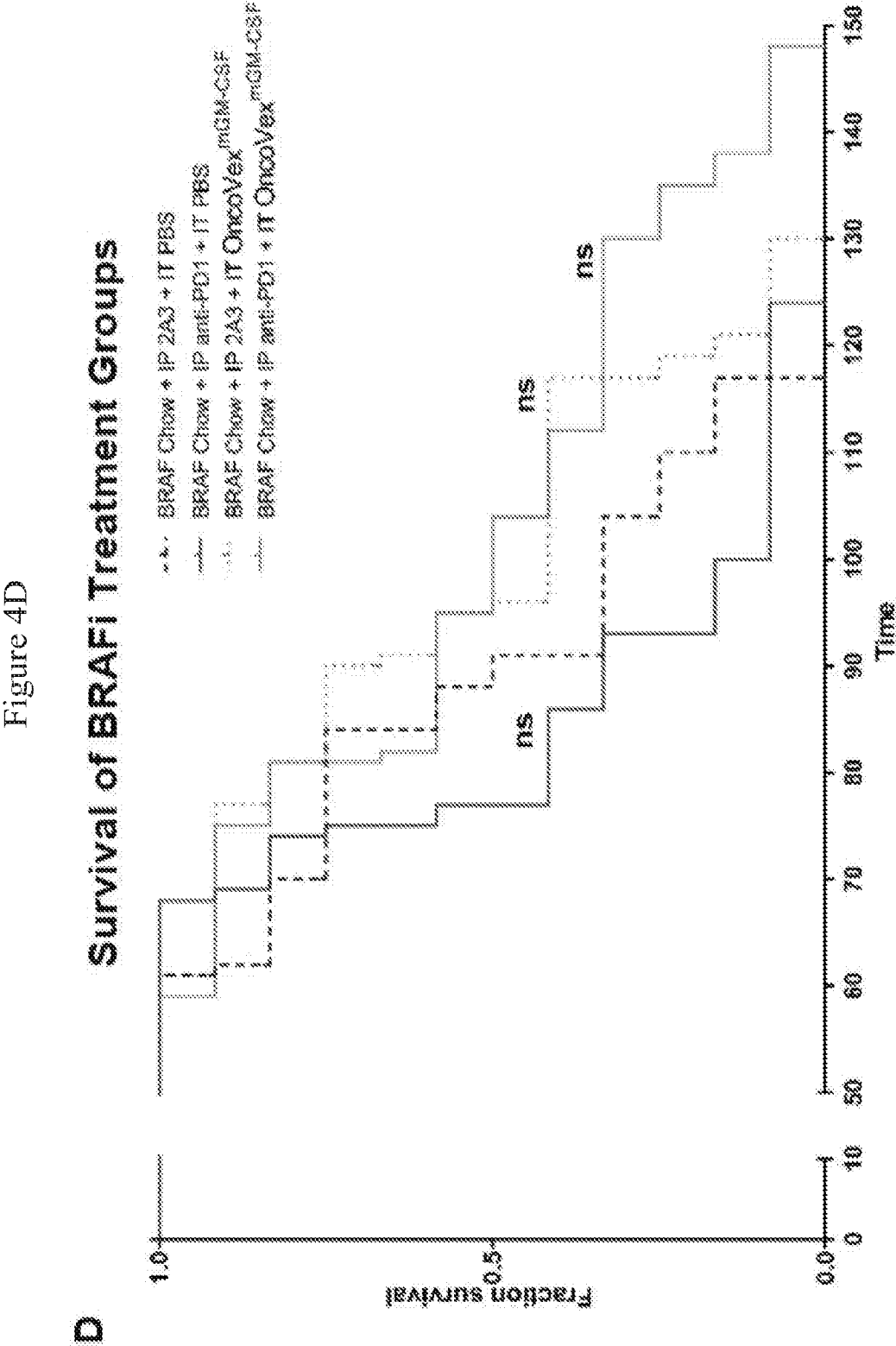


Figure 4E

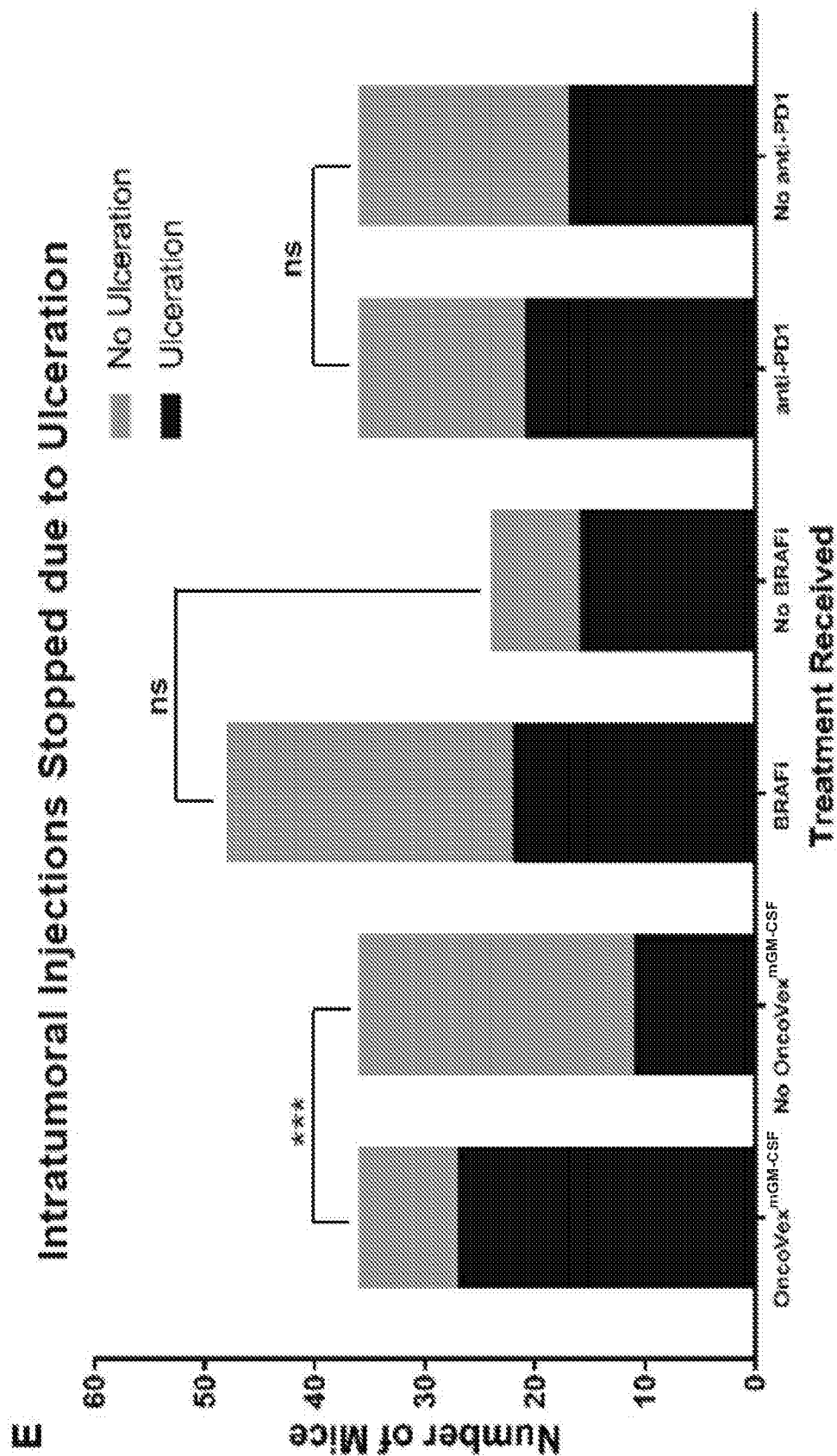


Figure 4F

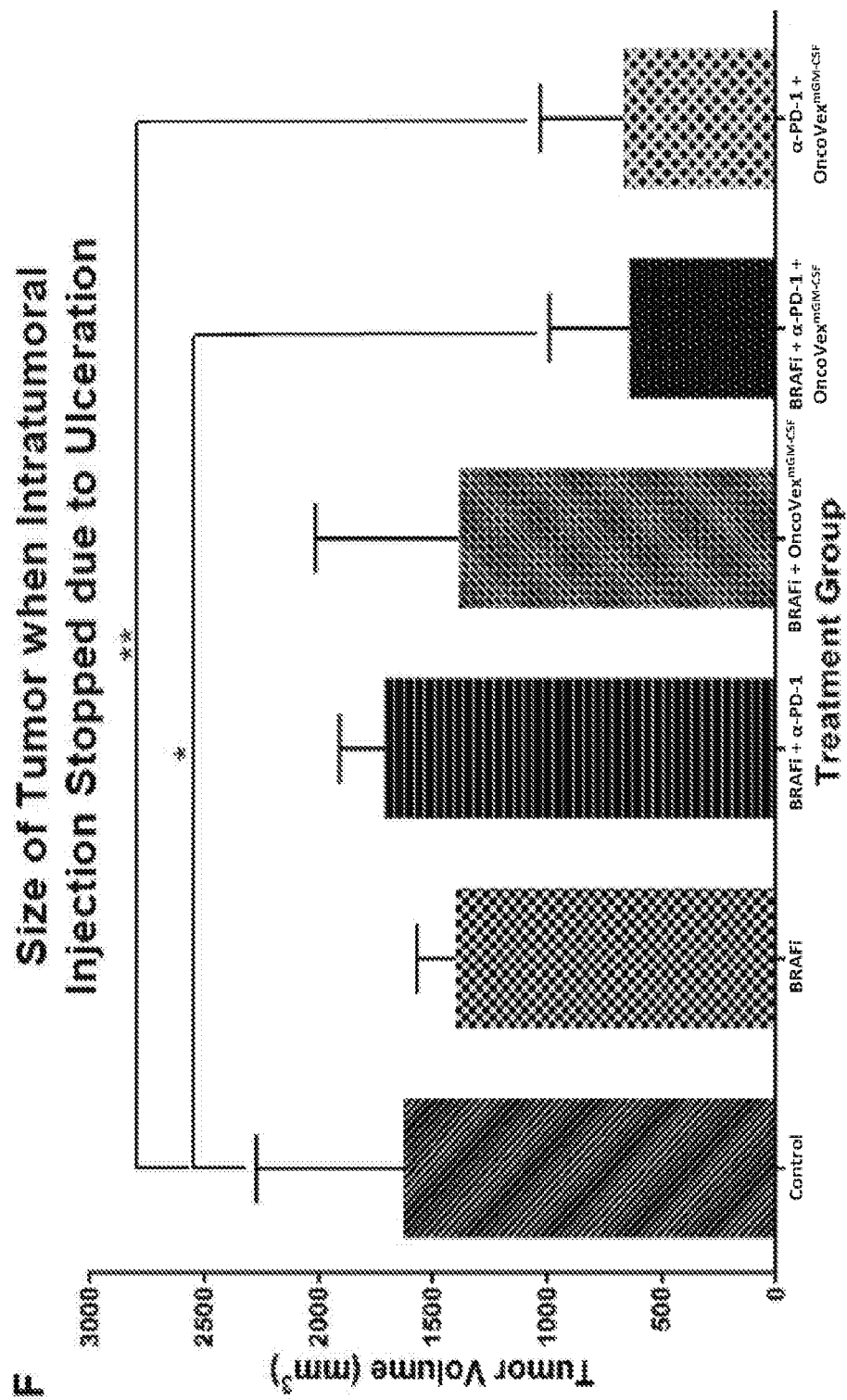


Figure 5
Tumor CD4+FOXP3+/CD4+

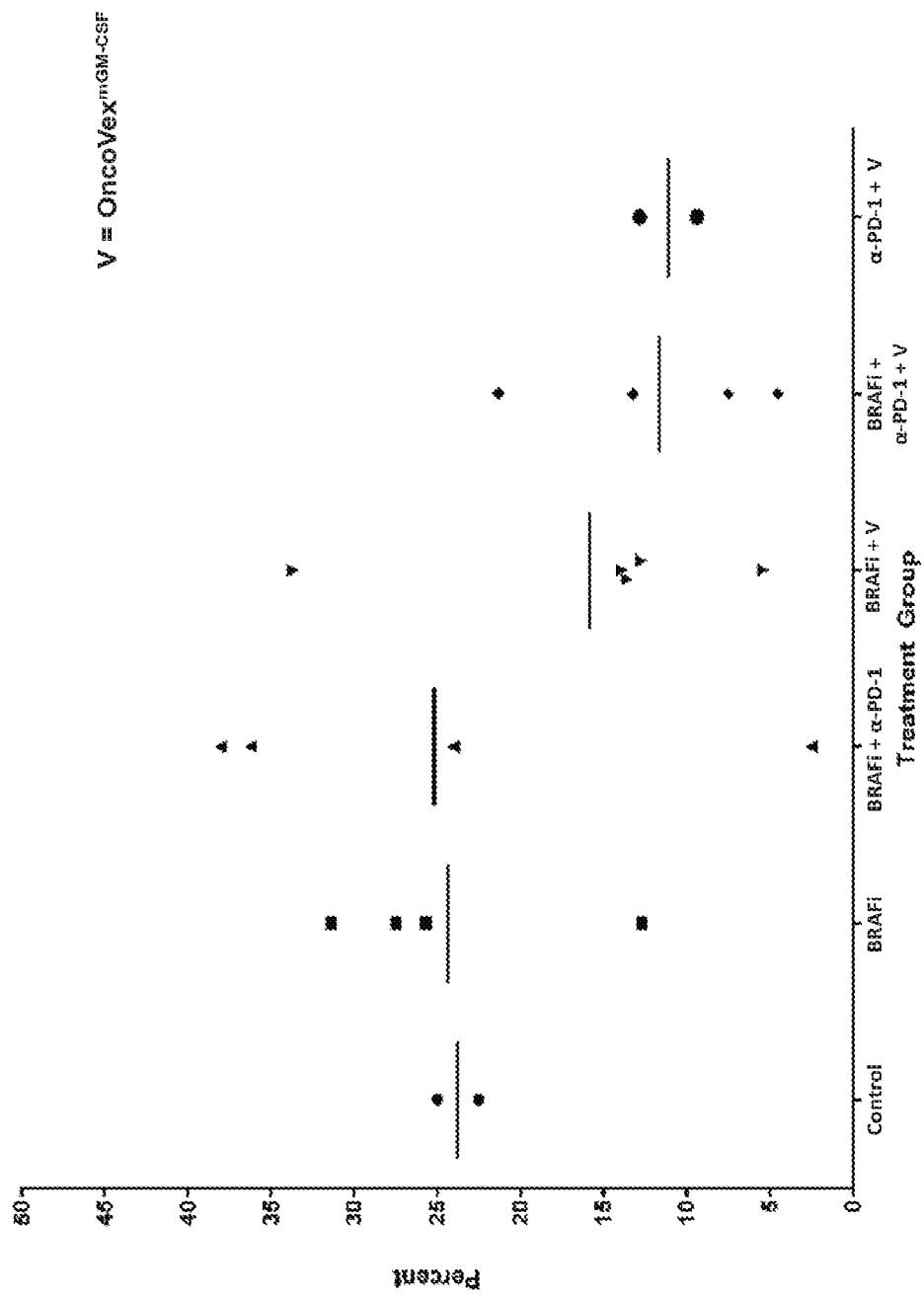


Figure 6A and 6B

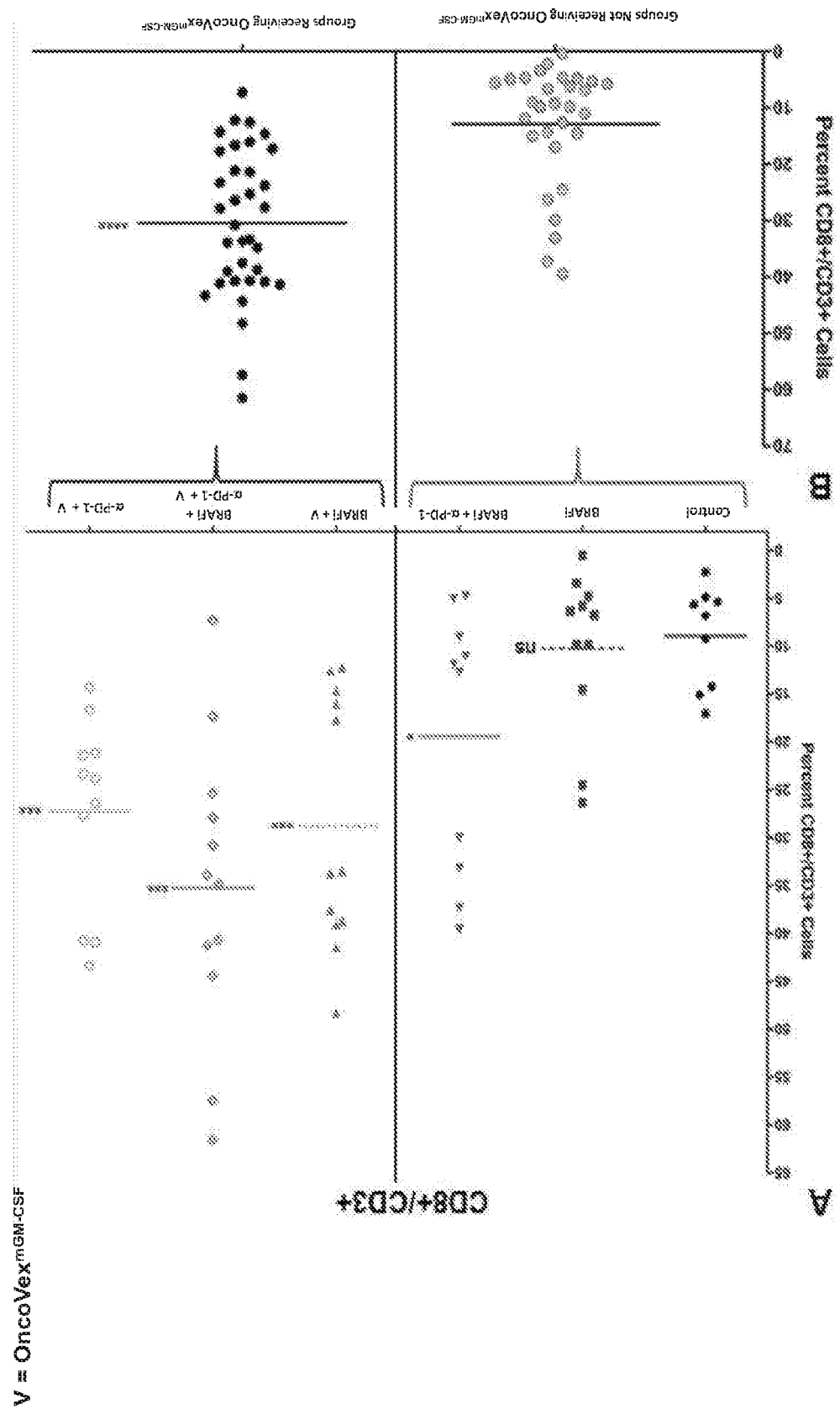
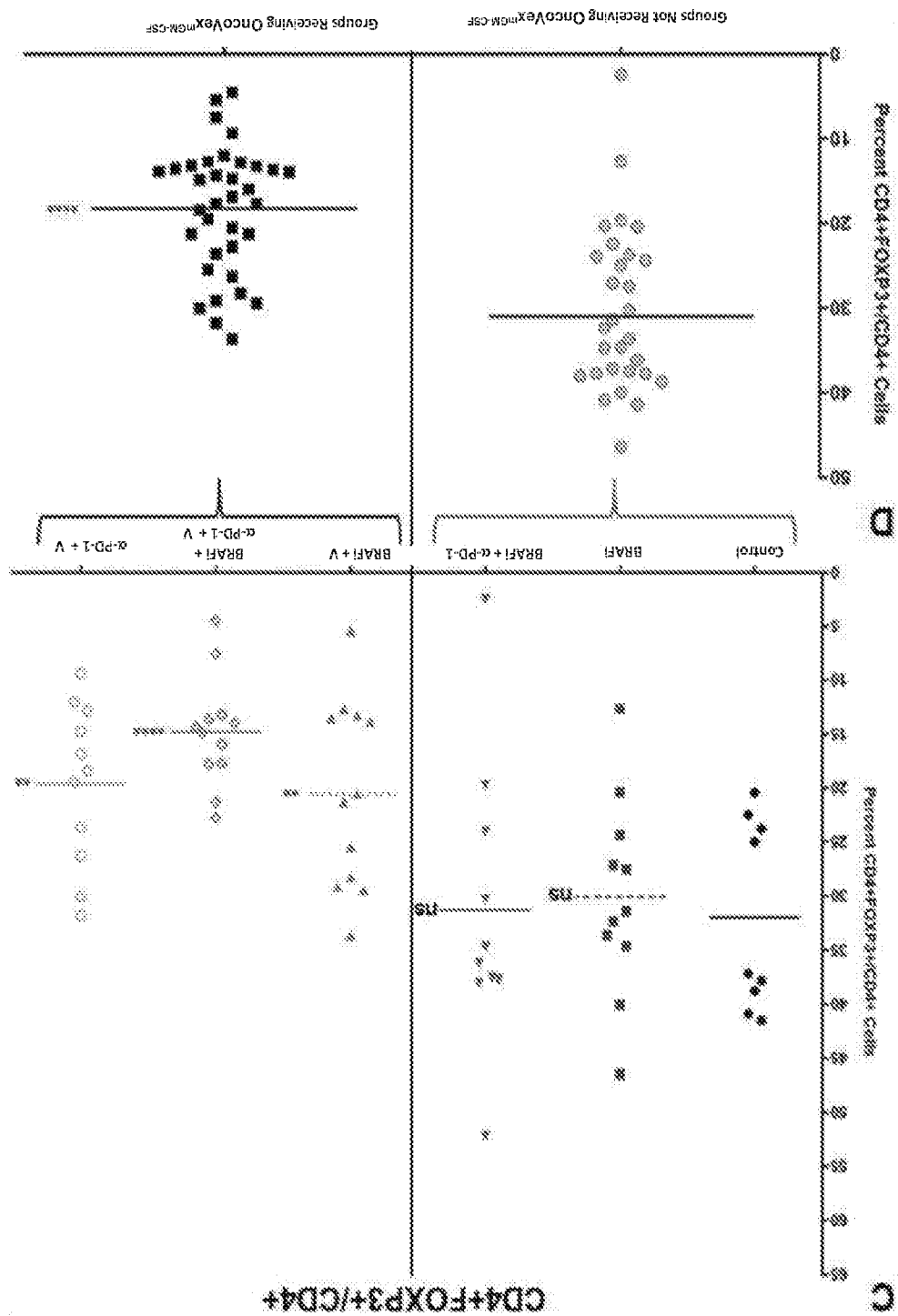


Figure 6C and 6D



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/056878

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/437 A61K35/763 A61K39/395 C12N7/01 A61P35/00
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 A61P C12N

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	WO 2014/036412 A2 (AMGEN INC [US]) 6 March 2014 (2014-03-06) claims 1, 9-15 -----	1-30
X	WO 2015/069770 A1 (COGNATE BIOSERVICES INC [US]; NORTHWEST BIOTHERAPEUTICS [US]; UNIV CAL) 14 May 2015 (2015-05-14) paragraph [0086] -----	1-30
X	EP 2 662 117 A1 (VIRTTU BIOLOG LTD [GB]) 13 November 2013 (2013-11-13) claims 3, 5 ----- -/-	1-30



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 December 2017

Date of mailing of the international search report

08/01/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Baumgärtner, Heike

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/056878

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C. MONTAGUT ET AL: "Elevated CRAF as a Potential Mechanism of Acquired Resistance to BRAF Inhibition in Melanoma", CANCER RESEARCH, vol. 68, no. 12, 15 June 2008 (2008-06-15) , pages 4853-4861, XP055279345, US ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-07-6787 the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/056878

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014036412	A2	06-03-2014	
		AU 2013308595 A1	05-03-2015
		CA 2881851 A1	06-03-2014
		CN 104704002 A	10-06-2015
		EA 201590451 A1	31-05-2016
		EP 2890714 A2	08-07-2015
		HK 1211601 A1	27-05-2016
		JP 2015526525 A	10-09-2015
		KR 20150047512 A	04-05-2015
		US 2015202290 A1	23-07-2015
		WO 2014036412 A2	06-03-2014
WO 2015069770	A1	14-05-2015	
		AU 2014346852 A1	16-06-2016
		CA 2929407 A1	14-05-2015
		CN 105828834 A	03-08-2016
		EA 201690912 A1	31-10-2016
		EP 3065772 A1	14-09-2016
		JP 2016540042 A	22-12-2016
		KR 20160093012 A	05-08-2016
		PH 12016500841 A1	04-07-2016
		US 2015202291 A1	23-07-2015
		US 2015273033 A1	01-10-2015
		WO 2015069770 A1	14-05-2015
EP 2662117	A1	13-11-2013	
		EP 2662117 A1	13-11-2013
		EP 2849852 A1	25-03-2015
		GB 2501991 A	13-11-2013
		US 2015125425 A1	07-05-2015
		WO 2013167909 A1	14-11-2013