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(71) Applicant: **IDERA PHARMACEUTICALS, INC.** [US/US]; 167 Sidney Street, Cambridge, MA 02139 (US).

(72) Inventors: **AGRAWAL, Sudhir**; 61 Lamplighter Drive, Shrewsbury, MA 01545 (US). **WANG, Daqing**; 7 Selfridge Road, Bedford, MA 01730 (US). **JIANG, Wayne**; 15 Lyman Terrace, Waltham, MA 02452 (US).

(74) Agent: **HAYMAN, Mark, L.** et al.; Morgan, Lewis & Bockius LLP, 1111 Pennsylvania Avenue, Nw, Washington, DC 20004 (US).

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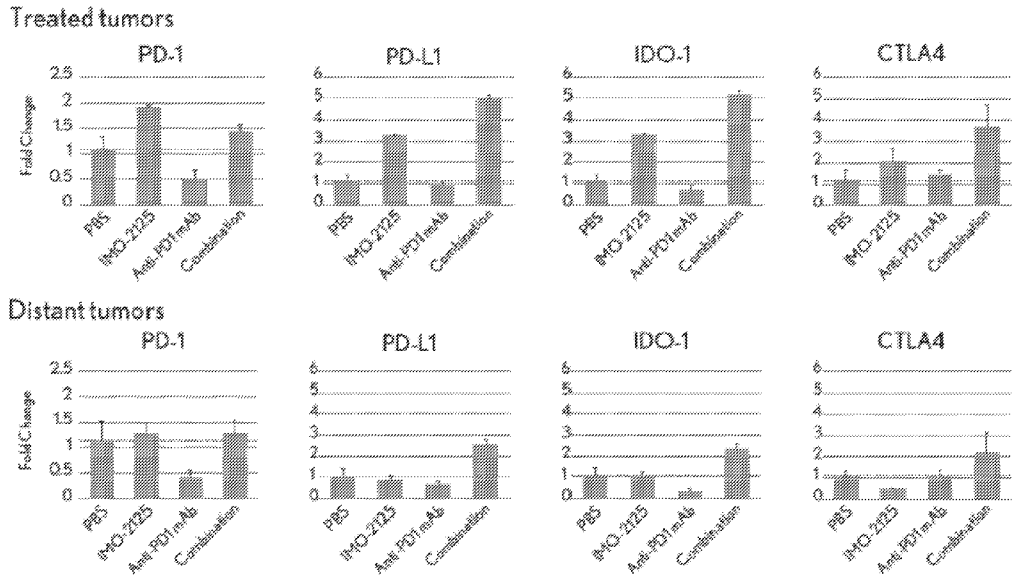
(54) Title: IMMUNE MODULATION WITH TLR9 AGONISTS FOR CANCER TREATMENT

FIG. 10 (CONT.)

D.



(57) Abstract: The present invention relates to methods for treating a tumor, including a metastatic tumor, with TLR9 agonist in combination with an immune checkpoint inhibitor therapy.

[Continued on next page]

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IMMUNE MODULATION WITH TLR9 AGONISTS FOR CANCER TREATMENT

PRIORITY

This Application claims priority to, and the benefit of, US Provisional Application No. 62/394,845 filed September 15, 2016, and US Provisional Application No. 62/486,738 filed April 18, 2017, each of which is hereby incorporated by reference in its entirety.

FIELD

The invention relates to the field of oncology, and use of immunotherapy in the treatment of cancer.

BACKGROUND

Toll-like receptors (TLRs) are present on many cells of the immune system and are involved in the innate immune response. In vertebrates, this family consists of eleven proteins called TLR1 to TLR11 that recognize pathogen associated molecular patterns from bacteria, fungi, parasites, and viruses. TLRs are a key mechanism by which vertebrates recognize and mount immune responses to foreign molecules and also provide a link between the innate and adaptive immune responses. Some TLRs are located on the cell surface to detect and initiate a response to extracellular pathogens and other TLRs are located inside the cell to detect and initiate a response to intracellular pathogens.

TLR9 recognizes unmethylated CpG motifs in bacterial DNA and in synthetic oligonucleotides. While agonists of TLR9, and other TLR agonists, can initiate anti-tumor immune responses, TLR agonists can also induce immune suppressive factors that may be counterproductive for effective tumor responses.

There is a need for cancer immunotherapies that induce antitumor responses, and keep the immune system productively engaged to improve the overall response.

SUMMARY

In various aspects, the present invention provides a method for treating a tumor, including, without limitation, metastatic melanoma, comprising intratumorally administering an oligonucleotide TLR9 agonist (e.g., IMO-2125 or other immunostimulatory oligonucleotides described herein) to a cancer patient in combination with immunotherapy with an immune checkpoint inhibitor therapy, such as a therapy targeting CTLA-4, PD-1/PD-L1/PD-L2, TIM3, LAG3, and/or IDO. The TLR9 agonist upon intratumoral injection induces global increases in expression of checkpoint genes, including IDO1, PDL1, PD1, IDO2, CEACAM1, OX40, TIM3, LAG3, CTLA4, and OX40L. By altering immune signaling in the tumor microenvironment, such changes in gene expression provide opportunities to improve responsiveness to checkpoint inhibitor therapy, including in some embodiments, a complete response. The invention further provides the opportunity to balance anti-tumor responses with inhibitory signals, thereby also minimizing immune-related adverse events (irAEs) of checkpoint inhibitor therapy.

In various embodiments, the patient has a cancer that was previously unresponsive to, or had become resistant to, a checkpoint inhibitor therapy, such as anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or anti-PD-L2 agent. The invention finds use for treating primary cancer or a metastatic cancer, including cancers that originate from skin, colon, breast, or prostate, among other tissues. In some embodiments, the cancer is progressive, locally advanced, or metastatic carcinoma. In some embodiments, the cancer is metastatic melanoma.

In accordance with embodiments of the invention, the immunostimulatory oligonucleotide (e.g., IMO-2125) is administered intratumorally. Intratumoral administration alters immune signaling in the tumor microenvironment, priming the immune system for an effective anti-tumor response, while inducing changes that are compatible with more effective checkpoint inhibitor therapy. For example, the TLR9 agonist (e.g., IMO-2125) may be administered intratumorally at from about 4 mg to about 64 mg per dose, with from about 3 to about 12 doses being administered over 10 to 12 weeks. For example, therapy may be initiated with 3 to 5 weekly doses of IMO-2125, optionally followed by 3 to 8 maintenance doses, which are administered about every three weeks.

During the regimen of IMO-2125 (or other TLR9 agonist), one or more checkpoint inhibitor therapies are administered to take advantage of the changes in immune signaling. In some embodiments, the patient receives an anti-CTLA-4 agent (e.g., ipilimumab or tremelimumab) and/or an anti-PD-1 agent (e.g., nivolumab or pembrolizumab). The immune checkpoint inhibitor can be administered parenterally, such as, in some embodiments, subcutaneously, intratumorally, intravenously. For example, in various embodiments the immune checkpoint inhibitor is administered at a dose of from about 1 mg/kg to about 5 mg/kg intravenously. The initial dose of the immune checkpoint inhibitor can be administered at least one week after the initial TLR9 agonist dose, for example in about weeks 2, 3 or 4. In some embodiments, the immunotherapy agent is administered from about 2 to about 6 times (e.g., about 4 times, preferably every three weeks).

In some embodiments, IMO-2125 is administered intratumorally to a metastatic melanoma patient previously found to be unresponsive or only partially responsive to PD-1 blockade therapy. For example, IMO-2125 is administered at a dose of from 4 to 32 mg per dose in weeks 1, 2, 3, 5, 8, and 11, with ipilimumab i.v. at 3 mg/kg. Ipilimumab can be administered every three weeks, beginning in week 2. Alternatively, pembrolizumab can be administered i.v. at 2 mg/kg every three weeks beginning on week 2.

The present methods in various embodiments allow for a robust anti-tumor immune response (which in some embodiments is a complete response), and which does not come at the expense of significant side effects, *e.g.* relative to side effects observed when one or more immunotherapies are used in the absence of the TLR9 agonist. Such side effects include commonly observed immune-related adverse events that affect various tissues and organs including the skin, the gastrointestinal tract, the kidneys, peripheral and central nervous system, liver, lymph nodes, eyes, pancreas, and the endocrine system; such as hypophysitis, colitis, hepatitis, pneumonitis, rash, and rheumatic disease (among others).

Other aspects and embodiments will be apparent from the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows tumor growth reduction in a CT26.CL25 tumor model with IMO-2125 monotherapy. Tumor volume for treated tumors and distant tumors is shown.

FIG. 2 shows, in panel A, tumor infiltrating lymphocytes in tumor nodules from Day 28 of the experiment shown in FIG. 1. Magnification is x 400. In panel B, FACS data shows $CD8^+$ T cells tumor infiltration with IMO-2125 monotherapy (0.5 mg/kg).

FIG. 3 shows assays to demonstrate specific cytotoxic T cell responses to tumor antigens.

FIG. 4 shows, in panel A, a study design to evaluate the relationship of intratumoral IMO-2125 antitumor activity and infiltrating $CD4^+$ and $CD8^+$ T cells. Panel B shows the impact of $CD4^+$ and $CD8^+$ T cell depletion in treated and distal tumors.

FIG. 5 shows, in panel A, a study design to evaluate the duration and specificity of the antitumor response induced by intratumoral IMO-2125 treatment. Panel B shows the tumor growth of mice rechallenged with CT26 or A20 and intratumoral IMO-2125.

FIG. 6 shows a tumor study in the A20 model comparing intratumor and subcutaneous administration. Panel A shows the study design and tumor kinetics while panel B shows the presence of tumor-infiltrating lymphocytes (TILs) and changes in gene expression of various checkpoint genes.

FIG. 7 shows, in panel A, a study design to evaluate the antitumor activity of intratumoral IMO-2125 in combination with anti-CTLA-4 mAb on treated tumors and systemic lung metastases. **FIG. 7**, panel B shows the anti-tumor effects of intratumoral IMO-2125 and anti-CTLA-4 mAb alone or in combination.

FIG. 8 shows anti-tumor activities of IMO-2125 and anti-CTLA-4 mAb alone or in combination on systemic lung metastasis. Panel A shows number of lung tumor nodules in the various treatment groups and panel B shows images of tumors in the various treatment groups (pictures taken on Day 13 after tumor implantation).

FIG. 9 shows TILs in metastatic nodules in the various treatment groups (CD3 IHC stain x 400).

FIG. 10 shows an evaluation of the antitumor activity of intratumoral IMO-2125 in combination with anti-PD-1 mAb in CT26 colon carcinoma tumor model. Panel A shows the study design. Panel B shows the impact of the combination on tumor growth kinetics at treated and distal sites. Panel C shows the impact of the combination on TILs (magnifications are shown). Panel D shows checkpoint gene expression at treated and distal sites after treatment with the combination.

FIG. 11 shows an evaluation of the antitumor activity of intratumoral IMO-2125 in combination with anti-PD-1 mAb on treated tumors and systemic lung metastases in a B16 melanoma model. Panel A shows the study design. Panel B shows the impact of the combination on tumor growth kinetics at treated sites. Panel C shows the combination's impact on lung metastases. Panel D shows histopathology of metastatic lung tumors (Circle: Large tumor nodule, Arrow: Small tumor nodule, Inset figures: HE stained (x 40), and Large figures: CD3 stained (x 400)).

FIG. 12 shows a study design to evaluate the antitumor activity of intratumoral IMO-2125 in combination with an IDO-1 inhibitor on treated tumors and systemic lung metastases.

FIG. 13 shows that intratumoral IMO-2125 anti-tumor activity is potentiated by co-treatment with an IDO-1 inhibitor. Panel A shows the number of lung tumor nodules in each treatment group. Panel B shows the change in tumor volume in each treatment group during the regimen.

FIG. 14 provides a dosing overview in a study population of adults with **unresectable or metastatic melanoma that progressed with ≥12 weeks of PD-1-directed** therapy (alone or in combination).

FIG. 15 shows dendritic cell maturation results pre-dose and 24 hours post i.t. IMO-2125 injection for patient 003 (4 mg doses of IMO-2125) (Panel A); and shows T-cell activation results in injected and distant tumors (Panel B).

FIG. 16 shows expansion of top cell clones in distant lesions and induction of **IFN-γ for patient 003 (4 mg IMO-2125).**

FIG. 17 shows tumor imaging pre- and post-therapy for patient 004 (8 mg 2125).

DETAILED DESCRIPTION

In various aspects, the present invention provides a method for treating a tumor, *e.g.* a metastatic tumor (including, without limitation, metastatic melanoma) comprising intratumorally administering an oligonucleotide TLR9 agonist (e.g., IMO-2125) to a cancer patient, in combination with immunotherapy with an immune checkpoint inhibitor therapy, such as a therapy targeting CTLA-4, PD-1/PD-L1/PD-L2, LAG3, TIM3, and/or IDO.

Exemplary immune checkpoint inhibitors include anti-PD-1, anti-PD-L1, anti-PD-L2, and anti-CTLA-4 agents. PD-1/PD-L1/PD-L2 antibodies inhibit the interaction between PD-1 and its ligands (PD-L1 and PD-L2) on tumor cells to promote immune-mediated tumor destruction. CTLA-4 antibodies block the inhibitory signals to T-cells transmitted by CTLA-4. While PD-1 antibodies and CTLA-4 antibodies have emerged as important therapeutic options for a variety of cancers, many patients fail to respond. For example, some melanoma patients show no response to anti-PD-1 treatment, or even progress, after 12 weeks of treatment. Further, immune checkpoint blockade is associated with various immune-related adverse events, which can affect various tissues and organs including the skin, the gastrointestinal tract, the kidneys, peripheral and central nervous system, liver, lymph nodes, eyes, pancreas, and the endocrine system. These immune-related adverse events (irAEs) can be severe, or even fatal, and may require discontinuation of therapy. Examples of common irAEs are hypophysitis, colitis, hepatitis, pneumonitis, rash, and rheumatic disease.

Expression of the various immune checkpoint molecules on cells of the immune system induces a complex series of events that determines whether an immune response will be effective to combat the tumor, or otherwise result in immune tolerance. For example, increased expression of PD-1 on dendritic cells (DCs) promotes apoptosis of activated DCs, a critical antigen presenting cell for anti-tumor immune responses. Park SJ, Negative role of inducible PD-1 on survival of activated dendritic cells, *J. Leukocyte Biology* 95(4):621-629 (2014). Further, expression of IDO, PD-L1, and CTLA-4 in the peripheral blood of melanoma patients and can be associated with advanced disease and negative outcomes, and are interconnected, suggesting that

multiple immune checkpoints might require targeting to improve therapy in some cases. Chevolet I, et al., Characterization of the *in vivo* immune networks of IDO, tryptophan metabolism, PD-L1, and CTLA-4 in circulating immune cells in melanoma, *Oncoimmunology* 4(3) e982382-7 (2015).

The TLR9 agonist known as IMO-2125, which is described more fully herein, upon intratumoral injection induces global increases in expression of checkpoint genes, including IDO1 (5.3 fold), PDL1 (2.6 fold), PD1 (2.5 fold), IDO2 (5.9 fold), CEACAM1 (2.1 fold), OX40 (1.4 fold), TIM3 (2.9 fold), LAG3 (1.9 fold), CTLA4 (1.8 fold), and OX40L (1.5 fold). See FIG. 6B. By altering immune signaling in the tumor microenvironment, such changes in gene expression provide opportunities to improve responsiveness with checkpoint inhibitor therapy, and to achieve lasting anti-tumor immunity. Further, by targeting a single immune checkpoint molecule selected from the stronger inhibitory signals of PD-1 or CTLA-4, in connection with the robust activation of antigen presenting cells (e.g., DCs) and priming of T cells with IMO-2125, the invention provides the opportunity to balance anti-tumor responses with inhibitory signals, thereby also minimizing irAEs of checkpoint inhibitor therapy.

In various embodiments, the patient has a cancer that was previously unresponsive to, or had become resistant to, a checkpoint inhibitor therapy. For example, the cancer may be refractory or insufficiently responsive to an immunotherapy, such as anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or PD-L2 agent, including for example, one or more of ipilimumab, tremelimumab, pembrolizumab and nivolumab. In various embodiments, the cancer patient has progressed after or during treatment with an anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or PD-L2 agent, including for example, one or more of ipilimumab, tremelimumab, pembrolizumab and nivolumab (or agents related thereto) or shown no response to such treatment for at least about 12 weeks.

Other immune checkpoint inhibitors can be administered alone (e.g, in place of) or in combination with anti-CTLA4 or anti-PD-1/anti-PD-L1, such as an inhibitor of IDO (e.g., IDO-1 or IDO-2), LAG3, TIM3, among others. These and other immune checkpoint inhibitors are described in US 2016-0101128, which is hereby incorporated by reference in its entirety. For example, the patient may further receive a regimen of an IDO-1 inhibitor such as Epacadostat.

In various embodiments, the cancer is a primary cancer or a metastatic cancer. A primary cancer refers to cancer cells at an originating site that become clinically detectable, and may be a primary tumor. "Metastasis" refers to the spread of cancer from a primary site to other places in the body. Cancer cells can break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasize) in normal tissues elsewhere in the body. Metastasis can be local or distant.

The cancer may have an origin from any tissue. The cancer may originate from skin, colon, breast, or prostate, and thus may be made up of cells that were originally skin, colon, breast, or prostate, respectively. The cancer may also be a hematological malignancy, which may be lymphoma. In various embodiments, the primary or metastatic cancer is lung cancer, kidney cancer, prostate cancer, cervical cancer, colorectal cancer, pancreatic cancer, ovarian cancer, urothelial cancer, gastric/GEJ cancer, head and neck cancer, glioblastoma, Merkel cell cancer, head and neck squamous cell carcinoma (HNSCC), non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer, prostate cancer (e.g. hormone-refractory) and hematologic malignancies.

In some embodiments, the cancer is progressive, locally advanced, or metastatic carcinoma. In some embodiments, the cancer is metastatic melanoma, and may be recurrent. In some embodiments, the metastatic melanoma is stage III or IV, and may be stage IVA, IVB, or IVC. The metastasis may be regional or distant.

IMO-2125 and related immunostimulatory oligonucleotides target TLR9, and act as TLR9 agonists to alter immune signaling in the tumor microenvironment, and induce anti-tumor T cell responses.

In accordance with various embodiments, the TLR9 agonist comprises at least two oligonucleotides linked together through their 3' ends, so as to have multiple accessible 5' ends. The linkage at the 3' ends of the component oligonucleotides is independent of the other oligonucleotide linkages and may be directly via 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker or a nucleoside, utilizing either the 2' or 3 ' hydroxyl positions of the nucleoside. Linkages may also employ a functionalized sugar or nucleobase of a 3' terminal nucleotide. Exemplary TLR9 agonists are described in US Patent Nos. 8,420,615, 7,566,702, 7,498,425, 7,498,426,

7,405,285, 7,427,405, including Tables 1 and 2A-2D of each, the entire contents of which are hereby incorporated by reference in their entireties.

In various embodiments, the TLR agonist is selected from:

5'-TCTGACG$_1$TTCT-X-TCTTG$_1$CAGTCT-5' (SEQ ID NO:1)

5'-TCTGTCG$_1$TTCT-X-TCTTG$_1$CTGTCT-5' (SEQ ID NO:2)

5'-TCG$_1$TCG$_1$TTCTG-X-GTCTTG$_1$CTG$_1$CT-5' (SEQ ID NO:3)

5'-TCG$_1$AACG$_1$TTCG$_1$-X-G$_1$CTTG$_1$CAAG$_1$CT-5' (SEQ ID NO:4)

5'-CTGTCoG$_2$TTCTC-X-CTCTTG$_2$oCTGTC-5' (SEQ ID NO:5)

5'-CTGTCG$_2$TTCTCo-X-oCTCTTG$_2$CTGTC-5' (SEQ ID NO:6)

5'-TCG$_1$AACG$_1$TTCG$_1$-X-TCTTG$_2$CTGTCT-5' (SEQ ID NO:7)

5'-TCG$_1$AACG$_1$TTCG$_1$-Y-GACAG$_1$CTGTCT-5' (SEQ ID NO:8)

5'-CAGTCG$_2$TTCAG-X-GACTTG$_2$CTGAC-5' (SEQ ID NO:9)

5'-CAGTCG$_1$TTCAG-X-GACTTG$_1$CTGAC-5' (SEQ ID NO:10)

5'-TCG$_1$AACG$_1$TTCoG-Z-GoCTTG$_1$CAAG$_1$CT-5' (SEQ ID NO:11)

5'-TCG$_1$AACG$_1$TTCG$_1$-Y$_2$-TCTTG$_1$CTGTCTTG$_1$CT-5' (SEQ ID NO:12)

5'-TCG$_1$AACG$_1$TTCG$_1$-Y$_2$-TCTTG$_1$CTGUCT-5' (SEQ ID NO:13)

5'-TCG$_1$AACG$_1$ToTCoG-m-GoCToTG$_1$CAAG$_1$CT-5' (SEQ ID NO:14)

5'-TCG$_1$AACG$_1$TTCoG-Y$_3$-GACTTG$_2$CTGAC-5' (SEQ ID NO:15)

5'-TCG$_1$AACG$_1$TTCG$_1$-Y$_4$-TGTTG$_1$CTGTCTTG$_1$CT-5' (SEQ ID NO:16)

5'-TCG$_2$TCG$_2$TTU$_1$Y-M-YU$_1$TTG$_2$CTG$_2$CT-5' (SEQ ID NO:17)

5'-CAGTCG$_2$TTCAG-Y$_3$-TCTTG$_1$CTGTCT-5' (SEQ ID NO:18)

5'-TCG$_1$TACG$_1$TACG$_1$-X-G$_1$CATG$_1$CATG$_1$CT-5' (SEQ ID NO:19)

5'-TCG$_1$AACG$_1$TTCG-Z-GCTTG$_1$CAAG$_1$CT-5' (SEQ ID NO:20)

5'-TCG$_1$AACG$_1$TTCoG-Y$_3$-CTTG$_2$CTGACTTG$_1$CT-5' (SEQ ID NO:21)

5'-TCG$_1$AACG$_1$oTTCG$_1$-X$_2$-G$_1$CTToG$_1$CAAG$_1$CT-5' (SEQ ID NO:22)

5'-TCG$_1$AACG$_1$TTCG$_1$-Y$_4$-CATTG$_1$CTGTCTTG$_1$CT-5' (SEQ ID NO:23)

5'-TCG$_1$AACG$_1$TTCG$_1$-m-G$_1$CTTG$_1$CAAG$_1$CT-5' (SEQ ID NO:24)

5'-TCoG$_1$oAACoG$_1$TTCoG$_1$o-X$_2$-oG$_1$oCTTG$_1$oCAAoG$_1$oCT-5' (SEQ ID NO:25)

5'-ToCG$_1$oAACoG$_1$TTCoG$_1$o-X$_2$-oG$_1$oCTTG$_1$oCAAoG$_1$CoT-5' (SEQ ID NO:26)

5'-TCoG$_1$oAACoG$_1$TTCoG$_1$o-m-oG$_1$oCTTG$_1$oCAAoG$_1$oCT-5' (SEQ ID NO:27)

5'-TCoG$_2$oAACoG$_2$TTCoG$_2$o-X$_2$-oG$_2$oCTTG$_2$oCAAoG$_2$oCT-5' (SEQ ID NO:28)

5'-TCoG$_1$oAACoG$_1$TTCoGo-Z-oGoCTTG$_1$oCAAoG$_1$oCT-5' (SEQ ID NO:29) and

5'-ToCG$_1$oAACoG$_1$TTCoGo-Z-oGoCTTG$_1$oCAAoG$_1$CoT-5' (SEQ ID NO:30),

where G$_1$ is 2'-deoxy-7-deazaguanosine; G$_2$ is 2'-deoxy-arabinoguanosine; G, C, or U are 2'-O-methylribonucleotides; U$_1$ is 2'-deoxy-U; o is a phosphodiester linkage; X is a glycerol linker; X$_2$ is a isobutanetriol linker, Y is C3-linker; m is cis,trans-1,3,5-cyclohexanetriol linker; Y$_2$ is 1,3-propanediol linker; Y$_3$ is 1,4-butanediol linker; Y$_4$ is 1,5-pentandiol linker; Z is 1,3,5-pentanetriol linker; and M is cis,cis-1,3,5-cyclohexanetriol linker.

In various embodiments, the TLR9 agonist is selected from 5'-TCG$_1$AACG$_1$TTCG$_1$-X-G$_1$CTTG$_1$CAAG$_1$CT-5' (SEQ ID NO:4), 5'-CTGTCoG$_2$TTCTC-X-CTCTTG$_2$oCTGTC-5' (SEQ ID NO:5), 5'-CTGTCG$_2$TTCTCo-X-oCTCTTG$_2$CTGTC-5' (SEQ ID NO:6), 5'-TCG$_1$AACG$_1$TTCG$_1$-Y-TCTTG$_2$CTGTCT-5' (SEQ ID NO:7), and 5'-TCG$_1$AACG$_1$TTCG$_1$-Y-GACAG$_1$CTGTCT-5' (SEQ ID NO:8), wherein X is a glycerol linker, Y is a C3-linker, G$_1$ **is 2'-deoxy-7-deazaguanosine,** G$_2$ is arabinoguanosine, and o is a phosphodiester linkage.

In various embodiments, the TLR9 agonist is 5'-TCG$_1$AACG$_1$TTCG$_1$-X-G$_1$CTTG$_1$CAAG$_1$CT-5' (SEQ ID NO:4), wherein X is a glycerol linker and G$_1$ **is 2'-**deoxy-7-deazaguanosine, otherwise known as IMO-2125.

Alternative TLR9 agonists are immune stimulatory oligonucleotides disclosed in US 8,871,732, which is hereby incorporated by reference in its entirety. Such

agonists comprise a palindromic sequence of at least 8 nucleotides and at least one CG dinucleotide.

In accordance with embodiments of the invention, the immunostimulatory oligonucleotide (e.g., IMO-2125) is administered intratumorally. In some embodiments, the intratumoral administration is in a primary or secondary tumor (e.g., metastatic melanoma lesion). Intratumoral administration alters immune signaling in the tumor microenvironment, priming the immune system for an effective anti-tumor response, while inducing changes that are compatible with more effective checkpoint inhibitor therapy.

Illustrative dosage forms suitable for intratumoral administration include solutions, suspensions, dispersions, emulsions, and the like. The TLR9 agonist may be provided in the form of sterile solid compositions (*e.g.* lyophilized composition), which can be dissolved or suspended in sterile injectable medium immediately before use. They may contain, for example, suspending or dispersing agents known in the art.

In various embodiments, the TLR9 agonist is IMO-2125 and is administered intratumorally at from about 4 mg to about 64 mg per dose, or in some embodiments from about 8 mg to about 64 mg per dose, or from about 12 mg to about 64 mg per dose, or from about 16 mg to about 64 mg per dose, or from about 20 mg to about 64 mg per dose. In some embodiments, IMO-2125 is administered at from about 20 mg to about 48 mg per dose, or about 20 mg to about 40 mg per dose. For example, in various embodiments, IMO-2125 is administered at about 4 mg, or about 8 mg, or about 12 mg, or about 16 mg, or about 20 mg, or about 24 mg, or about 28 mg, or about 32 mg, or about 36 mg, or about 40 mg, or about 44 mg, or about 48 mg, or about 52 mg, or about 56 mg, or about 60 mg, or about 64 mg per dose, *e.g.* intratumorally.

In various embodiments, about 3 to about 12 doses of the TLR9 agonist (*e.g.* IMO-2125) are administered (*e.g.* about 3 doses, or about 4 doses, or about 5 doses, or about 6 doses, or about 7 doses, or about 8 doses, or about 9 doses, or about 10 doses, or about 11 doses, or about 12 doses). In various embodiments, about 4 to about 8 doses are administered over 10 to 12 weeks. In some embodiments, about 6 doses are administered over 10 to 12 weeks. In some embodiments, therapy is initiated with 3 to 5 weekly doses of IMO-2125, optionally followed by 3 to 8 maintenance doses, which are administered about every three weeks. In some embodiments, an IMO-2125 dose is

administered in weeks 1, 2, 3, 5, 8, and 11. The IMO-2125 doses may be administered in the same or different lesions.

During the regimen of IMO-2125 (or other TLR9 agonist), one or more checkpoint inhibitor therapies are administered to take advantage of the changes in immune signaling. The one or more checkpoint inhibitors can be administered parenterally, including intravenously, intratumorally, or subcutaneously, among other methods. In some embodiments, the patient receives an anti-CTLA-4 agent. For example, the anti-CTLA-4 agent may be an antibody that targets CTLA-4, for instance an antagonistic antibody. In various embodiments, the anti-CTLA-4 is ipilimumab (*e.g.* YERVOY, BMS-734016, MDX-010, MDX-101). In various embodiments, the anti-CTLA-4 is tremelimumab (*e.g.* CP-675,206, MEDIMMUNE). In other embodiments, the immunotherapy agent is an anti-PD-1 agent. For example, the anti-PD-1 agent may be an antibody that targets the PD-1, for instance, inhibiting the interaction between PD-1 and PD-L1 (and/or PD-L2). In various embodiments, the anti-PD-1 agent is nivolumab (ONO-4538/BMS-936558, MDX1106 or OPDIVO). In various embodiments, the anti-PD-1 agent is pembrolizumab (KEYTRUDA or MK-3475). In various embodiments, the anti-PD-1 agent is pidilizumab (CT-011 or MEDIVATION).

In some embodiments, the present immunotherapy agent is an anti-PD-L1 and/or PD-L2 agent. For example, in various embodiments, the anti-PD-L1 and/or PD-L2 agent is an antibody that targets PD-L1 and/or PD-L2, for instance, inhibiting the interaction between PD-1 and PD-L1 and/or PD-L2. In various embodiments, the anti-PD-L1 and/or PD-L2 agent is atezolizumab (TECENTRIQ, ROCHE) BMS 936559 (BRISTOL MYERS SQUIBB), or MPDL328OA (ROCHE).

In various embodiments, the anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or PD-L2 agent (*e.g.* YERVOY, OPDIVO, or KEYTRUDA, or comparable agents thereto) is administered at a dose of about 1 mg/kg, or about 2 mg/kg, or about 3 mg/kg, or about 4 mg/kg, or about 5 mg/kg, *e.g.* intravenously. For example, in some embodiments, the dose of an anti-CTLA-4 agent, *e.g.* YERVOY, is about 3 mg/kg. For example, in some embodiments, the dose of an anti-PD-1 agent, *e.g.* OPDIVO, is about 3 mg/kg. For example, in some embodiments, the dose of an anti- PD-1 agent, *e.g.* KEYTRUDA, is about 2 mg/kg. In various embodiments, the initial dose of the anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or PD-L2 agent (*e.g.* YERVOY, OPDIVO, or KEYTRUDA, or

comparable agents thereto) is administered at least one week after the initial TLR9 agonist dose, for example in about weeks 2, 3 or 4.

In some embodiments, the immunotherapy agent is anti-CTLA-4 (*e.g.* YERVOY), anti-PD-1 (*e.g.* OPDIVO or KEYTRUDA), or anti-PD-L1 and/or anti-PD-L2 agent, which is administered from about 2 to about 6 times (*e.g.* about 2 times, or about 3 times, or about 4 times, or about 5 times, or about 6 times). In some embodiments, the immunotherapy agent, *e.g.* anti-CTLA-4 (*e.g.* YERVOY), anti-PD-1 (*e.g.* OPDIVO or KEYTRUDA), or anti-PD-L1 and/or PD-L2 agent is administered about 4 times.

In some embodiments, the immunotherapy agent is an anti-CTLA-4 agent such as YERVOY and is dosed at 3 mg/kg i.v. over about 90 minutes about every 3 weeks. In some embodiments, the immunotherapy agent is an anti-PD-1 agent such as OPDIVO and is dosed at about 3 mg/kg i.v. over about 60 minutes about every 2 weeks. In some embodiments, the immunotherapy agent is an anti-PD-1 agent such as KEYTRUDA and is dosed at about 2 mg/kg i.v. over about 30 minutes about every 3 weeks.

In some embodiments, maintenance doses of the TLR9 agonist (*e.g.* IMO-2125), along with dosing of anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or PD-L2 agent (*e.g.* YERVOY, OPDIVO, or KEYTRUDA, or comparable agents thereto) are administered about every 3 weeks.

In various embodiments, the present immunostimulatory oligonucleotides allow for a dose reduction of the immunotherapy to about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 100% of a monotherapy dose. For example, in some embodiments, an immunotherapy dose is about 0.1 mg/kg, or about 0.3 mg/kg, or about 0.5 mg/kg, or about 0.7 mg/kg, or about 1 mg/kg, or about 1.5 mg/kg, or about 2 mg/kg, or about 2.5 mg/kg, or about 3 mg/kg.

In some embodiments, IMO-2125 is administered intratumorally to a metastatic melanoma patient previously found to be unresponsive or only partially responsive to PD-1 blockade therapy. IMO-2125 is administered at a dose of from 4 to 32 mg per dose (e.g., about 16 mg, about 20 mg, about 24 mg, about 28 mg, or about 32 mg) in weeks 1, 2, 3, 5, 8, and 11, with ipilimumab i.v. at 3 mg/kg. Ipilimumab can be

administered every three weeks, beginning in week 2 (e.g., weeks 2, 5, 8, and 11). Alternatively, pembrolizumab can be administered i.v. at 2 mg/kg every three weeks beginning on week 2 (e.g., weeks 2, 5, 8, and 11).

In some embodiments, the patient further receives a regimen of Epacadostat (an IDO-1 inhibitor), which may be administered at from 25 mg to 300 mg orally, about twice daily. The regimen may be administered for about 5 day cycles. The first dose of Epacadostat may be administered starting at about one week following the initial IMO-2125 (or other TLR9 agonist) intratumoral injection.

In various embodiments, without wishing to be bound by theory, the invention provides for a more balanced immune response in a cancer patient, including cancer patients with advanced, metastatic disease. The combination therapy described herein can eliminate or reduce deficiencies that are observed in the respective monotherapies. For example, various patients are refractory to immunotherapies, or such monotherapies are hampered by extensive side effect profiles. Further as the field is moving to combinations of immunotherapies (*e.g.* YERVOY and OPDIVO), such side effects are likely to be more problematic.

In various embodiments, the combination therapy allows for activation and/or maturation of dendritic cells, *e.g.* plasmacytoid dendritic cells, and modulates the tumor microenvironment (TME) in both treated and distant tumors. For example, in various embodiments, the combination therapy provides for improvements in the amount or quality of TILs and/or $CD8^+$ T cells to promote anti-tumor activities. For example, primed T cells are observed to invade both the proximal and distal tumors. Such primed T cells are suited for tumor invasion, particularly at distal sites (*e.g.* secondary tumors), and, without wishing to be bound by theory, encounter a tumor environment that has reduced tolerance mechanisms in place. In various embodiments, the combination therapy provides for stimulation of interferons (*e.g.* IFN-α) and various Th1 type cytokines (*e.g.* **IFN-γ, IL-2, IL-12, and TNF-β).**

The invention provides, in various embodiments, methods for treating cancers, including metastatic cancers, in which the overall host immune milieu is reengineered away from tumor tolerance. For example, a local TME is created that both disrupts pathways of immune tolerance and suppression and allow for tumor regression. The

present methods provide in some embodiments, a TME capable of propagating a robust immune response.

In various embodiments, a cancer patient's DCs are immature and unable to take up, process, or present antigens. These DCs may also be inhibited from migrating to regional lymph nodes or may induce tolerance, especially when presenting self-antigens. The cancer patient's tumor site may also be infiltrated with regulatory T cells that are able to mediate suppression of antigen-primed T cells. The helper CD4 T cell response may also be skewed toward a Th2 phenotype, which inhibits the initiation of Th1 T cells and effective cellular immunity. The tumor cells may express aberrant **MHC class I molecules or β2-microglobulin, resulting in inadequate antigen** presentation and, thus, inefficient recognition of tumors by effector T cells. Finally, tumor cells and the surrounding stroma may release a number of suppressive cytokines, **such as IL-6, IL-10, and TGF-β. This creates an environment that is not conducive to** local immunity, which allows tumor cells to escape. In various embodiments, the present methods allow for an environment that is conducive to local immunity against tumors, *e.g.*, without limitation, maturation of DCs and/or reduction of regulatory T cells and Th2 CD4 T cells.

In some embodiments, the combination therapy according to the invention alters the balance of immune cells in favor of immune attack of a tumor. For instance, in some embodiments, the present methods shift the ratio of immune cells at a site of clinical importance, *e.g.* at the site of agent administration or a distal site, in favor of cells that can kill and/or suppress a tumor (*e.g.* T cells, cytotoxic T lymphocytes, T helper cells, natural killer (NK) cells, natural killer T (NKT) cells, anti-tumor macrophages (*e.g.* M1 macrophages), B cells, dendritic cells, or subsets thereof) and in opposition to cells that protect tumors (*e.g.* myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs); tumor associated neutrophils (TANs), M2 macrophages, tumor associated macrophages (TAMs), or subsets thereof). In some embodiments, the present methods increase a ratio of effector T cells to regulatory T cells. In various embodiments, this altered balance of immune cells is affected locally/proximally and/or systemically/distally. In various embodiments, this altered balance of immune cells is affected in the TME.

Further, in various embodiments, the present methods allow for a robust anti-tumor immune response that does not come at the expense of significant side effects

(e.g., irAEs), *e.g.* relative to side effects observed when one or more immunotherapies are used in the absence of the TLR9 agonist.

For example, the combination therapy reduces one or more side effects of an immunotherapy, *e.g.* an anti-CTLA-4, anti-PD-1, or anti-PD-L1 and/or PD-L2 agent, including for example, one or more of YERVOY, OPDIVO, and KEYTRUDA or agents related thereto. Such side effects include: fatigue, cough, nausea, loss of appetite, skin rash, itching pruritus, rash, and colitis. In some embodiments, the side effects are intestinal problems (*e.g.* colitis) that can cause perforations in the intestines. Signs and symptoms of the colitis may include: diarrhea or more bowel movements than usual; blood in the stools or dark, tarry, sticky stools; and abdominal pain or tenderness. In some embodiments, the side effects are liver problems (*e.g.* hepatitis) that can lead to liver failure. Signs and symptoms of hepatitis may include: yellowing of skin or the whites of the eyes; dark urine; nausea or vomiting; pain on the right side of the stomach; and bleeding or bruising more easily than normal. In some embodiments, the side effects are skin problems that can lead to severe skin reactions. Signs and symptoms of severe skin reactions may include: skin rash with or without itching; sores in the mouth; and the skin blisters and/or peels. In some embodiments, the side effects are nerve problems that can lead to paralysis. Symptoms of nerve problems may include: unusual weakness of legs, arms, or face; and numbness or tingling in hands or feet. In some embodiments, the side effects are hormone gland problems (*e.g.* pituitary, adrenal, and thyroid glands). Signs and symptoms include: persistent or unusual headaches; unusual sluggishness; feeling cold all the time; weight gain; changes in mood or behavior such as decreased sex drive, irritability, or forgetfulness; and dizziness or fainting. In some embodiments, the side effects are ocular problems. Symptoms may include: blurry vision, double vision, or other vision problems; and eye pain or redness.

In some embodiments, patients experience fewer incidences of colitis, crohn's disease, or other GI involved irAE in accordance with the present invention.

In some embodiments, the patient achieves longer progression-free interval or longer survival (e.g., as compared to monotherapy), or in some embodiments, achieves remission or complete response. A complete response refers to the disappearance of all signs of cancer in response to treatment.

This invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1: Anti-Tumor Effects of Immunostimulatory Oligonucleotides (IMO-2125)

Immunomers were synthesized as is known in the art (see, *e.g.*, International Patent Publication No. WO 2016/057898, the entire contents of which, inclusive of Example 1 and FIGs. 1 and 2 therein, are hereby incorporated by reference).

BALB/c mice (n=8 per group) were implanted s.c. with 2 x 10^6 CT26.WT cells on right flank (Tumor 1) and 2 x 10^6 CT26.CL25 cells on the left flank (Tumor 2). Treatment was initiated on Day 5 when tumor volume on right flank reached 50 to 150 mm^3.

Test compound was administered by intratumoral (i.t.) injection (100 µl) on right side tumor nodules (Tumor 1) only at Days 5, 8, 11 and 14. Tumor nodules were collected at Day 28. The text compounds were Control DNA, IMO-2125: 0.5 mg/kg, IMO-2125: 2.5 mg/kg, and IMO-2125: 5 mg/kg. As shown in **FIG. 1**, intratumoral IMO-2125 treatment led to dose-dependent decreases in tumor volume in both treated and distant tumors. FIG. 2 shows tumor nodules collected on Day 28 after tumor implantation. Immunohistochemical staining for $CD3^+$ T lymphocyte surface marker. $CD3^+$ cells stained brown color. While few $CD3^+$ cells presented inside tumor tissue bordering normal tissue from placebo-injected mice, a large number of $CD3^+$ cells presented in the tumor tissue from mice treated with IMO-2125, 2.5 mg/kg. Results are shown in **FIG. 2** panel A, which demonstrates *inter alia*, antitumor activity was associated with induction of tumor infiltrating lymphocytes (TILs). **FIG. 2** panel B, shows that intratumoral IMO-2125 treatment increased infiltration of $CD8^+$ T cells in tumors.

Further, T cells from spleens of placebo - and IMO-2125 (2.5 mg/kg) - treated tumor-bearing mice (n = 3) were collected on Day 28. IFN-secreting ELISPOT was used for determining T cells specifically against tumor internal antigen AH1 presented **in both CT26.WT and CT26.CL25 and β-gal presented only in CT26.CL25. FIG. 3** shows that intratumoral IMO-2125 treatment elicited specific cytotoxic T cell responses to tumor antigens. In **FIG. 4**, the key role of $CD8^+$ T cells in treated and distal tumors is demonstrated.

FIG. 5 shows a study demonstrating intratumoral IMO-2125 induced durable and tumor-specific immune memory. Six tumor-bearing mice (6 of 9) whose tumors completely or partially regressed (<150 mm3) after IMO-2125 (5 mg/kg, i.t.) treatments and 8 naïve BALB/c mice (n = 8) were rechallenged on Day 33 with 1 x 106 CT26 cells by s.c. injection at abdominal right and left flank. Naïve BALB/c mice inoculated same way were used as tumor growth control. The mice that rejected CT26 tumor cell rechallenge (5 of 6) were then inoculated on Day 73 with 106 syngeneic, non-organ-related B cell lymphoma A20 cells by s.c. inoculation at the upper back area. See the plan of **FIG. 5**, panel A. Results are shown in **FIG. 5**, panel B.

In **FIG. 6**, a study comparing intratumoral IMO-2125 is more effective than systemic (s.c.) treatment as demonstrated by antitumor activity in an A20 lymphoma model. BALB/c mice (n=10) were implanted s.c. with $3x10^6$ A20 cells on the right and left flank. Treatment was initiated on day 8 with intratumoral injection in the left flank with 2.5 mg/kg IMO-2125. IMO-2125 was given on days 8, 10, 12, and 14. Samples from placebo (PBS) control and IMO-2125 treated tumor-bearing mice were collected on day 21 after tumor implantation. Panel A shows the study design and tumor kinetics. In panel A, the tumor kinetics of subcutaneous administration is slightly better than control while intratumoral administration significantly slows tumor growth. Panel B shows the presence of TILs and changes in gene expression of various checkpoint genes. Importantly, IMO-2125 increased tumoral TILs and modulated tumor checkpoint expression thereby sensitizing the TME for combination with one or more checkpoint inhibitors

Example 2: Anti-Tumor Effects of Combination Therapy of IMO-2125 and an Anti-CTLA-4 Antibody

FIG. 7 shows an evaluation of the antitumor activity of intratumoral IMO-2125 in combination with anti-CTLA-4 mAb on treated tumors and systemic lung metastases. Study design is shown in **FIG. 7**, panel A and results are shown in **FIG. 7**, panel B.

BALB/c mice were implanted s.c. with 2 x 10^7 CT26 cells on right flank. The mice were than i.v. injected with 3 x 10^6 CT26 cells to establish lung metastases. Treatment was initiated on day 5. 2.5 mg kg IMO-2125 was administered intratumorally into CT26 solid tumors on the right flank and 10 mg/kg anti-CTLA-4

mAb was administered by interperitoneal (i.p.) injection. IMO-2125 and anti-CTLA-4 mAb were given either alone or co-administered on days 5, 6, 8 and 9. Lungs and T cells from spleens of PBS control, IMO-4, anti-CTLA-4 mAb or IMO-2125 and anti-CTLA-4 mAb treated tumor-bearing mice were collected.

Intratumoral IMO-2125 and anti-CTLA-4 mAb combination demonstrated improved growth inhibition in treated tumors versus monotherapy with either agent.

FIG. 8 shows anti-tumor activities of IMO-2125 and anti-CTLA-4 mAb alone or in combination on systemic lung metastasis.

FIG. 9 shows that intratumoral IMO-2125 and anti-CTLA-4 mAb combination increased TILs in metastatic nodules.

The combination of intratumoral IMO-2125 and an anti-CTLA-4 mAb resulted in improved inhibition of tumor growth, regression of systemic lung metastases and infiltration of TILs versus monotherapy with either agent. The effects were observed in directly treated tumors and systemic lung metastasis.

Example 3: Anti-Tumor Effects of Combination Therapy of IMO-2125 and an Anti-PD-1 Antibody

FIG. 10 shows an evaluation of the antitumor activity of intratumoral IMO-2125 in combination with anti-PD-1 mAb in CT26 colon carcinoma tumor model. Panel A shows the study design. BALB/c mice (n=8 per group) were implanted s.c. with 1 x 10^7 murine colon carcinoma CT26 cells in right flank (Tumor 1) and left flank (Tumor 2). Treatment was initiated on day 7 when tumor volume on reached 200 to 300 mm^3. **2.5 mg/kg IMO-2125 (50 µg in 100 µL PBS) was i.t injected at right tumor nodules and anti-PD-1 mAb (10 mg/kg, 200 µg/mouse) was administered by i.p.** injection either alone or co-administered on days 7, 8, 11 and 12 for total 4 times. Tumor nodules were collected at day 14. Tumor growth inhibition, TILs and checkpoint gene expression were evaluated at day 21. **FIG. 10,** panel B shows the impact of the combination on tumor growth kinetics at treated and distal sites. The combination of IMO-2125 and anti-PD-1 demonstrated growth inhibition in both treated and distal sites that was superior to either monotherapy. Panel C shows the impact of the combination on TILs. intratumoral IMO-2125 and anti-PD-1 mAb combination increased TILs. The PBS control group showed a few T cells (brown color); the IMO-2125 group showed large number of T cells; the PD-1 mAb group

showed slightly increased T cells over PBS treated group; the combination group showed abundant T cells - more than IMO-2125 treated group (magnification: top row x 100, mid row x 200, bottom row x 400). Panel D shows checkpoint gene expression at treated and distal sites after treatment with the combination of IMO-2125 and anti-PD-1.

IMO-2124 and anti-PD-1 were tested in combination on treated tumors and systemic lung metastases. *See* **FIG. 11**.

C57BL/6 mice (n=10) were implanted s.c. with 1 x 10^7 B 16.F 10 cells in the right flank (Tumor 1). The mice were than i.v. injected with 2x10^6 B16.F10 cells to establish lung metastases (Tumor 2). Treatment was initiated on day 5. 5 mg/kg IMO-2125 was administered intratumorally into B16 solid tumors on the right flank and 15 mg/kg anti-PD-1 mAb was administered by interperitoneal (i.p.) injection. IMO-2125 and anti-PD-1 mAb were given either alone or co-administered on days 5, 6, 7, 8, and 9. Samples from control, IMO-2125, anti-PD-1 mAb or IMO-2125 and anti-PD-1 mAb treated tumor-bearing mice were collected. **FIG. 11**, panel A shows the study design.

FIG. 11, panel B shows the impact of the combination on tumor growth kinetics at treated sites.

FIG. 11, panel C shows the combination's impact on lung metastases. Intratumoral injections of IMO-2125 in combination with anti-PD-1 mAb induced potent systemic immune responses against disseminated lung metastases

FIG. 11, panel D shows histopathology of metastatic lung tumors (Circle: Large tumor nodule, Arrow: Small tumor nodule, Inset figures: HE stained (x 40), and Large figures: CD3 stained (x 400)). Treatment with intratumoral IMO-2125 and anti-PD-1 mAb combination led to decreased lung tumor metastasis (inset and large figures) and creased TILs (large figure).

Treatment with a combination of intratumoral IMO-2125 with an anti-PD-1 antibody showed more potent antitumor activity than either agent alone. Antitumor activity was observed on treated as well as distant tumors. Infiltration levels of TILs increased in both treated and distant tumors. In preclinical models, IMO-2125 increased PD-L1 and other checkpoint expression in the treated and distant tumors.

Example 4: Anti-Tumor Effects of Combination Therapy of IMO-2125 and an IDO-1 inhibitor

FIG. 12 shows a study design to evaluate the antitumor activity of intratumoral IMO-2125 in combination with an IDO-1 inhibitor on treated tumors and systemic lung metastases in a mouse model. Solid tumors and lung metastasis are implanted on Day 0 (solid tumor, $1x10^7$ CT26, s.c., right flank; lung metastasis, $3x10^6$ CT26 i.v.), with IMO-2125 given intratumorally (2.5 mg/kg) on Days 4, 5, 7, and 8. An IDO-1 inhibitor is administered twice (75 mg/kg i.g.) on Days 4, 5, 7, and 8.

FIG. 13 shows that intratumoral IMO-2125 anti-tumor activity is potentiated by co-treatment with an IDO-1 inhibitor. Panel A shows the number of lung tumor nodules in each treatment group, showing the improvement of IMO-2125 and IDO-1 inhibitor in comparison to each agent alone. Panel B shows the change in tumor volume in each treatment group during the regimen.

Example 5: Study Population of Adults with Unresectable or Metastatic Melanoma that Progressed with ≥12 weeks PD-1 Directed Therapy (alone or in combination)

FIG. 14 provides a dosing overview in a study population of adults with **unresectable or metastatic melanoma that progressed with ≥12 weeks of PD-1-directed** therapy (alone or in combination). IMO-2125 was administered alone, intratumorally, in weeks 1 and 3. IMO-2125 was administered with ipilimumab or pembrolizumab in weeks 2, 5, 8, and 11. Administration of pembrolizumab continues every third week until time of progression.

FIG. 15 shows dendritic cell maturation results (CD1c, CD303, and HLA-DR expression) and pre-dose and 24 hours post i.t. IMO-2125 injection for patient 003 (4 mg doses of IMO-2125; ipilimumab) (Panel A); and shows T-cell activation results in injected and distant tumors (Panel B).

FIG. 16 shows expansion of top cell clones in distant lesions, and compares a non-responding patient with a responding patient (patient 003, 4 mg IMO-2125, **ipilimumab). The far right panel shows inductions of IFN-γ for patient 003.**

FIG. 17 shows tumor imaging pre- and post-therapy for patient 004 (8 mg 2125, 3mg ipilimumab). Injected and distant lesions are not visible after about 5 weeks of therapy.

EQUIVALENTS

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

INCORPORATION BY REFERENCE

All patents and publications referenced herein are hereby incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. A method for treating a cancer patient, comprising intratumorally administering an oligonucleotide TLR9 agonist to a cancer patient, and administering an immune checkpoint inhibitor therapy to the patient beginning one week or more after the initial TLR9 agonist dose.

2. The method of claim 1, wherein the immune checkpoint inhibitor targets PD-1, PD-L1, PD-L2, CTLA-4, LAG3, TIM3, and/or IDO.

3. The method of claim 1 or 2, wherein the patient showed no response to prior treatment with PD-1 blockade therapy.

4. The method of claim 3, wherein the patient experienced at least one immune-related adverse event to the prior PD-1 blockade therapy.

5. The method of claim 3 or 4, wherein the prior PD-1 blockade therapy includes therapy with nivolumab or pembrolizumab.

6. The method of any one of claims 1 to 5, wherein the cancer is a primary cancer.

7. The method of any one of claims 1 to 5, wherein the cancer is a metastatic cancer.

8. The method of claim 6 or 7, wherein the cancer originates from skin, colon, breast, or prostate.

9. The method of claim 6 or 7, wherein the cancer is melanoma, lung cancer, kidney cancer, prostate cancer, cervical cancer, colorectal cancer, pancreatic cancer, ovarian cancer, urothelial cancer, gastric/GEJ cancer, head and neck cancer, glioblastoma, Merkel cell cancer, head and neck squamous cell carcinoma (HNSCC), non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer, prostate cancer or hematologic malignancies.

10. The method of claim 9, wherein the cancer is metastatic melanoma.

11. The method of any one of claims 1 to 10, wherein the TLR9 agonist is IMO-2125.

12. The method of claim 11, wherein the IMO-2125 is administered intratumorally at from about 4 mg to about 64 mg per dose.

13. The method of claim 12, wherein the IMO-2125 is administered intratumorally at from about 4 to about 12 mg per dose.

14. The method of claim 11, wherein the IMO-2125 is administered intratumorally at about 8 mg per dose.

15. The method of claim 12, wherein the IMO-2125 is administered at from about 20 mg to about 64 mg per dose.

16. The method of claim 15, wherein the IMO-2125 is administered at from about 20 mg to about 48 mg per dose.

17. The method of any one of claim 1 to 16, wherein about 3 to about 12 doses of the TLR9 agonist are administered.

18. The method of claim 17, wherein about 4 to about 8 doses of the TLR9 agonist are administered over 10 to 12 weeks.

19. The method of claim 18, wherein about 6 doses of the TLR9 agonist are administered over 10 to 12 weeks.

20. The method of claim 18 or 19, wherein therapy is initiated with 3 to 5 weekly doses of the TLR9 agonist, followed by 3 to 8 maintenance doses administered about every three weeks.

21. The method of claim 20, wherein the TLR9 agonist is IMO-2125, which is administered in weeks 1, 2, 3, 5, 8, and 11.

22. The method of any one of claims 1 to 21, wherein the patient receives an anti-CTLA-4 agent beginning on week 2 or week 3.

23. The method of claim 22, wherein the anti-CTLA-4 agent is administered from 2 to 6 times, and optionally about 4 times.

24. The method of claim 23, wherein the anti-CTLA-4 agent is administered every three weeks.

25. The method of any one of claims 22 to 24, wherein the anti-CTLA-4 agent is ipilimumab.

26. The method of any one of claims 1 to 21, wherein the patient receives an anti-PD-1 agent beginning on week 2 or week 3.

27. The method of claim 26, wherein the PD-1 agent is administered from 2 to 6 times, and optionally about 4 times.

28. The method of claim 27, wherein the anti-CTLA-4 agent is administered every three weeks.

29. The method of any one of claims 26 to 28, wherein the anti-PD-1 agent is pembrolizumab or nivolumab.

30. The method of any one of claims 1 to 29, wherein the immune checkpoint inhibitor therapy is administered parenterally, and optionally by intravenous infusion, subcutaneous injection, or intratumoral injection.

31. A method for treating metastatic melanoma, comprising administering IMO-2125 intratumorally to a metastatic melanoma patient previously found to be unresponsive or only partially responsive to PD-1 blockade therapy; the IMO-2125 being administered at a dose of from 4 to 32 mg per dose in weeks 1, 2, 3, 5, 8, and 11; with ipilimumab or pembrolizumab administered intravenously at from 2 to 4 mg/kg every three weeks beginning in week 2.

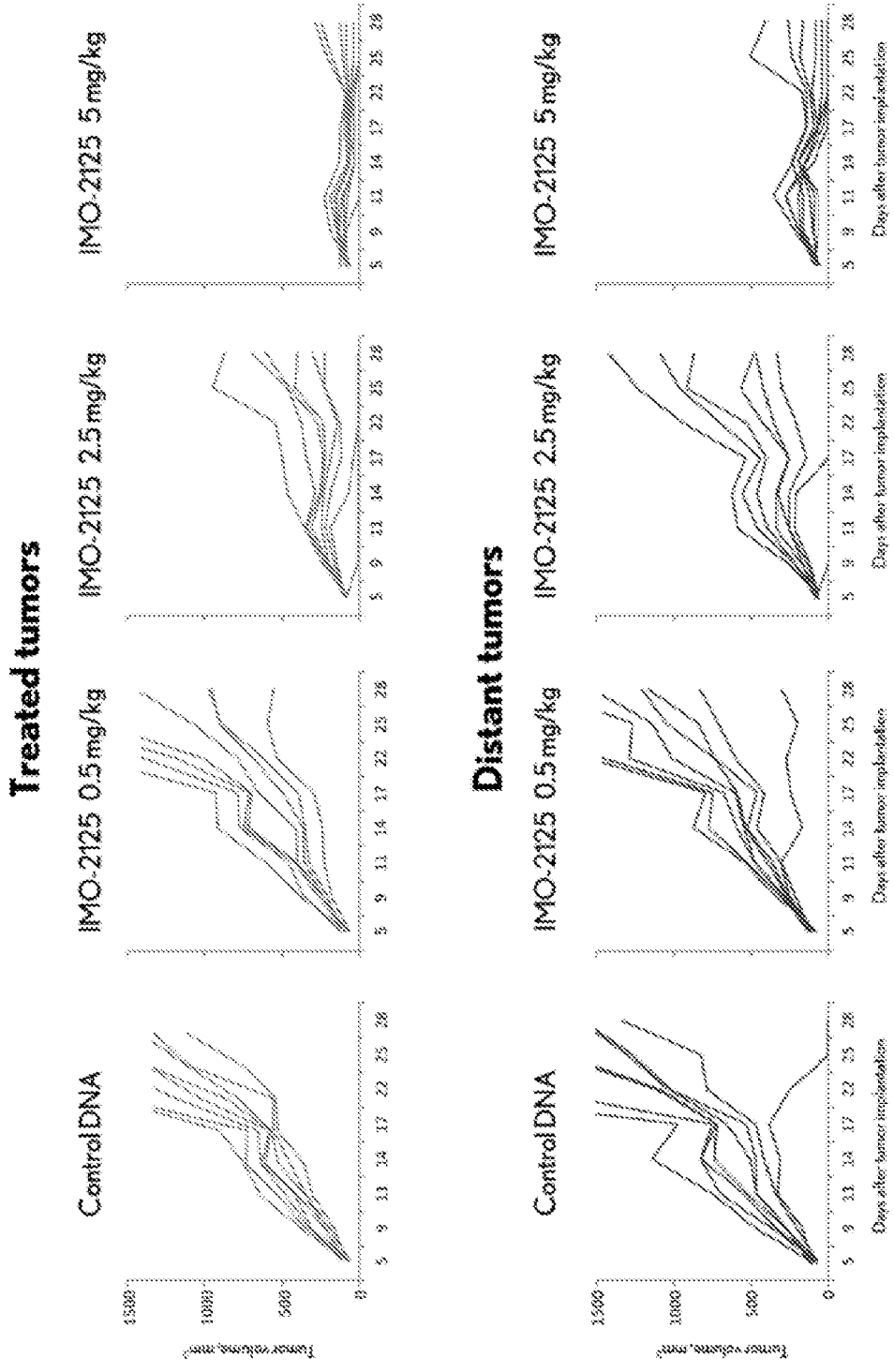

Treated tumors
Distant tumors
Control DNA
IMO-2125 0.5 mg/kg
IMO-2125 2.5 mg/kg
IMO-2125 5 mg/kg
Tumor volume, mm³
Days after tumor implantation

FIG. 1

FIG. 2

A.

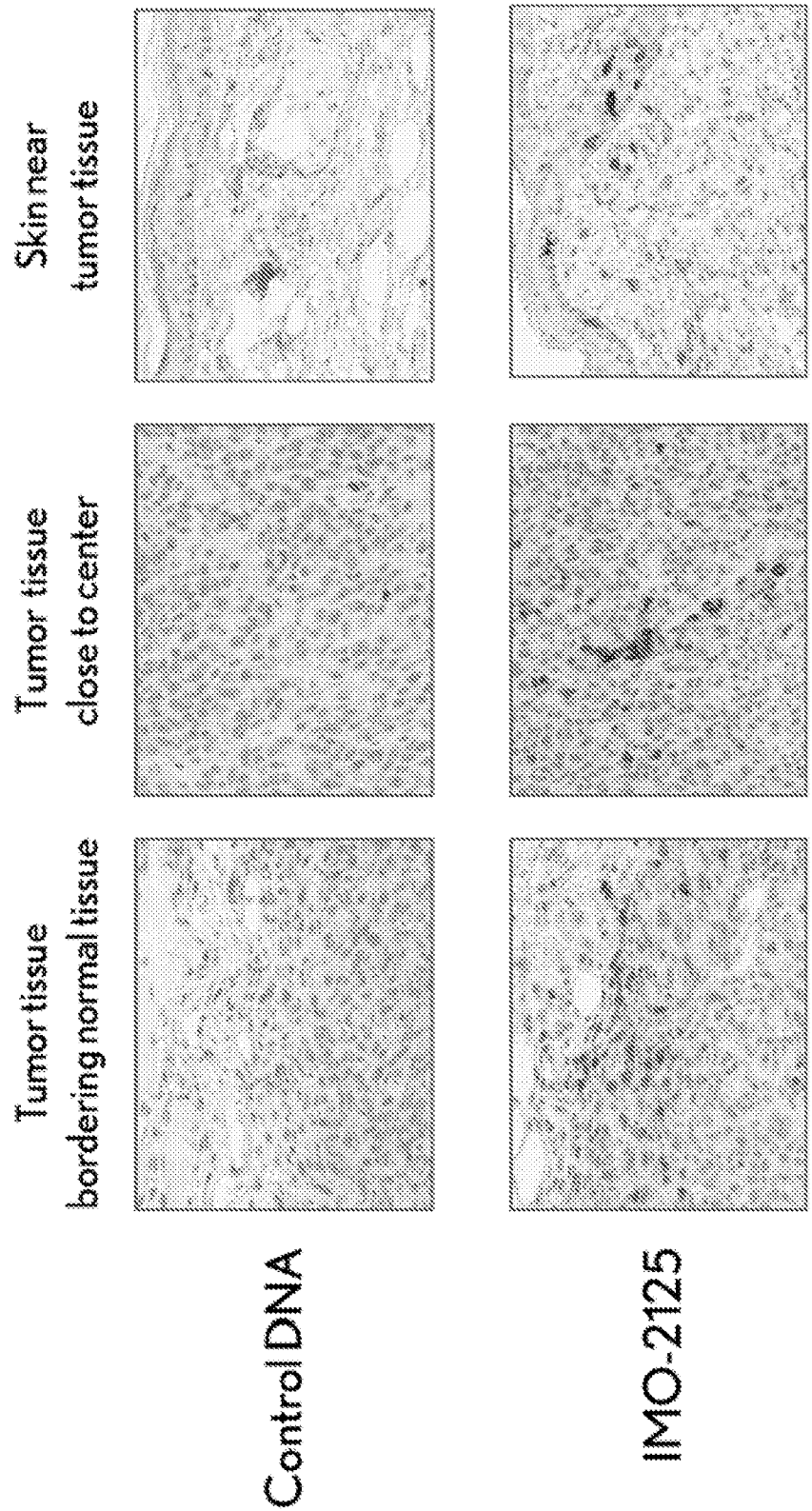
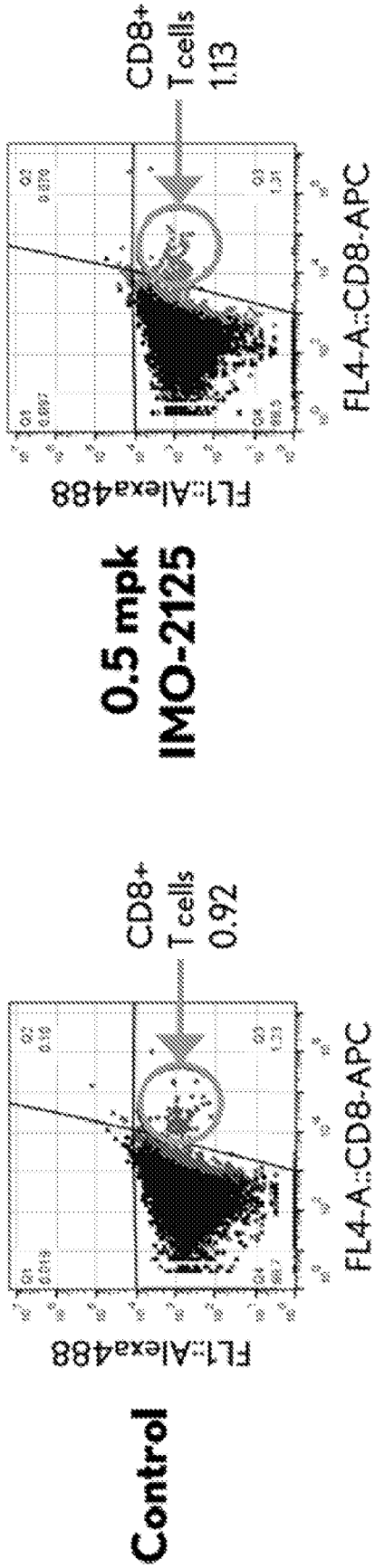

Tumor tissue bordering normal tissue
Tumor tissue close to center
Skin near tumor tissue
Control DNA
IMO-2125

FIG. 2 (CONT.)

B.


Control
CD8+ T cells 0.92
FL1-A::Alexa488
FL4-A::CD8-APC
0.5 mpk IMO-2125
CD8+ T cells 1.13
FL1-A::Alexa488
FL4-A::CD8-APC

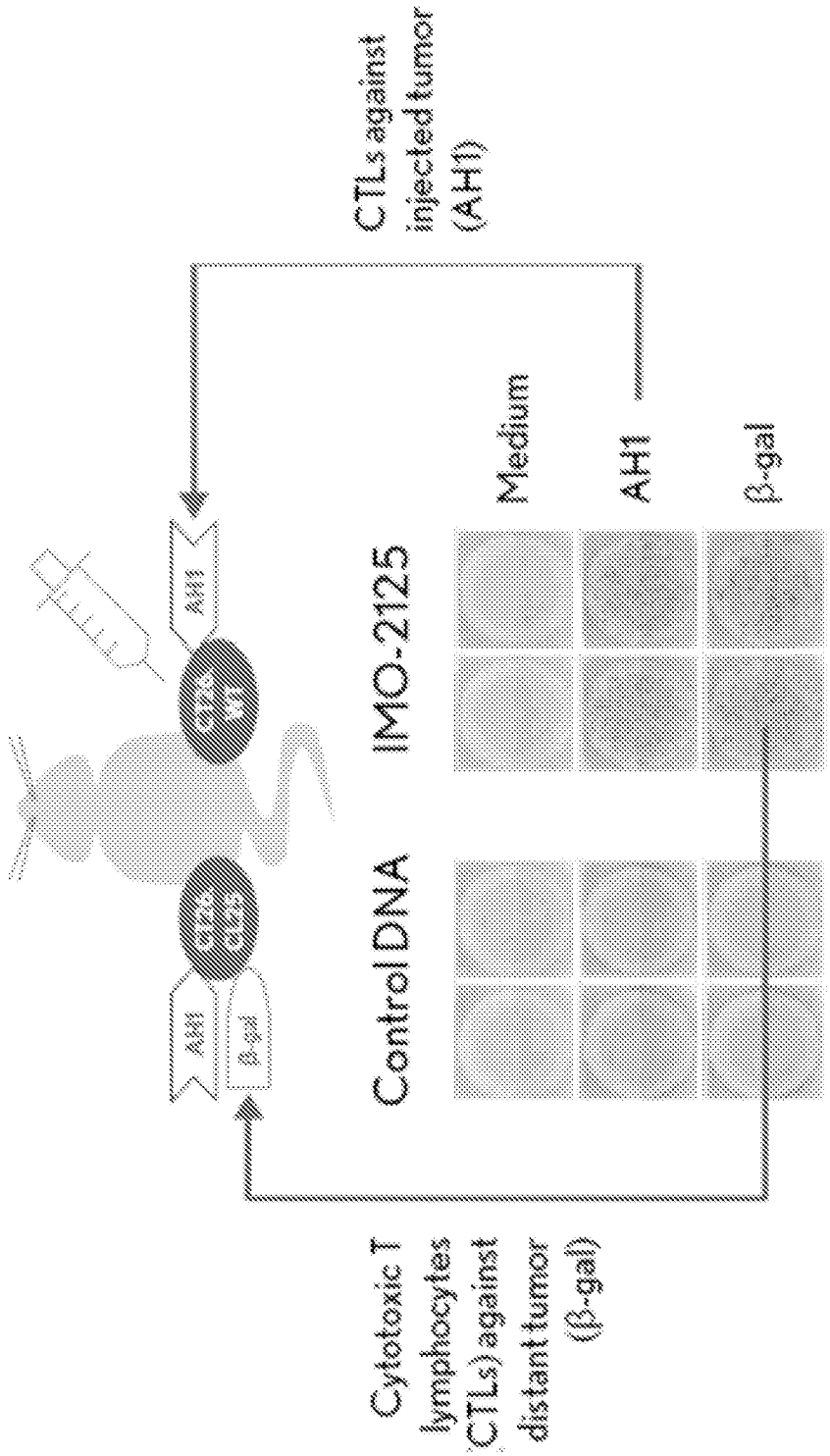
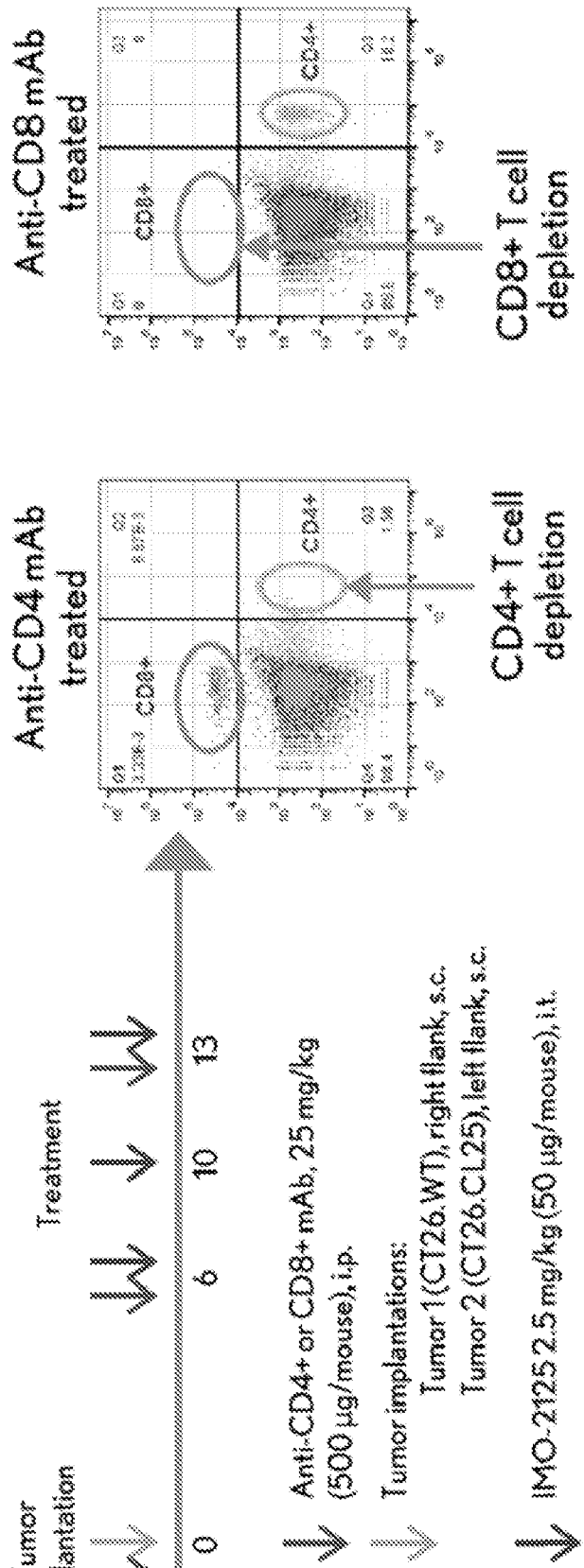

CTLs against injected tumor (AH1)
Medium
AH1
β-gal
IMO-2125
Control DNA
AH1
β-gal
Cytotoxic T lymphocytes (CTLs) against distant tumor (β-gal)

FIG. 3

FIG. 4

A.



CD4+ and CD8+ T cells were depleted by i.p. injections of 25 mg/kg (500 µg/mouse) anti-mouse CD4 mAb or anti-mouse CD8 mAb on Days 1, 6 and 13. Tumor-bearing mice were treated by i.t. injections with 2.5 mg/kg (50 µg/mouse) placebo or IMO-2125 at right tumor on Days 6, 10 and 13.

FIG. 4 (CONT.)

B.

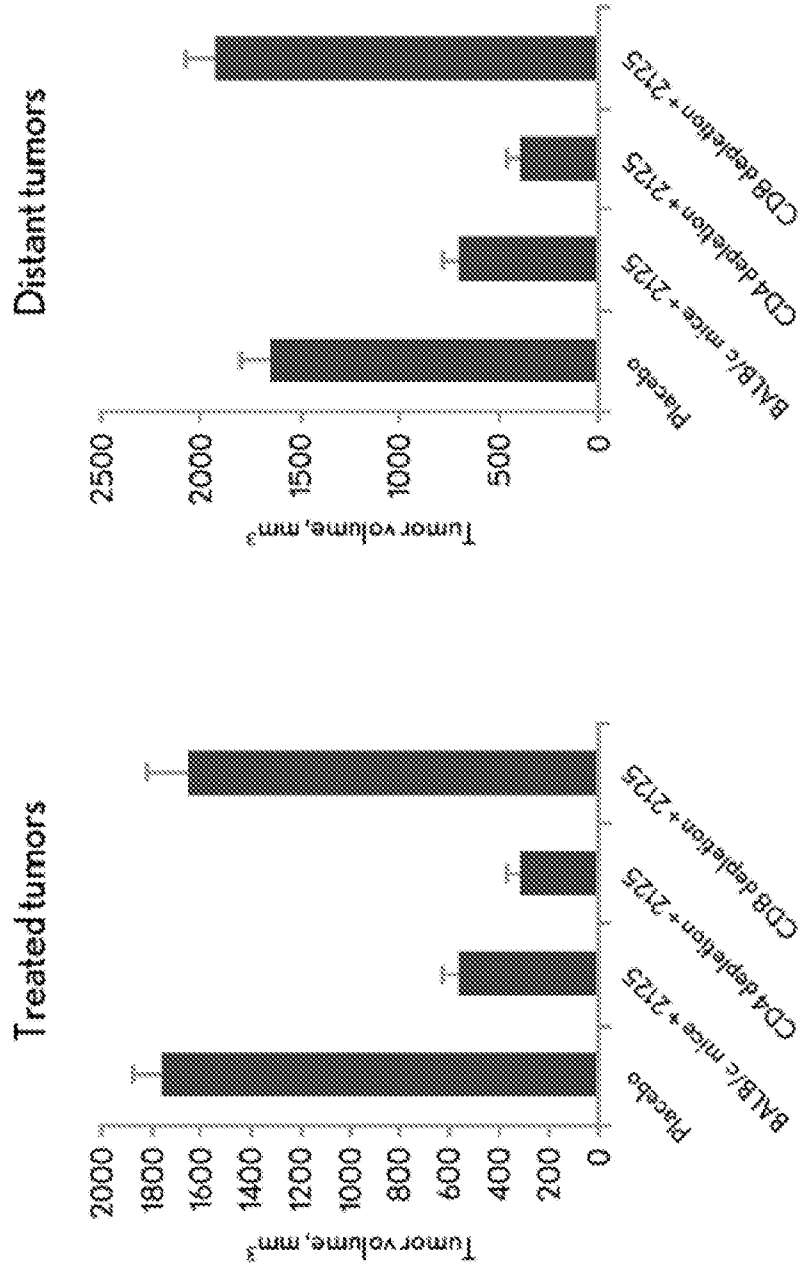

Distant tumors
Tumor volume, mm³
2500
2000
1500
1000
500
0
Placebo
BALB/c mice + 2125
CD4 depletion + 2125
CD8 depletion + 2125
Treated tumors
Tumor volume, mm³
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Placebo
BALB/c mice + 2125
CD4 depletion + 2125
CD8 depletion + 2125

FIG. 5

A.

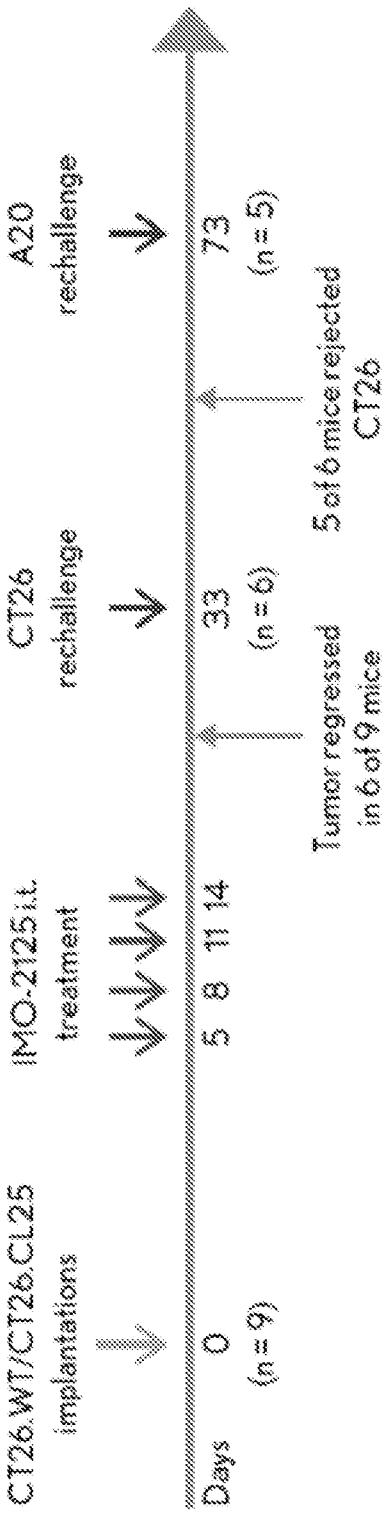

CT26.WT/CT26.CL25
implantations
IMO-2125 i.t.
treatment
CT26
rechallenge
A20
rechallenge
Days
0
(n = 9)
5 8 11 14
33
(n = 6)
73
(n = 5)
Tumor regressed
in 6 of 9 mice
5 of 6 mice rejected
CT26

FIG. 5 (CONT.)

B.

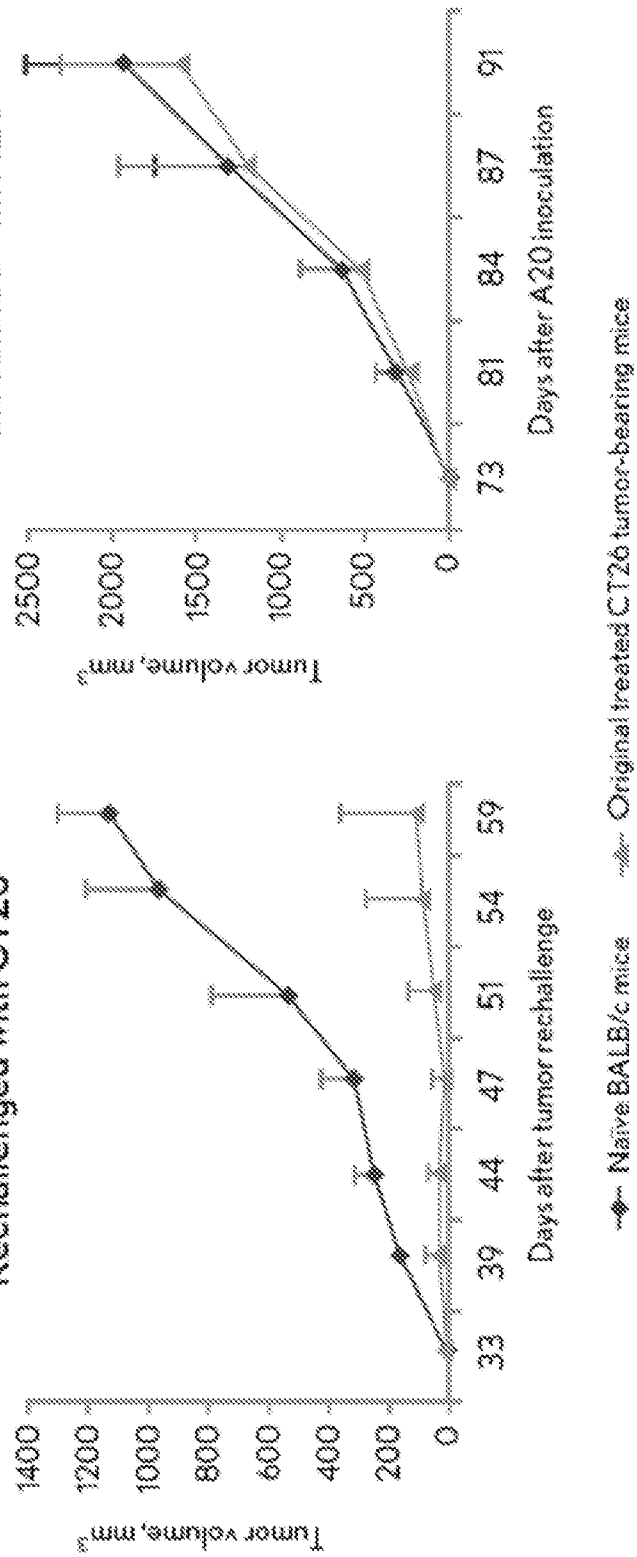

Rechallenged with CT26
Tumor volume, mm3
1400
1200
1000
800
600
400
200
0
33
39
44
47
51
54
59
Days after tumor rechallenge
Inoculated with A20
Tumor volume, mm3
2500
2000
1500
1000
500
0
73
81
84
87
91
Days after A20 inoculation
Naïve BALB/c mice
Original treated CT26 tumor-bearing mice

FIG. 6

A.

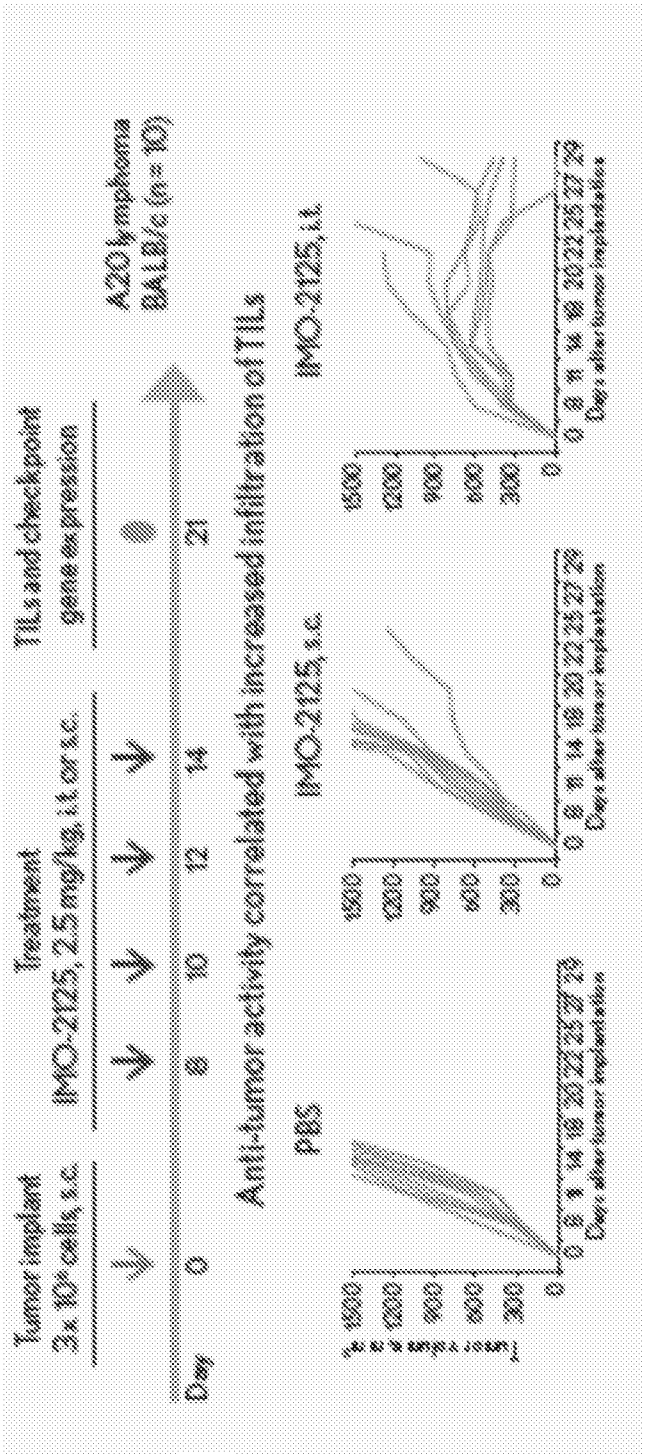

Tumor implant
2x 10^6 cells, s.c.
Treatment
IMO-2125, 2.5 mg/kg, i.t. or s.c.
TILs and checkpoint
gene expression
A20 lymphoma
BALB/c (n=10)
Day
0
6
10
12
14
21
Anti-tumor activity correlated with increased infiltration of TILs
PBS
IMO-2125, s.c.
IMO-2125, i.t.
1500
1200
900
600
300
0
Days after tumor implantation

FIG 6 (CONT.)

B.

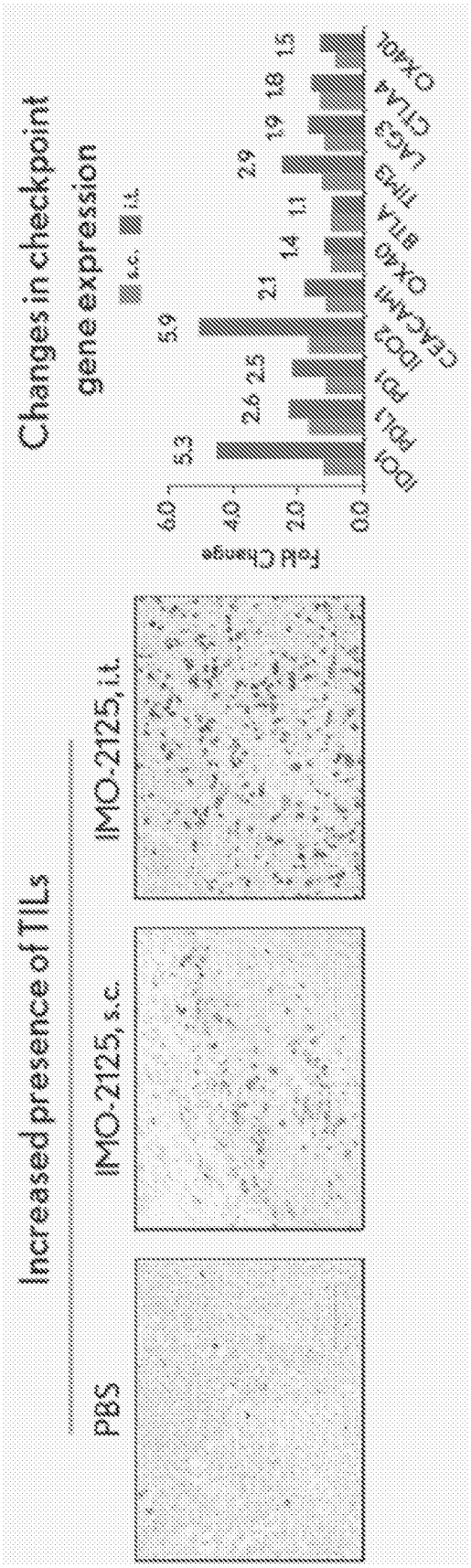

Increased presence of TILs
PBS
IMO-2125, s.c.
IMO-2125, i.t.
Changes in checkpoint gene expression
s.c.
i.t.
Fold Change
6.0
4.0
2.0
0.0
5.3
2.6
2.5
5.9
2.1
1.4
1.1
2.9
1.9
1.8
1.5
ICOS
PDL1
PD1
ICOS
CEACAM1
OX40
BTLA
TIM3
LAG3
CTLA4
OX40L

FIG. 7

A.

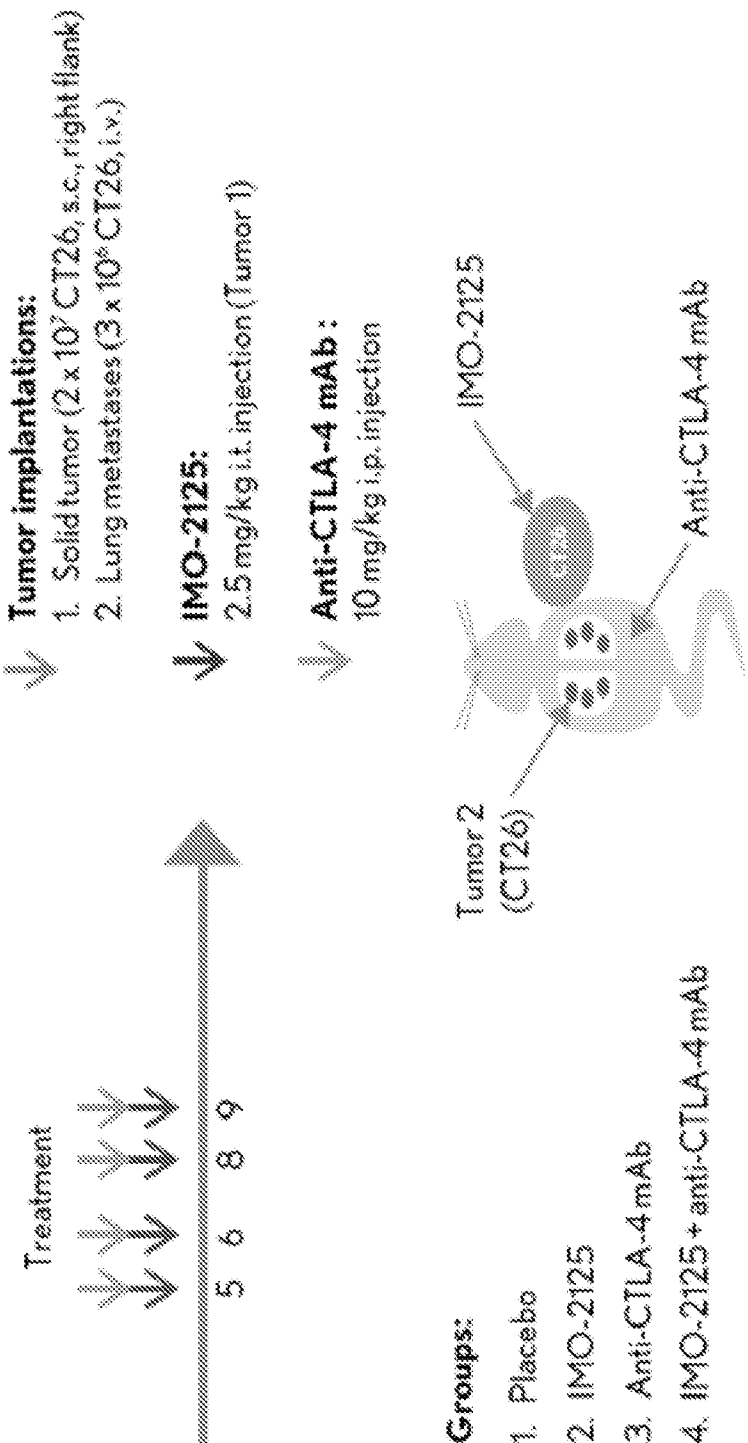
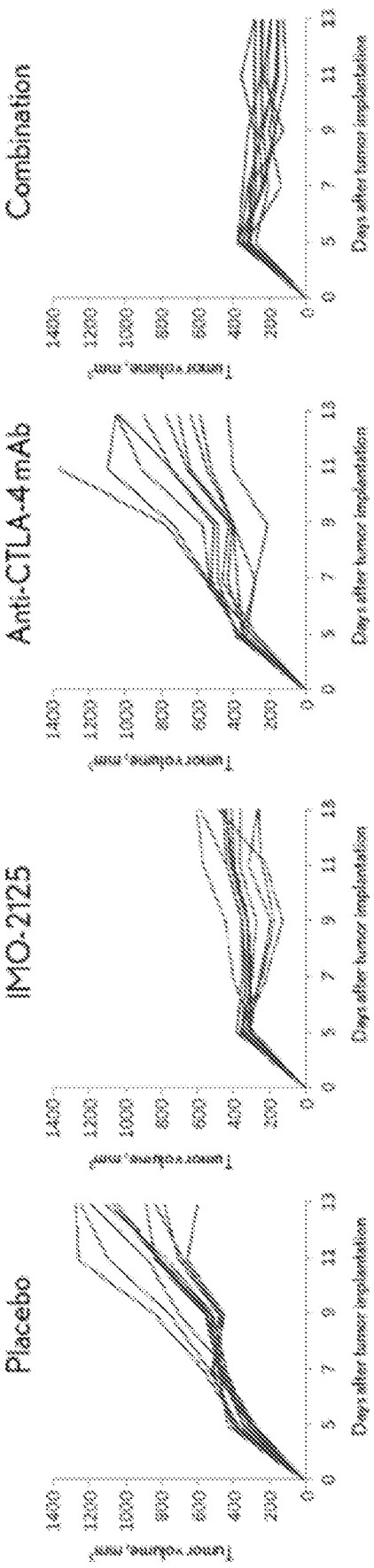

Tumor implantation
Treatment
Days 0 5 6 8 9
Tumor implantations:
1. Solid tumor (2 x 10^7 CT26, s.c., right flank)
2. Lung metastases (3 x 10^5 CT26, i.v.)
IMO-2125:
2.5 mg/kg i.t. injection (Tumor 1)
Anti-CTLA-4 mAb:
10 mg/kg i.p. injection
Groups:
1. Placebo
2. IMO-2125
3. Anti-CTLA-4 mAb
4. IMO-2125 + anti-CTLA-4 mAb
Tumor 2 (CT26)
IMO-2125
Anti-CTLA-4 mAb

FIG. 7 (CONT.)

B.


Placebo
IMO-2125
Anti-CTLA-4 mAb
Combination
Tumor volume, mm³
Days after tumor implantation
0
200
400
600
800
1000
1200
1400
0
5
7
9
11
13

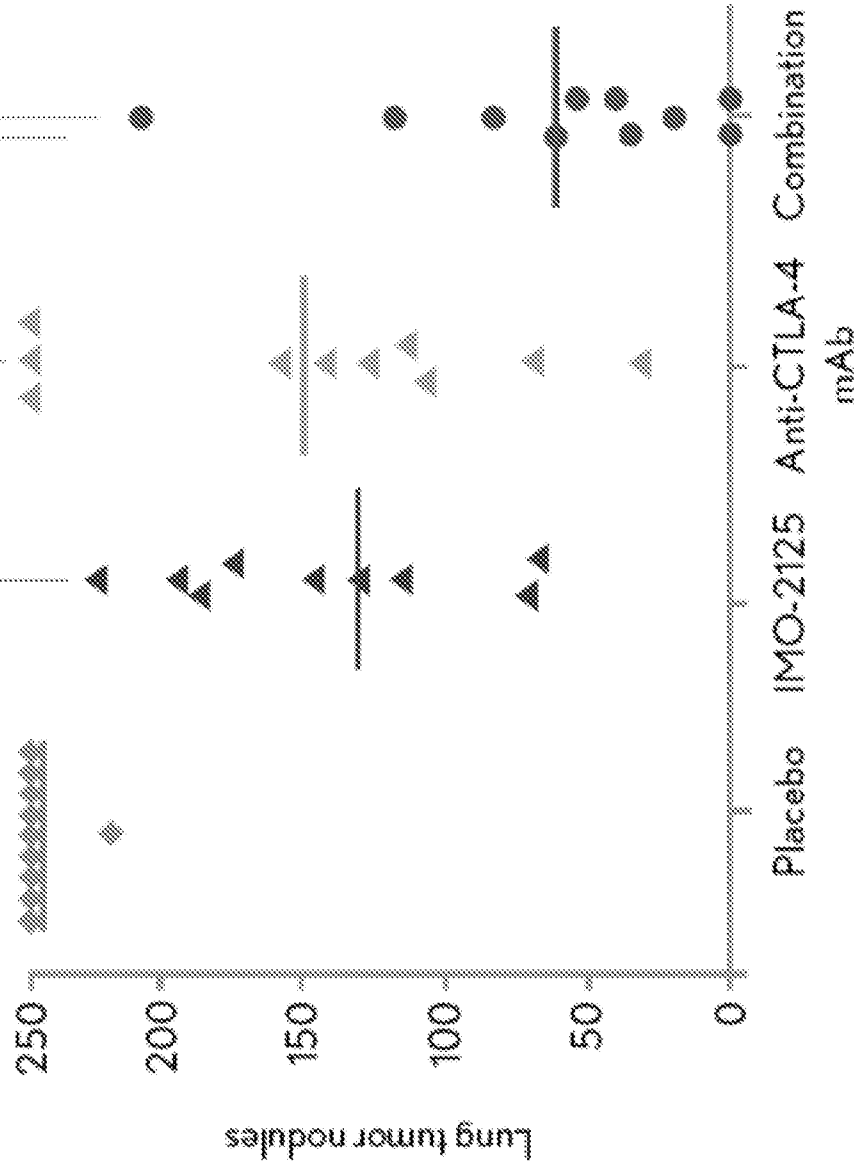
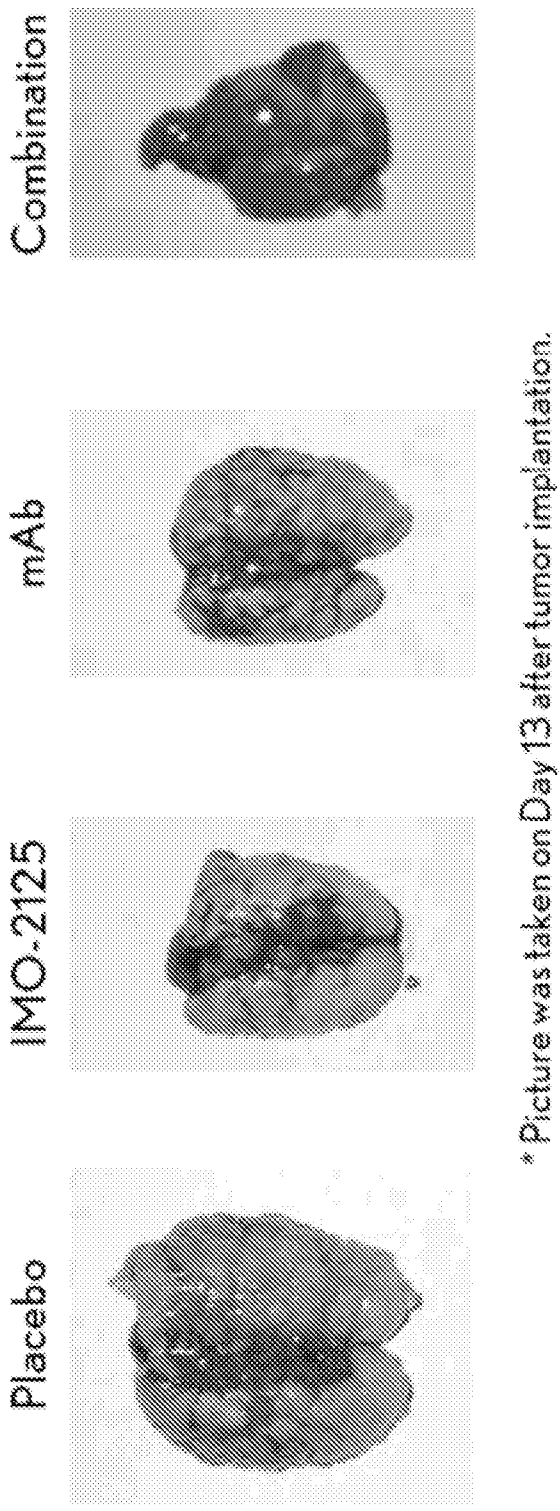

p=0.0115
p=0.0292
250
200
150
100
50
0
Lung tumor nodules
Placebo
IMO-2125
Anti-CTLA-4 mAb
Combination

FIG. 8

A.

FIG. 8 (CONT.)

B.


Placebo
IMO-2125
Anti-CTLA-4 mAb
Combination
* Picture was taken on Day 13 after tumor implantation.

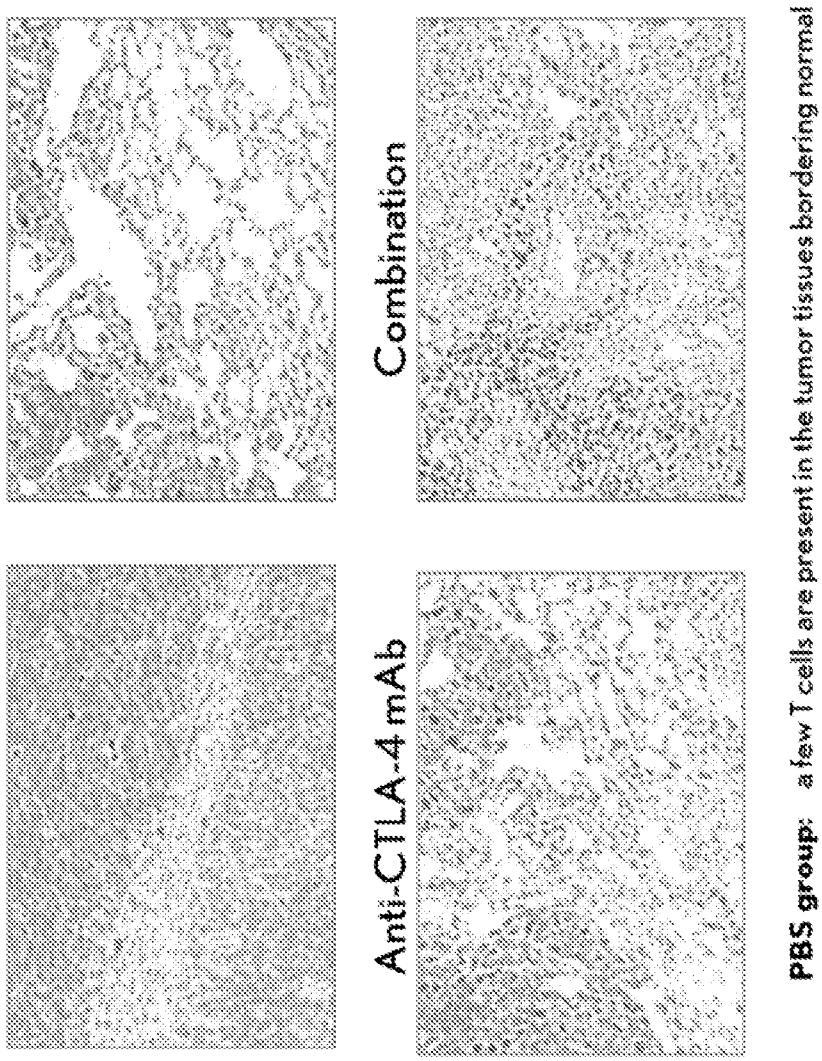

PBS
IMO-2125
Anti-CTLA-4 mAb
Combination
PBS group: a few T cells are present in the tumor tissues bordering normal tissue.
IMO-2125 group: increased T cells are infiltrating into tumor tissues.
Anti-CTLA-4 mAb group: increased T cells are infiltrating into tumor tissues.
Combination group: massive T cell infiltrate into tumor tissue.

FIG. 9

FIG. 10

A.

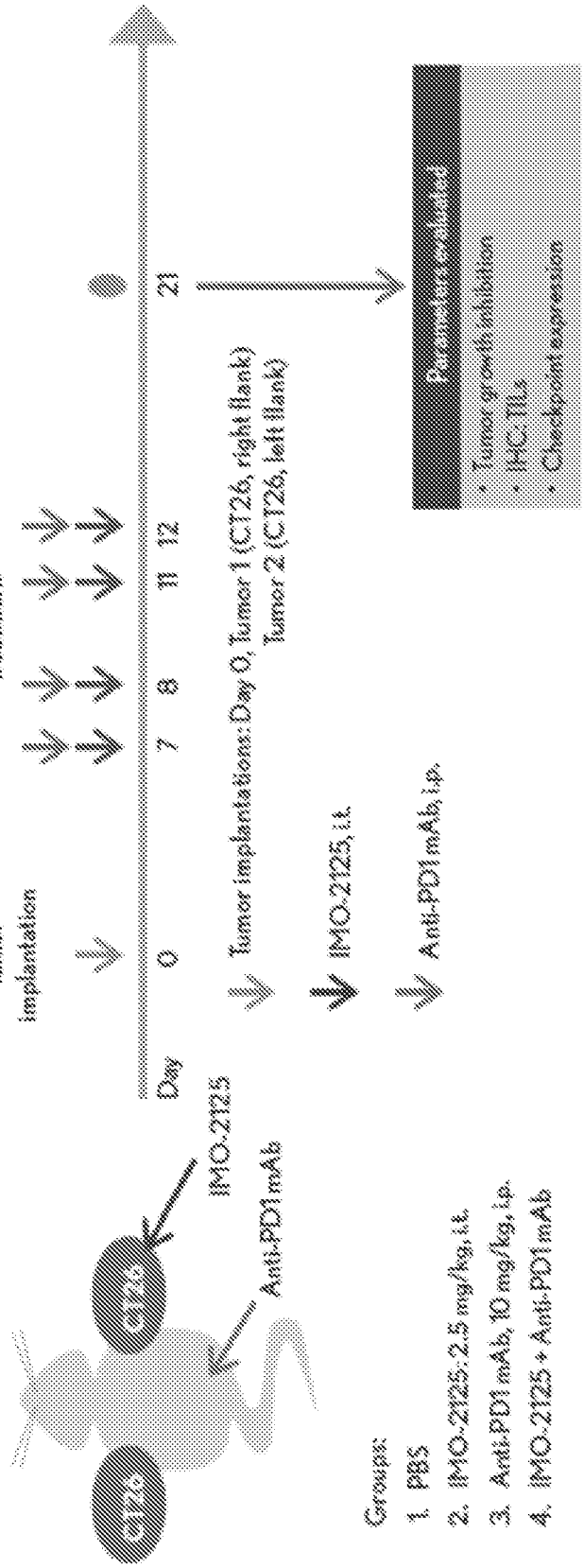

Tumor implantation
Treatment
Day
0
7
8
11
12
21
Tumor implantations: Day 0, Tumor 1 (CT26, right flank)
Tumor 2 (CT26, left flank)
IMO-2125, i.t.
Anti-PD1 mAb, i.p.
Pharmacodynamics
• Tumor growth inhibition
• IFN-γ ELISA
• Checkpoint expression
Groups:
1. PBS
2. IMO-2125, 2.5 mg/kg, i.t.
3. Anti-PD1 mAb, 10 mg/kg, i.p.
4. IMO-2125 + Anti-PD1 mAb
IMO-2125
Anti-PD1 mAb
CT26
CT26

FIG. 10 (CONT.)

B.

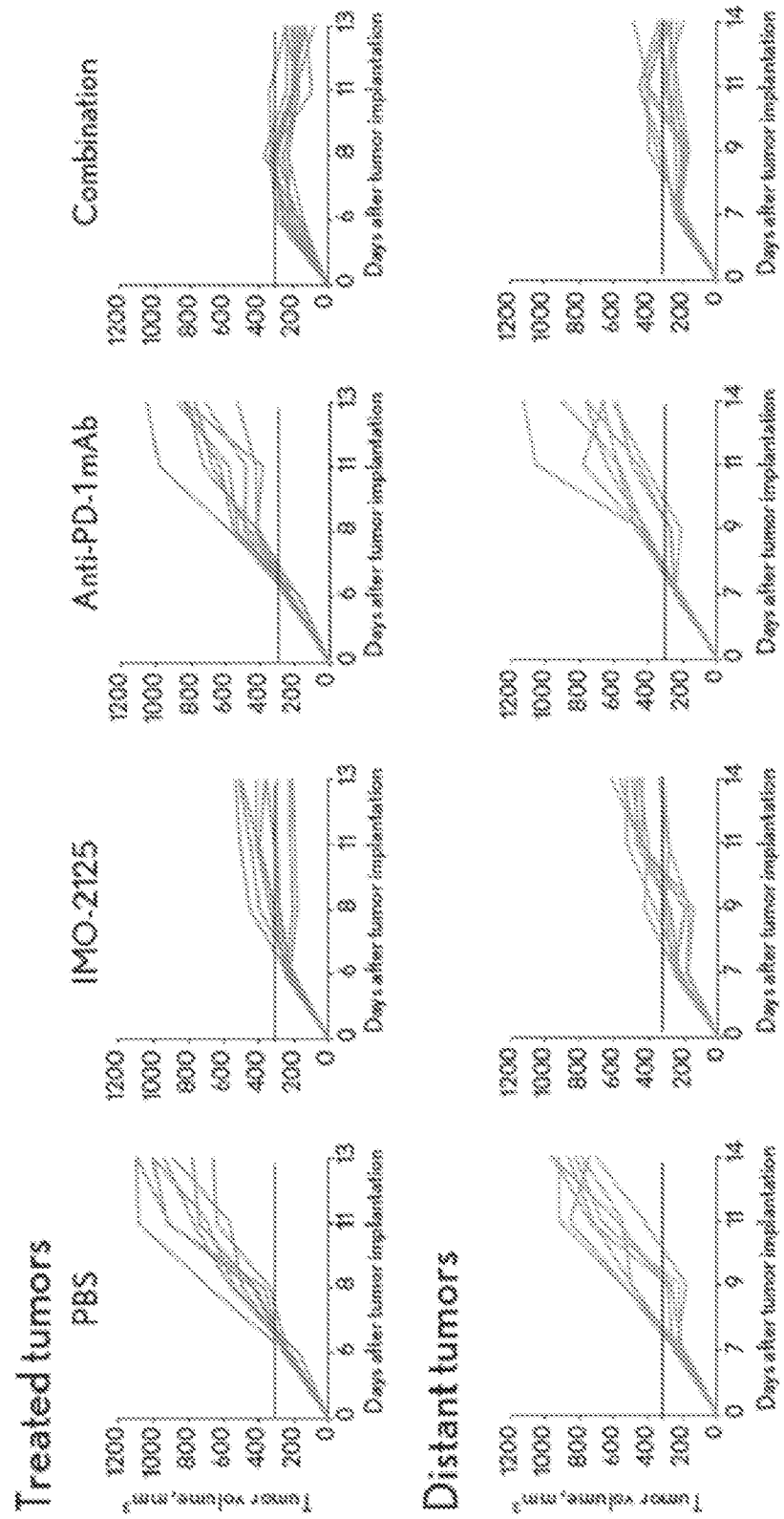

Treated tumors
Distant tumors
PBS
IMO-2125
Anti-PD-1 mAb
Combination
Tumor volume, mm³
Days after tumor implantation

FIG. 10 (CONT.)

C.

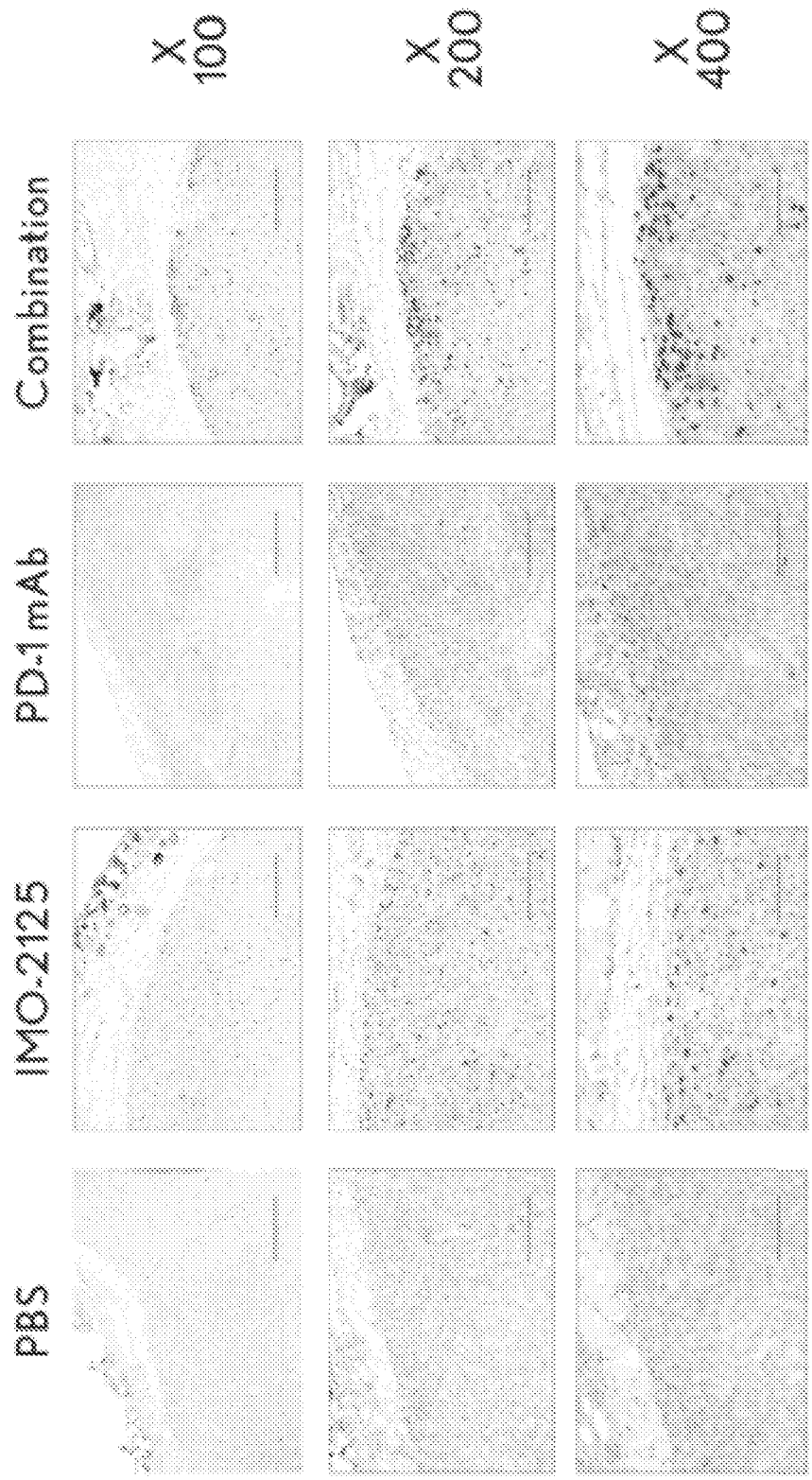


FIG. 10 (CONT.)

D.

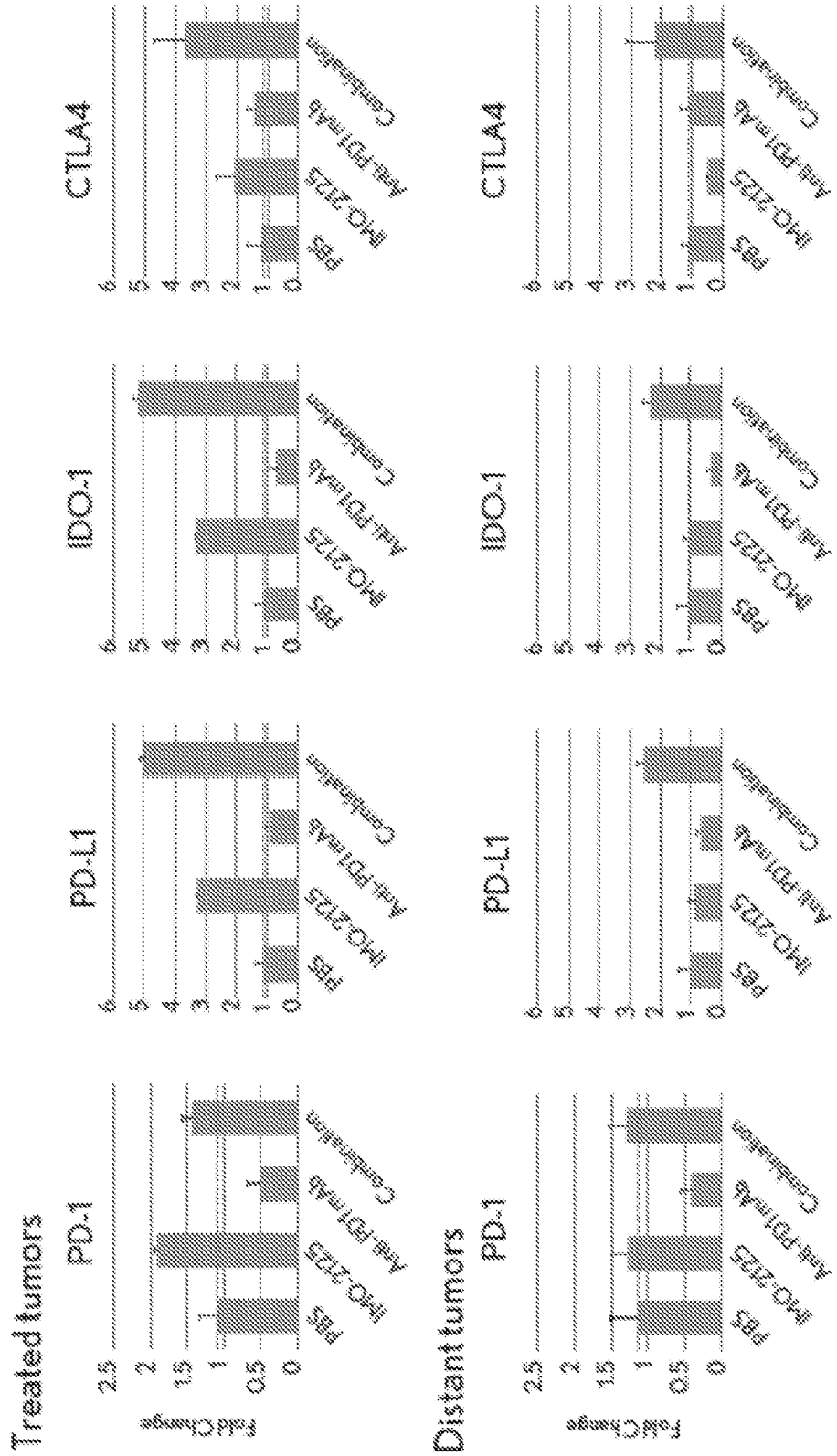
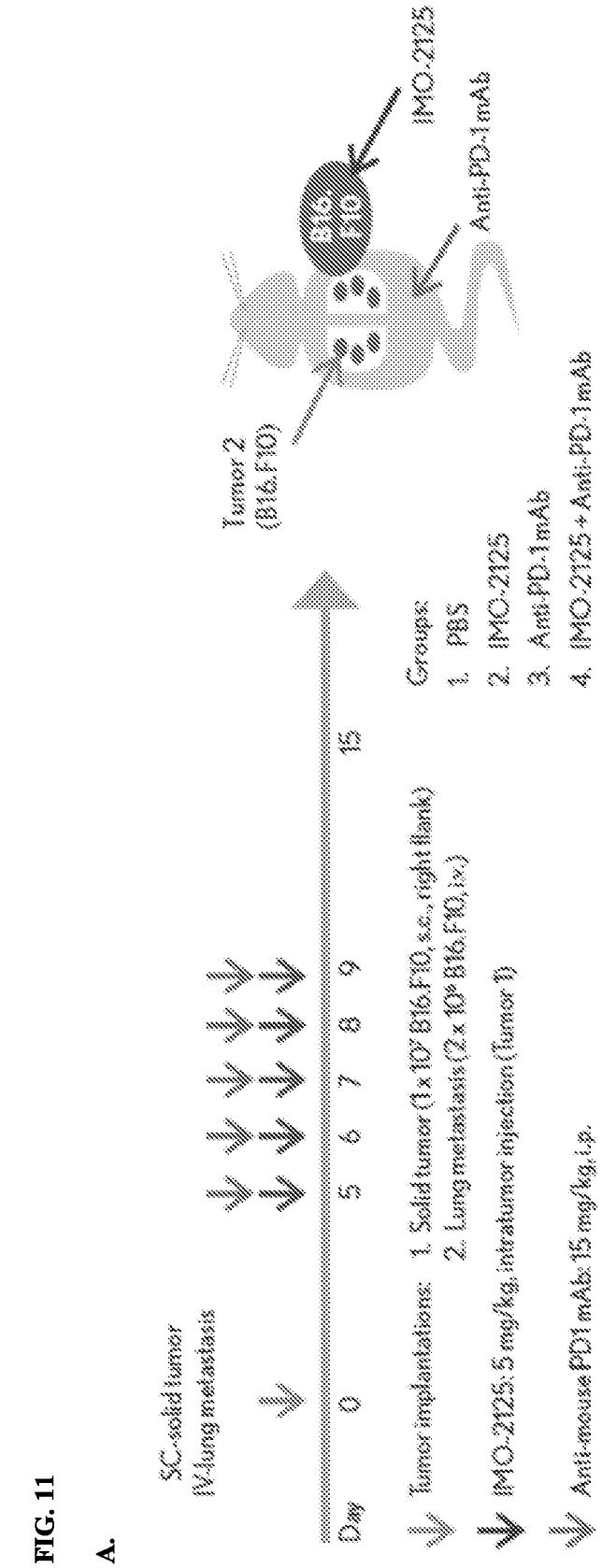

Treated tumors
Distant tumors
PD-1
PD-L1
IDO-1
CTLA4
Fold Change
PBS
IPI-D-2125
Anti-PD1 mAb
Combination

FIG. 11

A.


SC-solid tumor
IV-lung metastasis
Day 0 5 6 7 8 9 15
Tumor implantations: 1. Solid tumor (1 x 10⁵ B16.F10, s.c., right flank)
2. Lung metastasis (2 x 10⁵ B16.F10, i.v.)
IMO-2125: 5 mg/kg, intratumor injection (tumor 1)
Anti-mouse PD1 mAb: 15 mg/kg, i.p.
Groups:
1. PBS
2. IMO-2125
3. Anti-PD-1 mAb
4. IMO-2125 + Anti-PD-1 mAb
Tumor 2
(B16.F10)
B16
F10
IMO-2125
Anti-PD-1 mAb

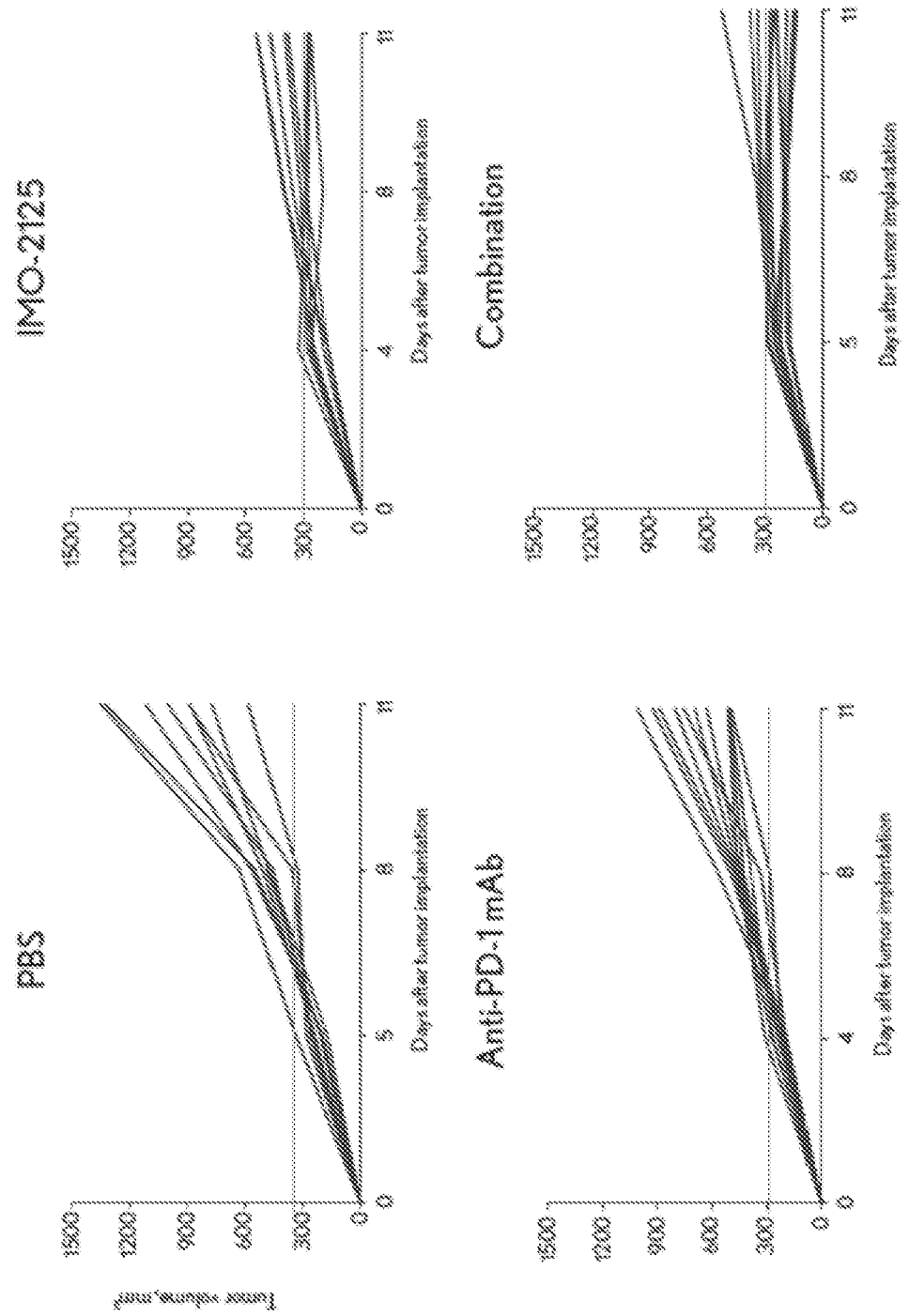

PBS
IMO-2125
Anti-PD-1 mAb
Combination
Tumor volume, mm³
1500
1200
900
600
300
0
0
4
8
11
Days after tumor implantation

FIG. 11

B.

FIG. 11

C.

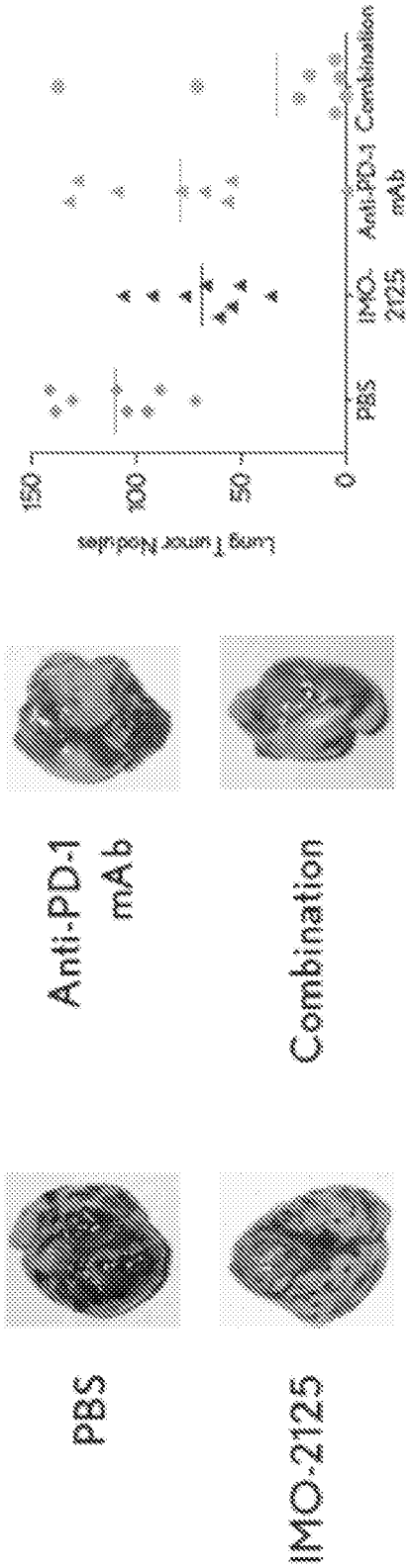

PBS
IMO-2125
Anti-PD-1 mAb
Combination
Lung Tumor Nodules
150
100
50
0
PBS
IMO-2125
Anti-PD-1 mAb
Combination

FIG. 11

D.

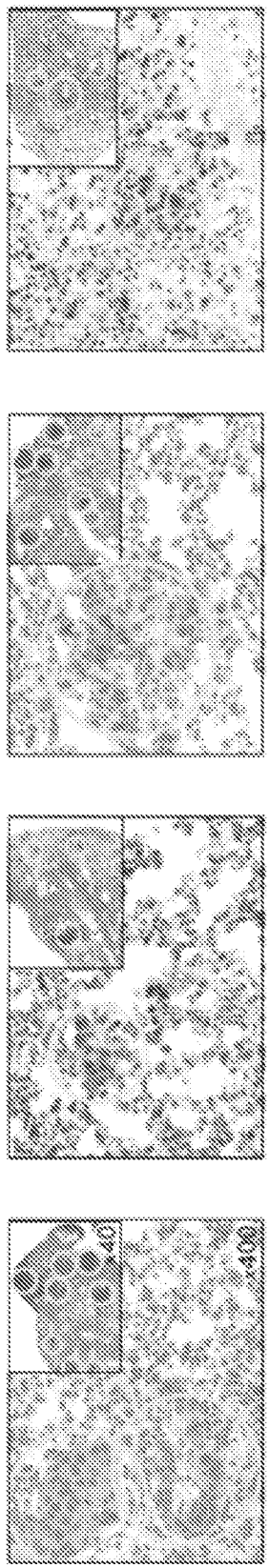
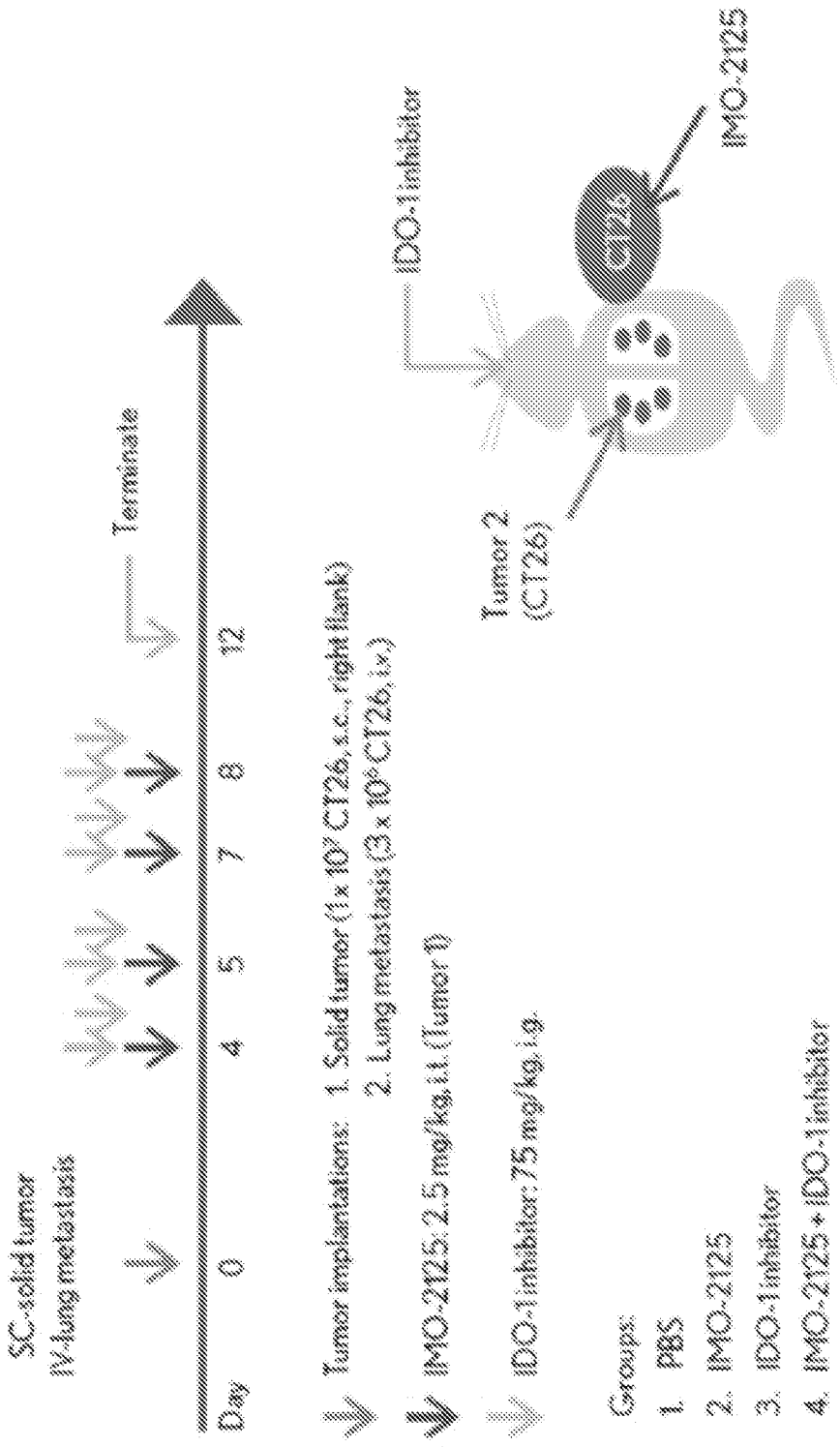

Placebo
IMO-2125
Anti-PD-1 mAb
Combination IMO-2125 and anti-PD-1 mAb
x40
x400
Legend
Circle: Large tumor nodule
Arrow: Small tumor nodule
Inset figures: HE stained (x40)
Large figures: CD3 stained (x400)

FIG. 12


SC-solid tumor
IV-lung metastasis
Terminate
Day 0 4 5 7 8 12
Tumor implantations: 1. Solid tumor (1 x 10⁷ CT26, s.c., right flank)
2. Lung metastasis (3 x 10⁵ CT26, i.v.)
IMO-2125: 2.5 mg/kg, i.t. (Tumor 1)
IDO-1 inhibitor: 75 mg/kg, i.g.
Groups:
1. PBS
2. IMO-2125
3. IDO-1 inhibitor
4. IMO-2125 + IDO-1 inhibitor
IDO-1 inhibitor
Tumor 2
(CT26)
IMO-2125

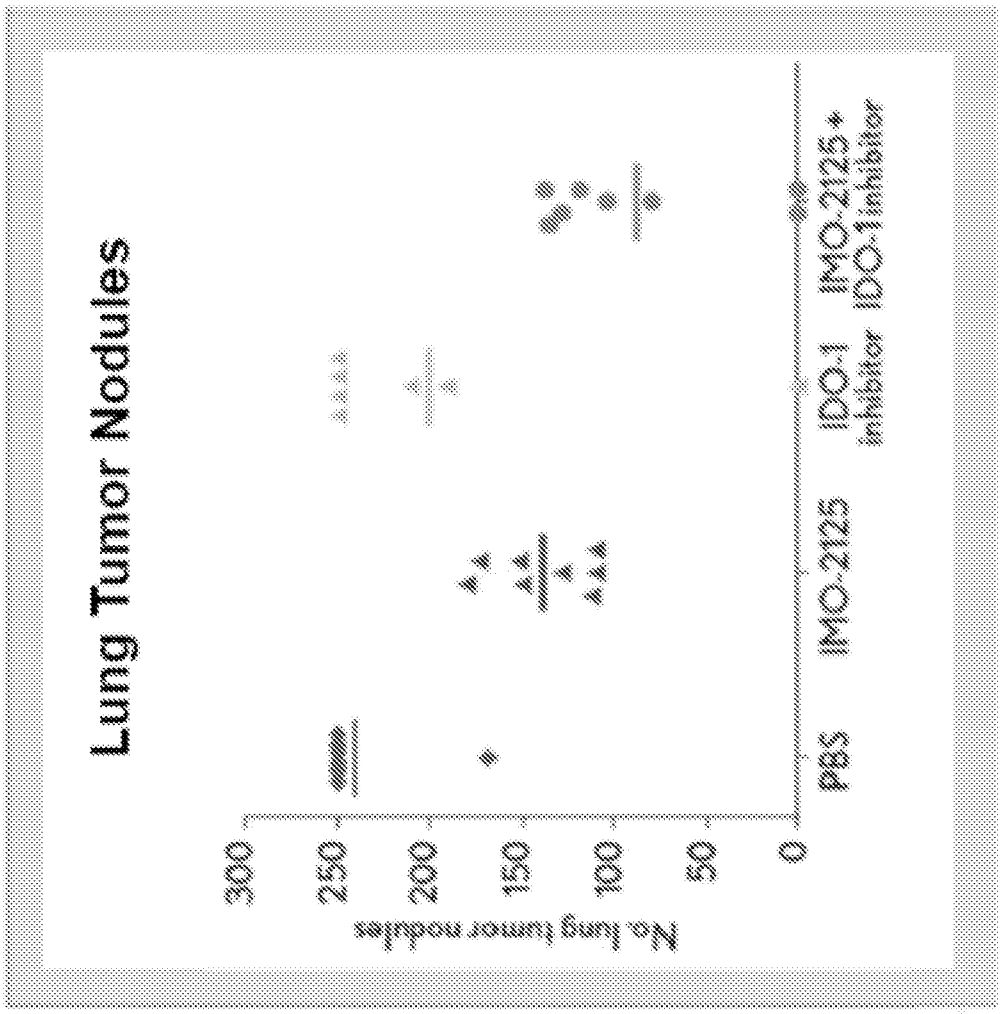

Lung Tumor Nodules
No. lung tumor nodules
300
250
200
150
100
50
0
PBS
IMO-2125
IDO-1 inhibitor
IMO-2125 + IDO-1 inhibitor

FIG. 13A

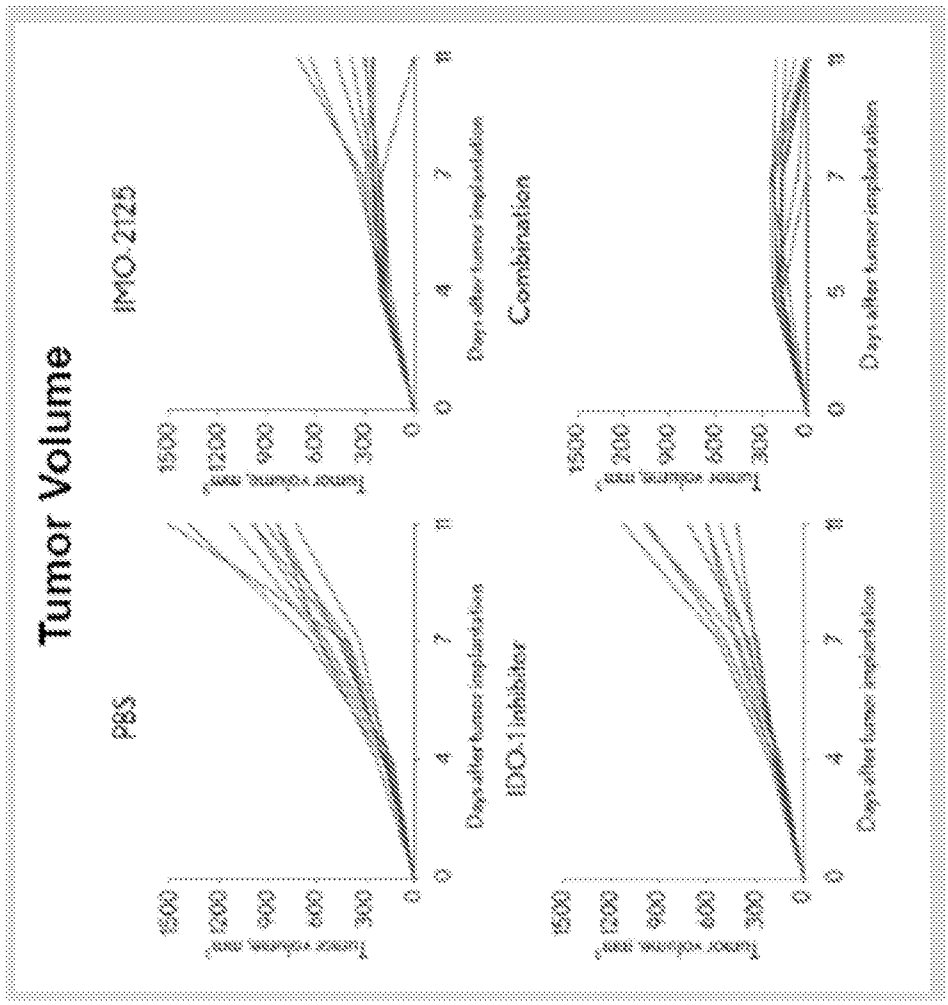

Tumor Volume
PBS
IMO-2125
IDO-1 Inhibitor
Combination
1500
1200
900
600
300
0
0
4
7
11
Days after tumor implantation

FIG. 13B

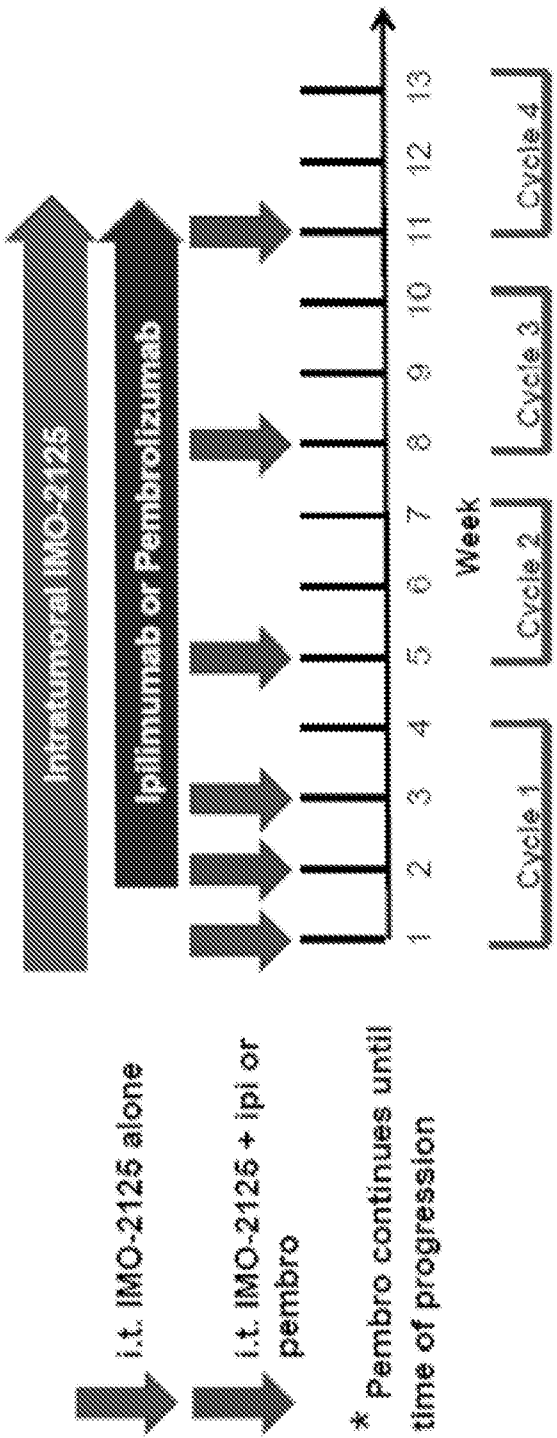

Intratumoral IMO-2125
Ipilimumab or Pembrolizumab
1 2 3 4 5 6 7 8 9 10 11 12 13
Week
Cycle 1
Cycle 2
Cycle 3
Cycle 4
i.t. IMO-2125 alone
i.t. IMO-2125 + ipi or pembro
* Pembro continues until time of progression

FIG. 14

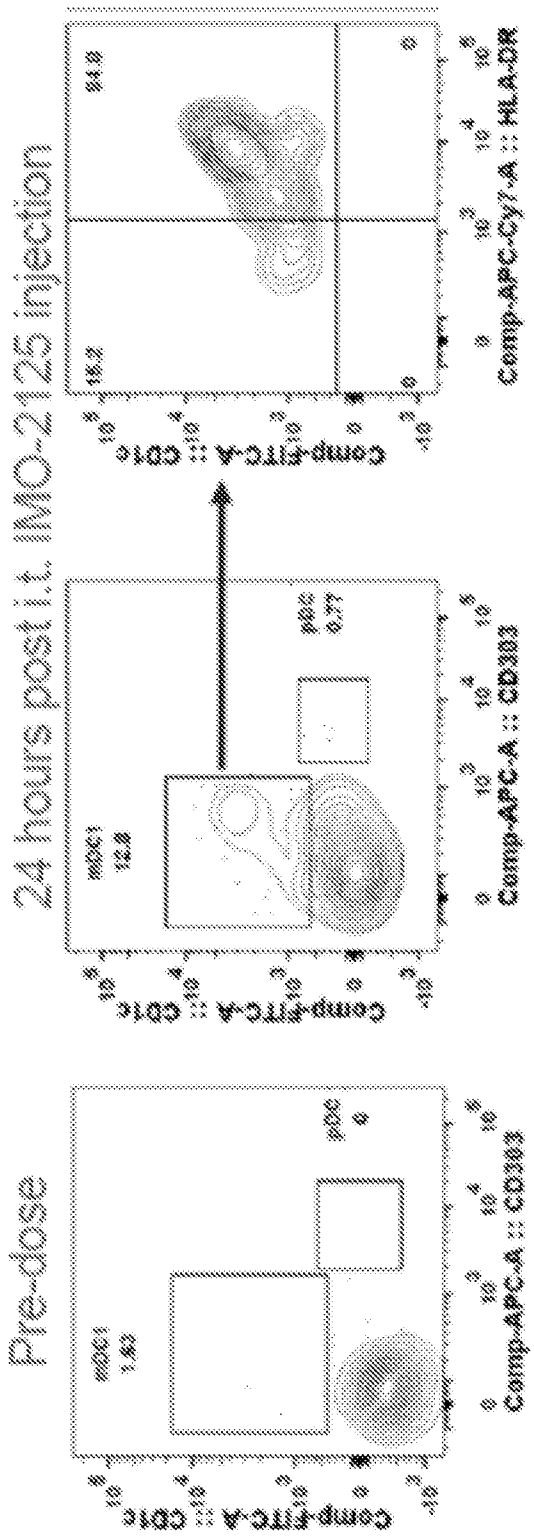

Pre-dose
24 hours post i.t. IMO-2125 injection
Comp-FITC-A :: CD1c
Comp-APC-A :: CD303
Comp-APC-Cy7-A :: HLA-DR

FIG. 15A

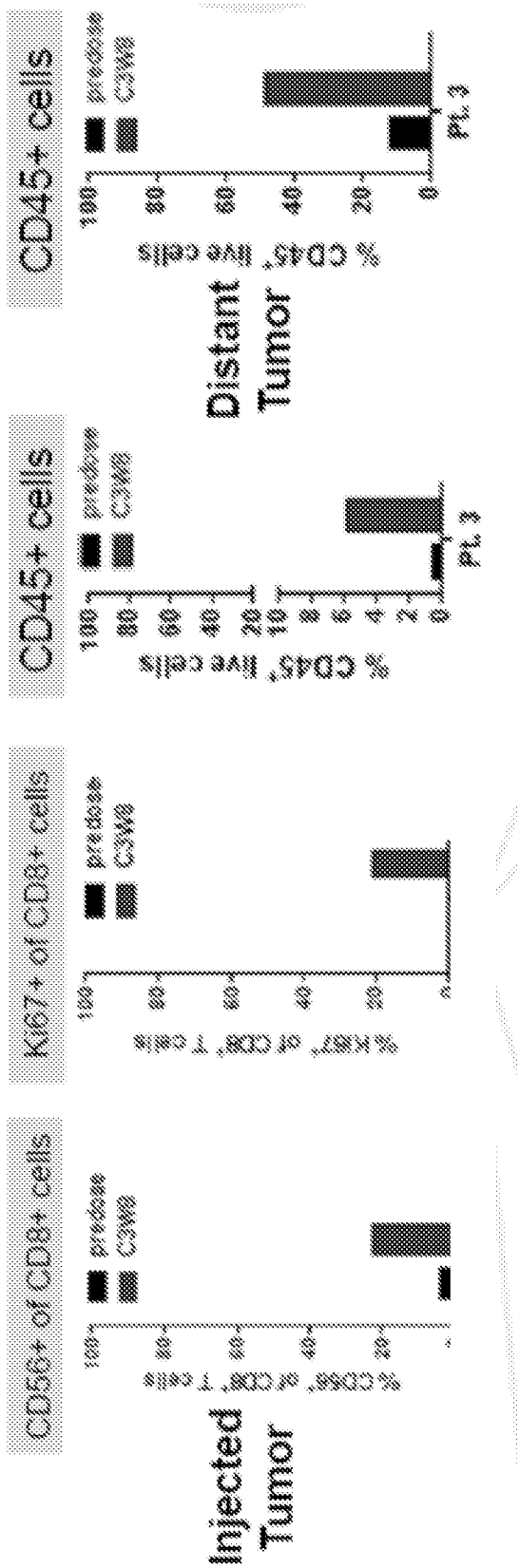

CD45+ cells
CD45+ cells
Ki67+ of CD8+ cells
CD56+ of CD8+ cells
predose
C3W8
% CD45+ live cells
% Ki67+ of CD8+ T cells
% CD56+ of CD8+ T cells
PL 3
Distant Tumor
Injected Tumor

FIG. 15B

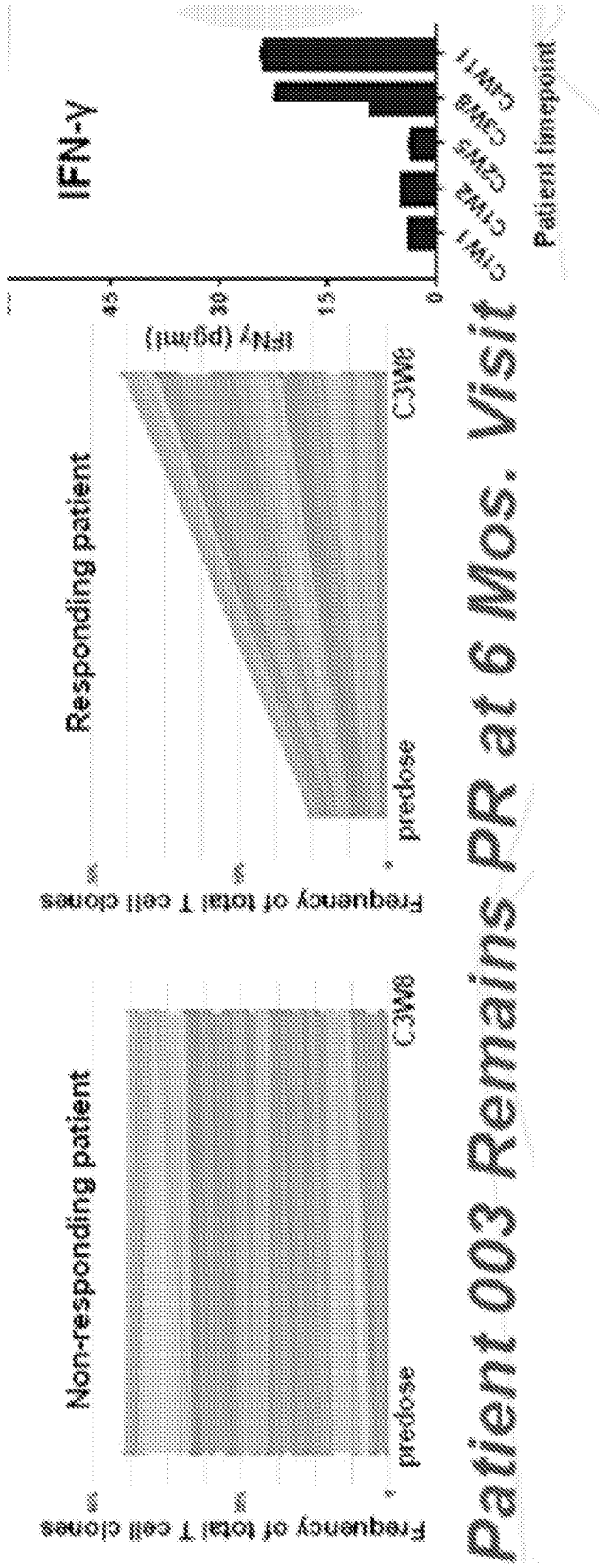

Patient 003 Remains PR at 6 Mos. Visit
IFN-γ
IFNγ (pg/ml)
45
30
15
0
CM1
CM2
CM3
CM4
CM5
Patient timepoint
Responding patient
C3W8
predose
Frequency of total T cell clones
Non-responding patient
C3W8
predose
Frequency of total T cell clones

FIG. 16

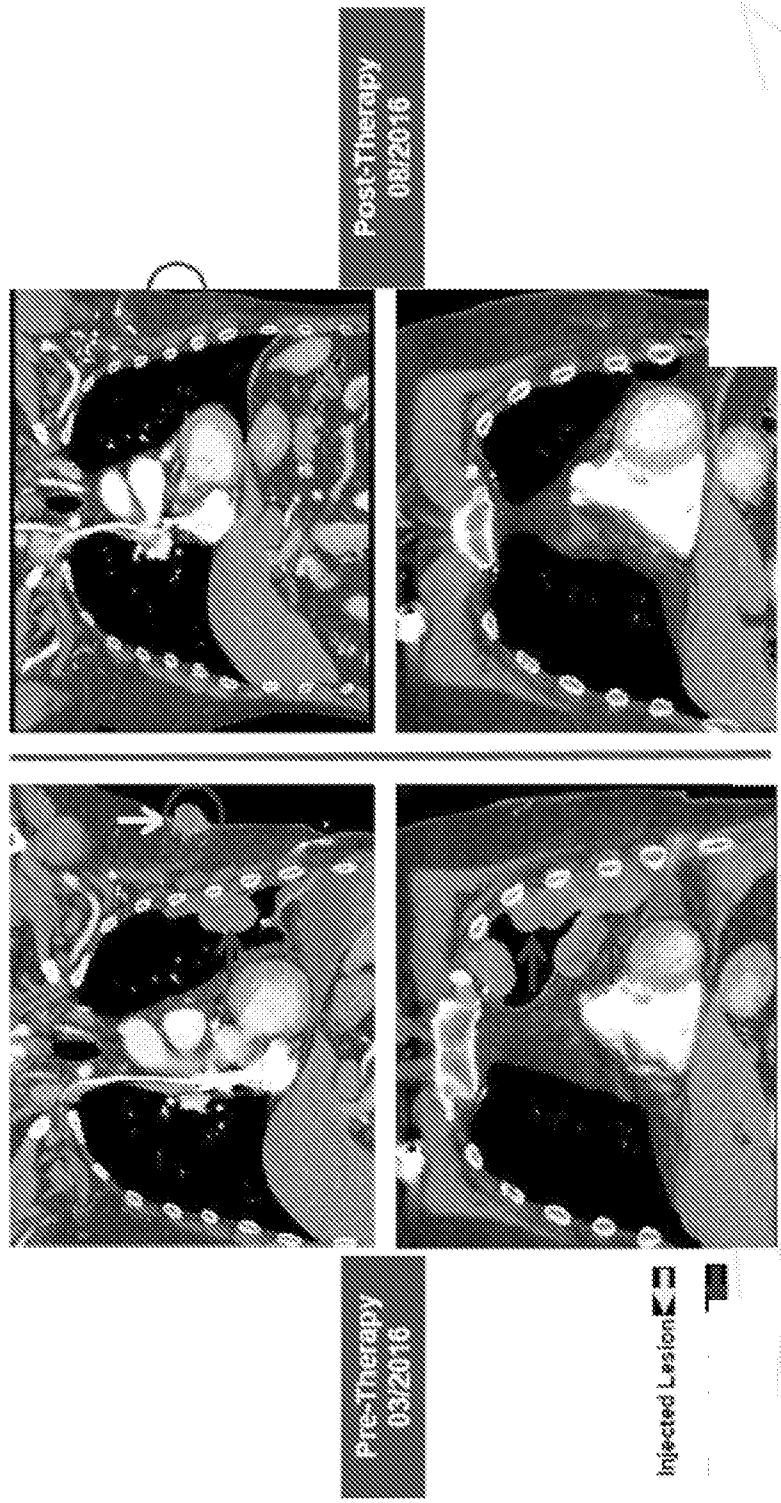

Ipilimumab 3mg plus i.t. IMO-2125 8 mg
Pre-Therapy 03/2016
Post-Therapy 08/2016
Injected Lesion

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/51742

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/51742

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/51742

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 9/00, 31/7088, 31/7115, 45/06 (2017.01)
CPC - A61K 9/0012, 9/0019, 31/7088, 31/7115, 45/06

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0101128 A1 (IDERA PHARMACEUTICALS) 14 April 2016; paragraphs [0011], [0012], [0071], [0087]	1-2, 3/1-2, 4/3/1-2
Y	WO 2016/128542 A1 (TRANSGENE SA) 18 August 2016; page 4, lines 29-32, page 35, lines 13-15, page 41, lines 11-19, page 42, lines 5-16, page 51, lines 2-10	1-2, 3/1-2, 4/3/1-2
Y	(PARDOLL, DM) The blockade of immune checkpoints in cancer immunotherapy. National Review Cancer. April 2012, Epub 22 March 2012, Vol. 12, No. 4; pages 252-264; page 9, 2nd paragraph; DOI: 10.1038/nrc3239	4/3/1-2
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22 November 2017 (22.11.2017)	18 DEC 2017
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