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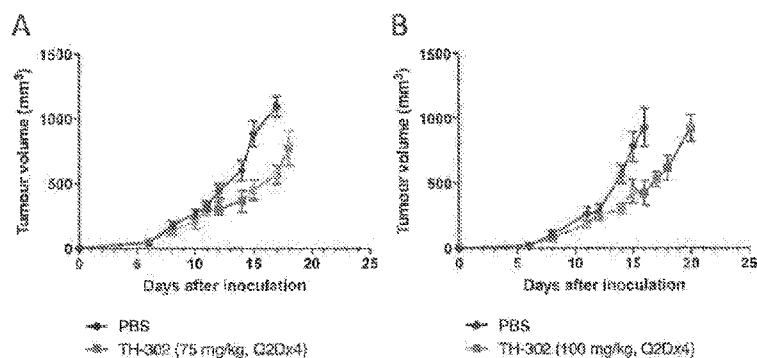


FIG. 1

(57) Abstract: Disclosed herein are combinations comprising a tyrosine kinase inhibitor (TKI) and an immunotherapeutic agent, formulations, and methods of treating cancer comprising the same.



COMBINATION OF A KINASE INHIBITOR AND AN IMMUNOTHERAPEUTIC AGENT, COMPOSITIONS AND METHODS COMPRISING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/751,355, filed October 26, 2018, the contents of which is incorporated by reference herein in its entirety.

BACKGROUND

[0002] Cancer continues to be a significant unmet clinical indication. Kinases represent a large family of enzymes that catalyse the phosphorylation of proteins, lipids and metabolites and play a central role in the regulation of a wide variety of cellular processes. Abnormal kinase activity has been related to a wide range of disorders, including cancers. This has led to the development of kinase inhibitors (TKIs) as therapeutics, including as anti-cancer agents. Immunotherapies have also been developed as anti-cancer agents. However, immunotherapy response rates remain low, and only a few patients ultimately benefit from this approach. Indeed, immunotherapy is only effective in a very small number of patients, and some patients who may initially respond show subsequent substantial and rapid disease progression. See, Liang, H et al. *OncoTargets and Therapy* 2018;11, 6189-96. Several combination therapies comprising TKIs and certain immunotherapeutic agents have been proposed; however, these combinations have had little to no clinical success for various reasons. First, the majority of patients treated with these combinations develop acquired resistance. Thress KS et al. *Nat Med.* 2015;21(6):560–562; Yu HA, et al. *Clin Cancer Res.* 2013;19(8):2240–2247; Jänne PA et al. *AZD9291 N Engl J Med.* 2015;372(18):1689–1699. Second, these combinations suffer from little to no efficacy for the treatment of cancer. Lee CK et al. *J. Thorac. Oncol.* 2017; 12(2): 403-407. Third, the safety profiles of these combinations are poor, with certain clinical trials discontinued due to reports of severe adverse effects. See, Liang, H et al. *OncoTargets and Therapy* 2018;11, 6189-96. No safe and effective TKI + immunotherapy combinations currently exist. Accordingly, new combination treatments are needed for the treatment of cancer that are exhibit high efficacy, low toxicity, and little acquired resistance.

SUMMARY

[0003] In one aspect, disclosed herein is a pharmaceutical combination comprising (a) (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (Compound

A), or a pharmaceutically acceptable salt or solvate thereof; and (b) an immunotherapeutic agent.

[0004] In one aspect, disclosed herein is a pharmaceutical combination comprising (a) (E)-N-(4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide (Compound B), or a pharmaceutically acceptable salt or solvate thereof; and (b) an immunotherapeutic agent.

[0005] In some embodiments, the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy. In some embodiments, the immunotherapeutic agent is an inhibitor of an immune checkpoint protein. In some embodiments, the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, or CTLA-4. In some embodiments, the combination is for simultaneous, separate, or sequential administration. In some embodiments, the pharmaceutical combination is a synergistic combination. In some embodiments, the combinations disclosed herein exhibit synergy. Known combinations of TKIs and immunotherapeutic agents do not have a synergistic tumour cell-killing effect. Accordingly, it is surprising and unexpected that the combinations disclosed herein exhibit such synergy.

[0006] In one aspect, disclosed herein is a method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, oesophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland or testicular cancer. In some embodiments, the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer. In some embodiments, the cancer is non-small cell lung cancer. In some embodiments, the cancer is a gastric tumor.

[0007] In one aspect, disclosed herein is a method of activating T-cells or a T-cell response in a subject comprising administering to the subject a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, T-cell proliferation is increased in the subject. In some embodiments, T-cell function is enhanced in the subject.

[0008] In one aspect, disclosed herein is a method of enhancing an immune response in a subject comprising administering to the subject having a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

[0009] In one aspect, disclosed herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD-1 ligand to human PD-1, comprising administering to a subject having the disease a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is an inhibitor of an immune checkpoint protein. In some embodiments, the immune checkpoint inhibitor is an inhibitor of an immune checkpoint protein selected from Programmed Death 1 (PD-1) and Programmed Death-Ligand 1 (PD-L1). In some embodiments, the disease is cancer.

[0010] In another aspect, disclosed herein is a method of inhibiting tumour cell growth comprising administering to the tumour a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, the tumour is a solid tumour. In some embodiments, the solid tumour is selected from bladder carcinoma, squamous cell carcinoma, transitional cell carcinoma, basal cell carcinoma, renal cell carcinoma, ductal cell carcinoma, and adenocarcinoma.

[0011] In one aspect, disclosed herein is a compound which is Compound A, or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment of cancer, wherein the treatment comprises administering the compound in combination with an immunotherapeutic agent.

[0012] In one aspect, disclosed herein is a pharmaceutical combination comprising Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for use in the treatment of cancer.

[0013] In one aspect, disclosed herein is a use of a combination comprising a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for the treatment of cancer in a patient.

[0014] In one aspect, disclosed herein is a use of a combination comprising a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent in the manufacture of a medicament for the treatment of cancer in a patient.

[0015] In yet another aspect, disclosed herein is a method of sensitizing a hypoxic tumour to a checkpoint inhibitor comprising administering to the hypoxic tumour a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The following detailed description of specific embodiments of the disclosure will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosure, specific embodiments are shown in the drawings. It should be understood, however, that the disclosure is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

[0017] **FIG. 1** shows average tumour volume ($\pm$ S.E.M.) of MB49 tumours treated with vehicle control (PBS), 75 mg/kg (A) or 100 mg/kg (B) TH-302.

[0018] **FIG. 2** shows average weight loss ($\pm$ S.E.M.) in C57BL/6 mice bearing MB49 tumours treated with vehicle control (PBS), 75 mg/kg (A) or 100 mg/kg (B) TH-302.

[0019] **FIG. 3** shows individual tumour volumes of MB49 tumours treated with vehicle control (PBS), 75 mg/kg, Q2Dx4 (top) or 100 mg/kg, Q2Dx4 (bottom) TH-302.

[0020] **FIG. 4** shows individual body weight changes of MB49 tumour bearing C57BL/6 mice treated with vehicle control (PBS), 75 mg/kg, Q2Dx4 (top) or 100 mg/kg, Q2Dx4 (bottom) TH-302.

[0021] **FIG. 5** shows survival analysis of MB49 tumours treated with vehicle control (PBS), 75 mg/kg, Q2Dx4 (A) or 100 mg/kg, Q2Dx4 (B) TH-302.

[0022] **FIG. 6** shows average tumour volumes ($\pm$ S.E.M.) in C57BL/6 mice bearing MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination.

[0023] **FIG. 7** shows average weight loss ($\pm$ S.E.M.) in C57BL/6 mice bearing MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination.

[0024] **FIG. 8** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination on day 14. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test. ****, $P < 0.0001$.

[0025] **FIG. 9** shows individual tumour volumes of MB49 tumours treated with 50 mg/kg tarloxotinib (Q2Dx4) and tarloxotinib/anti-PD-1 combination on day 24. Statistical significance between groups were determined using unpaired t-test. ***, $P = 0.0004$.

[0026] **FIG. 10** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination.

[0027] **FIG. 11** shows individual body weight changes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination.

[0028] **FIG. 12** shows survival analysis of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination. Log-rank (Mantel-Cox) test indicated that the survival curves were significantly different ($P < 0.0001$).

[0029] **FIG. 13** shows average tumour volumes ($\pm$ S.E.M.) in C57BL/6 mice bearing MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0030] **FIG. 14** shows average weight loss ($\pm$ S.E.M.) in C57BL/6 mice bearing MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0031] **FIG. 15** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations on day 19. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test. **, $P < 0.005$; ****, $P < 0.0001$.

[0032] **FIG. 16** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-

L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations on day 26. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test. **, $P<0.005$; ***, $P<0.0005$; ****, $P<0.0001$.

[0033] **FIG. 17** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0034] **FIG. 18** shows individual body weight changes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0035] **FIG. 19** shows survival analysis of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 100 mg/kg TH-302 (Q2Dx4), anti-PD-1 (Q3Dx3), tarloxotinib/anti-PD-1 combination and TH-302/anti-PD-1 combination. Log-rank (Mantel-Cox) test indicated that the survival curves were significantly different ($P<0.0001$).

[0036] **FIG. 20** shows tSNE plot of T-cells in the blood overlaid with the expression of CD3 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0037] **FIG. 21** shows tSNE plot of T-cells in the blood overlaid with the expression of CD4 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0038] **FIG. 22** shows tSNE plot of T-cells in the blood overlaid with the expression of CD8 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0039] **FIG. 23** shows percentage of CD3⁺, CD4⁺ and CD8⁺ T-cells in the blood of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations

[0040] **FIG. 24** shows tSNE plot of myeloid cells in the blood overlaid with the expression of CD11b marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0041] **FIG. 25** shows tSNE plot of myeloid cells in the blood overlaid with the expression of CD11c marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0042] **FIG. 26** shows tSNE plot of immune cells in the blood overlaid with the expression of CD45 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0043] **FIG. 27** shows tSNE plot of immune cells in the blood overlaid with the expression of CD69 cell activation marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0044] **FIG. 28** shows tSNE plot of macrophages in the blood overlaid with the expression of F4-80 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0045] **FIG. 29** shows tSNE plot of immune cells in the blood overlaid with the expression of IFN γ intracellular marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0046] **FIG. 30** shows percentage of IFN γ ⁺ CD4⁺ and CD8⁺ T-cells in the blood of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0047] **FIG. 31** shows tSNE plot of T-cells in the blood overlaid with the expression of FOXP3 intracellular marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0048] **FIG. 32** shows tSNE plot of immune cells in the blood overlaid with the expression of Ki67 intracellular marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0049] **FIG. 33** shows tSNE plot of immune cells in the blood overlaid with the expression of KLRG1 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0050] **FIG. 34** shows percentage of Ki67⁺ and KLRG1⁺ CD8⁺ T-cells, and FOXP3⁺ CD4⁺ T-cells in the blood of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0051] **FIG. 35** shows tSNE plot of myeloid cells in the blood overlaid with the expression of Ly6C marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0052] **FIG. 36** shows tSNE plot of myeloid cells in the blood overlaid with the expression of Ly6G marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0053] **FIG. 37** shows tSNE plot of myeloid cells in the blood overlaid with the expression of MHC-II marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0054] **FIG. 38** shows tSNE plot of immune cells in the blood overlaid with the expression of NK1.1 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0055] **FIG. 39** shows tSNE plot of immune cells in the blood overlaid with the expression of PD-1 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0056] **FIG. 40** shows tSNE plot of immune cells in the blood overlaid with the expression of PD-L1 marker of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1 (Q3Dx3), anti-CTLA4 (Q3Dx3), anti-PD-L1 (Q3Dx3), tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 and tarloxotinib/anti-PD-L1 combinations.

[0057] **FIG. 41** shows immunofluorescence staining of hypoxia (red, EF5-Cy5) and cell nuclei (blue, DAPI) in EL4 lymphoma tumours harvested 24 hours or 72 hours post treatment with tarloxotinib at 60 mg/kg.

[0058] **FIG. 42** shows the MFI of activation marker CD86 and exhaustion marker PD-L1 on dendritic cells and percentage expansion of OT-I cells over 10 days post-vaccination assessed by flow cytometry. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (**, $P < 0.005$; ***, $P < 0.0005$; ****, $P < 0.0001$).

[0059] **FIG. 43** shows average tumour volumes ($\pm$ S.E.M.) (A), body weight change (B) and survival curve (C) in C57BL/6 mice bearing EL4 tumours treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0060] **FIG. 44** shows Individual tumour volumes of EL4 tumours treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0061] **FIG. 45** shows individual body weight changes of EL4 tumours treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0062] **FIG. 46** shows average tumour volumes ($\pm$ S.E.M.) (A), body weight change (B) and survival curves (C) in C57BL/6 mice bearing EG7 tumours treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0063] **FIG. 47** shows individual tumour volumes of EG7 tumours treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0064] **FIG. 48** shows individual body weight changes of EL4 tumours treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0065] **FIG. 49** shows tumour volume and number of tumour infiltrating lymphocytes in EG7 tumour bearing mice treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0066] **FIG. 50** shows number of CD4⁺ and CD8⁺ T-cells and their corresponding PD-1 expression levels in the spleen in EG7 tumour bearing mice treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0067] **FIG. 51** shows number of IFN γ and TNF α producing CD4 $^{+}$ and CD8 $^{+}$ T-cells in the spleen in EG7 tumour bearing mice treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0068] **FIG. 52** shows percentage of regulatory T-cells (FOXP3 $^{+}$ CD4 $^{+}$ T-cells) in the spleen, draining lymph node and tumour in EG7 tumour bearing mice treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0069] **FIG. 53** shows dot plots showing the magnitude of OT-I and OT-II cell proliferation represented by the intensity of the CFSE dye.

[0070] **FIG. 54** shows number of IFN γ spots per million splenocytes re-stimulated with irradiated EG7.OVA cells from EG7 tumour bearing mice treated with vehicle control, 60 mg/kg tarloxotinib (Q2Dx3), anti-PD-1 and tarloxotinib/anti-PD-L1 combinations.

[0071] **FIG. 55** shows average weight ($\pm$ S.E.M.) of MB49 tumours harvested from MB49 tumour bearing mice, percentage and number of CD45 $^{+}$ immune cells in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination.

[0072] **FIG. 56** shows percentage and number of CD3 $^{+}$, CD4 $^{+}$ and CD8 $^{+}$ T-cells cells in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination.

[0073] **FIG. 57** shows percentage and number of regulatory T-cells (FoxP3 $^{+}$ CD4 $^{+}$ T-cells) in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination. CD8/Treg ratios were calculated based on the number of CD8 $^{+}$ T-cells over the number of Tregs in the tumour.

[0074] **FIG. 58** shows percentage and number of natural killer-like T-cells (Nk1.1 $^{+}$ CD3 $^{+}$ T-cells) and natural killer cells (Nk1.1 $^{+}$ CD3 $^{-}$) in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination.

[0075] **FIG. 59** shows percentage and number of myeloid cells and conventional dendritic cells (CD11c $^{+}$ MHC-II $^{+}$ cells) in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination.

[0076] **FIG. 60** shows percentage and number of granulocyte-like (G-MDSC) and monocytic (M-MDSC) myeloid derived suppressor cells, and conventional dendritic cells (CD11c $^{+}$ MHC-II $^{+}$ cells) in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg

tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination. CD8/MDSC ratios were calculated based on the number of CD8⁺ T-cells over the number of M-MDSC or G-MDSC in the tumour.

[0077] **FIG. 61** shows average tumour volumes ($\pm$ S.E.M.) in MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3), anti-PD-1/anti-CTLA4 (Q3Dx3), tarloxotinib/anti-PD-L1 combination and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0078] **FIG. 62** shows average body weight change ($\pm$ S.E.M.) in MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3), anti-PD-1/anti-CTLA4 (Q3Dx3), tarloxotinib/anti-PD-L1 combination and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0079] **FIG. 63** shows average tumour volumes (mean $\pm$ SEM) of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination on day 19 and day 30. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (*, $P < 0.05$; ****, $P < 0.0001$).

[0080] **FIG. 64** shows average tumour volumes (mean $\pm$ SEM) of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination on day 19 and day 30. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (****, $P < 0.0001$).

[0081] **FIG. 65** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3), anti-PD-1/anti-CTLA4 (Q3Dx3), tarloxotinib/anti-PD-L1 combination and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0082] **FIG. 66** shows individual body weight changes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3), anti-PD-1/anti-CTLA4 (Q3Dx3), tarloxotinib/anti-PD-L1 combination and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0083] **FIG. 67** shows survival analysis of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-L1 (Q3Dx3), anti-PD-1/anti-CTLA4 (Q3Dx3), tarloxotinib/anti-PD-L1 combination and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0084] **FIG. 68** shows average tumour volumes and body weight change ($\pm$ S.E.M.) in MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0085] **FIG. 69** shows average tumour volumes (mean $\pm$ SEM) of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination on day 18 and day 22. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

[0086] **FIG. 70** shows individual tumour volumes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0087] **FIG. 71** shows individual body weight changes of MB49 tumours treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0088] **FIG. 72** shows survival analysis of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0089] **FIG. 73** shows average weight ($\pm$ S.E.M.) of MB49 tumours harvested from MB49 tumour bearing mice, percentage and number of CD45⁺ immune cells in the tumour treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0090] **FIG. 74** shows percentage and number of CD3⁺, CD4⁺ and CD8⁺ T-cells cells in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0091] **FIG. 75** shows percentage and number of regulatory T-cells (FOXP3⁺ CD4⁺ T-cells) in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination. CD8/Treg ratios were calculated based on the number of CD8⁺ T-cells over the number of Tregs in the tumour.

[0092] **FIG. 76** shows percentage and number of conventional dendritic cells (CD11c⁺ MHC-II⁺ cells) in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg

tarloxotinib (Q2Dx4), anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination.

[0093] FIG. 77 shows percentage of granulocyte-like (G-MDSC) and monocytic (M-MDSC) myeloid derived suppressor cells in the tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), anti-PD-1/anti-CTLA4 (Q3Dx3) and tarloxotinib/anti-PD-1 + anti-CTLA4 combination. CD8/MDSC ratios were calculated based on the number of CD8⁺ T-cells over the number of M-MDSC or G-MDSC in the tumour.

[0094] FIG. 78 shows average concentration ($\pm$ S.E.M.) of IFN γ in the blood serum and tumour of MB49 tumour bearing mice treated with vehicle control, 50 mg/kg tarloxotinib (Q2Dx4), 200 μ g anti-PD-L1 (Q3Dx3) and tarloxotinib/anti-PD-L1 combination treatments. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (*, $P < 0.05$; **, $P < 0.01$).

[0095] FIG. 79 shows average percentage and mean fluorescence intensity (MFI) ($\pm$ S.E.M.) of EF5⁺ tumour cells in control and 50 mg/kg tarloxotinib (Q2Dx4) treated mice.

[0096] FIG. 80 shows average number and mean fluorescence intensity (MFI) ($\pm$ S.E.M.) of EF5⁺ CD4⁺ and CD8⁺ T-cells cells in the tumour of MB49 tumour bearing mice treated with vehicle control or 50 mg/kg tarloxotinib (Q2Dx4).

[0097] FIG. 81 shows average percentage and number ($\pm$ S.E.M.) of PD-L1⁺ CD45⁻ tumour cells in MB49 tumour bearing mice treated with vehicle control or 50 mg/kg tarloxotinib (Q2Dx4).

DETAILED DESCRIPTION

[0098] The present disclosure relates to the combinations of certain small molecule tyrosine kinase inhibitors (TKIs) and an immunotherapeutic agent that can be used as a combination therapy to treat or prevent certain cancers. The TKIs are nitromethylaryl quaternary ammonium salts (also referred to as NMQ prodrugs). In some embodiments, the cancer is non-small cell lung cancer. In some embodiments, the TKI is RN-4000 (also known as "(E)-4-((4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)amino)-N,N-dimethyl-N-((1-methyl-4-nitro-1H-imidazol-5-yl)methyl)-4-oxobut-2-en-1-aminium salt (bromide)"; also referred to herein as "(2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide"; also referred to herein as Compound A, RN-4000, TRLX, or tarloxotinib) and/or its active metabolite, RN-4000E (also known as "(E)-N-(4-((3-bromo-4-

chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide”; also referred to herein as “(2E)-N-[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide”; also referred to herein as Compound B).

[0099] In some embodiments, the combination comprises Compound A and an immunotherapeutic agent. In some embodiments, the combination comprises Compound B and an immunotherapeutic agent. In some embodiments, the combination comprises Compound A, Compound B and an immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is selected from an anti-PD1 antibody, an anti-PD-L1 antibody, and an anti-CTLA4 antibody. In some embodiments, the combinations of the TKI and the immunotherapeutic agent are used to treat or prevent certain cancers.

[0100] In some embodiments, the cancer is non-small cell lung cancer.

[0101] It will be understood by one of ordinary skill in the art that Compound A may exist as a cation or salt, for example, a bromide salt, as depicted below.

Definitions

[0102] 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. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present application, illustrative methods and materials are described. As used herein, each of the following terms has the meaning associated with it in this section.

[0103] As used herein, the articles “a” and “an” are used to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0104] As used herein, “about,” when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

[0105] A disease or disorder is “alleviated” if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

[0106] As used herein, the terms “alkyl”, “alkenyl”, “alkynyl” and “alkoxy” include both straight chain and branched chain groups, and unsubstituted and substituted groups. The optional substituents may include, without limitation, halogen, C₁-C₆ alkoxy, CN, OH, NH₂,

NO₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, CONH₂, CO(C₁-C₆ alkyl), SO₂NH₂ and SO₂(C₁-C₆ alkyl).

[0107] As used herein, the term “aromatic nitroheterocycle” means an aromatic heterocyclic moiety substituted at any ring position by one or more nitro (NO₂) groups. The aromatic heterocyclic moiety may be a monocyclic or bicyclic ring containing 4 to 12 atoms of which at least one atom is chosen from nitrogen, sulphur or oxygen. The aromatic heterocyclic moiety may be carbon or nitrogen linked. The aromatic heterocyclic moiety may additionally be substituted by one or more additional substituents at any available ring carbon or heteroatom. The substituents may include, but are not limited to the groups as defined for R₂₆ in Formula V.

[0108] As used herein, the term “aromatic nitrocarbocycle” means a benzene moiety substituted at any position by one or more nitro (NO₂) groups. In addition, two adjacent ring carbon atoms may optionally be linked to form a fused carbocyclic or heterocyclic ring. The benzene moiety (and optional fused ring) may additionally be substituted by one or more additional substituents at any available carbon or heteroatom. The substituents may include, but are not limited to, the groups as defined for R₂₆ in Formula V.

[0109] As used herein, the terms “co-administered” and “co-administration” refer to administering to the subject a compound contemplated herein or salt thereof along with a compound that may also treat the disorders or diseases contemplated herein. In one embodiment, the co-administered compounds are administered separately, or in any kind of combination as part of a single therapeutic approach. The co-administered compound may be formulated in any kind of combinations as mixtures of solids and liquids under a variety of solid, gel, and liquid formulations, and as a solution.

[0110] As used herein, the term “composition” or “pharmaceutical composition” refers to a mixture of at least one compound contemplated herein with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient or subject. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, nasal, pulmonary and topical administration.

[0111] The term “container” includes any receptacle for holding the pharmaceutical composition or to add protection to manage stability and or water-uptake. For example, in one embodiment, the container is the packaging that contains the pharmaceutical composition such as liquid (solution and suspension), semisolid, lyophilized solid, solution and powder or

lyophilized formulation present in dual chambers. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, *i.e.*, the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, *e.g.*, treating, preventing, or reducing a breathing disorder in a patient.

[0112] A “disease” as used herein is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

[0113] A “disorder” as used herein in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favourable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

[0114] As used herein, the terms “effective amount,” “pharmaceutically effective amount” and “therapeutically effective amount” refer to a nontoxic but sufficient amount of a compound or agent to provide the desired biological result. That result may be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An appropriate therapeutic amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. A therapeutic benefit or improvement need not be complete ablation of any one, most or all symptoms, complications, consequences or underlying causes associated with the disorder or disease. Thus, in some embodiments, a satisfactory endpoint is achieved when there is a transient, medium or long term, incremental improvement in a subject's condition, or a partial reduction in the occurrence, frequency, severity, progression, or duration, or inhibition or reversal, of one or more associated adverse symptoms or complications or consequences or underlying causes, worsening or progression (*e.g.*, stabilizing one or more symptoms or complications of the condition, disorder or disease), of the disorder or disease, over a duration of time (hours, days, weeks, months, and so forth).

[0115] “Instructional material,” as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness

of a composition and/or compound contemplated herein in a kit. The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition contemplated herein or be shipped together with a container that contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively. Delivery of the instructional material may be, for example, by physical delivery of the publication or other medium of expression communicating the usefulness of the kit, or may alternatively be achieved by electronic transmission, for example by means of a computer, such as by electronic mail, or download from a website.

[0116] As used herein, “likelihood”, “likely to”, and similar generally refers to an increase in the probability of an event. Thus, “likelihood”, “likely to”, and similar, when used in reference to responsiveness to cancer therapy, generally contemplates an increased probability that the individual will exhibit a reduction in the severity of cancer or the symptoms of cancer or the retardation or slowing of the cancer progression. The term “likelihood”, “likely to”, and similar, when used in reference to responsiveness to cancer therapy, can also generally mean the increase of indicators that may evidence an increase in cancer treatment.

[0117] The terms “patient,” “subject” or “individual” are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in situ*, amenable to the methods described herein. In non-limiting embodiments, the patient is a human. In some embodiments, the subject is a subject in need of treatment thereof.

[0118] As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0119] As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound disclosed herein or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, including compounds disclosed herein, and not injurious to the patient.

Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0120] As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of compounds disclosed herein, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions.

[0121] The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compounds disclosed herein. Other additional ingredients that may be included in the pharmaceutical compositions disclosed herein are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

[0122] As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compounds prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids, organic acids, solvates, hydrates, or clathrates thereof.

[0123] The term "prevent," "preventing" or "prevention," as used herein, means avoiding or delaying the onset of symptoms associated with a disease or condition in a subject that has not developed such symptoms at the time the administering of an agent or compound commences.

[0124] As used herein, the term "prodrug" refers to a compound that, after administration, is metabolised or otherwise converted to a biologically active or more active compound (or drug) with respect to at least one property. A prodrug, relative to the drug, is modified chemically in a manner that renders it, relative to the drug, less active or inactive, but the chemical modification is such that the corresponding drug is generated by metabolic or other biological processes after the prodrug is administered. A prodrug may have, relative to the active drug,

altered metabolic stability or transport characteristics, fewer side effects or lower toxicity, or improved flavour (for example, see the reference Nogrady, 1985, Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392, incorporated herein by reference). A prodrug may be synthesized using reactants other than the corresponding drug.

[0125] A “therapeutic” treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

[0126] The term “responsiveness” or “responsive,” when used in reference to a treatment, refers to the degree of effectiveness of the treatment in lessening or decreasing the symptoms of a disease, disorder, or condition being treated. For example, the term “increased responsiveness,” when used in reference to a treatment of a cell or a subject, refers to an increase in the effectiveness in lessening or decreasing the symptoms of the disease when measured using any methods known in the art. In some embodiments, the increase in the effectiveness is at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, or at least about 50%.

[0127] As used herein, the term “treatment” or “treating” is defined as the application or administration of a therapeutic agent, *i.e.*, a compound disclosed herein (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a condition contemplated herein, a symptom of a condition contemplated herein or the potential to develop a condition contemplated herein, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect a condition contemplated herein, the symptoms of a condition contemplated herein or the potential to develop a condition contemplated herein. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

[0128] In some embodiments, the term “treatment” or “treating” refers to an action that occurs while an individual is suffering from the specified cancer, which reduces the severity of the cancer or the symptoms of the cancer, and/or retards or slows the progression of the cancer. For instance, in some embodiments, “treatment” or “treat” refers to a 5%, 10%, 25%, 50%, or 100% decrease in the rate of progress of a tumour. In other embodiments, “treatment” refers to a 5%, 10%, 25%, 50%, or 100% decrease in determined tumour burden (*i.e.*, number of cancerous cells present in the individual, and/or the size of the tumour). In yet other embodiments, “treatment” refers to a 5%, 10%, 25%, 50%, or 100% decrease in any physical

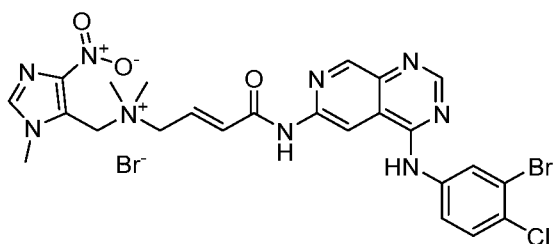
symptom(s) of a cancer. In yet other embodiments, “treatment” refers to a 5%, 10%, 25%, 50%, or 100% increase in the general health of the individual, as determined by any suitable means, such as cell counts, assay results, or other suitable means.

[0129] Throughout this disclosure, various aspects of the disclosure can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the disclosure herein. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.1, 5.3, 5.5, and 6. Thus, for example, reference to a range of 90-100% includes 91-99%, 92-98%, 93-95%, 91-98%, 91-97%, 91-96%, 91-95%, 91-94%, 91-93%, and so forth. Reference to a range of 90-100% also includes 91%, 92%, 93%, 94%, 95%, 96%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, and so forth. A series of ranges are disclosed throughout this document. The use of a series of ranges includes combinations of the upper and lower ranges to provide another range. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a series of ranges such as 5-10, 10-20, 20-30, 30-40, 40-50, 50-75, 75-100, 100-150, includes ranges such as 5-20, 5-30, 5-40, 5-50, 5-75, 5-100, 5-150, and 10-30, 10-40, 10-50, 10-75, 10-100, 10-150, and 20-40, 20-50, 20-75, 20-100, 20-150, and so forth. This applies regardless of the breadth of the range.

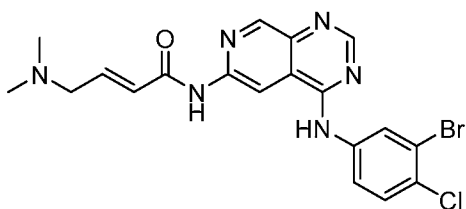
Tyrosine Kinase Inhibitors

[0130] The following non-limiting, exemplary compounds act as tyrosine kinase inhibitors (TKIs). As used herein, a “TKI” refers to a compound capable of acting as a tyrosine kinase inhibitor, for example, the compounds set forth herein.

[0131] Structures of Compound A and Compound B are provided below:



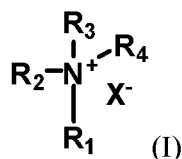
Compound A, RN-4000, TRLX, or tarloxotinib



Compound B, or RN-4000E, or TRLX-TKI

[0132] The present disclosure further contemplates the use of other NMQ prodrugs and/or small molecule EGFR inhibitors, including any other small molecule analogues of Compound A and/or Compound B, to treat or prevent certain HER-driven cancers. In some embodiments, the cancer is a HER-driven drug-resistant cancer. Such NMQ prodrugs and/or small molecule EGFR inhibitors include, but are not limited to those disclosed in WO2010104406, WO2011028135, US20120077811, and US20120202832, each of which is incorporated herein by reference in its entirety.

[0133] For example, the disclosure contemplates NMQ prodrugs of quaternary nitrogen salt compounds of Formula I:



wherein:

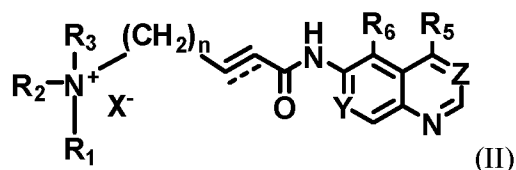
X is any negatively charged counterion;

R₁ is a group of the formula -(CH₂)_nTr, wherein Tr is an aromatic nitroheterocycle or an aromatic nitrocarbocycle and -(CH₂)_nTr acts as a reductively-activated fragmenting trigger (“reductive trigger”);

n is an integer from 0 to 6; and

R₂, R₃ and R₄ are each independently an aliphatic or an aromatic group of a tertiary amine kinase inhibitor (R₂)(R₃)(R₄)N, or two of R₂, R₃, and R₄ may form an aliphatic or aromatic heterocyclic amine ring of a kinase inhibitor, or one of R₂, R₃ and R₄ may be absent and two of R₂, R₃ and R₄ form an aromatic heterocyclic amine ring of a kinase inhibitor.

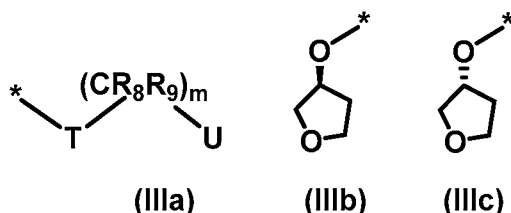
[0134] In some embodiments, the compounds are of Formula II:



wherein:

X is any negatively charged counterion;

Y is N or C-R₇, where R₇ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, and a group of one of the following Formulas IIIa, IIIb, and IIIc:



wherein * indicates the point of attachment;

T is selected from O, NH, N(C₁-C₆ alkyl), and a direct bond;

m is an integer from 0 to 6;

U is selected from OR₁₀, CF₃, OCF₃, CN, NR₁₁R₁₂, pyrrolidinyl, piperidinyl, piperazinyl, N¹-methylpiperazinyl, morpholinyl, CON(R₁₃)(R₁₄), SO₂N(R₁₅)(R₁₆), N(R₁₇)COR₁₈, N(R₁₉)SO₂R₂₀, COR₂₁, SOR₂₂, SO₂R₂₃, and COOR₂₄;

R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄ are independently selected from H and C₁-C₆ alkyl;

Z is N or C-CN;

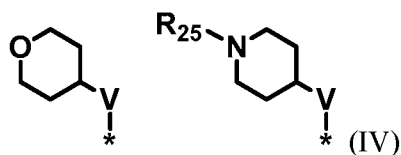
R₁ is a group of the formula (CH₂)_nTr wherein Tr is an aromatic nitroheterocycle or aromatic nitrocarbocycle and -(CH₂)_nTr acts as a reductive trigger;

n is an integer from 0 to 6;

R₂ and R₃ are independently selected from C₁-C₆ alkyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, CH₂CH₂OH, and CH₂CH₂O(C₁-C₆ alkyl), or R₂ and R₃ may together form a non-aromatic carbocyclic ring or non-aromatic heterocyclic ring comprising at least one heteroatom;

R₅ is selected from an aniline, an indole, an indoline, an amine, an aminoindole, and an aminoindazole, each of which may be optionally substituted with one or more substituents selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, CONH₂, CO(C₁-C₆ alkyl), SO₂NH₂, and SO₂(C₁-C₆ alkyl);

R₆ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, and a group of the following Formula IV:



wherein

* indicates the point of attachment;

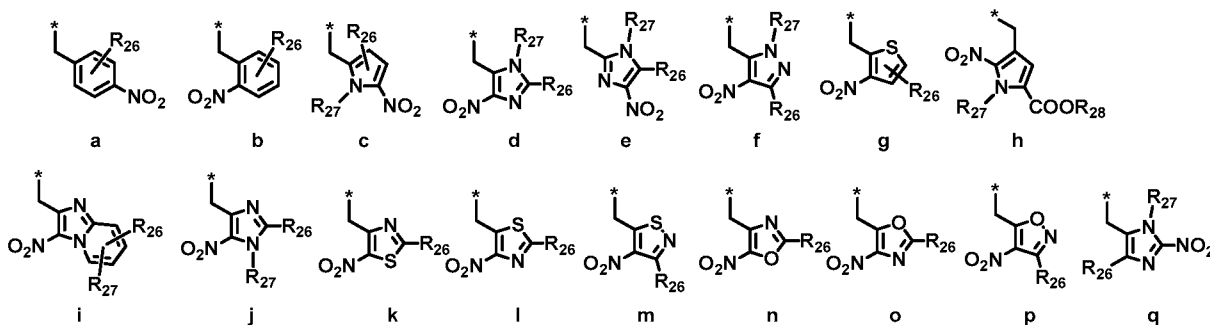
V is selected from $(CH_2)_k$, O, NH, and N(C₁-C₆ alkyl);

k is an integer from 0 to 6; and

R₂₅ is selected from H and C₁-C₆ alkyl.

[0135] In some embodiments, X is selected from halide (*e.g.*, fluoride, chloride, bromide, iodide), methanesulfonate, trifluoromethanesulfonate, acetate, trifluoroacetate, tosylate, lactate, citrate, and formate. In some embodiments, X is halide. In some embodiments, X is selected from fluoride, chloride, bromide, and iodide.

[0136] In some embodiments, R₁ is a group of one of the following Formulas Va-Vq:



wherein:

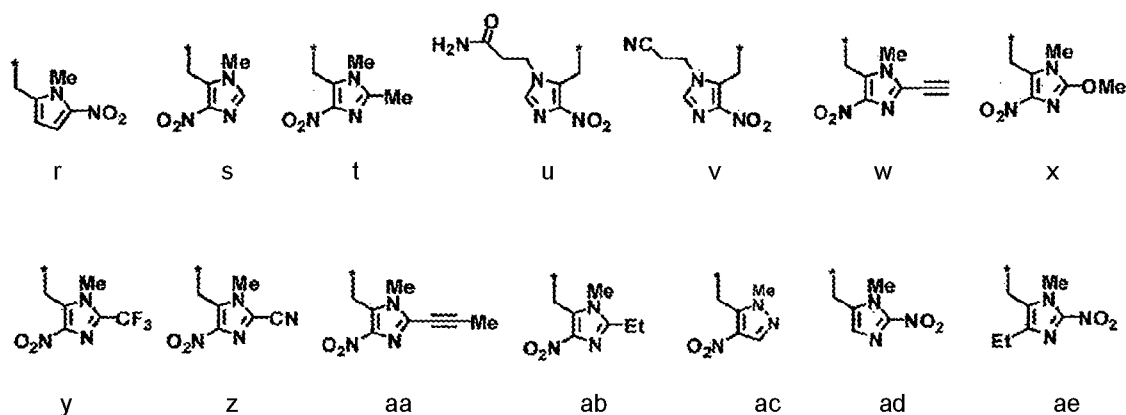
* indicates the point of attachment to the quaternary nitrogen of a compound of Formula II;

R₂₆ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₂-C₆ alkenyl, C₂-C₆ alkynyl, CF₃, OCF₃, F, Cl, Br, I, NO₂, CN, COOH, COO(C₁-C₆ alkyl), CONH₂, CONH(C₁-C₆ alkyl), CON(C₁-C₆ alkyl)₂, CO(C₁-C₆ alkyl), SO₂NH₂, SO₂NH(C₁-C₆ alkyl), SO₂N(C₁-C₆ alkyl)₂, SO₂(C₁-C₆ alkyl), and a group of Formula IIIa, as defined above, where * indicates the point of attachment to a group of Formula V;

R₂₇ is selected from H, C₁-C₆ alkyl, and a group of Formula IIIa, as defined above, where * indicates the point of attachment to a group of Formula V; and

R₂₈ is selected from H and C₁-C₆ alkyl.

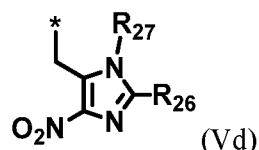
[0137] In some embodiments, R₁ is a group of one of the following Formulas Vr-Vae:



[0138] In some embodiments, R₁ is a group of Formula Vc, wherein R₂₆ is H; and R₂₇ is CH₃.

[0139] In some embodiments, R₁ is a group of Formula Vd, wherein R₂₆ is selected from H, C₁-C₆ alkyl (*e.g.*, methyl), C₁-C₆ alkoxy (*e.g.*, OCH₃), C₂-C₆ alkynyl (*e.g.*, ethynyl), CONH₂, CONHMe, CF₃, OCF₃, Br, NO₂, and CN; and R₂₇ is selected from CH₃, CH₂CH₂CONH₂, and CH₂CH₂CN.

[0140] In some embodiments, R₁ is a group of Formula Vd,



wherein * indicates a point of attachment; R₂₆ is selected from H and C₁-C₃ alkyl; and R₂₇ is selected from H and C₁-C₆ alkyl. In some embodiments, R₂₆ is selected from H, methyl, ethyl, trifluoromethyl, -CN, -CONH₂, and propyn-1-yl and R₂₇ is C₁-C₆ alkyl.

[0141] In some embodiments, R₂₆ is H and R₂₇ is C₁-C₃ alkyl.

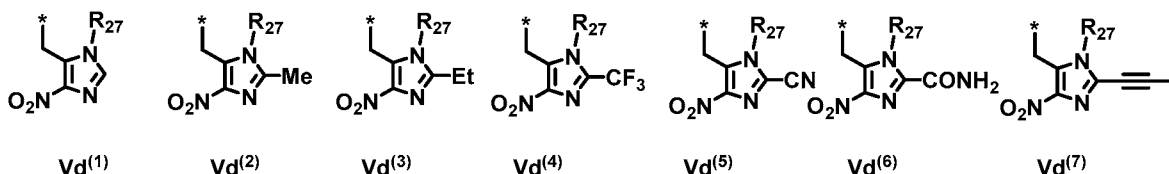
[0142] In some embodiments, R₂₆ is H and R₂₇ is methyl.

[0143] In some embodiments, R₁ is a group of Formula Vd, where R₂₆ is 1-propynyl and R₂₇ is CH₃.

[0144] In some embodiments, R₁ is a group of Formula Vq, where R₂₆ is selected from H, C₁-C₆ alkyl, and C₁-C₆ alkoxy, and R₂₇ is CH₃.

[0145] In some embodiments, R₁ is a group of Formula Vq, where R₂₆ is selected from H, methyl, ethyl, and OCH₃, and R₂₇ is CH₃.

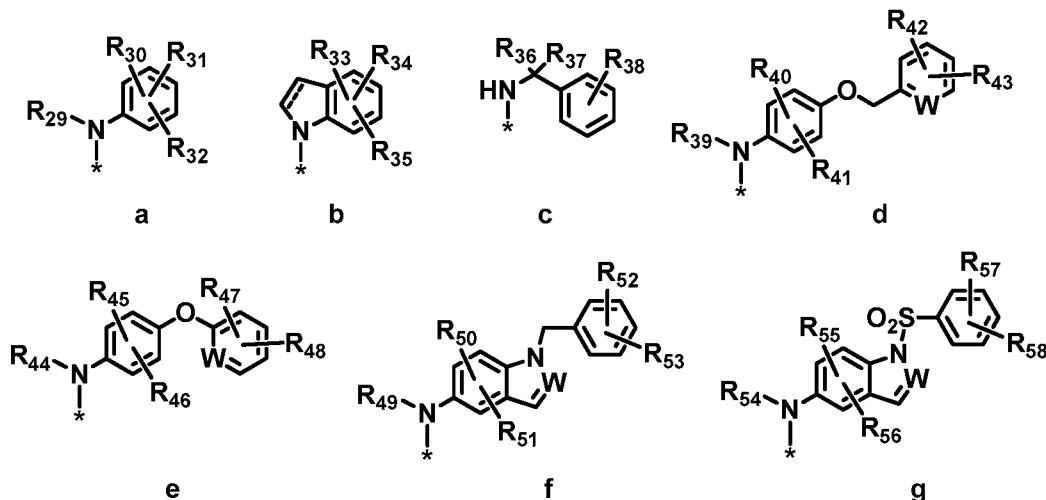
[0146] In some embodiments, R₁ is a group of any one of Formulas Vd⁽¹⁾-Vd⁽⁷⁾:



[0147] In some embodiments, R₂₇ is selected from methyl, ethyl, and propyl. In some embodiments R₂₇ is methyl.

[0148] In some embodiments, R₂ and R₃ form a ring selected from pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium.

[0149] In some embodiments, R₅ is a group of one of the following Formulas VIa-VIg:



wherein:

* indicates the point of attachment;

R₂₉, R₃₆, R₃₇, R₃₉, R₄₄, R₄₉ and R₅₄, are independently selected from H and C₁-C₆ alkyl;

R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₈, R₄₀, R₄₁, R₄₂, R₄₃, R₄₅, R₄₆, R₄₇, R₄₈, R₅₀, R₅₁, R₅₂, R₅₃, R₅₅, R₅₆, R₅₇ and R₅₈ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, CONH₂, CO(C₁-C₆ alkyl), SO₂NH₂, and SO₂(C₁-C₆ alkyl); and

W is N or C-H.

[0150] In some embodiments, Y is N, Z is N or C-CN;

R₁ is selected from the following:

- (a) a group of Formula Vc, wherein R₂₆ is H and R₂₇ is CH₃;
- (b) a group of Formula Vd, wherein (i) R₂₆ is selected from H, C₁-C₆ alkyl (*e.g.*, methyl), C₁-C₆ alkoxy (*e.g.*, OCH₃), C₂-C₆ alkynyl (*e.g.*, ethynyl), CF₃, OCF₃, Br, NO₂, and CN, and R₂₇ is selected from CH₃, CH₂CH₂CONH₂, and CH₂CH₂CN; or (ii) R₂₆ is 1-propynyl and R₂₇ is CH₃;
- (c) a group of Formula Vf, wherein R₂₆ is H and R₂₇ is CH₃; and
- (d) a group of Formula Vq, wherein R₂₆ is selected from H, C₁-C₆ alkyl (*e.g.*, methyl and ethyl) and C₁-C₆ alkoxy (*e.g.*, OCH₃), and R₂₇ is CH₃;

R₂ and R₃ are independently selected from C₁-C₆ alkyl, or together form a ring selected from pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium; and

R₅ is selected from the following:

(a) a group of Formula VIa, wherein * indicates the point of attachment, R₂₉ is H, and R₃₀, R₃₁, R₃₂ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂;

(b) a group of Formula VIId, wherein * indicates the point of attachment, R₃₉ is H, and R₄₀ and R₄₁ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂; R₄₂ and R₄₃ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂; and W is N or C-H; and

(c) a group of Formula VIIf, wherein * indicates the point of attachment, R₄₉ is H, and R₅₀ and R₅₁ are independently selected from H and F; R₅₂ and R₅₃ are independently selected from H, C₁-C₆ alkyl, F, Cl, Br, I, CH₂F, CHF₂, and CF₃; W is N or C-H; R₆ is H; X is any negatively charged counterion; and n=1 or 2.

[0151] In some embodiments, Y is C-H or C-(C₁-C₆ alkoxy), Z is N or C-CN;

R₁ is selected from the following:

(a) a group of Formula Vc, wherein R₂₆ is H, and R₂₇ is CH₃;

(b) a group of Formula Vd, wherein R₂₆ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₂-C₆ alkynyl, CF₃, OCF₃, Br, NO₂, and CN, and R₂₇ is selected from CH₃, CH₂CH₂CONH₂, and CH₂CH₂CN; or R₂₆ is 1-propynyl and R₂₇ is CH₃;

(c) a group of Formula Vf, wherein R₂₆ is H and R₂₇ is CH₃; and

(d) a group of Formula Vq, wherein R₂₆ is selected from H, C₁-C₆ alkyl (*e.g.*, methyl and ethyl), and C₁-C₆ alkoxy (*e.g.*, OCH₃), and R₂₇ is CH₃;

R₂ and R₃ are independently selected from C₁-C₆ alkyl, or together form a ring selected from pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium;

R₅ is selected from the following:

(a) a group of Formula VIa, wherein * indicates the point of attachment; R₂₉ is H; and R₃₀, R₃₁, R₃₂ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-

C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂;

(b) a group of Formula VI_d, wherein * indicates the point of attachment; R₃₉ is H; and R₄₀ and R₄₁ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂; R₄₂ and R₄₃ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂; and W is N or C-H; and

(c) a group of Formula VI_f, wherein * indicates the point of attachment; R₄₉ is H; and R₅₀ and R₅₁ are independently selected from H and F; R₅₂ and R₅₃ are independently selected from H, C₁-C₆ alkyl, F, Cl, Br, I, CH₂F, CHF₂, and CF₃; and W is N or C-H;

R₆ is H;

X is any negatively charged counterion; and

n=1 or 2.

[0152] In some embodiments, Y is C-R₇, wherein R₇ is a group of Formula III_b; Z is N or CN;

R₁ is selected from the following:

(a) a group of Formula V_c, wherein R₂₆ is H, and R₂₇ is CH₃;

(b) a group of Formula V_d, wherein R₂₆ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₂-C₆ alkynyl, CF₃, OCF₃, Br, NO₂, and CN, and R₂₇ is selected from CH₃, CH₂CH₂CONH₂, and CH₂CH₂CN; or R₂₆ is 1-propynyl; and R₂₇ is CH₃;

(c) a group of Formula V_f, wherein R₂₆ is H and R₂₇ is CH₃; and

(d) a group of Formula V_q, wherein R₂₆ is selected from H, C₁-C₆ alkyl (*e.g.*, methyl and ethyl) and C₁-C₆ alkoxy (*e.g.*, OCH₃); and R₂₇ is CH₃;

R₂ and R₃ are independently selected from C₁-C₆ alkyl, or together form a ring selected from pyrrolidinium, piperidinium, piperazinium, N1-methylpiperazinium, and morpholinium;

R₅ is selected from the following:

(a) a group of Formula VI_a, wherein * indicates the point of attachment to a compound of Formula II; R₂₉ is H; and R₃₀, R₃₁, R₃₂ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂;

(b) a group of Formula VI_d, wherein * indicates the point of attachment to a compound of Formula II; R₃₉ is H; and R₄₀ and R₄₁ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂; R₄₂ and R₄₃ are independently selected from H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, F, Cl, Br, I, CN, CH₂F, CHF₂, CF₃, OH, NH₂, NO₂, NH(C₁-C₆ alkyl), and N(C₁-C₆ alkyl)₂; and W is N or C-H; and

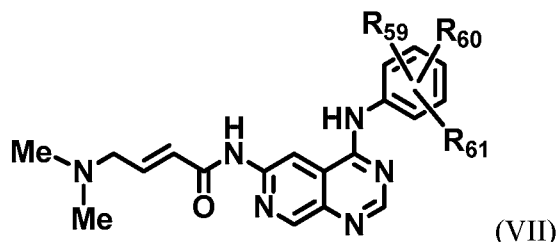
(c) a group of Formula VI_f, wherein * indicates the point of attachment to a compound of Formula II; R₄₉ is H; and R₅₀ and R₅₁ are independently selected from H or F; R₅₂ and R₅₃ are independently selected from H, C₁-C₆ alkyl, F, Cl, Br, I, CH₂F, CHF₂, and CF₃; and W is N or C-H;

R₆ is H;

X is any negatively charged counterion; and

n=1 or 2.

[0153] In some embodiments, the compounds are of Formula VII:



wherein either:

(1) R₅₉ is H, and

(a) R₆₀ is (3-chlorobenzyl)oxy- and R₆₁ is chloro;

(b) R₆₀ and R₆₁, together with the carbon atoms to which they are attached,

form 1-(3-fluorobenzyl)-1H-pyrazole;

(c) R₆₀ is 2-pyridinylmethoxy and R₆₁ is chloro;

(d) R₆₀ and R₆₁ are both chloro;

(e) R₆₀ is chloro and R₆₁ is bromo;

(f) R₆₀ and R₆₁ are both bromo;

(g) R₆₀ is fluoro and R₆₁ is ethynyl;

(h) R₆₀ is chloro and R₆₁ is ethynyl;

(i) R₆₀ is bromo and R₆₁ is ethynyl;

(j) other than when R₆₀ is in the 3-position in combination with R₆₁ in the 4-position, R₆₀ is bromo and R₆₁ is fluoro;

(k) R₆₀ is 2-pyridinylmethoxy and R₆₁ is fluoro; or

(l) R₆₀ is 2-pyridinylmethoxy and R₆₁ is bromo;

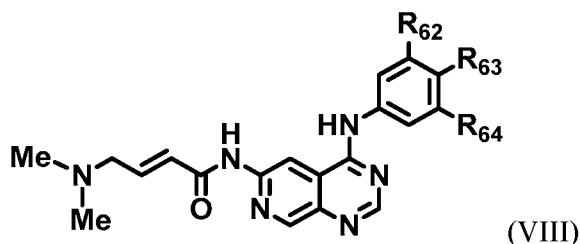
or

(2) at least one of R₅₉, R₆₀ and R₆₁ is selected from benzyloxy, 3-chlorobenzyloxy, and 2-pyridinylmethoxy, and when at least one of R₅₉, R₆₀ and R₆₁ is not benzyloxy, 3-chlorobenzyloxy or 2-pyridinylmethoxy, each of the others is independently selected from H, halogen, and C₂–C₄ alkynyl, with the proviso that when one of R₅₉, R₆₀ and R₆₁ is benzyloxy or 2-pyridinylmethoxy, the other two of R₅₉, R₆₀ and R₆₁ are not H;

or

(3) two of R₅₉, R₆₀ and R₆₁, together with the carbon atoms to which they are attached, form 1-(3-fluorobenzyl)-1*H*-pyrazole, and the other is selected from H, halogen, and C₂–C₄ alkynyl.

[0154] In some embodiments, the compound of Formula VII is a compound according to Formula VIII:



wherein R₆₂ is H, and either

(a) R₆₃ is (3-chlorobenzyl)oxy- and R₆₄ is chloro;

(b) R₆₃ and R₆₄, together with the carbon atoms to which they are attached, form 1-(3-fluorobenzyl)-1*H*-pyrazole;

(c) R₆₃ is 2-pyridinylmethoxy and R₆₄ is chloro;

(d) R₆₃ and R₆₄ are both chloro;

(e) R₆₃ is chloro and R₆₄ is bromo;

(f) R₆₃ is bromo and R₆₄ is chloro;

(g) R₆₃ and R₆₄ are both bromo;

(h) R₆₃ is fluoro and R₆₄ is ethynyl;

(i) R₆₃ is chloro and R₆₄ is ethynyl;

(j) R₆₃ is bromo and R₆₄ is ethynyl;

- (k) R₆₃ is bromo and R₆₄ is fluoro;
- (l) R₆₃ is 2-pyridinylmethoxy and R₆₄ is fluoro; or
- (m) R₆₃ is 2-pyridinylmethoxy and R₆₄ is bromo.

[0155] In some embodiments, the compound of Formula VII is selected from the group comprising:

(2*E*)-*N*-(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)-4-(dimethylamino)-2-butenamide (1);

(2*E*)-4-(dimethylamino)-*N*-(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)-2-butenamide (2);

(2*E*)-*N*-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(dimethylamino)-2-butenamide (3);

(2*E*)-*N*-[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (4);

(2*E*)-*N*-[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (5);

(2*E*)-*N*-[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (6);

(2*E*)-*N*-[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (7);

(2*E*)-4-(dimethylamino)-*N*-[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-2-butenamide (8);

(2*E*)-*N*-[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (9);

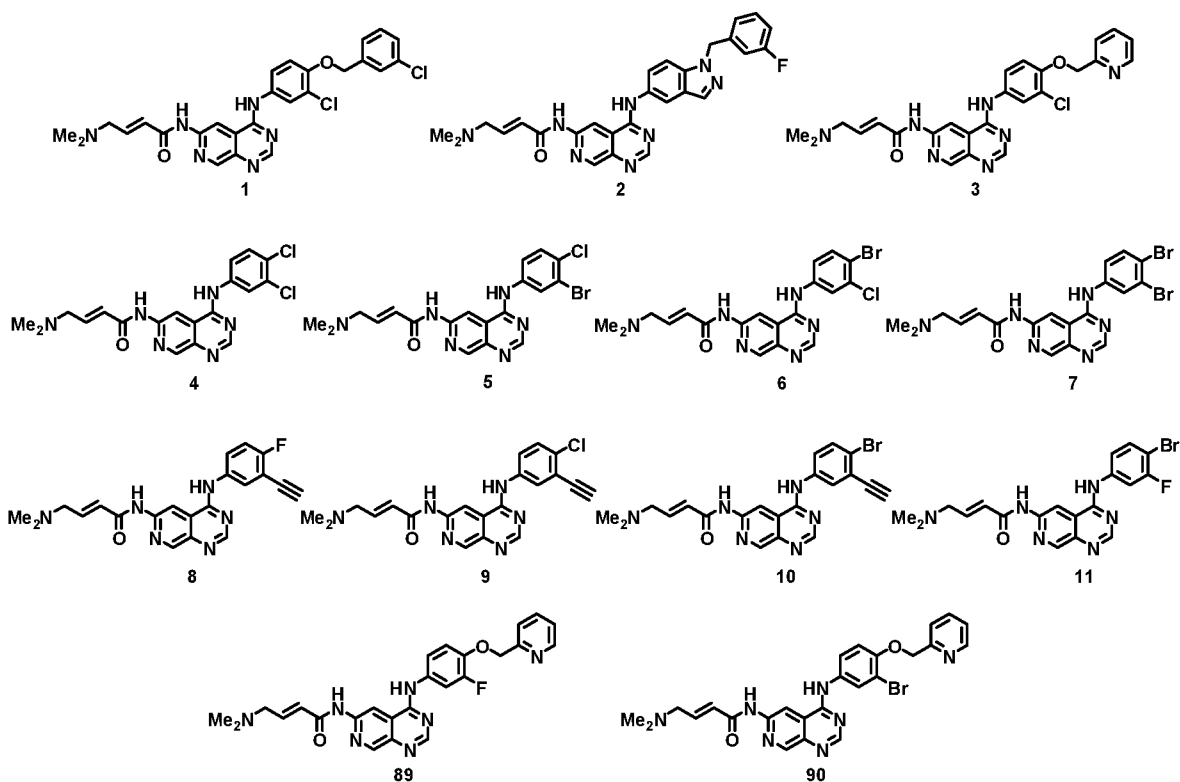
(2*E*)-*N*-[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (10);

(2*E*)-*N*-[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (11);

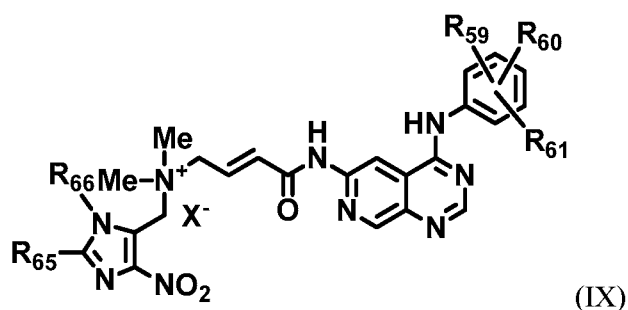
(2*E*)-4-(dimethylamino)-*N*-{4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-2-butenamide (89); and

(2*E*)-*N*-{4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(dimethylamino)-2-butenamide (90).

[0156] The structures of the compounds provided in the list above are depicted below:

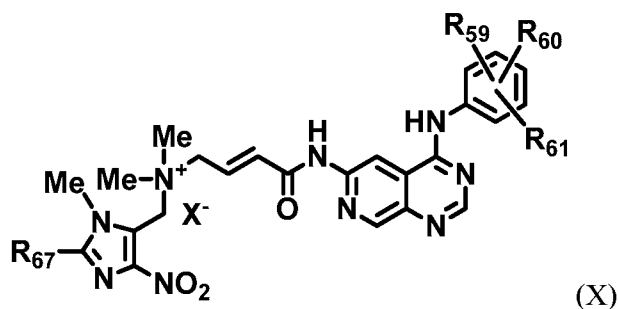


[0157] In some embodiments, the compounds are of Formula IX:



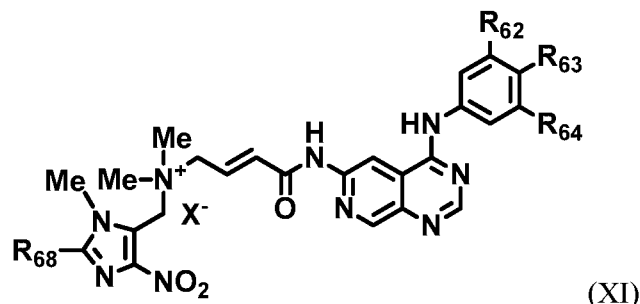
wherein X is any negatively charged counterion, R₅₉, R₆₀ and R₆₁ are as defined for Formula VII, R₆₅ is selected from H, methyl, ethyl, trifluoromethyl, -CN, -CONH₂, and propyn-1-yl, and R₆₆ is C₁-C₆ alkyl.

[0158] In some embodiments, the compounds are of Formula X:



wherein X is any negatively charged counterion, R₅₉, R₆₀ and R₆₁ are as defined for Formula VII and R₆₇ is selected from H, methyl, ethyl, trifluoromethyl, -CN, -CONH₂, and propyn-1-yl.

[0159] In some embodiments, the compounds are of Formula XI:



wherein X is any negatively charged counterion, R₆₂, R₆₃ and R₆₄ are as defined for Formula VIII and R₆₈ is selected from H, methyl, ethyl, trifluoromethyl, -CN, -CONH₂, and propyn-1-yl.

[0160] In some embodiments, X is selected from halide (*e.g.*, fluoride, chloride, bromide, iodide), methanesulfonate, trifluoromethanesulfonate, acetate, trifluoroacetate, tosylate, lactate, citrate, and formate.

[0161] In some embodiments, the compounds are selected from the group comprising:

(2*E*)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**12**);

(2*E*)-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**13**);

(2*E*)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**14**);

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**15**);

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**16**);

(2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**17**);

(2E)-4-{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**18**);

(2E)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**19**);

(2E)-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**20**);

(2E)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**21**);

(2E)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**22**);

(2E)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**23**);

(2E)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**24**);

(2E)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**25**);

(2E)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**26**);

(2E)-4-[(4-{3-chloro-4-[(3-chlorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**27**);

(2*E*)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**28**);

(2*E*)-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**29**);

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**30**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**31**);

(2*E*)-4-[(4-{[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]amino}pyrido[3,4-*d*]pyrimidin-6-yl)amino]-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**32**);

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**33**);

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**34**);

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**35**);

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**36**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**37**);

(2*E*)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**38**);

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**39**),

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**40**);

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**41**);

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**42**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**43**);

(2*E*)-4-{[4-(3,4-dichloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**44**);

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**45**);

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**46**);

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**47**);

(2*E*)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**48**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**49**);

(2*E*)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}}-4-oxo-2-buten-1-ammonium bromide (**50**);

(2*E*)-4-{{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**51**);

(2*E*)-4-{{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**52**);

(2*E*)-4-{{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}}-4-oxo-2-buten-1-ammonium bromide (**53**);

(2*E*)-4-{{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**54**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**55**);

(2*E*)-4-{{[4-(4-bromo-3-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}}-4-oxo-2-buten-1-ammonium bromide (**56**);

(2*E*)-4-{{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**57**);

(2*E*)-4-{{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**58**);

(2*E*)-4-{{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}}-4-oxo-2-buten-1-ammonium bromide (**59**);

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**60**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**61**);

(2*E*)-4-{[4-(3,4-dibromoanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**62**);

(2*E*)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**63**);

(2*E*)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**64**);

(2*E*)-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**65**);

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**66**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**67**);

(2*E*)-4-{[4-(3-ethynyl-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**68**);

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**69**);

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**70**);

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**71**);

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**72**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**73**);

(2*E*)-4-{[4-(4-chloro-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**74**);

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**75**);

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**76**);

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**77**);

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**78**);

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**79**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**80**);

(2*E*)-4-{[4-(4-bromo-3-ethynylanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**81**);

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**82**);

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**83**);

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**84**);

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**85**);

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**86**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**87**);

(2*E*)-4-{[4-(4-bromo-3-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]amino}-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**88**);

(2*E*)-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**91**);

(2*E*)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**92**);

(2*E*)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**93**);

(2*E*)-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide (**94**);

(2*E*)-*N*-[(2-cyano-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**95**);

(2*E*)-*N*-{[2-(aminocarbonyl)-1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**96**);

(2*E*)-4-({4-[3-fluoro-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(1-propynyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**97**);

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**98**);

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(1,2-dimethyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**99**);

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N*-[(2-ethyl-1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-*N,N*-dimethyl-4-oxo-2-buten-1-ammonium bromide (**100**);

(2*E*)-4-({4-[3-bromo-4-(2-pyridinylmethoxy)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}amino)-*N,N*-dimethyl-*N*-{[1-methyl-4-nitro-2-(trifluoromethyl)-1*H*-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide (**101**);

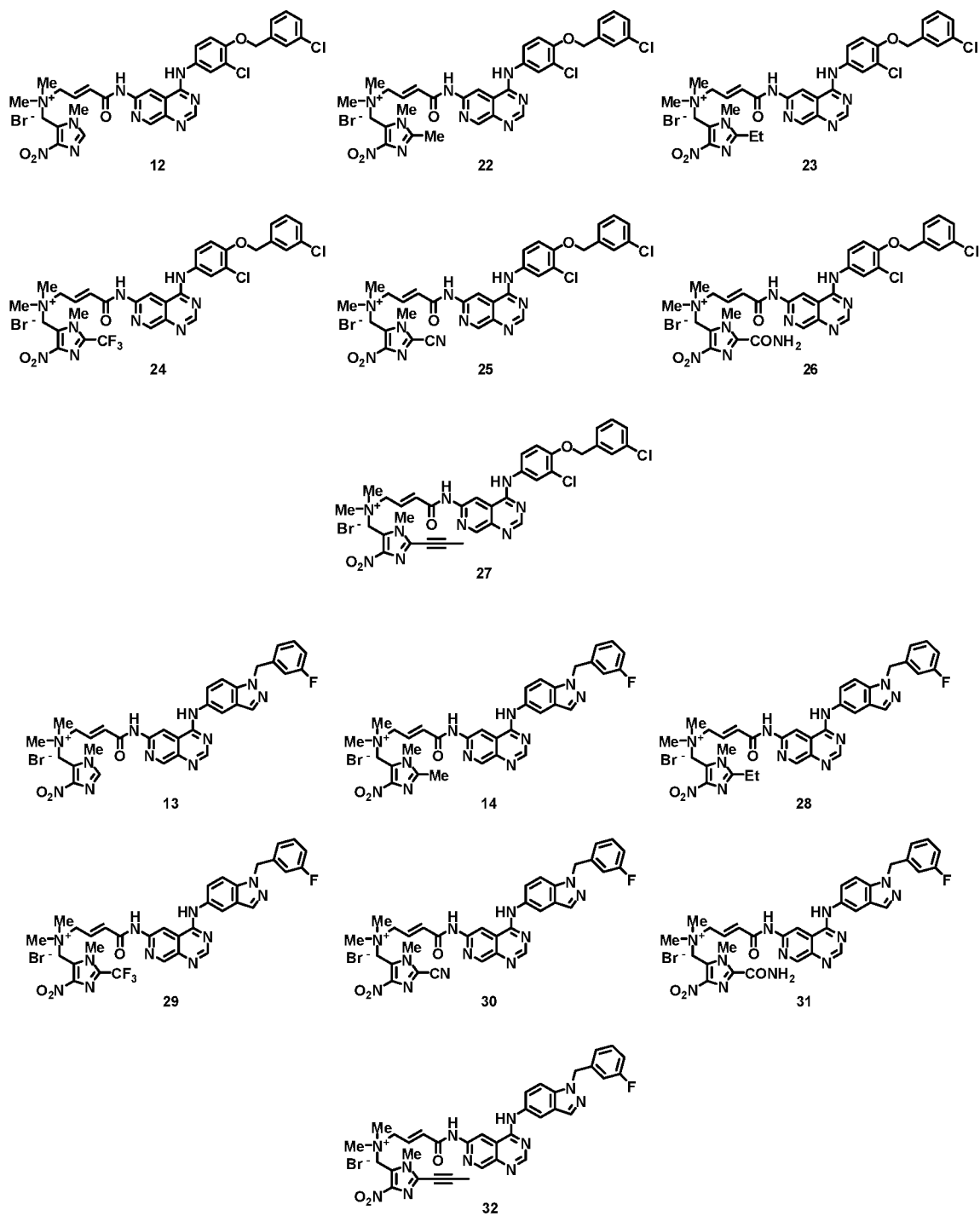
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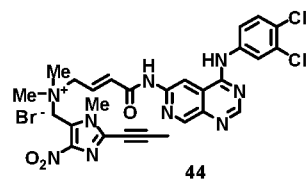
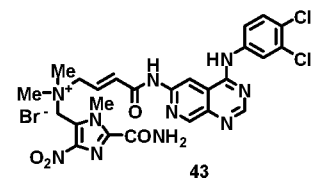
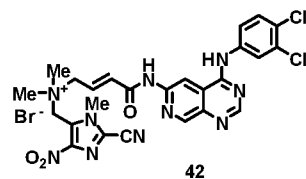
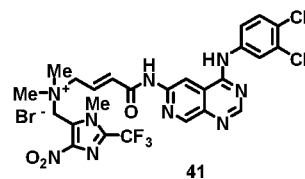
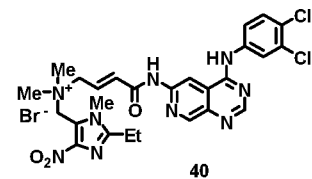
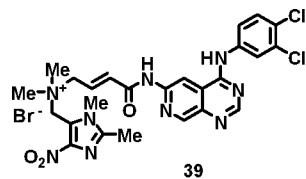
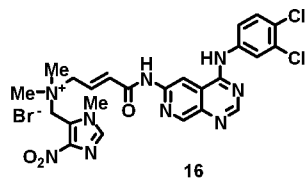
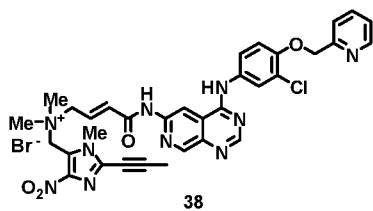
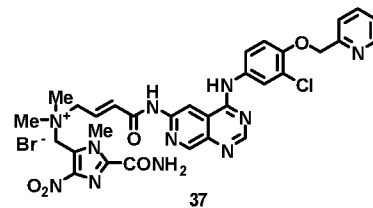
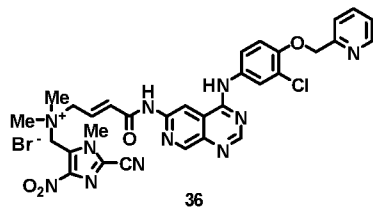
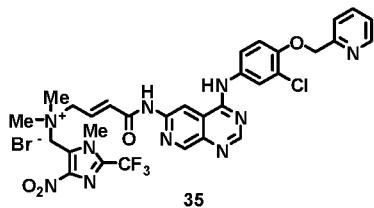
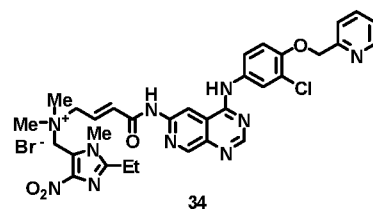
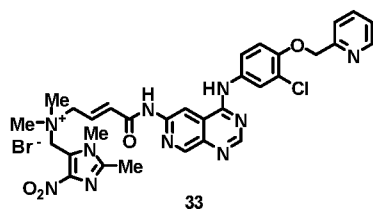
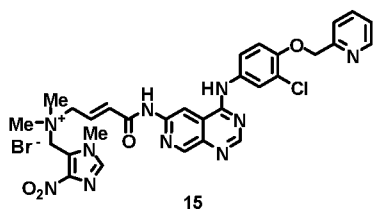
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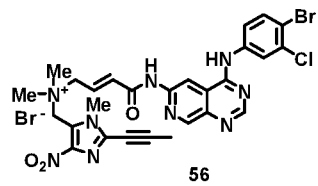
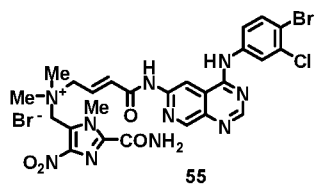
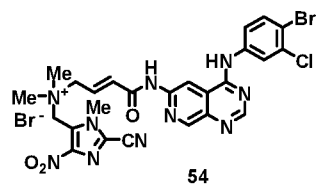
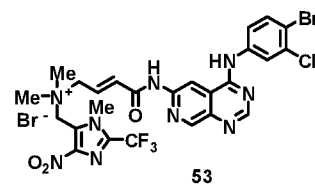
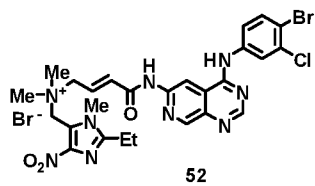
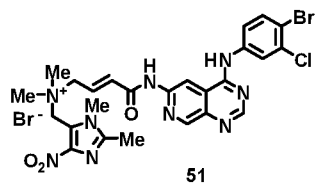
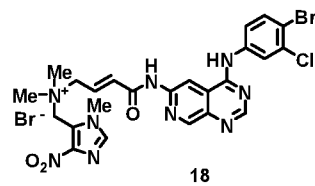
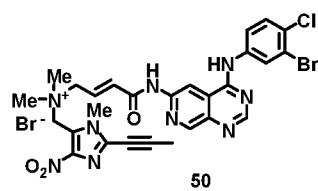
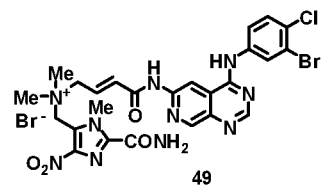
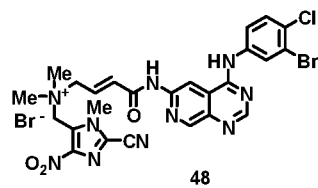
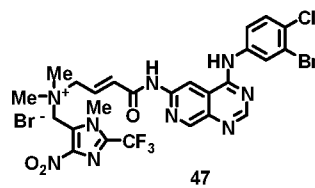
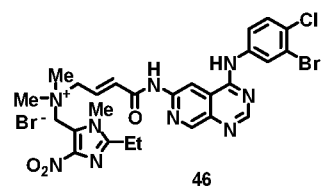
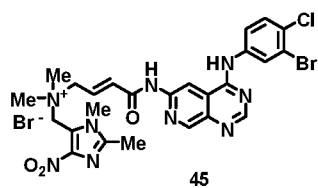
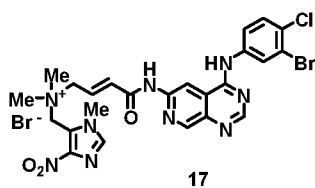
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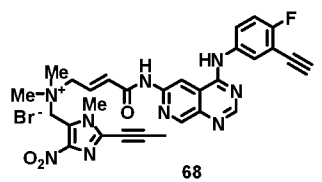
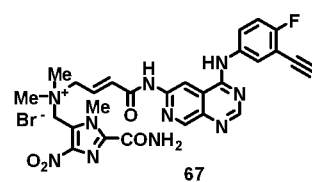
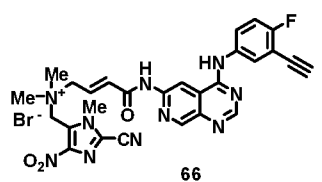
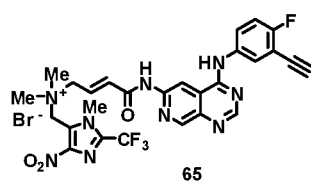
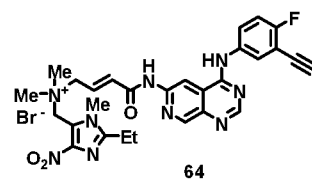
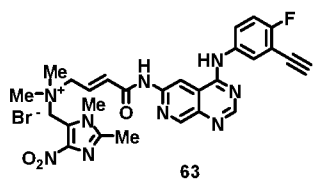
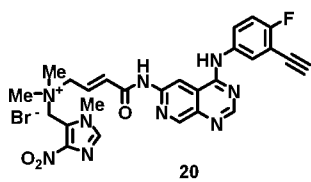
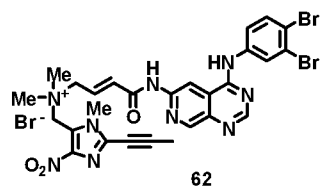
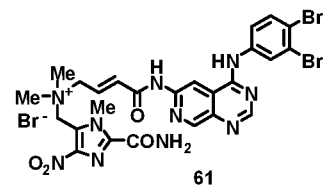
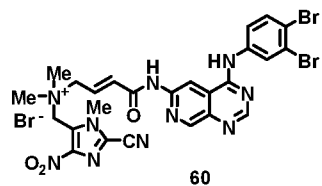
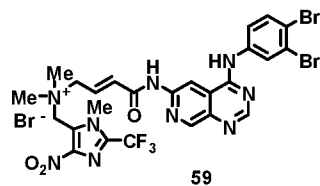
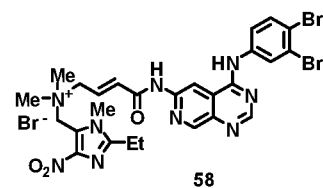
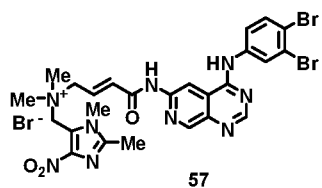
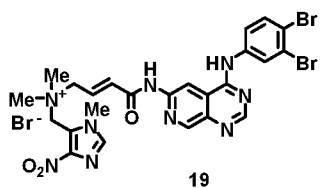
or any other salt thereof (*e.g.*, the listed counterion is exchanged for any other counterion, which in some embodiments is a pharmaceutically acceptable counterion, of same polarity – negative or positive), or any solvate thereof.

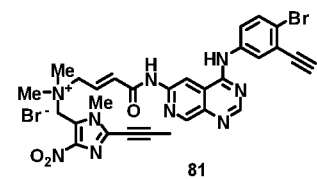
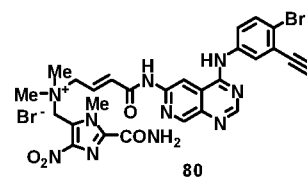
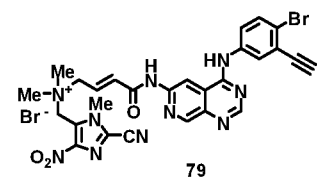
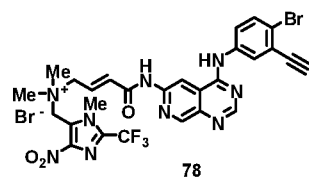
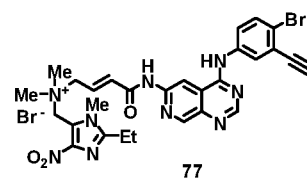
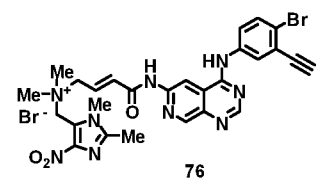
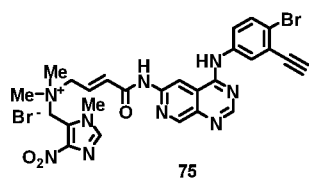
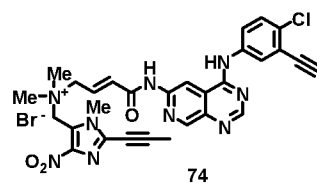
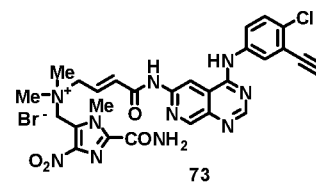
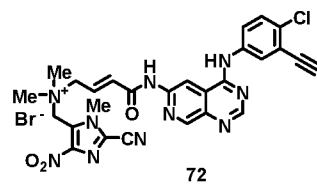
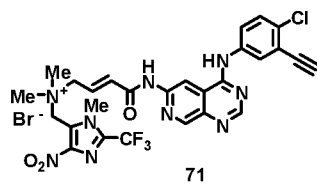
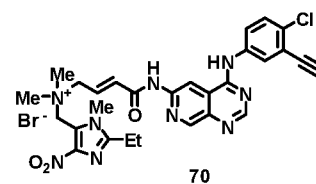
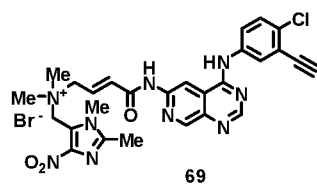
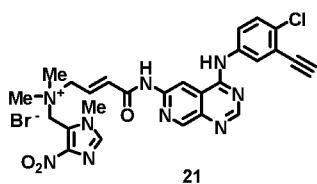
[0162] The structures of the compounds provided in the list above are depicted below:

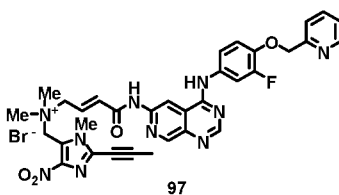
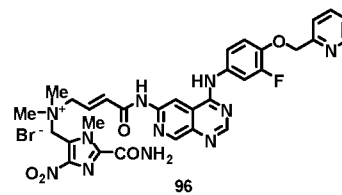
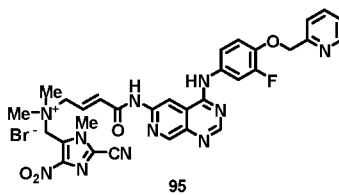
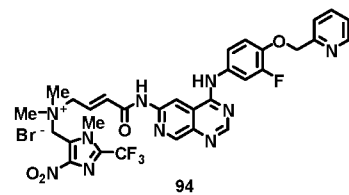
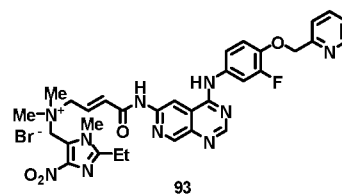
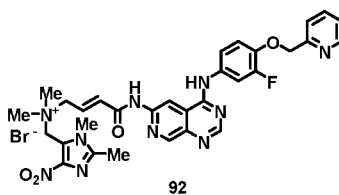
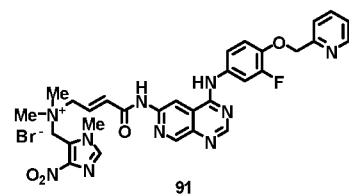
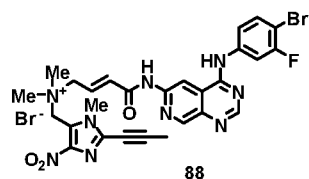
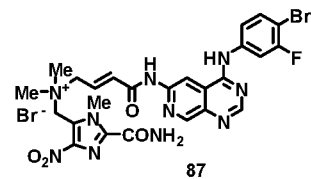
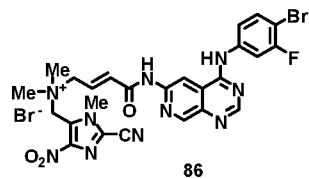
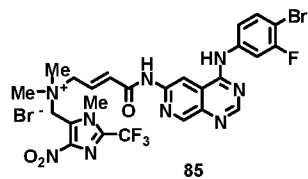
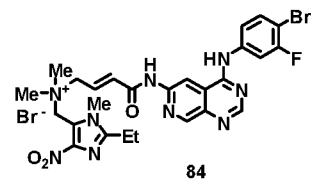
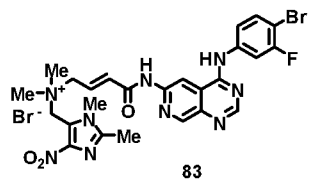
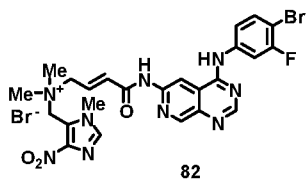


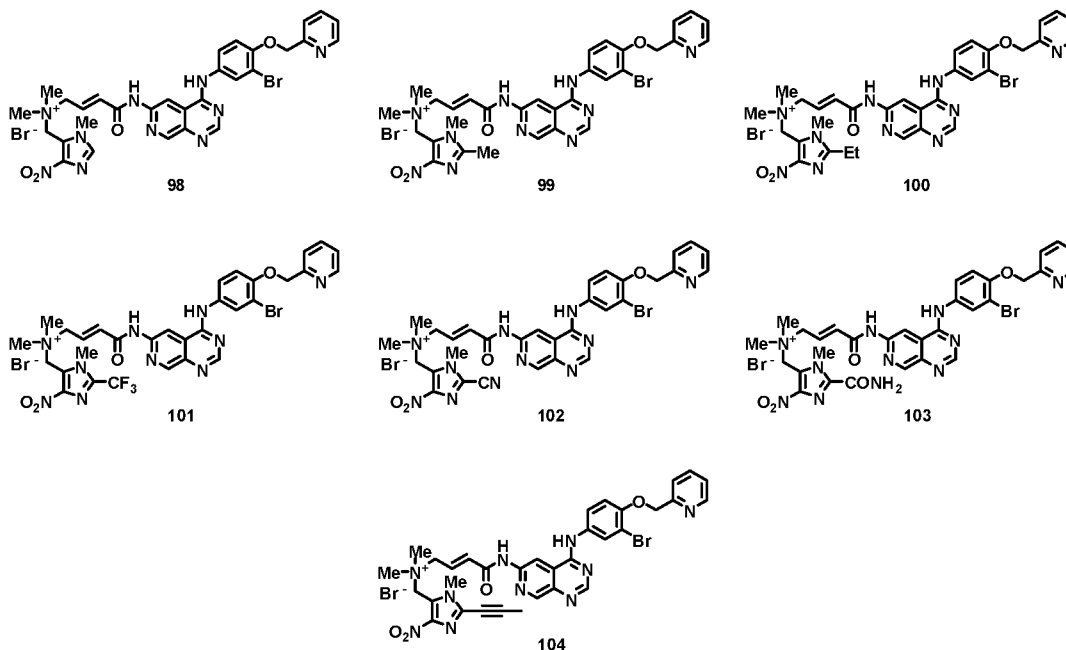












[0163] In some embodiments, the compounds are selected from the group comprising:

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-(4-nitrobenzyl)-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-(2-nitrobenzyl)-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-[(1-methyl-5-nitro-1H-pyrrol-2-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-2-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-pyrazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-N,N-dimethyl-N-[(3-nitroimidazo[1,2-a]pyridin-2-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

1-((2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-4-oxo-2-butenyl)-1-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]piperidinium bromide;

4-((2E)-4-{{[4-(3-bromoanilino)-6-quinazolinyl]amino}}-4-oxo-2-butenyl)-4-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]morpholin-4-ium formate;

(2E)-4-{[4-(3-chloro-4-fluoroanilino)-7-methoxy-6-quinazoliny]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)-6-quinazoliny]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(4-fluoro-3-methoxyanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-methoxy-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-ethynyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-{[1-methyl-4-nitro-2-(trifluoromethyl)-1H-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{[1-(3-amino-3-oxopropyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium trifluoroacetate;

(2E)-4-{[4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N-{[1-(2-cyanoethyl)-4-nitro-1H-imidazol-5-yl]methyl}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N-[(2-methoxy-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(2-ethynyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-N-{[1-methyl-4-nitro-2-(trifluoromethyl)-1H-imidazol-5-yl]methyl}-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{[1-(3-amino-3-oxopropyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{[1-(2-cyanoethyl)-4-nitro-1H-imidazol-5-yl]methyl}-4-({4-[4-fluoro-3-(trifluoromethyl)anilino]pyrido[3,4-d]pyrimidin-6-yl}amino)-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-4-{[4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N-[(2-methoxy-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N-[(2-ethynyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-{{1-methyl-4-nitro-2-(trifluoromethyl)-1H-imidazol-5-yl)methyl}-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{{1-(3-amino-3-oxopropyl)-4-nitro-1H-imidazol-5-yl)methyl}-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-[(2-cyano-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-N-{{1-(2-cyanoethyl)-4-nitro-1H-imidazol-5-yl)methyl}-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-({4-(3-chloro-4-fluoroanilino)-7-[(3S)-tetrahydro-3-furanyloxy]-6-quinazolinyll}amino)-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium trifluoroacetate;

(2E)-4-({4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyll}amino)-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium trifluoroacetate;

(2E)-4-{{4-(3-chloro-4-fluoroanilino)-3-cyano-7-ethoxy-6-quinolinyll}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

2-(4-{{6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl}amino}phenoxy)-N,N-diethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]ethan ammonium bromide;

2-(4-{{6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl}amino}phenoxy)-N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-diethylethan ammonium bromide;

4-{{6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl}amino}-1-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]pyridinium bromide;

1-[2-(4-{{6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl}amino}phenoxy)ethyl]-1-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]piperidinium bromide;

N,N-diethyl-2-[(5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrol-3-yl)carbonyl)amino]-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]ethan ammonium trifluoroacetate;

N-[(1,2-dimethyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-diethyl-2-[(5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrol-3-yl)carbonyl)amino]ethan ammonium bromide;

4-{{4-(4-bromo-2-fluoroanilino)-6-methoxy-7-quinazolinyl}oxy}methyl)-1-methyl-1-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]piperidinium trifluoroacetate;

(2E)-4-{{4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N-[(2-ethyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-{{1-methyl-4-nitro-2-(1-propynyl)-1H-imidazol-5-yl}methyl}-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-2-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide;

(2E)-4-{{4-(3-bromo-4-fluoroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N-[(4-ethyl-1-methyl-2-nitro-1H-imidazol-5-yl)methyl]-N,N-dimethyl-4-oxo-2-buten-1-ammonium bromide; and

(2E)-N-[(2-ethyl-1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-{{4-(3-ethynylanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-4-oxo-2-buten-1 ammonium bromide.

Immunotherapeutic Agents

[0164] Modulation of a subject's immune response has been identified as potentially useful for treatment of cancer. For example, modulation of programmed cell death protein-1 (PD-1) activity has been identified as one avenue by which such an immune response modulation can be achieved. PD-1, a member of the immunoglobulin superfamily, is expressed on immune

cells, including T cells, and functions as an immune checkpoint molecule. PD-1 signalling, which is facilitated by its binding to the ligands programmed cell death-ligand 1 (PD-L1) and programmed cell death-ligand 2 (PD-L2), downregulates the immune system. The therapeutic anti-PD-1 antibodies nivolumab and pembrolizumab, that block the interaction between PD-1 and PD-L1, have been approved for treatment of certain types of cancer.

[0165] Accordingly, disclosed herein are combinations of a TKI and an immunotherapeutic agent which are useful for treatment of cancer. In some embodiments, the immunotherapeutic agent is an inhibitor of an immune checkpoint molecule, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

[0166] In some embodiments, the immunotherapeutic agent is an inhibitor of an immune checkpoint molecule. The term “immune checkpoint molecule,” as used herein refers to molecules that modulate T cell activation or function, for example, totally or partially reduce or inhibit T cell activity or function. *See, e.g., Pardoll (2012) Nature Reviews Cancer 12: 252-264.* A non-limiting list of immune checkpoint molecules includes, for example, CD28, OX40, GITR, CD137 (4-1BB), CD27, HVEM, TIM3, LAG3, CTLA-4, VISTA, BTLA, PD-1, PD-L1, and PD-L2.

[0167] In some embodiments, the immunotherapeutic agent is an antibody that is an inhibitor of an immune checkpoint molecule, for example, an anti-immune-checkpoint inhibitor antibody. In some embodiments, the immunotherapeutic agent is an anti-cancer antibody therapy. Examples of anti-immune-checkpoint inhibitor antibodies include, but are not limited to, pembrolizumab (Keytruda®), and nivolumab (Opdivo®), and cemiplimab (Libtayo®), which target PD-1, and atezolizumab (Tecentriq®), avelumab (Bavencio®), and durvalumab (Imfinzi®), which target PD-L1, and ipilimumab (Yervoy®), which targets CTLA-4. In some embodiments, the immunotherapeutic agent is an immunostimulant, including, for example, sipuleucel-T (Provenge®). In some embodiments, the immunotherapeutic agent is an oncolytic virus, including, for example, Talimogene laherparepvec (Imlgic®). In some embodiments, the immunotherapeutic agent is an anti-19 CAR T cell therapy, including, for example, tisagenlecleucel (Kymriah®) and axicabtagene ciloleucel (Yescarta®).

[0168] The term “antibody” is a well-known term of art that refers to a molecule with an antigen binding site that specifically binds an antigen. The term “antibody” is used herein in the broadest sense and covers fully assembled antibodies, antibodies in substantially intact form (which can, for example, be referred to as “full-length antibodies,” “intact antibodies,” or “whole antibodies”), antibodies comprising at least one heavy chain that contains an Fc region,

antibody fragments which retain the ability to specifically bind to an antigen (*e.g.*, Fab, F(ab')₂, Fv, and other fragments), single chain antibodies, diabodies, antibody chimeras, hybrid antibodies, multispecific, *e.g.*, bispecific, antibodies, and humanized antibodies. The term “antibody” also covers both polyclonal and monoclonal antibodies.

[0169] In some embodiments, the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function, for example, by increasing T cell mediated IL-2 production and/or, increasing proliferation of CD4⁺ and/or CD8⁺ T cells. In some embodiments, the immunomodulatory molecule directly influences T-cell function by enhancing the ability of a T-cell to respond via its T-cell receptor to specific antigen, where a response can be defined as one or more of the following: enhanced rate of T-cell proliferation, enhanced ability to migrate into tissue, enhanced ability to manufacture or release cytokines, or enhanced ability to manufacture or release cytolytic granules.

[0170] In some embodiments, the immunotherapeutic agent is a vaccine.

[0171] In some embodiments, the immunotherapeutic agent is a cellular therapy.

[0172] In some embodiments, the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

[0173] In some embodiments, the immunotherapeutic agent targets a protein selected from Programmed Death 1 (PD-1), Programmed Death-Ligand 1 (PD-L1), Programmed Death-Ligand 2 (PD-L2), Cytotoxic T-Lymphocyte-associated Antigen-4 (CTLA-4), PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, T Lymphocyte Attenuator (BTLA), CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, Glucocorticoid-Induced Tumour-necrosis factor Receptor-related protein (GITR), HAVCR2, Herpes Virus Entry Mediator (HVEM), IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, T cell Immunoglobulin and Mucin-domain containing-3 (TIM3), V-domain Immunoglobulin Suppressor of T cell Activation (VISTA), B70H3, or any combination thereof. In some embodiments, the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, or CTLA-4. In some embodiments, the immunotherapeutic agent is a PD-1 antagonist which can inhibit binding of PD-1 with a PD-1 ligand, for example, PD-L1 or PD-L2.

[0174] In some embodiments, the immunotherapeutic agent is selected from pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab, cemiplimab, and ipilimumab.

In some embodiments, the immunotherapeutic agent is selected from Rituximab MabThera, (Rituxan®) (Anti-CD20); Trastuzumab (Herceptin®) (Anti-HER2); Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33); Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52); Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20); Cetuximab (Erbix®) (Anti-EGFR); Ibritumomab tiuxetan (Zevalin®) (Anti-CD20); Bevacizumab (Avastin®) (Anti-VEGF); Panitumumab (Vectibix®) (Anti-EGFR); Catumaxomab (Removab®) (Anti-EPCAM/CD3); Ofatumumab (Arzerra®) (Anti-CD20); Brentuximab vedotin (Adcetris®) (Anti-CD30); Pertuzumab (Perjeta®) (Anti-Her2); Obinutuzumab (Gazyva®) (Anti-CD20); Ramucirumab (Cyramza®) (Anti-VEGFR2); Siltuximab (Sylvant) (Anti-IL-6); Blinatumomab (Blincyto®) (Anti-CD3/CD19); Dinutuximab (Unituxin®) (Anti-GD2); Daratumumab (Darzalex®) (Anti-CD38); Necitumumab (Portanza®) (Anti-EGFR); and Elotuzumab (Explicit®) (Anti-CD319).

[0175] In some embodiments, the immune checkpoint inhibitor is pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab, cemiplimab, or ipilimumab, or any combination thereof.

[0176] In some embodiments, the immune checkpoint inhibitor is pembrolizumab. In some embodiments, the immune checkpoint inhibitor is nivolumab. In some embodiments, the immune checkpoint inhibitor is cemiplimab. In some embodiments, the immune checkpoint inhibitor is atezolizumab. In some embodiments, the immune checkpoint inhibitor is avelumab. In some embodiments, the immune checkpoint inhibitor is durvalumab. In some embodiments, the immune checkpoint inhibitor is cemiplimab. In some embodiments, the immune checkpoint inhibitor is ipilimumab.

[0177] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with pembrolizumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with pembrolizumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with pembrolizumab.

[0178] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with nivolumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with nivolumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with nivolumab.

[0179] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with cemiplimab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with cemiplimab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with cemiplimab.

[0180] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with atezolizumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with atezolizumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with atezolizumab.

[0181] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with avelumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with avelumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with avelumab.

[0182] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with durvalumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with durvalumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with durvalumab.

[0183] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with cemiplimab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with cemiplimab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with cemiplimab.

[0184] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with ipilimumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with ipilimumab. In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with ipilimumab.

[0185] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises

administering Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20).

[0186] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2).

[0187] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33).

[0188] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52).

[0189] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20).

[0190] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR). In some

embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Cetuximab (Erbix[®]) (Anti-EGFR). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Cetuximab (Erbix[®]) (Anti-EGFR).

[0191] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Ibritumomab tiuxetan (Zevalin[®]) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Ibritumomab tiuxetan (Zevalin[®]) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin[®]) (Anti-CD20).

[0192] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Bevacizumab (Avastin[®]) (Anti-VEGF). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Bevacizumab (Avastin[®]) (Anti-VEGF). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Bevacizumab (Avastin[®]) (Anti-VEGF).

[0193] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Panitumumab (Vectibix[®]) (Anti-EGFR). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Panitumumab (Vectibix[®]) (Anti-EGFR). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Panitumumab (Vectibix[®]) (Anti-EGFR).

[0194] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Catumaxomab (Removab[®]) (Anti-EPCAM/CD3). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Catumaxomab (Removab[®]) (Anti-EPCAM/CD3). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Catumaxomab (Removab[®]) (Anti-EPCAM/CD3).

[0195] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Ofatumumab (Arzerra[®]) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering

Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20).

[0196] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30).

[0197] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2).

[0198] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20).

[0199] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2).

[0200] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6). In some embodiments, the method of

treating cancer in a subject comprises administering Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6).

[0201] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19).

[0202] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2).

[0203] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38).

[0204] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR).

[0205] In some embodiments, the method of treating cancer in a subject comprises administering Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319). In some embodiments, the method of treating cancer in a subject comprises administering Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319). In some embodiments, the method of treating cancer in a subject comprises administering Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319).

[0206] In some embodiments, the pharmaceutical composition comprises Compound A in combination with pembrolizumab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with pembrolizumab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with pembrolizumab.

[0207] In some embodiments, the pharmaceutical composition comprises Compound A in combination with nivolumab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with nivolumab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with nivolumab.

[0208] In some embodiments, the pharmaceutical composition comprises Compound A in combination with cemiplimab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with cemiplimab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with cemiplimab.

[0209] In some embodiments, the pharmaceutical composition comprises Compound A in combination with atezolizumab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with atezolizumab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with atezolizumab.

[0210] In some embodiments, the pharmaceutical composition comprises Compound A in combination with avelumab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with avelumab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with avelumab.

[0211] In some embodiments, the pharmaceutical composition comprises Compound A in combination with durvalumab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with durvalumab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with durvalumab.

[0212] In some embodiments, the pharmaceutical composition comprises Compound A in combination with cemiplimab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with cemiplimab. In some embodiments, the

pharmaceutical composition comprises Compound A and Compound B in combination with cemiplimab.

[0213] In some embodiments, the pharmaceutical composition comprises Compound A in combination with ipilimumab. In some embodiments, the pharmaceutical composition comprises Compound B in combination with ipilimumab. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with ipilimumab.

[0214] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20).

[0215] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2).

[0216] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33).

[0217] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52).

[0218] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound B in combination with

Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20).

[0219] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR).

[0220] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20).

[0221] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF).

[0222] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR).

[0223] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3).

[0224] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Ofatumumab

(Arzerra®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20).

[0225] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30).

[0226] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2).

[0227] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20).

[0228] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2).

[0229] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6).

[0230] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19). In some embodiments, the pharmaceutical composition

comprises Compound A and Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19).

[0231] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2).

[0232] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38).

[0233] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR).

[0234] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319). In some embodiments, the pharmaceutical composition comprises Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319). In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319).

[0235] In some embodiments, the pharmaceutical composition comprises Compound A in combination with pembrolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with pembrolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with pembrolizumab for use in the treatment of cancer.

[0236] In some embodiments, the pharmaceutical composition comprises Compound A in combination with nivolumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with nivolumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises

Compound A and Compound B in combination with nivolumab for use in the treatment of cancer.

[0237] In some embodiments, the pharmaceutical composition comprises Compound A in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with cemiplimab for use in the treatment of cancer.

[0238] In some embodiments, the pharmaceutical composition comprises Compound A in combination with atezolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with atezolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with atezolizumab for use in the treatment of cancer.

[0239] In some embodiments, the pharmaceutical composition comprises Compound A in combination with avelumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with avelumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with avelumab for use in the treatment of cancer.

[0240] In some embodiments, the pharmaceutical composition comprises Compound A in combination with durvalumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with durvalumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with durvalumab for use in the treatment of cancer.

[0241] In some embodiments, the pharmaceutical composition comprises Compound A in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with cemiplimab for use in the treatment of cancer.

[0242] In some embodiments, the pharmaceutical composition comprises Compound A in combination with ipilimumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with ipilimumab for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with ipilimumab for use in the treatment of cancer.

[0243] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for use in the treatment of cancer.

[0244] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for use in the treatment of cancer.

[0245] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for use in the treatment of cancer.

[0246] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition

comprises Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for use in the treatment of cancer.

[0247] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for use in the treatment of cancer.

[0248] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for use in the treatment of cancer.

[0249] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for use in the treatment of cancer.

[0250] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for use in the treatment of cancer.

[0251] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and

Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for use in the treatment of cancer.

[0252] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for use in the treatment of cancer.

[0253] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for use in the treatment of cancer.

[0254] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for use in the treatment of cancer.

[0255] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for use in the treatment of cancer.

[0256] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and

Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for use in the treatment of cancer.

[0257] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for use in the treatment of cancer.

[0258] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for use in the treatment of cancer.

[0259] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for use in the treatment of cancer.

[0260] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for use in the treatment of cancer.

[0261] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and

Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for use in the treatment of cancer.

[0262] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for use in the treatment of cancer.

[0263] In some embodiments, the pharmaceutical composition comprises Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for use in the treatment of cancer. In some embodiments, the pharmaceutical composition comprises Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for use in the treatment of cancer.

[0264] In some embodiments, the pharmaceutical combination comprises Compound A in combination with pembrolizumab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with pembrolizumab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with pembrolizumab.

[0265] In some embodiments, the pharmaceutical combination comprises Compound A in combination with nivolumab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with nivolumab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with nivolumab.

[0266] In some embodiments, the pharmaceutical combination comprises Compound A in combination with cemiplimab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with cemiplimab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with cemiplimab.

[0267] In some embodiments, the pharmaceutical combination comprises Compound A in combination with atezolizumab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with atezolizumab. In some embodiments, the

pharmaceutical combination comprises Compound A and Compound B in combination with atezolizumab.

[0268] In some embodiments, the pharmaceutical combination comprises Compound A in combination with avelumab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with avelumab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with avelumab.

[0269] In some embodiments, the pharmaceutical combination comprises Compound A in combination with durvalumab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with durvalumab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with durvalumab.

[0270] In some embodiments, the pharmaceutical combination comprises Compound A in combination with cemiplimab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with cemiplimab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with cemiplimab.

[0271] In some embodiments, the pharmaceutical combination comprises Compound A in combination with ipilimumab. In some embodiments, the pharmaceutical combination comprises Compound B in combination with ipilimumab. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with ipilimumab.

[0272] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20).

[0273] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2).

[0274] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33).

[0275] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52).

[0276] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20).

[0277] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR).

[0278] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20).

[0279] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Bevacizumab

(Avastin®) (Anti-VEGF). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF).

[0280] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR).

[0281] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3).

[0282] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20).

[0283] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30).

[0284] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2).

[0285] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Obinutuzumab

(Gazyva®) (Anti-CD20). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20).

[0286] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2).

[0287] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6).

[0288] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19).

[0289] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2).

[0290] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38).

[0291] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Necitumumab

(Portanza®) (Anti-EGFR). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR).

[0292] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319). In some embodiments, the pharmaceutical combination comprises Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319). In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319).

[0293] In some embodiments, the pharmaceutical combination comprises Compound A in combination with pembrolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with pembrolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with pembrolizumab for use in the treatment of cancer.

[0294] In some embodiments, the pharmaceutical combination comprises Compound A in combination with nivolumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with nivolumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with nivolumab for use in the treatment of cancer.

[0295] In some embodiments, the pharmaceutical combination comprises Compound A in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with cemiplimab for use in the treatment of cancer.

[0296] In some embodiments, the pharmaceutical combination comprises Compound A in combination with atezolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with atezolizumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with atezolizumab for use in the treatment of cancer.

[0297] In some embodiments, the pharmaceutical combination comprises Compound A in combination with avelumab for use in the treatment of cancer. In some embodiments, the

pharmaceutical combination comprises Compound B in combination with avelumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with avelumab for use in the treatment of cancer.

[0298] In some embodiments, the pharmaceutical combination comprises Compound A in combination with durvalumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with durvalumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with durvalumab for use in the treatment of cancer.

[0299] In some embodiments, the pharmaceutical combination comprises Compound A in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with cemiplimab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with cemiplimab for use in the treatment of cancer.

[0300] In some embodiments, the pharmaceutical combination comprises Compound A in combination with ipilimumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with ipilimumab for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with ipilimumab for use in the treatment of cancer.

[0301] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for use in the treatment of cancer.

[0302] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for use in the treatment of cancer.

In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for use in the treatment of cancer.

[0303] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for use in the treatment of cancer.

[0304] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for use in the treatment of cancer.

[0305] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for use in the treatment of cancer.

[0306] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for use in the treatment of cancer.

[0307] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in

combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for use in the treatment of cancer.

[0308] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for use in the treatment of cancer.

[0309] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for use in the treatment of cancer.

[0310] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for use in the treatment of cancer.

[0311] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for use in the treatment of cancer.

[0312] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in

combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for use in the treatment of cancer.

[0313] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for use in the treatment of cancer.

[0314] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for use in the treatment of cancer.

[0315] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for use in the treatment of cancer.

[0316] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for use in the treatment of cancer.

[0317] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in

combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19) for use in the treatment of cancer.

[0318] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for use in the treatment of cancer.

[0319] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for use in the treatment of cancer.

[0320] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for use in the treatment of cancer.

[0321] In some embodiments, the pharmaceutical combination comprises Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for use in the treatment of cancer. In some embodiments, the pharmaceutical combination comprises Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for use in the treatment of cancer.

[0322] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with pembrolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical

combination comprising Compound B in combination with pembrolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with pembrolizumab for the treatment of cancer.

[0323] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with nivolumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with nivolumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with nivolumab for the treatment of cancer.

[0324] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with cemiplimab for the treatment of cancer.

[0325] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with atezolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with atezolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with atezolizumab for the treatment of cancer.

[0326] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with avelumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with avelumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with avelumab for the treatment of cancer.

[0327] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with durvalumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with durvalumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with durvalumab for the treatment of cancer.

[0328] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with cemiplimab for the treatment of cancer.

[0329] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with ipilimumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with ipilimumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with ipilimumab for the treatment of cancer.

[0330] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for the treatment of cancer.

[0331] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Trastuzumab

(Herceptin®) (Anti-HER2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for the treatment of cancer.

[0332] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for the treatment of cancer.

[0333] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for the treatment of cancer.

[0334] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for the treatment of cancer.

[0335] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present

disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for the treatment of cancer.

[0336] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for the treatment of cancer.

[0337] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for the treatment of cancer.

[0338] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for the treatment of cancer.

[0339] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising

Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for the treatment of cancer.

[0340] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for the treatment of cancer.

[0341] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for the treatment of cancer.

[0342] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for the treatment of cancer.

[0343] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and

Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for the treatment of cancer.

[0344] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for the treatment of cancer.

[0345] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for the treatment of cancer.

[0346] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for the treatment of cancer.

[0347] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for the treatment of cancer.

[0348] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for the treatment of cancer.

[0349] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for the treatment of cancer.

[0350] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for the treatment of cancer.

[0351] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with pembrolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with pembrolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with pembrolizumab for the treatment of cancer.

[0352] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with nivolumab for the treatment of

cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with nivolumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with nivolumab for the treatment of cancer.

[0353] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with cemiplimab for the treatment of cancer.

[0354] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with atezolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with atezolizumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with atezolizumab for the treatment of cancer.

[0355] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with avelumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with avelumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with avelumab for the treatment of cancer.

[0356] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with durvalumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with durvalumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with durvalumab for the treatment of cancer.

[0357] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with cemiplimab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with cemiplimab for the treatment of cancer.

[0358] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with ipilimumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with ipilimumab for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with ipilimumab for the treatment of cancer.

[0359] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) for the treatment of cancer.

[0360] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) for the treatment of cancer.

[0361] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for the treatment of cancer. In some embodiments, the present

disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) for the treatment of cancer.

[0362] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) for the treatment of cancer.

[0363] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) for the treatment of cancer.

[0364] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) for the treatment of cancer.

[0365] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with

Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) for the treatment of cancer.

[0366] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) for the treatment of cancer.

[0367] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) for the treatment of cancer.

[0368] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) for the treatment of cancer.

[0369] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present

disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) for the treatment of cancer.

[0370] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) for the treatment of cancer.

[0371] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) for the treatment of cancer.

[0372] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) for the treatment of cancer.

[0373] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A

and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) for the treatment of cancer.

[0374] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) for the treatment of cancer.

[0375] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) for the treatment of cancer.

[0376] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) for the treatment of cancer.

[0377] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) for the treatment of cancer.

[0378] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) for the treatment of cancer.

[0379] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) for the treatment of cancer.

[0380] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with pembrolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with pembrolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with pembrolizumab in the manufacture of a medicament for the treatment of cancer.

[0381] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with nivolumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with nivolumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with nivolumab in the manufacture of a medicament for the treatment of cancer.

[0382] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer.

[0383] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with atezolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with atezolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with atezolizumab in the manufacture of a medicament for the treatment of cancer.

[0384] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with avelumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with avelumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with avelumab in the manufacture of a medicament for the treatment of cancer.

[0385] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with durvalumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with durvalumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with durvalumab in the manufacture of a medicament for the treatment of cancer.

[0386] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer.

[0387] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with ipilimumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with ipilimumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with ipilimumab in the manufacture of a medicament for the treatment of cancer.

[0388] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0389] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in

combination with Trastuzumab (Herceptin®) (Anti-HER2) in the manufacture of a medicament for the treatment of cancer.

[0390] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) in the manufacture of a medicament for the treatment of cancer.

[0391] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) in the manufacture of a medicament for the treatment of cancer.

[0392] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0393] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments,

the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer.

[0394] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0395] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) in the manufacture of a medicament for the treatment of cancer.

[0396] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer.

[0397] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) in the manufacture of a medicament for the treatment of cancer.

[0398] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0399] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) in the manufacture of a medicament for the treatment of cancer.

[0400] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a

pharmaceutical combination comprising Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) in the manufacture of a medicament for the treatment of cancer.

[0401] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0402] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) in the manufacture of a medicament for the treatment of cancer.

[0403] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) in the manufacture of a medicament for the treatment of cancer.

[0404] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19) in the manufacture of a medicament for the treatment of cancer. In some

embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Blinatumomab (Blinicyto®) (Anti-CD3/CD19) in the manufacture of a medicament for the treatment of cancer.

[0405] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) in the manufacture of a medicament for the treatment of cancer.

[0406] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) in the manufacture of a medicament for the treatment of cancer.

[0407] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer.

[0408] In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical combination comprising Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) in the manufacture of a medicament for the treatment of cancer.

[0409] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with pembrolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with pembrolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with pembrolizumab in the manufacture of a medicament for the treatment of cancer.

[0410] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with nivolumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with nivolumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with nivolumab in the manufacture of a medicament for the treatment of cancer.

[0411] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer.

[0412] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with atezolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with atezolizumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with atezolizumab in the manufacture of a medicament for the treatment of cancer.

[0413] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with avelumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with avelumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with avelumab in the manufacture of a medicament for the treatment of cancer.

[0414] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with durvalumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with durvalumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with durvalumab in the manufacture of a medicament for the treatment of cancer.

[0415] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with cemiplimab in the manufacture of a medicament for the treatment of cancer.

[0416] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with ipilimumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with ipilimumab in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with ipilimumab in the manufacture of a medicament for the treatment of cancer.

[0417] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Rituximab MabThera, (Rituxan®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0418] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Trastuzumab (Herceptin®) (Anti-HER2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Trastuzumab (Herceptin®) (Anti-HER2) in the manufacture of a medicament for the treatment of cancer.

[0419] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A

and Compound B in combination with Gemtuzumab ozogamicin (Mylotarg®) (Anti-CD33) in the manufacture of a medicament for the treatment of cancer.

[0420] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Alemtuzumab MabCampath, (Campath-1H®) (Anti-CD52) in the manufacture of a medicament for the treatment of cancer.

[0421] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Tositumomab + 131I-Tositumomab (Bexxar®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0422] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Cetuximab (Erbix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Cetuximab (Erbix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer.

[0423] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some

embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Ibritumomab tiuxetan (Zevalin®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0424] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Bevacizumab (Avastin®) (Anti-VEGF) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Bevacizumab (Avastin®) (Anti-VEGF) in the manufacture of a medicament for the treatment of cancer.

[0425] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Panitumumab (Vectibix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Panitumumab (Vectibix®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer.

[0426] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Catumaxomab (Removab®) (Anti-EPCAM/CD3) in the manufacture of a medicament for the treatment of cancer.

[0427] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Ofatumumab (Arzerra®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Ofatumumab (Arzerra®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0428] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Brentuximab vedotin (Adcetris®) (Anti-CD30) in the manufacture of a medicament for the treatment of cancer.

[0429] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Pertuzumab (Perjeta®) (Anti-Her2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Pertuzumab (Perjeta®) (Anti-Her2) in the manufacture of a medicament for the treatment of cancer.

[0430] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Obinutuzumab (Gazyva®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides

use of a pharmaceutical composition comprising Compound A and Compound B in combination with Obinutuzumab (Gazyva®) (Anti-CD20) in the manufacture of a medicament for the treatment of cancer.

[0431] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Ramucirumab (Cyramza®) (Anti-VEGFR2) in the manufacture of a medicament for the treatment of cancer.

[0432] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Siltuximab (Sylvant) (Anti-IL-6) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Siltuximab (Sylvant) (Anti-IL-6) in the manufacture of a medicament for the treatment of cancer.

[0433] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Blinatumomab (Blincyto®) (Anti-CD3/CD19) in the manufacture of a medicament for the treatment of cancer.

[0434] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Dinutuximab (Unituxin®) (Anti-GD2) in the manufacture of a medicament for the treatment of cancer. In some embodiments,

the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Dinutuximab (Unituxin®) (Anti-GD2) in the manufacture of a medicament for the treatment of cancer.

[0435] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Daratumumab (Darzalex®) (Anti-CD38) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Daratumumab (Darzalex®) (Anti-CD38) in the manufacture of a medicament for the treatment of cancer.

[0436] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Necitumumab (Portanza®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Necitumumab (Portanza®) (Anti-EGFR) in the manufacture of a medicament for the treatment of cancer.

[0437] In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A in combination with Elotuzumab (Explicit®) (Anti-CD319) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) in the manufacture of a medicament for the treatment of cancer. In some embodiments, the present disclosure provides use of a pharmaceutical composition comprising Compound A and Compound B in combination with Elotuzumab (Explicit®) (Anti-CD319) in the manufacture of a medicament for the treatment of cancer.

Cancers

[0438] In some embodiments, the combinations disclosed herein are administered to treat cancer. In some embodiments, the cancer to be treated comprises lung cancer. In some embodiments, the lung cancer comprises non-small cell lung cancer. In some embodiments, the cancer comprises gastric cancer. In some embodiments, the cancer comprises breast cancer. In some embodiments, the cancer comprises head and neck squamous cell carcinoma (HNSCC). In some embodiments, the cancer comprises gastric/gastroesophageal (GE) junction cancer. In some embodiments, the cancer comprises oesophageal cancer. In some embodiments, the cancer comprises salivary cancer. In some embodiments, the cancer comprises ovarian cancer. In some embodiments, the cancer comprises endometrial cancer. In some embodiments, the cancer comprises uterine cancer. In some embodiments, the cancer comprises pancreatic cancer. In some embodiments, the cancer comprises biliary tract cancer. In some embodiments, the cancer comprises bladder cancer. In some embodiments, the cancer comprises colorectal cancer. In some embodiments, the cancer comprises renal cancer. In some embodiments, the cancer comprises brain and/or spinal cord cancer (glioblastoma). In some embodiments, the cancer comprises lymphoma, e.g., primary central nervous system lymphoma. In some embodiments, the cancer comprises leukaemia, e.g., acute lymphoblastic leukaemia.

[0439] In some embodiments, the cancer is selected from the group of lung cancer, gastric cancer, breast cancer, HNSCC, GE junction cancer, oesophageal cancer, salivary cancer, ovarian cancer, endometrial cancer, uterine cancer, prostate cancer, pancreatic cancer, colon cancer, biliary tract cancer, bladder cancer, colorectal, renal, glioblastoma, mesothelioma, adenocarcinoma, lymphoma, and leukaemia.

[0440] In some embodiments, the cancer is selected from bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, oesophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, and testicular cancer.

[0441] In some embodiments, the cancer is selected from chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the

vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, and non-small cell lung cancer.

[0442] In some embodiments, the cancer is non-small cell lung cancer.

[0443] In some embodiments, the cancer is breast cancer.

[0444] In some embodiments, the cancer is brain cancer.

[0445] In some embodiments, the cancer is spinal cord cancer.

[0446] In some embodiments, the cancer is a solid tumour. Solid tumours are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumours can be benign or malignant. Different types of solid tumours are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). The solid tumour can be sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumour, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, oesophageal adenocarcinoma, oesophageal squamous cell carcinoma, squamous cell carcinoma of the head and neck (HNSCC), oral carcinoma, gastric carcinoma, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumour, cervical cancer, testicular tumour, seminoma, bladder carcinoma, melanoma, and CNS tumours (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyngioma, ependymoma, pineaioma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, and brain metastases).

[0447] In some embodiments, the solid tumour is malignant melanoma, adrenal carcinoma, breast carcinoma, renal cell cancer, carcinoma of the pancreas, non-small-cell lung carcinoma (NSCLC), or carcinoma of unknown primary.

[0448] In some embodiments, the solid tumour is breast cancer, ovarian cancer, brain cancer, gastric cancer, oesophageal cancer prostate cancer, lung cancer, colon cancer, skin cancer, liver cancer, pancreatic cancer, or thyroid cancer. In some embodiments, the solid tumour is selected

from the groups consisting of carcinoma, melanoma, sarcoma, and chronic granulomatous disease.

[0449] In some embodiments, the therapeutic combinations disclosed herein can pass the blood-brain barrier. Accordingly, in some embodiments, the cancer is brain cancer or spinal cord cancer. In some embodiments, the cancer is selected from glioblastoma, glioma, brain stem and hypophtalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, neuroectodermal tumours, pineal tumours, primary central nervous system lymphoma, and acute lymphoblastic leukaemia that has crossed the blood-brain barrier. In some embodiments, the cancer is secondary brain metastases. In some embodiments, the cancer comprises NSCLC with brain metastases. In some embodiments, the concentration of the compounds disclosed herein in the brain is surprisingly sufficient to treat a CNS cancer or a spinal cord cancer.

[0450] In some embodiments, Compound A passes the blood-brain barrier. In some embodiments, Compound A passes the blood-brain barrier and converts to its active metabolite, Compound B. In some embodiments, Compound A converts to its active metabolite, Compound B, and Compound B then passes the blood-brain barrier. In some embodiments, Compound B passes the blood-brain barrier. In some embodiments, Compound A and Compound B pass the blood-brain barrier.

Pharmaceutical Combinations, Compositions, and Methods

[0451] Provided herein are pharmaceuticals combinations, compositions, and methods using a combination of compound of Formula I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and an immunotherapeutic agent, as disclosed herein. In some embodiments, the combination comprises Compound A and an immunotherapeutic agent. In some embodiments, the combination comprises Compound B and an immunotherapeutic agent.

[0452] In some embodiments, the combination is a synergistic combination. As used herein, the combination of the TKI, e.g. a compound of Formula I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and an immunotherapeutic agent, as disclosed herein may refer to a combination wherein the individual agents are physically mixed or wherein the individual agents are physically separated. A combination therapy encompasses administering the components separately to produce the desired additive, complementary or synergistic effects. In some embodiments, the combinations disclosed herein exhibit surprising synergistic effects.

[0453] In some embodiments, the combinations disclosed herein exhibit low toxicity effects. In some embodiments, administration of the combinations disclosed herein results in reduced

systemic inflammation, skin inflammation, and/or lung inflammation in the subject as compared to administration of known combinations of a TKI and an immunotherapeutic agent (*see*, Liang, H et al. *OncoTargets and Therapy* 2018;11, 6189-96, which is hereby incorporated by reference in its entirety). In some embodiments, administration of the combinations disclosed herein results in reduced interstitial lung disease (ILD) in a subject as compared to administration of known combinations of a TKI and an immunotherapeutic agent. In some embodiments, administration of the combinations disclosed herein results in reduced idiopathic pulmonary fibrosis (IPF) in a subject as compared to administration of known combinations of a TKI and an immunotherapeutic agent. In some embodiments, administration of the combinations disclosed herein results in reduced pneumonitis in a subject as compared to administration of known combinations of a TKI and an immunotherapeutic agent. In some embodiments, administration of the combinations disclosed herein results in reduced toxicity to a lung of a subject, which can be measured by various methods including, e.g., alveolar septal thickening, intra-alveolar fibrosis, increases in alveolar macrophages, and dilation of bronchioles and alveolar ducts that demonstrate a uniform fibrotic state, increased collagen deposition, patchy fibrosis, septal widening, pleural, subpleural, and septal fibrosis extending to interstitium, alveolar septal elastosis, which are disclosed in e.g., Tashiro, J et al. *Front. Med.* 2017: 4(118) 1-10, which is hereby incorporated by reference in its entirety.

[0454] In some embodiments, administration of the combinations disclosed herein results in reduced expression of certain biomarkers of inflammation, e.g., skin, lung and/or systemic inflammation. In some embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are markers of lung inflammation, pneumonitis or ILD, including such markers as, for example, POSTN, OPN, surfactant protein-A [SP-A], matrix metalloproteinase-9 [MMP-9], Krebs von den Lungen-6 [KL-6], Chemokine (C-C motif) ligand-18 [CCL-18], MMPs, CC-chemokine ligand 2 (CCL2), alpha-smooth muscle actin (α -SMA), Desmin, telomerase related genes (hTERT and TERC), (C-X-C motif) ligand 8 (CXCL8), tumour necrosis factor (TNF)- α and transforming growth factor (TGF)- β and chemokines, including, for example, CCL17, CCL22, CCL2, and CCL3. Without wishing to be bound by theory, it is thought that the altered balance between proangiogenic (CXCL8, CXCL5, and CXCL12) and antiangiogenic (CXCL9, CXCL10, and CXCL11) CXC chemokines promotes aberrant neoangiogenesis and lung recruitment of circulating fibrocytes. Accordingly, in some embodiments, administration of the combinations disclosed herein reduces altered balance between proangiogenic and antiangiogenic CXC chemokines. In some

embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are markers of lung damage, e.g., non-specific interstitial pneumonia and/or idiopathic pulmonary fibrosis, including, for example, IGFBP5, SLN, SYNPO2, MYH11, DES, NLGN4Y, FAM83D, ACTG2, TPM2, CNN1, PRUNE2, EIF1AY, RPS4Y1, AHNK2, DDX3Y, KDM5D, TXLNGY, ATP1A2, ACTA-2, TXLNGY, ZFY, PDLIM3, TAHLN, UTY, IGFBP6, LRP2, IFI44L, SLC39A8, SCN1A, LNX2, WARS, SLC6A14, FZD5, PCDH9, F11, RSAD2, NECAB1, MFSD2A, SDR16C5, MOP-1, OAS-2, PHACTR1, PIGA, IFIT3, C1orf162, ATP8A1, FMO5, GBP4, ZNF385B, and SLCO4C1 as disclosed in, e.g., Cecchini et al. *Respiratory Research* 2018; 19:153.

[0455] In some embodiments, administration of the combinations disclosed herein results in reduced expression of certain biomarkers of lung inflammation including, e.g., creatine phosphokinase-1 (CPK-1), creatine phosphokinase-2 (CPK-2), creatine phosphokinase-3 (CPK-3), surfactant proteins, such as, SP-A, SP-B, SP-C and SP-D, mucin-associated antigens, such as, Krebs von den Lungen-(KL)-6 and MUC1, Clara Cell secretory protein-(CC)-16, other lung epithelial markers, such as cytokeratin fragment 19 (CK19), cancer-associated antigens sialyl Lewis (a) (Ca 19-9) and sialyl Lewis (x) (SLX).

[0456] In some embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are markers of liver damage, including indirect markers, direct markers, and/or combinatorial markers. In some embodiments, the biomarker is an indirect marker of liver damage and/or fibrosis including such markers as, for example, albumin, bilirubin, aspartate aminotransferase (AST)/alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT)/alkaline phosphatase (ALP), and prothrombin time. In some embodiments, the biomarker is a direct marker of liver damage and/or fibrosis, including, for example, collagens (e.g., PICP, PIINP, type IV collagen), glycoproteins and polysaccharides (e.g., hyaluronic acid, laminin, YKL-40), collagenases (e.g., MMPs, TIMPs), and cytokines (e.g., TGF- β 1, PDGF, microfibril associated protein -4). In some embodiments, the biomarker is a combinatorial marker of liver damage and/or fibrosis, including, for example, APRI, AST/ALT, Bonacini index, ELF index, FIB-4, fibro index, fibrometer test, fibrospect II, forns test, hepascore, and Lok index.

[0457] In some embodiments, the liver inflammation biomarkers that are reduced upon administration of the disclosed combinations are selected from alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum bilirubin, AST/ALT ratio, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), 5'-nucleotidase (NTP),

ceruloplasmin, alpha-fetoprotein (AFP), as disclosed in Gowda et al. *The Pan African Medical Journal* 2009: 3 (17), which is hereby incorporated by reference in its entirety. Other biomarkers of liver damage may also be reduced, see, e.g., Nallagangula, KS et al. *Future Sci OA* 2018: 4(1): FSO250; Eguchi, A et al. *J. Hepatol.* 2014: 60(5) 1063-74, each of which are hereby incorporated by reference in their entirety.

[0458] In some embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are markers of skin inflammation, including, for example, myeloperoxidase (MPO), interleukin-8 (IL-8), and 25-Dihydroxyvitamin D3 (calcitriol).

[0459] In some embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are markers of systemic inflammation, including, for example, fibronectin, interleukin-8 (IL-8), interleukin-6 (IL-6), biotin, neutrophil gelatinase-associated lipocalin (NGAL), C-reactive protein (CRP), procalcitonin (PCT), and soluble urokinase plasminogen activator receptor (suPAR).

[0460] In some embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are known general inflammation biomarkers including, for example, 6Ckine, adiponectin, agouti-related protein, alpha-1-acid glycoprotein 1, alpha-1-antichymotrypsin, alpha-1-antitrypsin, alpha-2-macroglobulin, amphiregulin, angiopoietin-1, annexin a1, antileukoproteinase, apolipoprotein E, B cell-activating factor, B lymphocyte chemoattractant, beta amyloid 1-40, beta amyloid 1-42, beta-2-microglobulin, biotin, C-reactive protein (CRP), carcinoembryonic antigen-related cell adhesion molecule 1, CD 40 antigen, CD163, CD40 ligand, CD5 antigen-like, chemerin, complement C1q antibody, complement C3, complement component C1q receptor, cortisol, E-selectin, EN-RAGE, endothelin-1, eotaxin-1, eotaxin-2, eotaxin-3, epidermal growth factor, epidermal growth factor receptor, epithelial-derived neutrophil-activating protein, Factor VII, ferritin, fibrinogen, ficolin-3, galectin-3, Granulocyte Colony-Stimulating Factor (GCSF), Granulocyte-Macrophage Colony-Stimulating Factor (GMCSF), growth-regulated alpha protein, growth/differentiation factor 15, haptoglobin, heat shock protein 60, hemopexin, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, intercellular adhesion molecule 1, interferon alpha, interferon gamma, interferon gamma induced protein 10, interferon gamma induced protein 10, long, interferon gamma induced protein 10, short, interferon-inducible T-cell alpha chemoattractant, interleukin-1 alpha, interleukin-1 beta, interleukin-1 receptor antagonist, interleukin-10, interleukin-11, interleukin-12 subunit p40, interleukin-12 subunit p70, interleukin-13, interleukin-15, interleukin-16, interleukin-17, interleukin-18, interleukin-

18-binding protein, interleukin-2, interleukin-2 receptor alpha, interleukin-22, interleukin-23, interleukin-25, interleukin-3, interleukin-31, interleukin-4, interleukin-5, interleukin-6, interleukin-6 receptor, interleukin-6 receptor subunit beta, interleukin-7, interleukin-8, lactoferrin, lectin-like Oxidized LDL receptor 1, Macrophage Colony-Stimulating Factor (MCSF) 1, macrophage inflammatory protein 3 beta, macrophage inflammatory protein-1 alpha, macrophage inflammatory protein-1 beta, macrophage inflammatory protein-3 alpha, macrophage migration inhibitory factor, macrophage-derived chemokine, matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-9, MHC class I chain-related protein A, midkine, monocyte chemotactic protein 1, monocyte chemotactic protein 2, monocyte chemotactic protein 3, monocyte chemotactic protein 4, monokine induced by gamma interferon, myeloperoxidase, nerve growth factor beta, neutrophil gelatinase-associated lipocalin (NGAL), oncostatin-M, osteopontin, P-selectin, pentraxin-3, platelet endothelial cell adhesion molecule, procalcitonin (PCT), progesterone, progranulin, pulmonary and activation-regulated chemokine, receptor for advanced glycosylation end products, resistin, serotransferrin, serum amyloid a protein, serum glutamic oxaloacetic transaminase, soluble urokinase plasminogen activator receptor (suPAR), ST2, stem cell factor, T lymphocyte-secreted protein I-309, T-cell-specific protein RANTES, tenascin-x, thrombin-activatable fibrinolysis, thymus and activation-regulated chemokine, thymus-expressed chemokine, tissue inhibitor of metalloproteinases 1, transforming growth factor alpha, tumour necrosis factor alpha, tumour necrosis factor beta, tumour necrosis factor receptor 2, tumour necrosis factor receptor i, tyrosine kinase with Ig and EGF homology domains 1, vascular cell adhesion molecule-1, vascular endothelial growth factor, vitamin D-Binding Protein, von Willebrand Factor, and YKL-40.

[0461] In some embodiments, the biomarkers having reduced expression upon administration of the combinations disclosed herein are known general inflammation biomarkers including, those disclosed in, e.g., Brenner, D et al. *Cancer Epidemiol Biomarkers Prev*; 23(9): 1729-51, which is hereby incorporated by reference in its entirety.

[0462] In some embodiments, the biomarkers are measured by microarray analysis, immunohistochemistry, or serum measurements.

[0463] A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid- $E_{\max}$ equation (Holford & Scheiner, 19981, *Clin. Pharmacokinet.* 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, *Arch. Exp. Pathol Pharmacol.* 114: 313-326), the median-effect equation (Chou & Talalay, 1984, *Adv. Enzyme*

Regul. 22: 27-55), and through the use of isobolograms (Tallarida & Raffa, 1996, Life Sci. 58: 23-28). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

[0464] In some embodiments, the TKI and the immunotherapeutic agent are physically mixed in the combination. In some embodiments, the TKI and the immunotherapeutic agent are physically separated in the combination.

[0465] In some embodiments, the TKI and the immunotherapeutic agent are physically mixed in a pharmaceutical composition. In some embodiments, the TKI and the immunotherapeutic agent are physically separated in a pharmaceutical composition.

[0466] In some embodiments, the combination is for simultaneous, separate, or sequential administration. In this regard, the combination of the TKI and the immunotherapeutic agent may be administered: a) as a combination that is part of the same medicament formulation (e.g., part of the same dosage form, actives being for simultaneous administration); or b) as a combination of two units, each with one of them for simultaneous, sequential, or separate administration.

[0467] In some embodiments, the compound is RN-4000 [“(E)-4-(((4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)amino)-N,N-dimethyl-N-((1-methyl-4-nitro-1H-imidazol-5-yl)methyl)-4-oxobut-2-en-1-aminium salt (bromide)”]; also referred to herein as “(2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide” Compound A], and/or RN-4000E [“(2E)-N-[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide”]; also referred to herein “(E)-N-(4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide”; Compound B].

[0468] In some embodiments, the compounds of Formula I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and the immunotherapeutic agent, as disclosed herein, are part of a pharmaceutical composition comprising a pharmaceutically acceptable excipient.

[0469] The present disclosure further contemplates methods of treating a subject with cancer, (e.g., the cancers disclosed herein), with the combinations contemplated herein, wherein the treatment is part of a maintenance therapy for subjects with recurring or refractory cancer. For example, the present application contemplates a method of treating a resistant or refractory

cancer in a subject with the combinations disclosed herein. In some embodiments, the treatment leads to a full response, remission, and/or complete cure in the subject with recurring or refractory cancer. In some embodiments, the treatment maintains a stable disease, leads to a partial response (*e.g.*, some tumour regression), or prevents the return of tumours which have fully regressed.

[0470] In some embodiments, disclosed herein is a method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of a TKI, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, the TKI and the immunotherapeutic agent are administered separately, simultaneously, or sequentially. In some embodiments, the TKI and the immunotherapeutic agent are administered separately. In some embodiments, the TKI and the immunotherapeutic agent are administered simultaneously. In some embodiments, the TKI and the immunotherapeutic agent are administered in the same dosage form. In some embodiments, the TKI and the immunotherapeutic agent are administered in separate dosage forms. In some embodiments, the TKI and the immunotherapeutic agent are administered sequentially. In some embodiments, the TKI is administered prior to the immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is administered prior to the TKI. In some embodiments, the TKI is administered in a sub-therapeutically effective amount. In some embodiments, the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

[0471] In some embodiments, the TKI is a compound of Formula I, II, VII, VIII, IX, X and/or XI. In some embodiments, the compound is of Formula I, II, IX, X and/or XI. In some embodiments, the compound is of Formula VII and/or VIII. In some embodiments, the compound is one of compounds 12-88 and 91-104. In some embodiments, the compound is one of compounds 1-11, 89 and 90. In some embodiments, the compound is compound 17. In some embodiments, the compound is compound 5. In some embodiments, the compound is Compound A. In some embodiments, the compound is Compound B.

[0472] In some embodiments, the immunotherapeutic agent is an inhibitor of an immune checkpoint protein. In some embodiments, the immunotherapeutic agent is an anti-immune-checkpoint inhibitor antibody. In some embodiments, the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, or CTLA-4.

[0473] In one aspect, the present application provides a method of treating or preventing cancer in a subject, the method comprising administering to the subject in need thereof a

therapeutically effective amount of at least one compound selected from the group consisting of “(2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide” (Compound A), and (2E)-N-[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (Compound B), or a salt or solvate thereof, and an immunotherapeutic agent.

[0474] In some embodiments, disclosed herein is a method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, Compound A and the immunotherapeutic agent are administered separately, simultaneously, or sequentially. In some embodiments, Compound A and the immunotherapeutic agent are administered simultaneously. In some embodiments, Compound A and the immunotherapeutic agent are administered in the same dosage form. In some embodiments, Compound A and the immunotherapeutic agent are administered in separate dosage forms. In some embodiments, Compound A and the immunotherapeutic agent are administered sequentially. In some embodiments, Compound A is administered prior to the immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is administered prior to Compound A. In some embodiments, Compound A is administered in a sub-therapeutically effective amount. In some embodiments, the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

[0475] In some embodiments, the methods provided herein for treating cancer in a subject include administering a sub-therapeutically and up to therapeutically effective amount of Compound A. In some embodiments, a dose of Compound A that would be sub-therapeutic in the absence of co-administration with an immunotherapeutic agent can be rendered therapeutic by co-administration with an immunotherapeutic agent. For example, the co-administration of Compound A and an immunotherapeutic agent can result in a surprising synergistic interaction.

[0476] In some embodiments, the methods provided herein for treating cancer in a subject include administering a sub-therapeutically and up to therapeutically effective amount of an immunotherapeutic agent. In some embodiments, a dose of an immunotherapeutic agent that would be sub-therapeutic in the absence of co-administration with Compound A can be rendered therapeutic by co-administration with Compound A. For example, the co-administration of an immunotherapeutic agent and Compound A can result in a surprising synergistic interaction.

[0477] In some embodiments, either Compound A or the immunotherapeutic agent, or both combined in sub-therapeutically effective amounts, as defined in the art, still provide a therapeutically useful combination because of the surprising synergistic therapeutic effect of the drug combination. In some embodiments, a sub-therapeutic dose of Compound A and immunotherapeutic agent can be administered to provide superior efficacy of the combination. In some embodiments, a sub-therapeutic dose of Compound A and immunotherapeutic agent can be administered to control the side effects of the individual drugs. In some embodiments, a sub-therapeutic dose of Compound A and immunotherapeutic agent can be administered to control the side effects of Compound A. In some embodiments, a sub-therapeutic dose of Compound A and immunotherapeutic agent can be administered to control the side effects of the immunotherapeutic agent. In some embodiments, a sub-therapeutic dose of Compound A and immunotherapeutic agent can be administered to provide superior efficacy of the combination while controlling the side effects of the individual drugs.

[0478] In some embodiments, disclosed herein is a method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, Compound B and the immunotherapeutic agent are administered separately, simultaneously, or sequentially. In some embodiments, Compound B and the immunotherapeutic agent are administered simultaneously. In some embodiments, Compound B and the immunotherapeutic agent are administered in the same dosage form. In some embodiments, Compound B and the immunotherapeutic agent are administered in separate dosage forms. In some embodiments, Compound B and the immunotherapeutic agent are administered sequentially. In some embodiments, Compound B is administered prior to the immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is administered prior to Compound B. In some embodiments, Compound B is administered in a sub-therapeutically effective amount. In some embodiments, the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

[0479] In some embodiments, the methods provided herein for treating cancer in a subject include administering a sub-therapeutically and up to therapeutically effective amount of Compound B. In some embodiments, a dose of Compound B that would be sub-therapeutic in the absence of co-administration with an immunotherapeutic agent can be rendered therapeutic by co-administration with an immunotherapeutic agent. For example, the co-administration of Compound B and an immunotherapeutic agent can result in a surprising synergistic interaction.

[0480] In some embodiments, the methods provided herein for treating cancer in a subject include administering a sub-therapeutically and up to therapeutically effective amount of an immunotherapeutic agent. In some embodiments, a dose of an immunotherapeutic agent that would be sub-therapeutic in the absence of co-administration with Compound B can be rendered therapeutic by co-administration with Compound B. For example, the co-administration of an immunotherapeutic agent and Compound B can result in a surprising synergistic interaction.

[0481] In some embodiments, either Compound B or the immunotherapeutic agent, or both combined in sub-therapeutically effective amounts, as defined in the art, still provide a therapeutically useful combination because of the surprising synergistic therapeutic effect of the drug combination. In some embodiments, a sub-therapeutic dose of Compound B and immunotherapeutic agent can be administered to provide superior efficacy of the combination. In some embodiments, a sub-therapeutic dose of Compound B and immunotherapeutic agent can be administered to control the side effects of the individual drugs. In some embodiments, a sub-therapeutic dose of Compound B and immunotherapeutic agent can be administered to control the side effects of Compound B. In some embodiments, a sub-therapeutic dose of Compound B and immunotherapeutic agent can be administered to control the side effects of the immunotherapeutic agent. In some embodiments, a sub-therapeutic dose of Compound B and immunotherapeutic agent can be administered to provide superior efficacy of the combination while controlling the side effects of the individual drugs.

[0482] In some embodiments, disclosed herein is a method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, Compound A, Compound B, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially. In some embodiments, Compound A, Compound B, and the immunotherapeutic agent are administered simultaneously. In some embodiments, Compound A, Compound B, and the immunotherapeutic agent are administered in the same dosage form. In some embodiments, Compound A, Compound B, and the immunotherapeutic agent are administered in separate dosage forms. In some embodiments, Compound A, Compound B, and the immunotherapeutic agent are administered sequentially. In some embodiments, Compound A and Compound B are administered prior to the immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is administered prior to Compound B. In some embodiments, Compound A and Compound B are administered in a sub-therapeutically

effective amount. In some embodiments, the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

[0483] In some embodiments, the methods provided herein for treating cancer in a subject include administering a sub-therapeutically and up to therapeutically effective amount of Compound A and Compound B. In some embodiments, a dose of Compound A and Compound B that would be sub-therapeutic in the absence of co-administration with an immunotherapeutic agent can be rendered therapeutic by co-administration with an immunotherapeutic agent. For example, the co-administration of Compound A, Compound B, and an immunotherapeutic agent can result in a surprising synergistic interaction.

[0484] In some embodiments, the methods provided herein for treating cancer in a subject include administering a sub-therapeutically and up to therapeutically effective amount of an immunotherapeutic agent. In some embodiments, a dose of an immunotherapeutic agent that would be sub-therapeutic in the absence of co-administration with Compound A and Compound B can be rendered therapeutic by co-administration with Compound A and Compound B. For example, the co-administration of an immunotherapeutic agent, Compound A, and Compound B can result in a surprising synergistic interaction.

[0485] In some embodiments, Compound A, Compound B, or the immunotherapeutic agent, or combined in sub-therapeutically effective amounts, as defined in the art, still provide a therapeutically useful combination because of the surprising synergistic therapeutic effect of the drug combination. In some embodiments, a sub-therapeutic dose of Compound A, Compound B, and immunotherapeutic agent can be administered to provide superior efficacy of the combination. In some embodiments, a sub-therapeutic dose of Compound A, Compound B, and immunotherapeutic agent can be administered to control the side effects of the individual drugs. In some embodiments, a sub-therapeutic dose of Compound A, Compound B, and immunotherapeutic agent can be administered to control the side effects of Compound A or Compound B. In some embodiments, a sub-therapeutic dose of Compound A or Compound B and immunotherapeutic agent can be administered to control the side effects of the immunotherapeutic agent. In some embodiments, a sub-therapeutic dose of Compound A or Compound B and immunotherapeutic agent can be administered to provide superior efficacy of the combination while controlling the side effects of the individual drugs.

[0486] In some embodiments, provided herein is a method of activating T-cells or a T-cell response in a subject comprising administering to the subject a therapeutically effective amount of a TKI and an immunotherapeutic agent. In some embodiments, provided herein is a

method of activating T-cells or a T-cell response in a subject comprising administering to the subject a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

[0487] In some embodiments, provided herein is a method of enhancing an immune response in a subject comprising administering to the subject having a therapeutically effective amount of a TKI, as described herein, and an immunotherapeutic agent, as described herein. In some embodiments, provided herein is a method of enhancing an immune response in a subject comprising administering to the subject having a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of enhancing an immune response in a subject comprising administering to the subject having a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of enhancing an immune response in a subject comprising administering to the subject having a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

[0488] In some embodiments, T-cell proliferation is increased in the subject. In some embodiments, T-cell function is enhanced in the subject. In some embodiments, the T-cell function enhancement is characterized as an increase in cytokine release and/or an increase in cytotoxic capacity. In some embodiments, the cytokine is IFN γ . In some embodiments, the T-cells are CD3⁺ T-cells. In some embodiments, the T-cells are selected CD4⁺ T-cells or CD8⁺ T-cells. In some embodiments, an amount of cytokine and/or T-cells in the subject with enhanced function after administration is greater than an amount of cytokine and/or T-cells in the subject with enhanced function prior to administration. In some embodiments, an amount cytokine and/or T-cells in the blood, lymphoid tissue, or tumour of the subject after administration is greater than an amount of cytokine and/or T-cells in the blood, lymphoid tissue, or tumour of the subject prior to administration. In some embodiments, an amount cytokine and/or T-cells in the blood, of the subject after administration is greater than an amount of cytokine and/or T-cells in the blood, of the subject prior to administration.

[0489] In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD-1 ligand to human PD-1, comprising administering to a subject having the disease a therapeutically effective amount of a TKI, as described herein, and

an immunotherapeutic agent, as described herein. In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD-1 ligand to human PD-1, comprising administering to a subject having the disease a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD-1 ligand to human PD-1, comprising administering to a subject having the disease a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD-1 ligand to human PD-1, comprising administering to a subject having the disease a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

[0490] In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD-L1 ligand to human PD- L1, comprising administering to a subject having the disease a therapeutically effective amount of a TKI, as described herein, and an immunotherapeutic agent, as described herein. In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD- L1 ligand to human PD- L1, comprising administering to a subject having the disease a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD- L1 ligand to human PD- L1, comprising administering to a subject having the disease a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of treating a disease ameliorated by inhibiting the binding of a human PD- L1 ligand to human PD- L1, comprising administering to a subject having the disease a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

[0491] In some embodiments, the immunotherapeutic agent targets a protein selected from Programmed Death 1 (PD-1), Programmed Death-Ligand 1 (PD-L1), CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1,

ID02, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

[0492] In some embodiments, the immunotherapeutic agent targets a protein selected from Programmed Death 1 (PD-1) and Programmed Death-Ligand 1 (PD-L1), or any combination thereof.

[0493] In some embodiments, the immunotherapeutic agent targets Programmed Death 1 (PD-1).

[0494] In some embodiments, the immunotherapeutic agent targets Programmed Death-Ligand 1 (PD-L1),

[0495] In some embodiments, the immunotherapeutic agent targets CTLA-4. In some embodiments, the immunotherapeutic agent targets PD-L2. In some embodiments, the immunotherapeutic agent targets LAG3. In some embodiments, the immunotherapeutic agent targets TIM3. In some embodiments, the immunotherapeutic agent targets 2B4. In some embodiments, the immunotherapeutic agent targets A2aR. In some embodiments, the immunotherapeutic agent targets B7H1. In some embodiments, the immunotherapeutic agent targets B7H3. In some embodiments, the immunotherapeutic agent targets B7H4. In some embodiments, the immunotherapeutic agent targets BTLA. In some embodiments, the immunotherapeutic agent targets CD2. In some embodiments, the immunotherapeutic agent targets CD27. In some embodiments, the immunotherapeutic agent targets CD28. In some embodiments, the immunotherapeutic agent targets CD30. In some embodiments, the immunotherapeutic agent targets CD40. In some embodiments, the immunotherapeutic agent targets CD70. In some embodiments, the immunotherapeutic agent targets CD80. In some embodiments, the immunotherapeutic agent targets CD86. In some embodiments, the immunotherapeutic agent targets CD137. In some embodiments, the immunotherapeutic agent targets CD160. In some embodiments, the immunotherapeutic agent targets CD226. In some embodiments, the immunotherapeutic agent targets CD276. In some embodiments, the immunotherapeutic agent targets DR3. In some embodiments, the immunotherapeutic agent targets GAL9. In some embodiments, the immunotherapeutic agent targets GITR. In some embodiments, the immunotherapeutic agent targets HAVCR2. In some embodiments, the immunotherapeutic agent targets HVEM. In some embodiments, the immunotherapeutic agent targets IDO1. In some embodiments, the immunotherapeutic agent targets ID02. In some

embodiments, the immunotherapeutic agent targets ICOS (inducible T cell costimulator). In some embodiments, the immunotherapeutic agent targets KIR. In some embodiments, the immunotherapeutic agent targets LAIR1. In some embodiments, the immunotherapeutic agent targets LIGHT. In some embodiments, the immunotherapeutic agent targets MARCO (macrophage receptor with collageneous structure). In some embodiments, the immunotherapeutic agent targets PS (phosphatidylserine). In some embodiments, the immunotherapeutic agent targets OX-40. In some embodiments, the immunotherapeutic agent targets SLAM. In some embodiments, the immunotherapeutic agent targets TIGIT. In some embodiments, the immunotherapeutic agent targets VISTA. In some embodiments, the immunotherapeutic agent targets VTCN1. In some embodiments, the immunotherapeutic agent targets intrinsic checkpoint blockades. In some embodiments, the immunotherapeutic agent targets CISH. In some embodiments, the immunotherapeutic agent targets BMS-202. In some embodiments, the immunotherapeutic agent targets BMS-8. In some embodiments, the immunotherapeutic agent targets AUPM170/CA327. In some embodiments, the immunotherapeutic agent targets LAG3. In some embodiments, the immunotherapeutic agent targets TIM3. In some embodiments, the immunotherapeutic agent targets VISTA. In some embodiments, the immunotherapeutic agent targets B70H3.

[0496] In some embodiments, provided herein is a method of inhibiting tumour cell growth comprising administering to the tumour a therapeutically effective amount of a TKI, as described herein, and an immunotherapeutic agent, as described herein. In some embodiments, provided herein is a method of inhibiting tumour cell growth comprising administering to the tumour a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of inhibiting tumour cell growth comprising administering to the tumour a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of inhibiting tumour cell growth comprising administering to the tumour a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, the tumour is a solid tumour. In some embodiments, the solid tumour is selected from bladder carcinoma, squamous cell carcinoma, transitional cell carcinoma, basal cell carcinoma, renal cell carcinoma, ductal cell carcinoma, and adenocarcinoma.

[0497] In some embodiments, provided herein is a compound which is a TKI, as described herein, for use in the treatment of cancer, wherein the treatment comprises administering the compound in combination with an immunotherapeutic agent. In some embodiments, provided herein is a compound which is Compound A, or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment of cancer, wherein the treatment comprises administering the compound in combination with an immunotherapeutic agent. In some embodiments, provided herein is a compound which is Compound B, or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment of cancer, wherein the treatment comprises administering the compound in combination with an immunotherapeutic agent. In some embodiments, provided herein is a compound which is Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment of cancer, wherein the treatment comprises administering the compound in combination with an immunotherapeutic agent.

[0498] In some embodiments, provided herein is a combination comprising a TKI, as described herein, and an immunotherapeutic agent for use in the treatment of cancer. In some embodiments, provided herein is a combination comprising Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for use in the treatment of cancer. In some embodiments, provided herein is a combination comprising Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for use in the treatment of cancer. In some embodiments, provided herein is a combination comprising Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for use in the treatment of cancer.

[0499] In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of a TKI, as described herein, and an immunotherapeutic agent for the treatment of cancer in a patient. In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for the treatment of cancer in a patient. In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for the treatment of cancer in a patient. In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of Compound A, or a

pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for the treatment of cancer in a patient.

[0500] In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of a TKI, as described herein, and an immunotherapeutic agent in the manufacture of a medicament for the treatment of cancer in a patient. In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent in the manufacture of a medicament for the treatment of cancer in a patient. In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent in the manufacture of a medicament for the treatment of cancer in a patient. In some embodiments, provided herein is a use of a combination comprising a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent in the manufacture of a medicament for the treatment of cancer in a patient.

[0501] In some embodiments, provided herein is a method of sensitizing a hypoxic tumour to a checkpoint inhibitor comprising administering to the hypoxic tumour a therapeutically effective amount of a TKI, as described herein, and an immunotherapeutic agent. In some embodiments, provided herein is a method of sensitizing a hypoxic tumour to a checkpoint inhibitor comprising administering to the hypoxic tumour a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of sensitizing a hypoxic tumour to a checkpoint inhibitor comprising administering to the hypoxic tumour a therapeutically effective amount of Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent. In some embodiments, provided herein is a method of sensitizing a hypoxic tumour to a checkpoint inhibitor comprising administering to the hypoxic tumour a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, Compound B, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

[0502] In some embodiments, the subject is further administered at least one additional agent, or a salt or solvate thereof, that treats or prevents the cancer, as described herein.

[0503] In some embodiments, at least one of a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and the immunotherapeutic agent is administered by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, optic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical. In some embodiments, at least one of Compound A and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, intramuscularly, subcutaneously, or intraperitoneally. In some embodiments, at least one of Compound A and the immunotherapeutic agent is administered intraperitoneally. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the subject is a human in need of treatment thereof.

[0504] In some embodiments, Compound A is administered by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, optic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical. In some embodiments, Compound A is administered orally, parenterally, rectally, topically, intravenously, intramuscularly, subcutaneously, or intraperitoneally. In some embodiments Compound A is administered intraperitoneally. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the subject is a human in need of treatment thereof.

[0505] In some embodiments, the immunotherapeutic agent is administered by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, intratracheal, optic, intraocular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical. In some embodiments, the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, intramuscularly, subcutaneously, or intraperitoneally. In some embodiments the immunotherapeutic agent is administered intraperitoneally. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the subject is a human in need of treatment thereof.

[0506] In some embodiments, provided herein is a kit comprising a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, an applicator and instructional material for use thereof, wherein the instructional material comprises instructions for preventing or treating HER-driven cancers. In some embodiments, the cancer is a HER-driven drug-resistant cancer.

[0507] In some embodiments, provided herein is a kit comprising Compound A or Compound B, an applicator and instructional material for use thereof, wherein the instructional material comprises instructions for preventing or treating HER-driven cancers. In some embodiments, the cancer is a HER-driven drug-resistant cancer.

Salts

[0508] The compounds (e.g., the TKI and/or the immunotherapeutic agent) described herein may form salts with acids, and such salts are included in the present application. In some embodiments, the salts are pharmaceutically acceptable salts. The term “salts” embraces addition salts of free acids that are useful within the methods disclosed herein. The term “pharmaceutically acceptable salt” refers to salts that possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present application, such as for example utility in process of synthesis, purification or formulation of compounds useful within the methods disclosed herein.

[0509] Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include sulphate, hydrogen sulphate, hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulphuric, and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β -hydroxybutyric, salicylic, galactaric, galacturonic acid, glycerophosphonic acids, and saccharin (e.g., saccharinate, saccharate). Salts may be comprised of a fraction of one, one or more than one molar equivalent of acid or base with respect to any compound contemplated herein.

[0510] Suitable pharmaceutically acceptable base addition salts of compounds contemplated herein include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound by reacting, for example, the appropriate acid or base with the compound.

Additional Agents

[0511] In some embodiments, the combinations contemplated herein are useful in the methods of the present application when used concurrently with at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein.

[0512] In some embodiments, the combinations contemplated herein are useful in the methods of present application in combination with at least one additional agent useful for preventing and/or treating diseases and/or disorders contemplated herein.

[0513] In some embodiments these additional agents may comprise compounds of the present application or other compounds, such as commercially available compounds, known to treat, prevent, or reduce the symptoms of diseases and/or disorders contemplated herein.

[0514] In a non-limiting example, the combinations contemplated herein, or a salt or solvate thereof, can be used concurrently or in combination with one or more agents known to be useful in treating or preventing cancer, such as the cancers described herein. Non-limiting examples of additional anti-proliferative agents contemplated include, but are not limited to, compounds listed on the cancer chemotherapy drug regimens in the 14th Edition of the Merck Index (2006), which is hereby incorporated by reference, such as asparaginase, bleomycin, carboplatin, carmustine, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, doxorubicin (adriamycin), epirubicin, etoposide, 5-fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, irinotecan, leucovorin, lomustine, mechlorethamine, 6-mercaptopurine, mesna, methotrexate, mitomycin C, mitoxantrone, prednisolone, prednisone, procarbazine, raloxifen, streptozocin, tamoxifen, thioguanine, topotecan, vinblastine, vincristine, and vindesine. Additional anti-proliferative agents include other molecular targeted agents that modulate parallel pathways such as MEK 1/2 inhibitors, AKT inhibitors and mTOR inhibitors, monoclonal antibodies (such as Cetuximab), oxaliplatin, gemcitabine, gefinitib, taxotere, ara A, ara C, herceptin, BCNU, CCNU, DTIC, and

actinomycin D. Still further anti-proliferative agents include but are not limited to those compounds acknowledged to be used in the treatment of neoplastic diseases in Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (Eleventh Edition), editor Molinoff et al., publ. by McGraw-Hill, pages 1225-1287 (2006), which is hereby incorporated by reference, such as aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine cladribine, busulfan, diethylstilbestrol, 2',2'-difluorodeoxycytidine, docetaxel, erythrohydroxynonyladenine, ethinyl estradiol, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, fludarabine phosphate, fluoxymesterone, flutamide, hydroxyprogesterone caproate, idarubicin, interferon, medroxyprogesterone acetate, megestrol acetate, melphalan, mitotane, paclitaxel, pentostatin, N-phosphonoacetyl-L-aspartate (PALA), plicamycin, semustine, tenipdside, testosterone propionate, thiotepa, trimethylmelamine, uridine, and vinorelbine.

[0515] In some embodiments, a combination of a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and an immunotherapeutic agent is co-administered with at least one additional agent. In some embodiments, a combination of a compound of any one of Formulas I, II, VII, VIII, IX, X and/or XI, as disclosed herein, and an immunotherapeutic agent is co-formulated with at least one additional agent.

Administration/Dosage/Formulations

[0516] The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the subject either prior to or after the onset of a disease or disorder contemplated herein. In some embodiments, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. In some embodiments, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

[0517] Administration of the compositions of the present application to a subject, for example a mammal, wherein the mammal may be human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder contemplated herein. In some embodiments, the subject is a patient. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the state of the disease or disorder in the patient; the age, sex, and weight of the patient; and the ability of the therapeutic compound to treat a disease or disorder contemplated herein. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as

indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic combination contemplated herein is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

[0518] Actual dosage levels of the active ingredients in the pharmaceutical compositions disclosed herein may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[0519] The therapeutically effective amount or dose of a combination of the present application depends on the age, sex and weight of the patient, the current medical condition of the patient and the progression of a disease or disorder contemplated herein.

[0520] A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the combinations contemplated herein employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0521] A suitable dose of a combination of the present application may be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses.

[0522] Combinations contemplated herein for administration may be in the range of from about 1 µg to about 10,000 mg, about 20 µg to about 9,500 mg, about 40 µg to about 9,000 mg, about 75 µg to about 8,500 mg, about 150 µg to about 7,500 mg, about 200 µg to about 7,000 mg, about 3050 µg to about 6,000 mg, about 500 µg to about 5,000 mg, about 750 µg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 30 mg to about 1,000 mg, about 40 mg to about 900 mg, about 50 mg to about 800 mg, about 60 mg to about 750 mg, about 70 mg to about 600 mg, about 80 mg to about 500 mg, and any and all whole or partial increments there between.

[0523] In some embodiments, the dose of a combination contemplated herein is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound contemplated herein used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of an additional agent in the combination as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

[0524] In some embodiments, the combination comprises a dosage of TKI of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject; and

[0525] a dosage of the immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 0.1 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 20 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 15 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 10 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 5 mg/kg of body weight of a subject, or from about 0.1 mg/kg of body weight of a subject to about 1 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 1 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 20 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 15 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 10 mg/kg of body weight of a subject, or from about 1 mg/kg of body weight of a

subject to about 5 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 10 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 10 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, from about 10 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject, from about 10 mg/kg of body weight of a subject to about 20 mg/kg of body weight of a subject, or from about 10 mg/kg of body weight of a subject to about 15 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 20 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 20 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, or from about 20 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject. In some embodiments, the TKI is Compound A. In some embodiments, the TKI is Compound B. In some embodiments, the combination comprises a dosage of Compound A of about 50 mg/kg of body weight of a subject.

[0526] In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 20 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 15 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 10 mg/kg of body weight of a subject, from about 0.1 mg/kg of body weight of a subject to about 5 mg/kg of body weight of a subject, or from about 0.1 mg/kg of body weight of a subject to about 1 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 1 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 20 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 15 mg/kg of body weight of a subject, from about 1 mg/kg of body weight of a subject to about 10 mg/kg of body weight of a subject, or from about 1 mg/kg of body weight of a subject to about 5 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 10 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 10 mg/kg of

body weight of a subject to about 50 mg/kg of body weight of a subject, from about 10 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject, from about 10 mg/kg of body weight of a subject to about 20 mg/kg of body weight of a subject, or from about 10 mg/kg of body weight of a subject to about 15 mg/kg of body weight of a subject. In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 20 mg/kg of body weight of a subject to about 100 mg/kg of body weight of a subject, from about 20 mg/kg of body weight of a subject to about 50 mg/kg of body weight of a subject, or from about 20 mg/kg of body weight of a subject to about 25 mg/kg of body weight of a subject.

[0527] In some embodiments, the combination comprises a dosage of TKI of from about 0.1 mg/kg of body weight of a subject to about 300 mg/m² of body weight of a subject; and

[0528] a dosage of the immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 0.1 mg/m² of body weight of a subject to about 200 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 150 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 20 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 15 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 10 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 5 mg/m² of body weight of a subject, or from about 0.1 mg/m² of body weight of a subject to about 1 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 1 mg/m² of body weight of a subject to about 150 mg/m² of body weight of a subject, about 1 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 20 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 15 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 10 mg/m² of body weight of a subject, or from about 1 mg/m² of body weight of a subject to about 5 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 10 mg/m² of body

weight of a subject to about 150 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 20 mg/m² of body weight of a subject, or from about 10 mg/kg of body weight of a subject to about 15 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 20 mg/m² of body weight of a subject to about 150 mg/m² of body weight of a subject, from about 20 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 20 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, or from about 20 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 50 mg/m² of body weight of a subject to about 150 mg/m² of body weight of a subject, from about 50 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 50 mg/m² of body weight of a subject to about 80 mg/m² of body weight of a subject, or from about 50 mg/m² of body weight of a subject to about 75 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 75 mg/m² of body weight of a subject to about 150 mg/m² of body weight of a subject, or from about 75 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of from about 80 mg/m² of body weight of a subject to about 150 mg/m² of body weight of a subject, or from about 80 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of TKI of about 0.1 mg/m², about 1 mg/m², about 5 mg/m², about 10 mg/m², about 15 mg/m², about 20 mg/m², about 25 mg/m², about 30 mg/m², about 40 mg/m², about 50 mg/m², about 60 mg/m², about 70 mg/m², about 75 mg/m², about 80 mg/m², about 100 mg/m², about 150 mg/m², about 200 mg/m², or about 300 mg/m².

[0529] In some embodiments, the TKI is Compound A. In some embodiments, the TKI is Compound B. In some embodiments, the combination comprises a dosage of Compound A of about 50 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of Compound A of from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of Compound A of about 150 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of Compound A of about 75 mg/m² of body weight of a subject. In some embodiments,

the combination comprises a dosage of Compound A of about 80 mg/ m² of body weight of a subject. In some embodiments, the combination comprises a dosage of Compound A of about 120 mg/ m² of body weight of a subject.

[0530] In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 300 mg/m² of body weight of a subject; and a dosage of the immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 200 mg/m² of body weight of a subject, the combination comprises a dosage of immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 20 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 15 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 10 mg/m² of body weight of a subject, from about 0.1 mg/m² of body weight of a subject to about 5 mg/m² of body weight of a subject, or from about 0.1 mg/m² of body weight of a subject to about 1 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 1 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 20 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 15 mg/m² of body weight of a subject, from about 1 mg/m² of body weight of a subject to about 10 mg/m² of body weight of a subject, or from about mg/m² of body weight of a subject to about 5 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage of immunotherapeutic agent of from about 10 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject, from about 10 mg/m² of body weight of a subject to about 20 mg/m² of body weight of a subject, or from about 10 mg/kg of body weight of a subject to about 15 mg/m² of body weight of a subject. In some embodiments, the combination comprises a dosage

of immunotherapeutic agent of from about 20 mg/m² of body weight of a subject to about 100 mg/m² of body weight of a subject, from about 20 mg/m² of body weight of a subject to about 50 mg/m² of body weight of a subject, or from about 20 mg/m² of body weight of a subject to about 25 mg/m² of body weight of a subject.

[0531] It should be understood that the combinations described herein can be administered simultaneously, separately, or sequentially. In some embodiments, the TKI is independently administered from the immunotherapeutic agent (*e.g.*, in two units, but at the same time). In some embodiments, the TKI is administered first, and then the immunotherapeutic agent is separately or sequentially administered. In some embodiments, the immunotherapeutic agent is administered first, and then the TKI is administered, separately or sequentially, as defined.

[0532] In some embodiments, the combinations contemplated herein are administered to the patient in dosages that range from one to five times per day or more. In some embodiments, the combinations contemplated herein are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions contemplated herein varies from individual to individual depending on many factors including, but not limited to, age, disease or disorder to be treated, gender, overall health, and other factors. Thus, the present disclosure should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient is determined by the attending physical taking all other factors about the patient into account.

[0533] It is understood that the amount of combination dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on.

[0534] In some embodiments, Compound A is administered once, twice, three times, four times, five times, or six times a day. In another embodiment, Compound A is administered once, twice, three times, four times, five times, or six times a week. In some embodiments, Compound A is administered every week, every two weeks, every three weeks, every four weeks, every five weeks, or every six weeks.

[0535] In some embodiments, the immunotherapeutic agent is administered once, twice, three times, four times, five times, or six times a day. In another embodiment, the

immunotherapeutic agent is administered once, twice, three times, four times, five times, or six times a week. In some embodiments, the immunotherapeutic agent is administered every week, every two weeks, every three weeks, every four weeks, every five weeks, or every six weeks.

[0536] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the combination contemplated herein is optionally given continuously; alternatively, the dose of drug being administered is temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday optionally varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday includes from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0537] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is reduced, as a function of the disease or disorder, to a level at which the improved disease is retained. In one embodiment, patients require intermittent treatment on a long-term basis upon any recurrence of symptoms and/or infection.

[0538] The combinations for use in the method disclosed herein may be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosage for patients undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

[0539] Toxicity and therapeutic efficacy of such therapeutic regimens are optionally determined in cell cultures or experimental animals, including, but not limited to, the determination of the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD₅₀ and ED₅₀. The data obtained from cell culture assays and animal studies are optionally used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal toxicity. The

dosage optionally varies within this range depending upon the dosage form employed and the route of administration utilized.

[0540] In some embodiments, the combinations contemplated herein are formulated in a pharmaceutical composition using one or more pharmaceutically acceptable excipients or carriers. In some embodiments, the pharmaceutical compositions contemplated herein comprise a therapeutically effective amount of a combination contemplated herein and a pharmaceutically acceptable carrier.

[0541] The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition.

[0542] In the compositions, either effective concentrations of the TKI or the immunotherapeutic agent are each separately mixed with a suitable pharmaceutical carrier or vehicle. In some embodiments, effective combined concentrations of the TKI or the immunotherapeutic agent are mixed with a suitable pharmaceutical carrier or vehicle. In some embodiments, the combined concentrations of the TKI or the immunotherapeutic agent in the compositions are effective for delivery of an amount, upon administration, that treats, prevents, or ameliorates one or more of the symptoms and/or progression of cancer, including haematological cancers and solid tumours. In some embodiments, separate concentrations of the TKI or the immunotherapeutic agent in the compositions are effective for delivery of an amount, upon administration, that treats, prevents, or ameliorates one or more of the symptoms and/or progression of cancer, including haematological cancers and solid tumours.

[0543] In some embodiments, the compositions can be formulated for single dosage administration. In some embodiments, to formulate a composition, the TKI or the immunotherapeutic agent are dissolved, suspended, dispersed or otherwise mixed in a selected vehicle at an effective combined concentration such that the treated condition is relieved or ameliorated. In some embodiments, the TKI alone is dissolved, suspended, dispersed or otherwise mixed in a selected vehicle and the immunotherapeutic agent alone is dissolved,

suspended, dispersed or otherwise mixed in a selected vehicle. The TKI or the immunotherapeutic agent are then administered, simultaneously or sequentially, at an effective combined concentration such that the treated condition is relieved or ameliorated. Pharmaceutical carriers or vehicles suitable for administration of the TKI or the immunotherapeutic agent provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.

[0544] In some embodiments, the present application is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound contemplated herein, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of a disease or disorder contemplated in the present disclosure.

[0545] Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for any suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, analgesic agents.

Additional Administration Forms

[0546] Additional dosage forms include dosage forms as described in U.S. Patents Nos. 6,340,475; 6,488,962; 6,451,808; 5,972,389; 5,582,837; and 5,007,790. Additional dosage forms also include dosage forms as described in U.S. Patent Applications Nos. 20030147952; 20030104062; 20030104053; 20030044466; 20030039688; and 20020051820. Further dosage forms include dosage forms as described in PCT Applications Nos. WO 03/35041; WO 03/35040; WO 03/35029; WO 03/35177; WO 03/35039; WO 02/96404; WO 02/32416; WO 01/97783; WO 01/56544; WO 01/32217; WO 98/55107; WO 98/11879; WO 97/47285; WO 93/18755; and WO 90/11757.

Controlled Release Formulations and Drug Delivery Systems

[0547] In some embodiments, the formulations of the present application may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

[0548] The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and

that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release which is longer than the same amount of agent administered in bolus form.

[0549] For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material that provides sustained release properties to the compounds. As such, the compounds for use in the methods disclosed herein may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation.

[0550] In some embodiments, the compounds contemplated herein are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

[0551] The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours.

[0552] The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration.

[0553] The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

[0554] As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration after drug administration.

[0555] As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of the present disclosure and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*,

nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

[0556] It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present application. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

[0557] The following examples further illustrate aspects of the present application. However, they are in no way a limitation of the teachings or disclosure of the present application as set forth herein.

EXAMPLES

Example 1. Growth delay of TH-302 as a single agent in the MB49 murine bladder cancer model

[0558] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups in two independent experiments:

- (1) Vehicle (Phosphate-buffered saline), IP, Q2Dx4; and
- (2) 1-Methyl-2-nitro-1H-imidazol-5-yl)methyl N,N'-bis(2-bromoethyl)phosphorodiamidate, (TH-302; evofosfamide; 2-bromo-N-[(2-bromoethylamino)-[(3-methyl-2-nitroimidazol-4-yl)methoxy]phosphoryl]ethanamine), 75 mg/kg or 100 mg/kg, IP, Q2Dx4.

Table 1. Experimental Parameters for Example 1

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	2.5 x 10 ⁵ cells
Drug solutions	TH-302 in PBS
Injection volume	15 mL/kg (0.015 mL/g)
Drug administration route	TH-302 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 8 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 8).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Criteria for culling mice	Volume of the tumour exceeded 200 mm ³ or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 8 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ³ or 1300 mm ³ Extensive tumour ulceration

Anti-tumour efficacy

[0559] The administration of TH-302 every two days for 4 doses showed marginal efficacy in the MB49 tumour model. The anti-tumour activities were similar at 75 mg/kg and 100 mg/kg dose levels (FIG. 1). Treatment with TH-302 delayed MB49 tumour growth but was unable to induce tumour regression nor provide significant survival advantage over vehicle control (FIGs. 3 and 5).

Drug toxicity

[0560] Both dose levels of TH-302 treatment were reasonably well tolerated with weight loss not exceeding 10% of body weight from weight on the day of treatment initiation (FIGs. 2 and 4).

[0561] The anti-tumour activity of two dose levels of TH-302 as a single agent in syngeneic MB49 tumour model was compared. TH-302 at 75 mg/kg and 100 mg/kg showed similar anti-tumour activity but induced greater body weight loss at 100 mg/kg. Treatment with TH-302 at both dose levels did not result in tumour regression but TH-302 was slightly more active at the 100 mg/kg dose level as the tumour volumes on day 20 in the corresponding experiment were smaller compared with the tumour volumes of mice dosed at 75 mg/kg.

Example 2. Growth delay of tarloxotinib or TH-302 as a single agent or in combination with anti-PD-1 in the MB49 murine bladder cancer model

[0562] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib (Compound A; ((2*E*)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-*d*]pyrimidin-6-yl}amino}-*N,N*-dimethyl-*N*-(1-methyl-4-nitro-1*H*-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide), 50 mg/kg, IP, Q2Dx4;
- (3) (2-bromo-*N*-[(2-bromoethylamino)-[(3-methyl-2-nitroimidazol-4-yl)methoxy]phosphoryl]ethanamine), TH-302, 100 mg/kg, IP, Q2Dx4;
- (4) Anti-PD-1 (*InVivo*Plus anti-mouse PD-1 (CD279)), 200 μ g, IP, Q3Dx3;
- (5) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-1, 200 μ g, IP, Q3Dx3; and
- (6) TH-302, 100 mg/kg, IP, Q2Dx4 in combination with anti-PD-1, 200 μ g, IP, Q3Dx3.

Table 2. Experimental Parameters for Example 2.

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	2.5×10^5 cells
Drug solutions	Tarloxotinib in WFI containing 20% 2-hydroxypropyl- β -cyclodextrin TH-302 in PBS Anti-PD-1 in PBS
Injection volume	Tarloxotinib and TH-302 – 15 mL/kg (0.015 mL/g) Anti-PD-1 – 100 μ L
Drug administration route	Tarloxotinib – intraperitoneal (IP) TH-302 – intraperitoneal (IP) Anti-PD-1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 7 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 7).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Criteria for culling mice	Volume of the tumour exceeded 200 mm ² or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 7 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ² or 1300 mm ³

Anti-tumour efficacy

[0563] Tarloxotinib administration at 50 mg/kg every two days for 4 doses (Q2Dx4) was highly active as a single agent in MB49 tumours (FIG. 6). Compared to vehicle treated controls (710.7 mm³), TH-302 treated (507.2 mm³) and anti-PD-1 treated mice (663.7 mm³), the average tumour volume on day 14 of tarloxotinib alone treated mice (104.5 mm³) was significantly smaller (FIG. 8). In comparison to tarloxotinib, the administration of 100 mg/kg TH-302 every two days for 4 doses as a single agent was marginally active with slight delay in tumour progression (FIG. 6). Treatment with TH-302 did not result in tumour regression but provided survival advantage over vehicle control (FIG. 12). Treatment with anti-PD-1 alone every three days for 3 doses at 200 μ g per mouse was ineffective in delaying MB49 tumour progression (FIG. 6). Anti-PD-1 treatment also did not prolong overall survival (FIG. 12). The combination of tarloxotinib and anti-PD-1 was more active than tarloxotinib or anti-PD-1 alone. Upon treatment cessation, tumours in the combination group regrew at a significantly

slower rate (FIGs. 9 and 10) compared to tarloxotinib monotherapy. This combination treatment also significantly prolonged survival (FIG. 12). The combination of TH-302 and anti-PD-1 did not improve anti-tumour activity or overall survival (FIGs. 6 and 12).

Drug toxicity

[0564] All treatment options were well tolerated across all groups with weight loss not exceeding 10% of body weight from weight at the start of dosing, except one mouse in the TH-302 alone group which had temporarily lost 13.9% of its initial body weight on day 17 (FIG. 11).

[0565] The anti-tumour activity of tarloxotinib/anti-PD-1 combination to the combination of TH-302/anti-PD-1 was examined and compared to determine if these combinations would be more efficacious than tarloxotinib, TH-302 or anti-PD-1 as single agents. Anti-PD-1 was ineffective as a single agent and tarloxotinib alone was shown to be highly efficacious in this study, the combination of the two treatments led to more robust delay in tumour progression upon treatment cessation. The combination of tarloxotinib with anti-PD-1 also led to longer survival of MB49 tumour bearing mice before reaching ethical endpoints. The combination treatment was also well tolerated with no additional side effects and minimal weight loss. Conversely, the combination of TH-302 with anti-PD-1 was not synergistic, which showed no improvement in anti-tumour activity and survival. Tarloxotinib but not TH-302 can be combined effectively with anti-PD-1 to delay MB49 tumour growth. Tarloxotinib being more active than TH-302 indicates that a hypoxia activated EGFR-targeted inhibitor has superior anti-tumour activity than a hypoxia activated alkylating agent in the EGFR driven MB49 tumours.

Example 3. Growth delay of tarloxotinib as a single agent or in combination with anti-PD-1, anti-CTLA4 or anti-PD-L1 in the MB49 murine bladder cancer model.

[0566] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxylpropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib, (Compound A;) 50 mg/kg, IP, Q2Dx4;
- (3) Anti-PD-1 (InVivoPlus anti-mouse PD-1 (CD279)), 200 μ g, IP, Q3Dx3;
- (4) Anti-CTLA4 (InVivoPlus anti-mouse CTLA4 (CD152)), 200 μ g, IP, Q3Dx3;
- (5) Anti-PD-L1 (InVivoPlus anti-mouse PD-L1 (B7-H1)), 200 μ g, IP, Q3Dx3;

(6) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-1, 200 µg, IP, Q3Dx3;

(7) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-CTLA4, 200 µg, IP, Q3Dx3; and

(8) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-L1, 200 µg, IP, Q3Dx3.

Table 3. Experimental Parameters for Example 3.

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5×10^5 cells
Drug solutions	Tarloxotinib in WFI containing 20% 2-hydroxypropyl- β -cyclodextrin Anti-PD-1 in PBS Anti-CTLA4 in PBS Anti-PD-L1 in PBS
Injection volume	Tarloxotinib – 15 mL/kg (0.015 mL/g) Anti-PD1 – 100 µL Anti-CTLA4 – 100 µL Anti-PD-L1 – 100 µL
Drug administration route	Tarloxotinib – intraperitoneal (IP) Anti-PD-1 – intraperitoneal (IP) Anti-CTLA4 – intraperitoneal (IP) Anti-PD-L1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Criteria for culling mice	Volume of the tumour exceeded 200 mm ² or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 6 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ² or 1300 mm ³

Anti-tumour efficacy.

[0567] Tarloxotinib administration at 50 mg/kg every two days for 4 doses (Q2Dx4) was modestly active as a single agent in MB49 tumours (FIG. 13). Compared to vehicle treated controls (1069 mm³), anti-PD-1 treated (649.3 mm³), anti-CTLA4 treated mice (564 mm³), and

anti-PD-L1 treated mice (552.6 mm³) the average tumour volume on day 19 of tarloxotinib alone treated mice (226.3 mm³) was significantly smaller (FIG. 15).

[0568] Treatment with immune checkpoint inhibitors (ICI), anti-PD-1, anti-CTLA4 or anti-PD-L1 alone every three days for 3 doses at 200 µg per mouse was marginally efficacious, delaying MB49 tumour growth by 20% - 32.5% (FIG. 13). Mice treated with checkpoint blockade monotherapies survived slightly longer than vehicle control treated mice (FIG. 19). However median time to endpoint was not significantly improved by anti-PD-1, anti-CTLA4 (4 days; $P < 0.05$) and marginally improved by anti-PD-L1 (6.5 days; $p = 0.0308$). MB49 is considered refractory to ICI treatment as single agent. The combinations of tarloxotinib with all three tested checkpoint blockades were more active than tarloxotinib, anti-PD-1, anti-CTLA4 or anti-PD-L1 alone. Upon treatment cessation, tumours in the combination group regrew at a significantly slower rate (FIG. 16) compared to tarloxotinib alone. Combination treatments also significantly prolonged survival of MB49 tumour bearing mice (FIG. 19). The combination of tarloxotinib with anti-PD-L1 is the most efficacious combination treatment in this study. Compared to tarloxotinib/anti-PD-1 treated (596.5 mm³) and tarloxotinib/anti-CTLA4 treated mice (455.6 mm³) the average tumour volume on day 26 of tarloxotinib/anti-PD-L1 treated mice (320.5 mm³) was the lowest (FIG. 16). Tarloxotinib provided 8 days improvement in median time to endpoint, anti-PD-L1 provided 6.5 days, whereas the combination resulted in a greater than additive effect, with 19 days improvement in median time to endpoint.

Drug toxicity

[0569] All treatment options were well tolerated across all groups with weight loss not exceeding 10% of body weight from weight at the start of dosing, except one mouse in the tarloxotinib/anti-PD-L1 combination group which had temporarily lost 11.5% of its initial body weight on day 21 (FIGs. 17 and 18).

[0570] The anti-tumour activity of tarloxotinib in combination with different checkpoint blockades was examined and compared to determine whether or not these combinations would be more efficacious than tarloxotinib, anti-PD-1, anti-CTLA4 or anti-PD-L1 monotherapies. While tarloxotinib alone was highly efficacious in this study, its combination with weakly active checkpoint blockades led to more robust delay in tumour progression upon treatment cessation, with tarloxotinib/anti-PD-L1 being the most efficacious combination. The combination of tarloxotinib with checkpoint blockade also led to longer survival of MB49 tumour bearing mice before reaching ethical endpoints. These combination treatments were

well tolerated with no additional side effects and minimal weight loss. In conclusion, tarloxotinib can be combined effectively with checkpoint blockades for example with anti-PD-L1 to delay MB49 tumour growth. To explore the mechanism behind such synergy, whole blood (6-10 drops by submandibular bleed) were collected from mice and examined by flow cytometry for changes in number and phenotype of immune cells in the periphery (study report RF51B).

Example 4. Growth delay of murine bladder cancer after administration with tarloxotinib as a single agent or in combination with anti-PD-L1 or anti-PD-1 plus anti-CTLA4 in the MB49 murine bladder cancer model using a protracted dosing schedule.

[0571] The anti-tumour activity of multiple cycles of tarloxotinib in combination with anti-PD-L1 or with anti-PD-1/anti-CTLA4 ‘dual blockade’ was examined in the syngeneic MB49 tumour model, with reference to monotherapy treatment with tarloxotinib, anti-PD-L1, anti-PD-1/anti-CTLA4 or vehicle control. The toxicity of prolonged dosing of tarloxotinib, checkpoint blockade and tarloxotinib/checkpoint blockade combination was also assessed.

[0572] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxylpropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib, 50 mg/kg, IP, Q2Dx4, 3 cycles with 3 days of resting period between cycles;
- (3) Anti-PD-1 and anti-CTLA4 ‘dual blockade’, 200 μ g of each, IP, Q3Dx3, 3 cycles with 3 days of resting period between cycles;
- (4) Anti-PD-L1, 200 μ g, IP, Q3Dx3, 3 cycles with 3 days of resting period between cycles;
- (5) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-1/anti-CTLA4, 200 μ g of each, IP, Q3Dx3, 3 cycles with 3 days of resting period between cycles; and
- (6) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-L1, 200 μ g, IP, Q3Dx3, 3 cycles with 3 days of resting period between cycles.

Table 4. Experimental Parameters for Example 4

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49

Cells per inoculation	1.5 x 10 ⁵ cells
Drug solutions	• Tarloxotinib in WFI containing 20% 2-hydroxypropyl-β-cyclodextrin • Anti-PD-1 in PBS • Anti-CTLA4 in PBS • Anti-PD-L1 in PBS
Injection volume	• Tarloxotinib – 15 mL/kg (0.015 mL/g) • Anti-PD1 – 100 μL • Anti-CTLA4 – 100 μL • Anti-PD-L1 – 100 μL
Drug administration route	• Tarloxotinib – intraperitoneal (IP) • Anti-PD-1 – intraperitoneal (IP) • Anti-CTLA4 – intraperitoneal (IP) • Anti-PD-L1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Criteria for culling mice	Volume of the tumour exceeded 200 mm² or 1300 mm³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 6 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ² or 1300 mm ³

Anti-tumour efficacy

[0573] Three cycles of tarloxotinib administration at 50 mg/kg every two days for 4 doses (Q2Dx4) was active as a single agent in MB49 tumours (FIG. 61). Compared to vehicle treated controls (951 $\pm$ 114 mm³), anti-PD-L1 (277 $\pm$ 65.2 mm³) and anti-PD-1 + anti-CTLA4 treated (164.1 $\pm$ 52.4 mm³) mice, the average tumour volume on day 19 of the tarloxotinib alone group (157 $\pm$ 24.7 mm³) was the smallest (FIG. 63 and 64). Three cycles of anti-PD-1 + anti-CTLA4 every three days for 3 doses at 200 μ g showed similar activity as tarloxotinib (FIG. 64). Mice treated with tarloxotinib on average survived 10 days longer than vehicle control treated mice (TGI 63%; Log rank test P = 0.0007) (FIG. 67 and Table 5).

[0574] Three cycles of treatment with anti-PD-L1 ($277 \pm 65.2 \text{ mm}^3$) or anti-PD-1/anti-CTLA4 ($164.1 \pm 52.4 \text{ mm}^3$) every three days for 3 doses at 200 μg per mouse was effective at delaying MB49 tumour growth compared to vehicle treated control ($951 \pm 114 \text{ mm}^3$) (FIG. 61). Mice treated with anti-PD-L1 or anti-PD-1/anti-CTLA4 survived longer than vehicle control treated mice (TGI 50%; Log rank test $P = 0.0005$ and TGI 75%; Log rank test $P = 0.0018$, respectively) (FIG. 67 and Table 5).

[0575] MB49 tumours escaped tarloxotinib treatment toward the end of the 3rd cycle of treatment, whereas the control on MB49 tumour growth was maintained in tarloxotinib/checkpoint inhibitor combination treated mice (FIG. 61). The combinations of tarloxotinib with checkpoint inhibitors were more active than single treatments. Upon treatment cessation, tumours in the tarloxotinib/anti-PD-L1 ($138 \pm 32.9 \text{ mm}^3$) and tarloxotinib/anti-PD-1+anti-CLA4 ($58.9 \pm 23.3 \text{ mm}^3$) groups regrew at a slower rate (FIG. 63 and 64) compared to tarloxotinib alone ($976 \pm 98.2 \text{ mm}^3$) on day 30. Combination treatment also significantly prolonged the survival of MB49 tumour bearing mice (FIG. 67). Mice treated with tarloxotinib plus anti-PD-L1 (TGI 162%; Log rank test $P = 0.0001$) or plus 'dual blockade' (TGI 213%; Log rank test $P = 0.0018$) combination on average survived 26 and 34 days longer than vehicle control treated mice, respectively (FIG. 67 and Table 5).

Table 5. Example 4 Data by Group

Group	Drug	Dose (mg/kg/dose)	Schedule	Animal number	Average Weight Loss Nadir (+/- SEM) [Day]	Endpoint (days)	Days to endpoint from treatment initiation	Days to reach endpoint	% Tumor Growth Delay	Log Rank test (vs control)
A	Vehicle (20%CDW) (PBS)		Q2Dx4 Q3Dx3	7	None	19, 21, 22, 22, 23, 23, 23	13, 15, 16, 16, 17, 17, 17	16	-	
B	Tarloxotinib	50	Q2Dx4	6	0.38 ± 1.5 [9]	30, 32, 32, 32, 32, 34	24, 26, 26, 26, 26, 28	26	63 %	0.00 07
C	Anti-PD-L1	200 µg	Q3Dx3	8	None	23, 27, 29, 30, 30, 32, 34, 34	17, 21, 23, 24, 24, 26, 28, 28	24	50 %	0.00 05
D	Tarloxotinib + Anti-PD-L1	50 200 µg	Q2Dx4 Q3Dx3	8	1.8 ± 0.84 [11]	46, 46, 46, 48, 48, 50, 52, 52	40, 40, 40, 42, 42, 44, 46, 46	42	163 %	0.00 01
E	Anti-PD-1 + Anti-CTLA4	200 µg	Q3Dx3	5	None	29, 32, 34, 36, 40	23, 26, 28, 30, 34	28	75 %	0.00 18
F	Tarloxotinib + Anti-PD-1 and anti-CTLA4	50 200 µg	Q2Dx4 Q3Dx3	5	None	54, 54, 56, 56, one long term control	48, 48, 50, 50, one long term control	50	213 %	0.00 18

Vehicle: 20% 2-hydroxylpropyl-β-cyclodextrin, dissolved in water for injection PBS; Schedule: (a) Tarloxotinib: Q2Dx4 = every 2 days for 4 doses (IP), (b) Anti-PD-1: Q3Dx3 = every 3 days for 3 doses (IP), (c) Anti-CTLA4: Q3Dx3 = every 3 days for 3 doses (IP), (d) Anti-PD-L1: Q3Dx3 = every 3 days for 3 doses (IP); Deaths independent (excludes mice culled prior to endpoint that had already reached endpoint (e.g. mice culled early due to grade II ulceration) of drug toxicity); Reasons for Death: Drug toxicity related: a - Found dead, b - Evidence of morbidity, c - Evidence of treatment-related adverse effect, d - Excessive weight loss (>20% from starting size), Independent of drug toxicity: e - Irregular tumor growth, f - Tumor ulceration grade III, g - Tumor metastasis, h - Dosing aberration, i - Treatment-independent sickness, j - Animal husbandry concern; Long term control: Failed to reach endpoint within specified duration of experiment; % Tumour growth delay: Percent of tumour growth delay; relative delay in median survival versus control expressed as percent.

[0576] Three cycles of tarloxotinib plus anti-PD-1/anti-CTLA4 was an efficacious combination treatment in this study. Compared to tarloxotinib/anti-PD-L1 treated (138 ± 32.9 mm³), the average tumour volume on day 30 of tarloxotinib/anti-PD-1+anti-CTLA4 treated mice (58.9 ± 23.3 mm³) was the lowest (FIG. 63 and 64). Mice also survived longer as compared to tarloxotinib/anti-PD-L1 (56 versus 48 days post-inoculation) (FIG. 67).

Drug toxicity

[0577] All treatment options were well tolerated across all groups with weight loss not exceeding 10% of body weight from weight at the start of dosing (FIG. 62 and 66).

[0578] While tarloxotinib alone was highly efficacious, toward the end of the 3rd cycle of treatment, MB49 tumour growth showed low suppression. The low suppression was not observed with mice treated with tarloxotinib/checkpoint inhibitor combinations. The combination of tarloxotinib with checkpoint blockade further delayed tumour growth and led to longer survival of MB49 tumour bearing mice compared to one cycle of treatment (See, Example 3). All treatments were well tolerated with no additional side effects. Protracted dosing schedule of tarloxotinib in combination with checkpoint blockades can be used safely to further delay MB49 tumour growth. The protracted dosing schedule of tarloxotinib in combination with anti-PD-L1 or anti-PD-1/anti-CTLA4 ‘dual blockade’ provided extended control on MB49 tumour growth and significantly prolonged survival without excessive body weight loss. Mice treated with tarloxotinib/anti-PD-1+anti-CTLA4 combination showed delay in tumour regrowth upon treatment cessation.

Example 5. Growth delay analysis of murine bladder cancer after administration with tarloxotinib as a single agent or in combination with anti-PD-1 plus anti-CTLA4 in the MB49 murine bladder cancer model.

[0579] Tarloxotinib in combination with anti-PD-1/anti-CTLA4 ‘dual blockade’ was examined in the syngeneic MB49 tumour model, with reference to monotherapy treatment with tarloxotinib or anti-PD-1/anti-CTLA4 dual blockade, or a vehicle control. The toxicity of tarloxotinib in combination with anti-PD-1/anti-CTLA4 in MB49 tumours was also assessed.

[0580] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib, 50 mg/kg, IP, Q2Dx4;
- (3) Anti-PD-1 and anti-CTLA4 (‘Dual blockade’), 200 μ g of each, IP, Q3Dx3; and
- (4) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-1/anti-CTLA4, 200 μ g of each, IP, Q3Dx3.

Table 6. Experimental Parameters for Example 5

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5×10^5 cells
Drug solutions	• Tarloxotinib in WFI containing 20% 2-hydroxypropyl-β-cyclodextrin • Anti-PD-1 in PBS • Anti-CTLA4 in PBS
Injection volume	• Tarloxotinib – 15 mL/kg (0.015 mL/g) • Anti-PD1 – 100 μL • Anti-CTLA4 – 100 μL
Drug administration route	• Tarloxotinib – intraperitoneal (IP) • Anti-PD-1 – intraperitoneal (IP) • Anti-CTLA4 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Criteria for culling mice	Volume of the tumour exceeded 200 mm² or 1300 mm³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 6 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ² or 1300 mm ³

[0581] Vehicle treated (control) MB49 tumours grew at a rapid rate and passed endpoint 20 days after implantation. Tarloxotinib administration at 50 mg/kg every two days for four doses (Q2Dx4) was active as a single agent in MB49 tumours (FIG. 68). In comparison to vehicle treated controls (1034 ± 167 mm³) and anti-PD-1/anti-CTLA4 treated (384 ± 219 mm³) mice, the average tumour volume on day 18 of tarloxotinib alone group (250 ± 56 mm³) was significantly smaller (FIG. 69). Mice treated with tarloxotinib survived, on average, 7 days longer than vehicle control treated mice (TGI 50%; Log rank test $P = 0.018$) (FIG. 72 and Table 7).

[0582] 'Dual blockade' treatment with anti-PD-1 and anti-CTLA4 every three days for three doses at 200 µg per mouse showed modest anti-tumour activity (FIG. 68). Mice treated with checkpoint inhibitor combination survived, on average, 9 days longer than vehicle control treated mice (TGI 64%; Log rank test $P = 0.028$) (FIG. 72 and Table 7).

Table 7. Experimental Analysis for Example 5

Group	Drug	Dose (mg/kg/dose)	Schedule	Animal number	Day of Death (includes all deaths)	Reason for Death	Average Weight Loss Nadir (+/- SEM) [Day]	Endpoint (days)	Days to endpoint from treatment initiation	Median days to reach endpoint	% Tumor Growth Delay	Log Rank test (vs control)
A	Vehicle (20%CDW) (PBS)	-	Q2Dx4 Q3Dx3	5	None		0.66 ± 0.31 [7]	18, 18, 20, 22, 24	12, 12, 14, 16, 18	14	-	
B	Tarloxotinib	50	Q2Dx4	5	None		6.0 ± 0.17 [16]	22, 24, 27, 27, 27	16, 18, 21, 21, 21	21	50%	0.0180
C	Anti-PD-1 + Anti-CTLA4	200 µg	Q3Dx3	5	None		None	20, 24, 29, 29, 33	14, 18, 23, 23, 27	23	64%	0.0278
D	Tarloxotinib + Anti-PD-1 and anti-CTLA4	50 200 µg	Q2Dx4 Q3Dx3	5	None		0.32 ± 0.21 [9]	35, 37, 43, 43, 43, 43	29, 31, 37, 37, 37, 37	37	164%	0.0017

Vehicle 20% 2-hydroxypropyl-β-cyclodextrin, dissolved in water for injection PBS
 Schedule Tarloxotinib: Q2Dx4 = every 2 days for 4 doses (IP); Anti-PD-1: Q3Dx3 = every 3 days for 3 doses (IP); Anti-CTLA4: Q3Dx3 = every 3 days for 3 doses (IP)
 Deaths independent Excludes mice culled prior to endpoint that had already reached endpoint (e.g. mice culled early due to grade II ulceration) of drug toxicity
 Reasons for Death Drug toxicity related: a - Found dead, b - Evidence of morbidity, c - Evidence of treatment-related adverse effect, d - Excessive weight loss (>20% from starting size)
 Independent of drug toxicity: e - Irregular tumor growth, f - Tumor ulceration grade III, g - Tumor metastasis, h - Dosing aberration, i - Treatment-independent sickness, j - Animal husbandry concern
 Long term control Failed to reach endpoint within specified duration of experiment
 % Tumour growth delay Percent of tumour growth delay; relative delay in median survival versus control expressed as percent.

[0583] The combinations of tarloxotinib with anti-PD-1 and anti-CTLA4 were more active than other treatments. Upon treatment cessation, tumours in the tarloxotinib with 'dual blockade' combination group ($120 \pm 73 \text{ mm}^3$) regrew at a significantly slower rate (FIG. 70) compared to tarloxotinib alone ($767 \pm 173 \text{ mm}^3$). Combination treatment also prolonged the survival of MB49 tumour bearing mice (FIG. 72 and Table 7). Mice treated with tarloxotinib plus checkpoint inhibitor combination on average survived 23 days longer than vehicle control treated mice (TGI 165%; Log rank test $P = 0.0017$) (FIG. 72 and Table 7).

[0584] All treatment options were well tolerated across all groups with weight loss not exceeding 10% of body weight from weight at the start of dosing (FIG. 68). C57BL/6 mice treated with the combination of tarloxotinib and anti-PD-1/anti-CTLA4 showed an MB49 tumour regrowth delay (TGI = 165%) in comparison to mice treated with either monotherapy treatment alone (50% and 64%, respectively).

[0585] While tarloxotinib alone was efficacious in this study, its combination with a checkpoint inhibitor led to a more robust delay in tumour progression upon treatment cessation. The combination of tarloxotinib with checkpoint blockade also led to longer survival of MB49 tumour bearing mice before reaching ethical endpoints. All treatments were well tolerated with no additional side effects or evidence of toxicity. Tarloxotinib can be combined effectively with checkpoint blockades to significantly delay MB49 tumour growth.

Example 6. Flow cytometry analysis of tarloxotinib as a single agent or in combination with anti-PD-1, anti-CTLA4 or anti-PD-L1 in the MB49 murine bladder cancer model.

[0586] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib (Compound A), 50 mg/kg, IP, Q2Dx4;
- (3) Anti-PD-1, 200 μ g, IP, Q3Dx3;
- (4) Anti-CTLA4, 200 μ g, IP, Q3Dx3;
- (5) Anti-PD-L1, 200 μ g, IP, Q3Dx3;
- (6) Tarloxotinib (Compound A), 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-1, 200 μ g, IP, Q3Dx3;
- (7) Tarloxotinib (Compound A), 50 mg/kg, IP, Q2Dx4 in combination with anti-CTLA4, 200 μ g, IP, Q3Dx3; and
- (8) Tarloxotinib (Compound A), 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-L1, 200 μ g, IP, Q3Dx3.

Table 8. Experimental Parameters for Example 6.

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49

Cells per inoculation	1.5 x 10 ⁵ cells
Drug solutions	Tarloxotinib in WFI containing 20% 2-hydroxypropyl- β -cyclodextrin Anti-PD-1 in PBS Anti-CTLA4 in PBS Anti-PD-L1 in PBS
Injection volume	Tarloxotinib – 15 mL/kg (0.015 mL/g) Anti-PD1 – 100 μ L Anti-CTLA4 – 100 μ L Anti-PD-L1 – 100 μ L
Drug administration route	Tarloxotinib – intraperitoneal (IP) Anti-PD-1 – intraperitoneal (IP) Anti-CTLA4 – intraperitoneal (IP) Anti-PD-L1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Tissue type	Mouse peripheral blood collected by submandibular bleed
Instrument	Aurora flow cytometer (Cytek TM)

[0587] One day after treatment cessation, peripheral blood were collected from mice by submandibular bleed for flow cytometry analysis. Blood samples were incubated with red blood cell lysis (Qiagen) for 2 minutes before centrifugation at 4000 rpm for 4 minutes to allow isolation of immune cells. Cells were then stained with surface antibodies for 15 minutes at 4°C and then washed with FACS buffer (PBS + 0.5% FBS). For intracellular staining, cells were subsequently fixed and permeabilised using the transcription factor buffer set (BD PharmingenTM). Stained samples were stored at 4°C and analysed on the Aurora (CytekTM) flow cytometer.

Table 9. List of antibodies used for flow cytometry analysis of peripheral blood.

Chanel	Antibody	Clones	Fluorochrome	Dilution
B2	CD3	145-2C11	FITC	1:400
B3	FoxP3	FGK-165	AF532	1:100
B5	IFN γ	XMG1.2	PE	1:100
B6	PDL1 (CD274)	B7-H1	PE/Dazzle 594	1:600
B8	B220	RA3-6B2	PerCP	1:600
B8	CD44	IM7	PE-Cy5	1:800
B9	CD11b	M1-70	BB700	1:800
B10	CD4	RM4-5	PerCP eFluor 710	1:800

Chanel	Antibody	Clones	Fluorochrome	Dilution
B14	PD-1 (CD279)	29F-1A12	PE-Cy7	1:500
R1	KLRG1	2F1	APC	1:900
R2	F4/80	BM8	AF647	1:600
R4	CD8	53-6.7	AF700	1:600
R5	Viability	/	Zombie NIR from BioLegend	1:1000
R7	CD45.2	A20	APC-Fire750	1:600
V3	MHCII	M5/114.15.2	eFluor450	1:900
V4	Ki67	B56	BV480	1:200
V8	Ly6C	HK1.4	BV570	1:500
V10	CD69	H1-2F3	SB600	1:600
V11	NK1.1	PK136	BV650	1:500
V13	Ly6G	1A8	BV711	1:600
V15	CD11c	NK18	BV786	1:300

[0588] The bar graph shows statistical analysis performed between checkpoint blockade treatment alone and tarloxotinib/checkpoint blockade combination for tidier presentation. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$; ****, $P < 0.0001$).

[0589] The addition of tarloxotinib (50 mg/kg, Q2Dx4) to checkpoint blockades did not significantly change the percentage of CD3⁺ and CD4⁺ T-cells in the blood (FIGs. 20, 21 and 23) but the combination of tarloxotinib with anti-PD-L1 resulted in significant reduction in the percentage of CD8⁺ T-cells in the blood (FIGs. 22 and 23).

[0590] The addition of tarloxotinib (50 mg/kg, Q2Dx4) to checkpoint blockades increased the percentage of IFN γ ⁺ CD4⁺ and CD8⁺ T-cells in the blood (FIG. 30). This can be observed visually on the tSNE plots as increased intensity or heat of populations located on the bottom left of the tSNE plots (FIG. 29). This increase in the percentage of IFN γ ⁺ CD4⁺ and CD8⁺ T-cells was most prominent in the blood of mice treated with tarloxotinib/anti-PD-L1 combination. Tarloxotinib or checkpoint blockade alone did not affect this population of T-cells (FIG. 30).

[0591] The combinations of tarloxotinib with all three tested checkpoint blockades did not significantly change the percentage of Ki67⁺ CD8⁺ T-cells in the blood (FIGs. 24 and 32). Blood from mice treated with the combination of tarloxotinib with anti-PD-1 had significantly

increased percentage of KLRG1⁺ CD8⁺ T-cells and FOXP3⁺ CD4⁺ T-cells (FIGs. 31, 33 and 34).

[0592] The combinations of tarloxotinib with all three tested checkpoint blockades resulted in increased PD-1 expression of CD8⁺ T-cells as shown by the increase in marker intensity or heat of the cell population located in the bottom middle of the tSNE plots (FIG. 39).

[0593] The immune cells in the blood of mice that received anti-PD-L1 as a part of the treatment had reduced expression levels of PD-L1 on all immune cell types as shown by the reduction in marker intensity on the anti-PD-L1 and tarloxotinib/anti-PD-L1 tSNE plots (FIG. 40).

[0594] No noticeable differences were observed in all other cell markers examined (FIGs. 24-28 and 35-38).

[0595] The study primarily aimed to examine and compare the percentage and phenotype of immune cells in the blood of mice that received anti-PD-1, anti-CTLA4, anti-PD-L1 monotherapies to mice that have received tarloxotinib/anti-PD-1, tarloxotinib/anti-CTLA4 or tarloxotinib/anti-PD-L1 combination therapies in the syngeneic MB49 tumour model. Changes in percentage and phenotype of immune cells can be analysed by using RStudio to generate tSNE (t-distributed stochastic nearest neighbour embedding) plots which allows visual identification of subtle and substantial changes in cell number and marker expression levels of collected flow samples. tSNE is a computational tool that can be used to map multi-parametric data on two dimensions, whilst conserving the high-dimensional structure of the data and retains single cell resolution. Each dot on a tSNE plot represents a single cell and phenotypically related cells will be located closer to each other on a tSNE plot thus forming a cluster of dots. The colour (or density or heat) on the tSNE plot represents marker expression level with markers expressed at a higher level coloured toward red/brown and lower level toward blue.

[0596] By combining FlowJo and tSNE analysis, it was found that the combination of tarloxotinib with anti-PD-L1 resulted in significant reduction in the percentage of CD8⁺ T-cells in the blood (FIGs. 22 and 23). This could be due to more CD8⁺ T-cells moving away from the periphery into the tumour microenvironment to exhibit their cytotoxicity. Experiments examining the immune cell populations inside the tumour is warranted to confirm such hypothesis. The addition of tarloxotinib to checkpoint blockades significantly increased the percentage of IFN γ ⁺ CD4⁺ and CD8⁺ T-cells in the blood (FIGs. 29 and 30). IFN γ is a crucial cytokine involved in generating a robust anti-tumour immune response as it directly enhances

CD8⁺ T-cell function as well as driving macrophage activation, increasing expression of MHC molecules and antigen processing components. IFN γ from CD4⁺ T-cells also enhances CD8⁺ T-cell activation. This phenomenon was only observed in mice treated with combination treatment but not in mice treated single agent treatments thus suggesting a synergy between tarloxotinib and checkpoint blockade(s).

[0597] The combinations of tarloxotinib with all three tested checkpoint blockades did not significantly change the percentage of Ki67⁺ (proliferation marker) CD8⁺ T-cells in the blood (FIGs. 32 and 34) which suggests combination treatment had no impact on the proliferation status of CD8⁺ T-cells. Blood from mice treated with the combination of tarloxotinib with anti-PD-1 had significantly increased percentage of KLRG1⁺ CD8⁺ T-cells (FIG. 34) which indicates there were more activated effector CD8⁺ T-cells in the blood of mice treated with tarloxotinib/anti-PD-1 combination. The regulatory T-cell population which typically has been regarded as immunosuppressive were also elevated with tarloxotinib/anti-PD-1 treatment (FOXP3⁺ CD4⁺ T-cells) (FIG. 34).

[0598] The combinations of tarloxotinib with all three tested checkpoint blockades resulted in increased PD-1 expression on CD8⁺ T-cells (FIG. 39). Although PD-1 has been commonly perceived as an exhausting marker, its expression has been found to be upregulated in more activated T-cells. This results therefore suggests that these CD8⁺ T-cells were more activated. The immune cells in the blood of mice that received anti-PD-L1 as a part of the treatment had reduced expression levels of PD-L1 on all immune cell types as anti-PD-L1 antibody has blocked all signalling via PD-1/PD-L1 axis and this inhibition was persistent even days after last dose of anti-PD-L1 (FIG. 40).

[0599] No noticeable differences were observed in all other cell markers examined which includes macrophages (F4-80), monocytes (CD11b), dendritic cells (CD11c and MHC-II), natural killer cells (NK1.1) and myeloid derived suppressor cells (Ly6C and Ly6G).

Example 7. Flow cytometry analysis of tarloxotinib as a single agent or in combination with anti-PD-1 plus anti-CTLA4 in the MB49 murine bladder cancer model

[0600] The frequency and phenotype of immune cells in the MB49 tumour model was examined in mice that have received tarloxotinib in combination with anti-PD-1/anti-CTLA4 ‘dual blockade’, with reference to monotherapy treatment and vehicle control. Changes contributing to the improved anti-tumour activity in mice treated with tarloxotinib/anti-PD-1+anti-CTLA4 as observed in experiment RF61 (report RN19-006) were identified.

Table 10. Experimental Parameters for Example 7

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5×10^5 cells
Drug solutions	• Tarloxotinib in WFI containing 20% 2-hydroxypropyl-β-cyclodextrin • Anti-PD-1 in PBS • Anti-CTLA4 in PBS
Injection volume	• Tarloxotinib – 15 mL/kg (0.015 mL/g) • Anti-PD1 – 100 μL • Anti-CTLA4 – 100 μL
Drug administration route	• Tarloxotinib – intraperitoneal (IP) • Anti-PD-1 – intraperitoneal (IP) • Anti-CTLA4 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Tissue type	MB49 tumours collected from MB49 tumour bearing mice
Instrument	Aurora flow cytometer (Cytek™)
Criteria for culling mice	Volume of the tumour exceeded 200 mm² or 1300 mm³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 6 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Day 14 post-inoculation

[0601] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib, 50 mg/kg, IP, Q2Dx4;
- (3) Anti-PD-1 and anti-CTLA4 ('dual blockade'), 200 μ g of each, IP, Q3Dx3; and
- (4) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-1/anti-CTLA4, 200 μ g of each, IP, Q3Dx3.

[0602] One day after treatment cessation, mice were culled by asphyxiation, follow by cervical dislocation. Tumours were collected and placed in Iscove's Modified Dulbecco's Medium (IMDM) on ice, finely minced and incubated with digestion buffer contacting Liberase (100 µg/mL) and DNase (100 µg/mL) at 37°C for 30 min before dissociation into single cell suspensions through 70 µm cell strainers (Becton Dickinson). Samples were washed with IMDM and centrifuged at 13,000 rpm for 4 min and resuspended in 2 mL of RBC lysis buffer (Qiagen) to remove red blood cells from the samples. Cells were pelleted again by centrifugation and washed with PBS before stained with surface antibodies at 4°C for 20 min. For intracellular staining, cells were subsequently fixed and permeabilised using the transcription factor buffer set (BD Pharmingen). Stained samples were stored at 4°C and analysed on the Aurora (Cytex) flow cytometer.

Table 11: List of antibodies used for flow cytometry analysis.

Chanel	Antibody	Clones	Fluorochrome	Dilution
B2	B220	RA3-6B2	FITC	1:600
B3	FoxP3	FGK-165	AF532	1:100
B5	IFN γ	XMG1.2	PE	1:100
B6	PDL1 (CD274)	B7-H1	PE/Dazzle 594	1:600
B8	CD44	IM7	PE-Cy5	1:800
B9	CD11b	M1-70	BB700	1:800
B10	CD4	RM4-5	PerCP eFluor 710	1:800
B14	PD-1 (CD279)	29F-1A12	PE-Cy7	1:500
R1	KLRG1	2F1	APC	1:900
R2	F4/80	BM8	AF647	1:600
R4	CD8	53-6.7	AF700	1:600
R5	Viability	/	Zombie NIR from BioLegend	1:1000
R7	CD45.2	A20	APC-Fire750	1:600
V3	MHCII	M5/114.15.2	eFluor450	1:900
V4	Ki67	B56	BV480	1:200
V5	CD3	145-2C11	BV510	1:100
V8	Ly6C	HK1.4	BV570	1:500
V10	CD69	H1-2F3	SB600	1:600
V11	NK1.1	PK136	BV650	1:500
V13	Ly6G	1A8	BV711	1:600
V15	CD11c	NK18	BV786	1:300

Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

[0603] Tarloxotinib in combination with anti-PD-1/anti-CTLA4 showed a trend toward increasing number of CD45⁺ immune cells ($1.2 \times 10^5 \pm 0.17 \times 10^5$ cells) per mg of tumour compared to vehicle treated control ($7.4 \times 10^4 \pm 0.16 \times 10^5$ cells), the percentage of CD45⁺ cells were comparable between different treatment groups (FIG. 73).

[0604] Tarloxotinib in combination with anti-PD-1/anti-CTLA4 significantly increased the percentage of CD8⁺ T-cells ($23.0 \pm 5.5\%$) as compared to anti-PD-1/anti-CTLA4 ($8.0 \pm 1.1\%$) and the number of CD4⁺ T-cells (4385 ± 2258 cells) in the tumour compared to vehicle treated control (1580 ± 344 cells) (FIG. 74). There was also a trend toward an increasing number of CD8⁺ T-cells in the tumour of the combination treated mice (8220 ± 3253 cells) (FIG. 74).

[0605] Tarloxotinib treatment significantly increased the percentage of FOXP3⁺ CD4⁺ regulatory T-cells (Tregs) in the tumour ($25.5 \pm 2.3\%$) as compared to vehicle treated control ($11.9 \pm 1.2\%$) (FIG. 75). An increasing number of Tregs per mg of tumour of mice that received tarloxotinib (369 ± 80 cells) compared to vehicle treated control (192 ± 42 cells) and anti-PD-1/anti-CTLA4 (174 ± 65 cells) was seen.

[0606] The combination of tarloxotinib with anti-PD-1/anti-CTLA4 decreased the percentage of Ly6G⁺ CD11b⁺ G-MDSC ($6.73 \pm 1.41\%$) and Ly6C⁺ CD11b⁺ M-MDSC ($9.82 \pm 0.73\%$) in the tumour as compared to vehicle treated control ($35.7 \pm 5.9\%$ and $13.4 \pm 2.1\%$, respectively) (FIG. 76). The CD8/G-MDSC ratio was higher in the tarloxotinib/anti-PD-1+anti-CTLA4 treated mice (20.8 ± 9.0) as compared to vehicle treated control (0.25 ± 0.07).

[0607] Tarloxotinib in combination with checkpoint inhibitors resulted in significant increase in the number of CD4⁺ T-cells and dendritic cells in the tumour (FIG. 74 and 76). Increasing number of CD8⁺ T-cells in the tumour was also seen in this study. The addition of tarloxotinib to checkpoint inhibitors increased the proportion of tumour localised dendritic cells (FIG. 76) and reduced the percentage of Ly6G⁺ and Ly6C⁺ CD11b⁺ cells (markers typically found on MDSCs) in the tumour (FIG. 77). The CD8/Treg and CD8/MDSC ratios were higher in the tarloxotinib/anti-PD-1+anti-CTLA4 treated mice which indicates an elevation in infiltration of cytotoxic CD8⁺ T-cells to the tumour as compared to Tregs and MDSCs. The CD8/Treg ratio, which may be used as an indicator for treatment efficacy, was higher in the tumour of mice in the combination group. Thus, combination treatment resulted in increased immune infiltrates, increased T-cell activation, and enhanced functional activity.

[0608] Tarloxotinib was shown to reduce the hypoxic fraction in MB49 tumours (study RF79 reported in RN19-010). Tumour hypoxia promotes the recruitment of Tregs and may enhance

the suppressive function of MDSCs. This indicates there may be a relationship between changes in tumour hypoxia and immune cell quantity and composition. The improved anti-tumour activity of tarloxotinib with checkpoint blockades observed in RF61 may be attributed to treatment-induced increase in tumour infiltration of immune cells including CD4⁺ and CD8⁺ T-cells, dendritic cells, and reduction in the percentage of regulatory T-cells (Tregs) and myeloid derived suppressor cells (MDSC).

Example 8. Evaluation of the therapeutic efficacy of tarloxotinib in combination with anti-PD-1 in the syngeneic EL4/EG7 murine lymphoma tumour model.

Table 12. Experimental Parameters for Example 8.

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5 x 10 ⁵ cells
Drug solutions	- Tarloxotinib in WFI containing 20% 2-hydroxylpropyl-β-cyclodextrin (Q2Dx3) - Anti-PD-1 in PBS (200 μg/mouse)
Injection volume	- Tarloxotinib – 15 mL/kg (0.015 mL/g) - Anti-PD1 – 100 μL
Drug administration route	- Tarloxotinib – intraperitoneal (IP) - Anti-PD-1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Criteria for culling mice	Volume of the tumour exceeded 200 mm ² or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 7 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ² or 1300 mm ³

Table 13. List of antibodies used for dendritic cell maturation assay.

Antibody	Clones	Fluorochrome	Dilution
CD80	16-10A1	APC	1:200
CD86	GL1	PE	1:1000
MHC-II (IA/IE)	M5/114.15.2	PeCy7	1:2000
PD-L1 (CD274)	MIH5	BV711	1:100
PD-L2 (CD273)	TY25	BV421	1:400
CD11c	HL3	BV786	1:100
CD8 α	53-6.7	FITC	1:200
Live/Dead fixable blue	/	UV	1:400

Table 14. List of antibodies used for T-cell expansion assay.

Antibody	Clones	Fluorochrome	Dilution
CD8 α (VG)	53-6.7	BV421	1:1000
CD44 (VB)	1M7	BV711	1:800
CD45.1 (GC)	A20	PE	1:500
V α 2 (RC)	B20.1	APC	1:1000
CD122 (BB)	TM- β 1	FITC	1:1000
DAPI	/	UV	1:2000

[0609] 2-hydroxylpropyl- β -cyclodextrin (Sigma Aldrich), formic acid (Sigma Aldrich), acetonitrile (Merck), dimethyl sulfoxide (ECP Ltd, New Zealand). All reagents used in the experiments were of LC-MS grade or better. Tarloxotinib (TRLX), tarloxotinib-TKI (T-TKI; activated TKI of tarloxotinib) and deuterated (D6) internal standards (TRLX-D6 and T-TKI-D6).

[0610] For *in vitro* experiment, tarloxotinib was formulated in DMSO. For *in vivo* experiments, tarloxotinib was formulated in water for injection containing 20% 2-hydroxylpropyl- β -cyclodextrin.

[0611] Specific pathogen-free male C57BL/6 mice were obtained from the Vernon Jansen Unit (VJU), The University of Auckland or the Biomedical Research Unit, Malaghan Institute of Medical Research. To minimize exogenous infection, mice were maintained in individually ventilated cages at 6 mice per cage (Sealsafe Plus Mouse AERO).

Immunofluorescent detection of EF5 adducts for hypoxic fraction visualisation

[0612] Male C57BL/6 mice were injected with 5×10^5 EL4 cells subcutaneously on the right flank. When tumour size exceeds 42 mm², mice were assigned to receive either vehicle control

or tarloxotinib at 60 mg/kg. Treatment was administered every 2 days for 3 doses (Q2D×3) via intraperitoneal route. Twenty-four hours or 72 hours after the last dose, mice were injected intraperitoneally with EF5, an immunohistochemical probe for hypoxia (60 mg/kg, formulated in PBS). EF5 contains a nitroimidazole group which confers hypoxia-selective reduction of EF5 and its subsequent binding to macromolecules in hypoxia regions of the tumour. Tumours were harvested 90 minutes after EF5 injection, fixed in 10% neutral buffered formalin (10% (v/v) of formalin in 79 mM sodium phosphate buffer, pH 7.0) for 48 hours and stored in 70% histology alcohol (63% ethanol, 3.5% methanol and 3.5% 2-propanol in water) at 4 °C before being embedded in paraffin. Sections (5 µm) were cut and mounted onto poly-L-lysine-coated slides. Slides were heat fixed for 30 minutes at 58 °C before the paraffin was removed and the sections were rehydrated using sequential immersions in 100% xylene, 100% ethanol, 95% ethanol, 100% distilled water and lastly 100% tris-buffered saline (TBS, pH 7.6). For antigen retrieval, slides were immersed in 0.01 M sodium citrate (dihydrate) buffer (pH 6) and high temperature heat-denaturation performed using a pressure cooker. Sectioned tumour slides were stained overnight with the EF5 Cy5-conjugated primary antibody and left overnight at 4 °C in a humidified StainTray slide staining system. The following day, primary antibody was removed and counterstained with DAPI. ProLong® Gold Antifade Mountant was applied to each section and mounted with coverslips. Stained slides were imaged using Olympus FV1200 laser scanning confocal microscope (Hugh Green Cytometry Core, Malaghan Institute of Medical Research).

Tissue preparation for flow cytometry analysis

Blood

[0613] Blood samples were collected from mice via submandibular bleed using a 4 mm lancet into 1.5 mL Eppendorf tubes containing 200 µl of 10 mM EDTA-PBS, to prevent cell aggregation. Cells were pelleted at 4000 rpm for 4 min in a microcentrifuge (Heraeus, ThermoFisher Scientific) and the supernatant discarded. Samples were incubated with 800 µl of red blood cell lysis buffer (Qiagen) for 20 min at 37°C to yield a pellet of pure white blood cells, pelleted, with supernatant removed and resuspended in 200 µl fluorescence-activated cell sorting (FACS) buffer (PBS + 0.1% FBS) for flow cytometric analysis.

Spleen

[0614] Mice were culled and spleens were collected and placed in IMDM on ice and injected with digestion buffer contacting Liberase (100 µg/mL) and DNase (100 µg/mL). Spleens were incubated 37°C for 30 min and then dissociated to single cell suspensions through 70 µm cell

strainer (Becton Dickinson). Samples were washed with IMDM and pelleted at 13,000 rpm for 4 min in a centrifuge and supernatant discarded. Cell pellets were resuspended in 2 mL of RBC lysis buffer and incubated for 2 min to remove red blood cells from the samples. Cells were pelleted again by centrifugation and washed with PBS.

Lymph nodes

[0615] Mice were culled and the outer skin layer was cut and peeled away to allow collection of inguinal lymph nodes with tweezers. All lymph nodes were collected in cold IMDM, dissociated through 70 µm cell strainer (Becton Dickinson) and washed with IMDM to prepare single cell suspensions. Samples were then centrifuged at 13,000 rpm for 4 min and washed with PBS.

Tumour

[0616] Mice were culled and tumours were collected and placed in IMDM on ice, finely minced and incubated with digestion buffer containing Liberase (100 µg/mL) and DNase (100 µg/mL) at 37°C for 30 min before dissociation into single cell suspensions through 70 µm cell strainers (Becton Dickinson). Samples were washed with IMDM and centrifuged at 13,000 rpm for 4 min and resuspended in 2 mL of RBC lysis buffer to remove red blood cells from the samples. Cells were pelleted again by centrifugation and washed with PBS.

Flow cytometry

Cell surface staining

[0617] All flow cytometry antibodies were purchased from eBioscience, BD Pharmingen and Biolegend, and titrated for optimal performance prior to being used in experiments. Single cell suspensions obtained from various tissues (outlined above) were washed and resuspended in FACS buffer. The cell suspensions were distributed to a 96-well U-bottom plate and incubated with anti-CD32/16 (clone 2.4G2) in flow buffer for 5 min at 4°C to block FcγRII/III and prevent Fc-mediated non-specific binding. Samples were centrifuged at 1600 rpm for 4 min and resuspended in 50 µl FACS buffer containing cell surface antibodies with incubation at 4°C for 20 min. Cells were then washed with FACS buffer, centrifuged and resuspended in 200 µl FACS buffer. A further 100 µl of flow buffer containing the viability dyes DAPI or Zombie NIR, was added to each sample to allow identification of live cells with intact cell membranes in the final analysis. Flow cytometric analysis of resuspended cells was performed using a BD LSR II, BD LSRFortessa or Cytex Aurora instrument and analysed using FlowJo software version 10 (TreeStar, Inc., OR, USA). Fluorescence minus one (FMO) controls were used

where required. FMO includes full staining panel of antibodies minus one antibody, therefore allowing for comparison to background fluorescence from other fluorophores.

Intracellular staining

[0618] For intracellular staining, cells were first prepared and stained with surface antibodies as outlined above. The cells were then stained intracellularly using the transcription factor buffer set (BD Pharmingen) by following the manufacturer's protocols. In brief, surface antibody stained samples were centrifuged and washed with Fix/Perm (TFP) buffer containing TFP solution and TFP diluent at the ratio of 1:4. Cells were then incubated with TFP buffer for 40 min at 4°C in the dark followed by washing with Perm/Wash buffer containing Perm/Wash solution and deionized water at the ratio of 1:5. Cells were stained with intracellular antibodies mixed in Perm/Wash solution for 40 min at 4°C. After incubation, cells were washed with Perm/Wash buffer and resuspended in FACS buffer until analysis using flow cytometers stated above.

[0619] For intracellular staining involving re-stimulation, 4×10^6 splenocytes or tumour cells were plated into 24-well plates and incubated for 2 hours at 37°C in IMDM containing 2 µg/mL anti-CD3 (clone 2C11) and 2 µg/mL anti-CD28 (clone 37.51) before 3 µg/mL Brefeldin A and 2 µM monensin were added. Cells were further incubated for 4 hours and then harvested and resuspended in FACS buffer containing CD4 and CD8 antibodies. The cells were then fixed using the transcription factor buffer set according to the manufacturer's protocol and stained with intracellular antibodies as stated above.

In vivo proliferation assay

[0620] In order to analyse the proliferation of antigen-specific CD4⁺ and CD8⁺ T-cells *in vivo*, CFSE (Carboxyfluorescein succinimidyl ester) was used to bind green fluorescent dye to all of the proteins within the T-cells. As T-cells proliferate, the CFSE stain intensity would be diluted evenly between the daughter cells, providing an incremental dilution of the CFSE dye per proliferation cycle. Lymph nodes were harvested from OTIxB6.SJL-Pyptv^aPep3^b/BoyJArc and OTIIxB6.SJL-Pyptv^aPep3^b/BoyJArc mice and processed to single cell suspension. The cells were then suspended at 2×10^7 cells/mL in PBS and filtered through a 70 µm filter. CFSE was added to the cells to reach a final concentration of 1 µM and incubated for 8 mins at room temperature in the dark before an equal volume of FCS was added to stop the staining. Cells were then washed twice with IMDM (5% FCS) and resuspended at a concentration of 1×10^7 cells/mL. A volume of 200 µl (2×10^6 cells) was injected intravenously into each mouse. Peripheral blood were collected two days later, white blood cells were isolated as described

above. T-cell proliferation was examined by flow cytometry, measuring CFSE dilution as indicated by the decrease in fluorescence intensity in the green channel.

Quantification of IFN γ producing T-cells in the spleen by enzyme linked immunospot (ELISpot) assay

[0621] Spleens were harvested from mice and processed to single-cell suspensions as described above. EG7.OVA cells were irradiated at 500 Grays using Gamacell 3000 Elan Cell Irradiator and seeded into ELISpot plate pre-coated with IFN γ antibody. 2×10^5 of splenocytes were added to appropriate wells before the plate is wrapped in tin foil and incubated at 37°C for 16 hours. Detection antibody R4-6A2-biotin were prepared at 1 μ g/ml in PBS + 0.5% FCS solution and added to each well for a 2-hour incubation at room temperature. The plate was then washed with PBS and diluted Streptavidin-alkaline phosphatase were added to the wells. IFN γ spots were developed by adding the substrate BCIP/NBT-plus solution and incubated for 2 minutes. Spots were counted using ELISpot plate reader.

Subcutaneous tumour challenge

[0622] Cells were harvested and resuspended in incomplete IMDM to achieve an inoculum of 5×10^5 cells in a volume of 0.1 mL per mouse. The inoculum was injected subcutaneously on the right flank of each mouse. When tumours reached the desired volume, drug treatment commenced or tumours harvested for various purposes as described. Tumour volumes were calculated assuming a prolate ellipsoid shape: $\pi/6 \times (\text{length} \times \text{width} \times \text{width})$.

Tumour growth delay

[0623] Tumour bearing mice were randomised into the appropriate treatment groups and tumour size and body weight recorded. Where possible, sample sizes were calculated and minimised in keeping with the 'Three R' guidelines of the Animal Welfare Act (1999) to 'Replace, Reduce and Refine' the use of animals for research purposes.

[0624] Drugs were formulated, aliquoted and stored at -20 °C until required. The following formulations were used for the various test compounds: (1) Tarloxotinib – 20% (w/v) (2-Hydroxypropyl)- β -cyclodextrin in Water For Injection; and (2) Anti-PD-1 – PBS. Tarloxotinib and anti-PD-1 were both administered by intraperitoneal injection. The injection volume for tarloxotinib was 0.015 mL/g. Mice were administered with the appropriate treatments at schedules indicated in the results section. Tumour size and body weight was monitored daily during periods of drug treatment and every 1-2 days thereafter. Mice were euthanized before completion of the study if body weight loss exceeded 15%, tumour volume exceeds 1300 mm³, if extensive tumour ulceration occurred or if any signs of adverse drug toxicity were observed.

Immunofluorescence staining of tumour hypoxia

[0625] There was a reduction in total hypoxic regions shown in red in EL4 tumours harvested from mice that received 3 doses of tarloxotinib at 60 mg/kg. This reduction of tumour hypoxia can be detected 24 hours post-treatment and persists 72 hours later. This demonstrates that tarloxotinib can modulate tumour hypoxia by eliminating hypoxic EL4 tumour cells (FIG. 41).

Effect of tarloxotinib on normal immune function

[0626] Undamaged lymphoid organs with intact population of immune cells are essential in generating a robust anti-tumour immune response. The spleen and lymph nodes contain regions of hypoxia with the concentration of oxygen in the spleen varying between 0.5% to 4.5%, meaning tarloxotinib can become activated in the spleen if the required electron reductase or EGFR are present, to become damaging to these secondary lymphoid organs. Results from flow cytometry show that the mean fluorescence intensity (MFI) levels of CD86 which are indicative of the magnitude of dendritic cell activation are comparable with or without tarloxotinib administration. (FIG. 42) The percentage change of OT-I cells (OVA antigen-specific CD8⁺ T-cells) over a period of 10 days were also similar across different treatment groups. This demonstrates that tarloxotinib has no apparent negative impact on the immune system in C57BL/6 mice.

Table 15: Study outcomes – EL4

10	Drug	Dose (mg/kg/dose)	Schedule	Animal number	Deaths independent of drug toxicity	Day of Death (includes all deaths)	Reason for Death	Average Weight Loss Nadir (+/- SEM) [Day]	Days to reach endpoint	% Tumour Growth Delay	Log Rank test (vs control)
A	Vehicle (20%CDW)		Q2Dx3	5				None	20	-	
B	Tarloxotinib	60	Q2Dx3	5				2.0 ± 0.7 [11]	24	20%	0.1250
C	Anti-PD-1	200 µg	Single dose	5				None	24	20%	0.6036
D	Tarloxotinib + Anti-PD-1	60 200 µg	Q2Dx3 Single dose	5		42	f	4.4 ± 0.6 [10]	Long term control	-	0.0018

Table 16: Study outcomes – EG7

Group	Drug	Dose (mg/kg/dose)	Schedule	Animal number	Deaths independent of drug toxicity	Day of Death (includes all deaths)	Reason for Death	Average Weight Loss Nadir (+/- SEM) [Day]	Days to reach endpoint	% Tumour Growth Delay	Log Rank test (vs control)
A	Vehicle (20%CDW)		Q2Dx3	5				None	17	-	
B	Tarloxotinib	60	Q2Dx3	5				4.7 ± 1.2 [12]	15	-	0.8978
C	Anti-PD-1	200 µg	Single dose	5				None	17	0%	0.5821
D	Tarloxotinib + Anti-PD-1	60 200 µg	Q2Dx3 Single dose	5		42	f	4.5 ± 0.6 [12]	23	35%	0.0031

Table 17: Notes/abbreviations from Study Outcomes

Vehicle	20% 2-hydroxypropyl-β-cyclodextrin, dissolved in water for injection PBS
Schedule	Tarloxotinib: Q2Dx3 = every 2 days for 3 doses (IP) Anti-PD-1: single dose (IP)
Deaths independent of drug toxicity	Excludes mice culled prior to endpoint that had already reached endpoint (e.g. mice culled early due to grade II ulceration)
Reasons for Death	<u>Drug toxicity related:</u> a - Found dead, b - Evidence of morbidity, c - Evidence of treatment-related adverse effect, d - Excessive weight loss (>20% from starting size) <u>Independent of drug toxicity:</u> e - Irregular tumour growth, f – Tumour ulceration grade III, g - Tumour metastasis, h - Dosing aberration, i - Treatment-independent sickness, j - Animal husbandry concern
Long term control	Failed to reach endpoint within specified duration of experiment
% Tumour growth delay	Percent of tumour growth delay; relative delay in median RTV ⁴ versus control expressed as percent.

Immune profiling of tumour bearing mice post-treatment

[0627] FIG. 49 shows that mice in the tarloxotinib plus anti-PD-1 combination treatment group have smallest tumours volumes and increased tumour infiltration of CD3⁺, CD4⁺ and CD8⁺ T-cells per mm³ of tumour. This suggests that relief from tumour hypoxia due to tarloxotinib treatment and the inhibition of PD-1 signalling in T-cells together have combined efficiently to improve tumour infiltration of T-cells which in turn improved the anti-tumour immunity. Although the number of CD4⁺ and CD8⁺ T-cells were similar across different treatment groups, the expression levels of PD-1 (depicted as MFI) were higher in mice treated with tarloxotinib (FIG. 50). PD-1 is an exhaustion marker but its expression is upregulated on activated T-cells, therefore the increased expression of PD-1 suggests that tarloxotinib enhanced T-cell activation. FIG. 51 shows trends toward increasing number of IFN γ and TNF α producing CD4⁺ and CD8⁺ upon treatment with tarloxotinib indicating that the improved anti-tumour activity of anti-PD-1 observed was due to tarloxotinib-mediated stimulation of IFN γ and TNF α production by T-cells. The percentages of regulatory T-cells (Tregs) were also increased in the spleen, draining lymph node and the tumour (FIG. 52) which are against the hypothesis that tumour hypoxia promotes recruitment of Tregs via CCL28 chemokine. However, this increased Treg percentage can be due to the immune system functioning to prevent over-activation of T-cells and the immune response. Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$; ****, $P < 0.0001$).

Table 18. List of antibodies used for T-cell proliferation assay.

Antibody	Clones	Fluorochrome	Dilution
T-cell panel			
CD45.2	/	APC	1:1000
CD4	RM4-5	BV605	1:200
CD8	53-6.7	BUV395	1:500
CD3	145-2C11	FITC	1:500
NK1.1	PK136	BV650	1:300
B220	RA3-6B2	PerCP	1:1000
PDL1	10F.9G2	BV711	1:800
PD1	RMP1-30	Pe-Cy7	1:200
FOXP3	FJK-16s	PE	1:400
Viability	/	LD/Blue	1:800

Antibody	Clones	Fluorochrome	Dilution
Myeloid cell panel			
CD45.2	/	PE	1:400
CD11b	M1/70	BUV737	1:500
CD11c	HL3	BV786	1:200
B220	RA3-6B2	PerCP	1:1000
MHC-II (I-A/I-E)	M5/114.15.2	BV421	1:500
Ly-6G	1A8	APC	1:200
Ly-6C	HK1.4	Pe-Cy7	1:1500
F4/80	T45-2342	BUV395	1:200
PD-L1	10F.9G2	BV711	1:800
Viability	/	LD/Blue	1:800

T-cell proliferation status of tumour bearing mice post-treatment

[0628] Monitoring of the proliferation of transferred CFSE-labelled transgenic T-cells for the model tumour antigen OVA can be used to assess the presence of tumour antigens in the lymphoid tissues. Where antigen is present in the lymph nodes, T-cells undergo clonal proliferation, which can be identified as a reduction of CFSE dye intensity. This analysis showed that treatment with tarloxotinib alone caused release of antigen from the tumour to the lymph nodes which enhanced the proliferation of OT-I cells (OVA specific CD8⁺ T-cell) but not OT-II cells (OVA specific CD4⁺ T-cell). Combined treatment with anti-PD-1 did not further increase OT-I proliferation (FIG. 53).

Quantification of IFN γ producing T-cells

[0629] ELISpot assay allows quantification of IFN γ producing T-cells upon re-stimulation with irradiated EG7.OVA tumour cells. Each stained spot represents production of IFN γ by a single T-cell. There were significantly higher number of spots produced by splenocytes from mice treated with the combination therapy compared with tarloxotinib or anti-PD-1 treatment alone groups which suggests that the combination treatment enhanced T-cell function (FIG. 54).

[0630] Administration of tarloxotinib reduced the percentage of hypoxic fractions in EL4 tumours *in vivo*. Tarloxotinib also showed no negative impact on the normal immunological function of C57BL/6 mice. Growth delay experiment showed that the combination of tarloxotinib plus anti-PD-1 is the most efficacious treatment against EL4 and EG7 tumours as both tarloxotinib and anti-PD-1 alone could not delay tumour growth. Such improved anti-tumour activity may be attributed to the increased T-cell proliferation, increased tumour T-cell infiltration, enhanced T-cell activation and increased IFN γ production by CD8⁺ T-cells (FIGs. 43-48).

Example 9. Serum and tumour IFN γ concentration analysis of tarloxotinib alone or in combination with anti-PD-L1 in the MB49 murine bladder cancer model.

[0631] The IFN γ levels were examined in the serum and tumour of mice treated with tarloxotinib/anti-PD-L1 combination treatment in the syngeneic MB49 tumour model, with reference to monotherapy treatment with tarloxotinib, anti-PD-L1, or a vehicle control.

[0632] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib at 50 mg/kg, IP, Q2Dx4;
- (3) Anti-PD-L1 at 200 μ g, IP, Q3Dx3; and
- (4) Tarloxotinib at 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-L1 at 200 μ g, IP, Q3Dx3.

[0633] Analysis of cytokine concentration in serum or tumour was performed using the mouse ELISA kit (Invitrogen) following the manufacturer's protocols. Wells in a transparent 96-well plate were first pre-coated with capture antibody (1:1000) in 1x coating buffer at 37°C for 18 hours. The plate was washed 4 times with wash buffer (PBS + 0.05% Tween-20) and blocked with 1x ELISA diluent for 1 hour at room temperature with shaking at 200 rpm. The plate was washed and 100 μ L of the serially diluted standards was aliquoted in duplicate into the allocated wells and 50 μ L of serum or tumour lysate samples were added into the appropriate sample wells. Tumours were submerged into cell lysis buffer containing protease inhibitor and homogenised using TissueLyzer. The plate was sealed, protected from light, and incubated for 2 hours (RT, 200 rpm). The plate was then washed 4 times and incubated with 100 μ L of biotinylated detection antibody (1:1000) for 1 hour at RT. After washing, diluted Avidin-HRP solution was added to each well of the plate, sealed, and incubated for 30 minutes at room

temperature with shaking. The plate was washed 5 times with wash buffer before 100 μ L of TMB solution was added to the wells and incubated in the dark at room temperature for up to 15 minutes. 50 μ L of Stop solution (2N sulfuric acid) was added to the wells and the absorbance was recorded at 450 nm.

Table 19. Experimental Parameters

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5×10^5 cells
Drug solutions	• Tarloxotinib in WFI containing 20% 2-hydroxypropyl-β-cyclodextrin • Anti-PD-L1 in PBS
Injection volume	• Tarloxotinib – 15 mL/kg (0.015 mL/g) • Anti-PD-L1 – 100 μL
Drug administration route	• Tarloxotinib – intraperitoneal (IP) • Anti-PD-L1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Tissue types	Blood serum and tumour
Instrument	Tecan Infinite M1000 pro plate reader and TissueLyzer II
Criteria for culling mice	Volume of the tumour exceeded 200 mm ² or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 6 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Day of tissue collection	Day 14

[0634] The concentration of IFN γ in the serum from mice treated with tarloxotinib in combination with anti-PD-L1 (199 ± 59.6 pg/mL) was higher compared to monotherapy of anti-PD-L1 (5.9 ± 4.1 pg/mL), tarloxotinib (34.6 ± 11.7 pg/mL), and vehicle treated control (0 pg/mL) (FIG. 78). MB49 tumours from mice showed an increased IFN γ concentration when

treated with tarloxotinib alone (9.7 ± 3.4 pg/mg) or tarloxotinib in combination with anti-PD-L1 (7.7 ± 1.4 pg/mg), as compared to the vehicle treated control (1.2 ± 0.16 pg/mg) (FIG. 78). [0635] In both the blood and tumour, the concentration of IFN γ was higher in mice treated with tarloxotinib/anti-PD-L1 combination therapy. The combination of tarloxotinib and anti-PD-L1 increased the production of IFN γ in the serum of mice. Tarloxotinib treatment alone also increased the IFN γ levels in the tumour. Tarloxotinib in combination with anti-PD-L1 enhanced the production of IFN γ by immune in the blood and tumour of MB49 tumour bearing mice. Tumours from mice treated with tarloxotinib alone or in combination with anti-PD-L1 showed increased intra-tumoural IFN γ concentration.

Example 10. Flow cytometry analysis of tarloxotinib as a single agent or in combination with anti-PD-L1 in the MB49 murine bladder cancer model.

[0636] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups:

- (1) Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4;
- (2) Tarloxotinib, 50 mg/kg, IP, Q2Dx4;
- (3) Anti-PD-L1, 200 μ g, IP, Q3Dx3; and
- (4) Tarloxotinib, 50 mg/kg, IP, Q2Dx4 in combination with anti-PD-L1, 200 μ g, IP, Q3Dx3.

Table 20. Experimental Parameters for Example 10.

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5×10^5 cells
Drug solutions	• Tarloxotinib in WFI containing 20% 2-hydroxypropyl-β-cyclodextrin • Anti-PD-L1 in PBS
Injection volume	• Tarloxotinib – 15 mL/kg (0.015 mL/g) • Anti-PD-L1 – 100 μL

Drug administration route	• Tarloxotinib – intraperitoneal (IP) • Anti-PD-L1 – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 6 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 6).
Evaluations	Tumour size and bodyweight were measured every 1-2 days.
Tissue type	MB49 tumours collected from MB49 tumour bearing mice
Instrument	Aurora flow cytometer (Cytek™)
Criteria for culling mice	Volume of the tumour exceeded 200 mm ² or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Body weight change reached 15% below the day 6 measurement (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Tumour size exceeds 200 mm ² or 1300 mm ³

[0637] One day after treatment cessation, mice were culled by asphyxiation, follow by cervical dislocation. Tumours were collected and placed in IMDM on ice, finely minced and incubated with digestion buffer contacting Liberase (100 µg/mL) and DNase (100 µg/mL) at 37°C for 30 min before dissociation into single cell suspensions through 70 µm cell strainers (Becton Dickinson). Samples were washed with IMDM and centrifuged at 13,000 rpm for 4 min and resuspended in 2 mL of RBC lysis buffer (Qiagen) to remove red blood cells from the samples. Cells were pelleted again by centrifugation and washed with PBS before stained with surface antibodies at 4°C for 20 min. For intracellular staining, cells were subsequently fixed and permeabilised using the transcription factor buffer set (BD Pharmingen). Stained samples were stored at 4°C and analysed on the Aurora (Cytek) flow cytometer.

Table 21: List of antibodies used for flow cytometry analysis of peripheral blood.

Chanel	Antibody	Clones	Fluorochrome	Dilution
B2	CD3	145-2C11	FITC	1:75
B3	FoxP3	FGK-165	AF532	1:100
B5	IFN γ	XMG1.2	PE	1:100
B6	PDL1 (CD274)	B7-H1	PE/Dazzle 594	1:600
B8	B220	RA3-6B2	PerCP	1:600
B8	CD44	IM7	PE-Cy5	1:800
B9	CD11b	M1-70	BB700	1:800

Chanel	Antibody	Clones	Fluorochrome	Dilution
B10	CD4	RM4-5	PerCP eFluor 710	1:800
B14	PD-1 (CD279)	29F-1A12	PE-Cy7	1:500
R1	KLRG1	2F1	APC	1:900
R2	F4/80	BM8	AF647	1:600
R4	CD8	53-6.7	AF700	1:600
R5	Viability	/	Zombie NIR from BioLegend	1:1000
R7	CD45.2	A20	APC-Fire750	1:600
V3	MHCII	M5/114.15.2	eFluor450	1:900
V4	Ki67	B56	BV480	1:200
V8	Ly6C	HK1.4	BV570	1:500
V10	CD69	H1-2F3	SB600	1:600
V11	NK1.1	PK136	BV650	1:500
V13	Ly6G	1A8	BV711	1:600
V15	CD11c	NK18	BV786	1:300

[0638] Statistical significance between groups were determined using one-way ANOVA with Turkey's multiple comparison test (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$; ****, $P < 0.0001$).

[0639] The addition of tarloxotinib (50 mg/kg, Q2Dx4) to anti-PD-L1 significantly increased the number of CD45⁺ immune cells per mg of tumour although the percentage of CD45⁺ cells were comparable between different treatment groups (FIG. 55).

[0640] The addition of tarloxotinib (50 mg/kg, Q2Dx4) to anti-PD-L1 significantly increased the percentage and number of CD3⁺ and CD8⁺ T-cells in the tumour (FIG. 56) but resulted in significant reduction in the percentage of CD4⁺ T-cells in the blood but not the number of CD4⁺ T-cells per mg of tumour (FIG. 56).

[0641] There was significant reduction in the percentage of FoxP3⁺ CD4⁺ regulatory T-cells (Tregs) in the tumour of mice that received tarloxotinib/anti-PD-L1 combination treatment (FIG. 57). There was a trend toward increasing number of Tregs per mg of tumour of mice received tarloxotinib or tarloxotinib/anti-PD-L1 treatments. The CD8/Treg ratios were significantly higher in the tarloxotinib/anti-PD-L1 treated mice.

[0642] The addition of tarloxotinib (50 mg/kg, Q2Dx4) to anti-PD-L1 increased the number of Nk1.1⁺ CD3⁺ natural killer like T-cells and Nk1.1⁺ CD3⁻ natural killer cells in the tumour (FIG. 58). The addition of tarloxotinib (50 mg/kg, Q2Dx4) to anti-PD-L1 increased the number of CD11b⁺ monocytes and CD11c⁺ MHC-II⁺ conventional dendritic cells in the tumour (FIG. 59). The addition of tarloxotinib (50 mg/kg, Q2Dx4) to anti-PD-L1 decreased the percentage of

Ly6G⁺ CD11b⁺ G-MDSC and Ly6C⁺ CD11b⁺ M-MDSC in the tumour (FIG. 60). The CD8/G-MDSC and CD8/M-MDSC ratios were significantly higher in the tarloxotinib/anti-PD-L1 treated mice.

[0643] This study examined and compared the percentage and phenotype of immune cells in the tumours of MB49 tumour-bearing mice that received tarloxotinib, anti-PD-L1 monotherapies to mice that have received tarloxotinib/anti-PD-L1 combination therapy. It was found that the combination of tarloxotinib with anti-PD-L1 resulted in significant increase in the number of CD3⁺, CD4⁺, CD8⁺ T-cells, NKT cells, NK cells and dendritic cells in the tumour (FIGs. 56, 58, and 59). This increased infiltration of immune cells into the tumour microenvironment may be due to tarloxotinib-induced reduction in tumour hypoxia. Hypoxia has been shown to prevent T-cell infiltration into the tumour, reduction in tumour hypoxia therefore allows T-cells to enter the tumour.

[0644] The addition of tarloxotinib to checkpoint blockades significantly reduced the percentage of Tregs and MDSCs in the tumour (FIGs. 57 and 60). There was a trend toward increased number of Tregs and MDSCs in tumour from mice that received the tarloxotinib/anti-PD-L1 treatment, which is mostly attributed to the increase in total number of immune cells in the tumour of combination treated mice (FIG. 55). Despite this, the ratio between the number of CD8⁺ T-cells and the number of Tregs, and the ratio between the number of CD8⁺ T-cells and the number of MDSCs per mg of tumour was significantly higher in the tarloxotinib/anti-PD-L1 treated mice. This shows that there was a more profound infiltration of cytotoxic CD8⁺ T-cells to the tumour compared to the increased infiltration of Tregs and MDSCs. Tumour hypoxia promotes the recruitment of Tregs and enhance the suppressive function of MDSCs. Reduction in hypoxia as a result of tarloxotinib treatment therefore reduced the number of Tregs and MDSCs in the tumour microenvironment.

Example 11. Effects of a combination of Compound A and an immunotherapeutic agent on lung damage/inflammation.

[0645] A combination of Compound A and an immunotherapeutic agent, as disclosed herein, is administered to a subject. Blood, serum, urine or other tissue is collected and prepared according to standardized protocols known to one of ordinary skill in the art. For example, specimens are obtained from the lung of the subject, e.g., from the peripheral area of the lower lobe of each lung. The levels of various lung damage/inflammation biomarkers (as disclosed herein) in the subjects are measured and/or quantified using known methods, for example,

microarray analysis, immunohistochemistry, and serum measurements, which are performed using standard techniques. Differential expression of the markers in the samples from patients administered the combinations disclosed herein as compared to patients administered combinations in the prior art is analyzed by statistical methods known in the art. The decreased expression of one or more of the biomarkers upon administration of the combinations disclosed herein as compared to the combinations in the prior art indicates an improved toxicity profile.

Example 12. Effects of a combination of Compound A and an immunotherapeutic agent on liver damage/inflammation.

[0646] A combination of Compound A and an immunotherapeutic agent, as disclosed herein, is administered to a subject. Blood, serum, urine or other tissue is collected and prepared according to standardized protocols known to one of ordinary skill in the art. For example, urine is collected from the subject and analyzed for various enzymes, e.g., ALT and bilirubin.

[0647] Additionally, levels of various other liver damage/inflammation biomarkers (as disclosed herein) in the subjects are measured and/or quantified using known methods, for example, microarray analysis, immunohistochemistry, and serum measurements, which are performed using standard techniques. Differential expression of the markers in the samples from patients administered the combinations disclosed herein as compared to patients administered combinations in the prior art is analyzed by statistical methods known in the art. The decreased expression of one or more of the biomarkers upon administration of the combinations disclosed herein as compared to the combinations in the prior art indicates an improved toxicity profile.

Example 13. Effects of a combination of Compound A and an immunotherapeutic agent on skin damage/inflammation.

[0648] A combination of Compound A and an immunotherapeutic agent, as disclosed herein, is administered to a subject. Blood, serum, urine or other tissue is collected and prepared according to standardized protocols known to one of ordinary skill in the art. Additionally, levels of various skin damage/inflammation biomarkers (as disclosed herein) in the subjects are measured and/or quantified using known methods, for example, microarray analysis, immunohistochemistry, and serum measurements, which are performed using standard techniques. Differential expression of the markers in the samples from patients administered the combinations disclosed herein as compared to patients administered combinations in the

prior art is analyzed by statistical methods known in the art. The decreased expression of one or more of the biomarkers upon administration of the combinations disclosed herein as compared to the combinations in the prior art indicates an improved toxicity profile.

Example 14. Effects of a combination of Compound A and an immunotherapeutic agent on systemic inflammation.

[0649] A combination of Compound A and an immunotherapeutic agent, as disclosed herein, is administered to a subject. Blood, serum, urine or other tissue is collected and prepared according to standardized protocols known to one of ordinary skill in the art.

[0650] Additionally, levels of various systemic damage/inflammation biomarkers (as disclosed herein) in the subjects are measured and/or quantified using known methods, for example, microarray analysis, immunohistochemistry, and serum measurements, which are performed using standard techniques. Differential expression of the markers in the samples from patients administered the combinations disclosed herein as compared to patients administered combinations in the prior art is analyzed by statistical methods known in the art. The decreased expression of one or more of the biomarkers upon administration of the combinations disclosed herein as compared to the combinations in the prior art indicates an improved toxicity profile.

Example 15. Effects of a combination of Compound A and an immunotherapeutic agent on general inflammation.

[0651] A combination of Compound A and an immunotherapeutic agent, as disclosed herein, is administered to a subject. Blood, serum, urine or other tissue is collected and prepared according to standardized protocols known to one of ordinary skill in the art.

[0652] Additionally, levels of various general damage/inflammation biomarkers (as disclosed herein) in the subjects are measured and/or quantified using known methods, for example, microarray analysis, immunohistochemistry, and serum measurements, which are performed using standard techniques. Differential expression of the markers in the samples from patients administered the combinations disclosed herein as compared to patients administered combinations in the prior art is analyzed by statistical methods known in the art. The decreased expression of one or more of the biomarkers upon administration of the combinations disclosed herein as compared to the combinations in the prior art indicates an improved toxicity profile.

Example 16. Tarloxotinib reduces tumour hypoxia, intra-tumoural T-cell hypoxia and tumour PD-L1 expression in the MB49 murine bladder cancer model.

[0653] The effect of tarloxotinib administration on modulating tumour hypoxia and the level of hypoxia experienced by the immune cells within the tumour and changes in PD-L1 expression post-treatment was examined.

[0654] MB49 tumour bearing C57BL/6 mice were randomized to one of the following treatment groups.

- Vehicle (WFI containing 20% 2-hydroxypropyl- β -cyclodextrin or PBS), IP, Q2Dx4
- Tarloxotinib, 50 mg/kg, IP, Q2Dx4

Table 22. Experimental Parameters

Gender	Male
Mouse strain	C57BL/6
Tumour injection site	Subcutaneous
Cell line	MB49
Cells per inoculation	1.5×10^5 cells
Drug solutions	• Tarloxotinib in WFI containing 20% 2-hydroxypropyl-β-cyclodextrin
Injection volume	• Tarloxotinib – 15 mL/kg (0.015 mL/g)
Drug administration route	• Tarloxotinib – intraperitoneal (IP)
Treatment dates	Mice were randomly assigned into treatment groups 11 days after tumour inoculation and drug administration for each animal began on the day of assignment (day 11).
Tissue type	MB49 tumours collected from MB49 tumour bearing mice
Instrument	LSR-II (BD™)
Criteria for culling mice	Volume of the tumour exceeded 200 mm ² or 1300 mm ³ (ethical criteria) Mean tumour diameter exceeds 20 mm (ethical criteria) Evidence of metastasis or extensive tumour ulceration (ethical criteria)
Endpoints	Day 18

[0655] Treatment with tarloxotinib reduced the percentage of hypoxic (EF5⁺) and PD-L1⁺ MB49 tumour cells *in vivo*. Tarloxotinib also reduced the level of hypoxia experienced by both CD4⁺ and CD8⁺ T-cells in the tumour.

[0656] One day after treatment cessation, mice were culled by cervical dislocation. Tumours were collected and placed in IMDM on ice, finely minced and incubated with digestion buffer contacting Liberase (100 µg/mL) and DNase (100 µg/mL) at 37°C for 30 min before dissociation into single cell suspensions through 70 µm cell strainers (Becton Dickinson). Samples were washed with Iscove's Modified Dulbecco's Medium (IMDM), centrifuged at 13,000 rpm for 4 min and resuspended in 2 mL of RBC lysis buffer (Qiagen) to remove red blood cells from the samples. Cells were pelleted again by centrifugation and washed with PBS before stained with surface antibodies at 4°C for 20 min. For EF5 staining, cells were fixed and permeabilised using the transcription factor buffer set (BD Pharmingen) following the manufacturer's protocols. Stained samples were stored at 4°C and analysed on the LSR-II (BD™) flow cytometer.

Table 23: List of antibodies used for flow cytometry analysis.

Antibody	Clones	Fluorochrome	Dilution
CD3	145-2C11	FITC	1:100
CD4	RM4-5	PerCP eFluor 710	1:800
CD8	5.3-6.7	BUV395	1:500
CD45.2	104	APC fire 750	1:600
H2kb – MHC-I	AF6-58.5	BV421	1:500
PD-L1	10.F.9G2	PE/Dazzle 594	1:600
Ly6G	145-2C11	BV711	1:600
CD11b	RM4-5	BUV737	1:500
EF5		Cy5	30 µL

Statistical significance between groups were determined using unpaired t test (, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

[0657] In this example, 68.6% ($\pm 3.5\%$) of vehicle treated control MB49 tumour cells (CD45⁻) were EF5 avid (EF5⁺) with a hypoxia intensity score (MFI) of $1.1 \times 10^4 \pm 299$ (Mean Fluorescence Intensity; MFI $1.1 \times 10^4 \pm 299$) (FIG. 79). In vehicle treated MB49 tumour bearing mice, the proportion of PD-L1 expressing (PD-L1⁺) tumour cells was $67.9 \pm 5.8\%$ (total cell count $6.7 \times 10^5 \pm 1.1 \times 10^5$) (FIG. 79).

[0658] Tarloxotinib at 50 mg/kg (Q2Dx4) reduced the percentage of EF5 avid (EF5⁺) MB49 tumour cells (CD45⁻) to 25.3% ($\pm 5.3\%$) and the hypoxia intensity (Mean Fluorescence

Intensity; MFI 5813 ± 318) (FIG. 80). Tarloxotinib treatment increased the tumour infiltration of CD4⁺ ($3.0 \times 10^4 \pm 6086$) and CD8⁺ T-cells ($7.6 \times 10^4 \pm 14524$), and reduced the level of hypoxia (EF5 MFI 1519 ± 166 and 1773 ± 200 , respectively) experienced by the T-cell populations in the tumour compared to vehicle treated control (CD4: $1.1 \times 10^4 \pm 1771$ and MFI: 2259 ± 219 ; CD8: $3.2 \times 10^4 \pm 3954$ and MFI: 3021 ± 338) (FIG. 81). Tarloxotinib treatment decreased the percentage of PD-L1 expressing (PD-L1⁺) MB49 tumour cells to 20.3% ($\pm 3.5\%$) (total cell count $1.3 \times 10^5 \pm 0.295 \times 10^5$) compared to vehicle treated control (FIG. 81).

[0659] Tarloxotinib administration reduced the percentage of hypoxic cells in the tumour. Due to reduced hypoxia intensity in the tumour, T-cells in the tumour were exposed to a less hypoxic tumour microenvironment, which is more favourable for their activation. Studies have shown that tumour hypoxia may promote the expression of PD-L1 on tumour cells. Thus, tarloxotinib-induced reduction in tumour hypoxia resulted in reduced tumour PD-L1 expression in MB49 tumour bearing mice.

EQUIVALENTS

[0660] The details of one or more embodiments of the disclosure are set forth in the accompanying description above. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. 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 disclosure belongs. All patents and publications cited in this specification are incorporated by reference.

[0661] The foregoing description has been presented only for the purposes of illustration and is not intended to limit the disclosure to the precise form disclosed, but by the claims appended hereto.

CLAIMS

We claim:

1. A pharmaceutical combination comprising:
 - (a) (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof; or (E)-N-(4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide, or a pharmaceutically acceptable salt or solvate thereof; and
 - (b) an immunotherapeutic agent.
2. The pharmaceutical combination of claim 1, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.
3. The pharmaceutical combination of claim 1, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.
4. The pharmaceutical combination of claim 3, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.
5. The pharmaceutical combination of claim 1, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.
6. The pharmaceutical combination of claim 1, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.
7. The pharmaceutical combination of claim 1, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT,

VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

8. The pharmaceutical combination of claim 7, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

9. The pharmaceutical combination of claim 8, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

10. The pharmaceutical combination of any one of claims 1-9, wherein the combination is a synergistic combination.

11. The pharmaceutical combination of any one of claims 1-10, wherein the combination is for simultaneous, separate, or sequential administration.

12. The pharmaceutical combination of any one of claims 1-11, wherein the combination comprises:

a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

13. The pharmaceutical combination of claim 12, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 50 mg/kg of body weight of a subject.

14. The pharmaceutical combination of any one of claims 1-11, wherein the combination comprises:

a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

15. The pharmaceutical combination of claim 14, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

16. The pharmaceutical combination of claim 15, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 mg/m² to about 150 mg/m² of body weight of a subject.

17. The pharmaceutical combination of claim 15, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/m² of body weight of a subject.

18. The pharmaceutical combination of claim 15, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/m² of body weight of a subject.

19. The pharmaceutical combination of claim 15, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/m² of body weight of a subject.

20. The pharmaceutical combination of claim 15, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/ m² of body weight of a subject.
21. A pharmaceutical combination comprising the pharmaceutical combination of any of claims 1-20 and a pharmaceutically acceptable excipient.
22. A method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.
23. The method of claim 22, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially.
24. The method of any one of claims 22-23, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered simultaneously.
25. The method of any one of claims 22-24, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in the same dosage form.
26. The method of any one of claims 22-25, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-

imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in separate dosage forms.

27. The method of any one of claims 22-23, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered sequentially.

28. The method of claim 27, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered prior to the immunotherapeutic agent.

29. The method of claim 27, wherein the immunotherapeutic agent is administered prior to (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof.

30. The method of any one of claims 22-29, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

31. The method of claim 30, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

32. The method of claim 31, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

33. The method of claim 30, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

34. The method of claim 30, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

35. The method of claim 30, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B7-H3, or any combination thereof.

36. The method of claim 35, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

37. The method of claim 36, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

38. The method of any one of claims 22-37, wherein the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, oesophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

39. The method of any one of claims 22-37, wherein the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer.

40. The method of claim 39, wherein the cancer is non-small cell lung cancer.

41. The method of any one of claims 22-40, wherein at least one of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, intramuscularly, subcutaneously, or intraperitoneally.

42. The method of any one of claims 22-40, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered in a sub-therapeutically effective amount.

43. The method of any one of claims 22-40, wherein the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

44. The method of any one of claims 22-40, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

45. The method of claim 44, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dosage of about 50 mg/kg.

46. The method of any one of claims 22-40, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and the immunotherapeutic agent of

from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

47. The method of claim 46, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

48. The method of claim 46, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 to about 150 mg/ m² of body weight of a subject.

49. The method of claim 46, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/ m² of body weight of a subject.

50. The method of claim 46, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/ m² of body weight of a subject.

51. The method of claim 46, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/ m² of body weight of a subject.

52. The method of claim 46, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/m² of body weight of a subject.

53. A method of activating T-cells or a T-cell response in a subject comprising administering to the subject a therapeutically effective amount of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.
54. The method of claim 53, wherein T-cell proliferation is increased in the subject.
55. The method of claim 53, wherein T-cell function is enhanced in the subject.
56. The method of claim 53, wherein the T-cell function enhancement is characterized as an increase in cytokine release and/or an increase in cytotoxic capacity.
57. The method of claim 56, wherein the cytokine is IFN γ .
58. The method of any one of claims 53-57, wherein the T-cells are CD3⁺ T-cells.
59. The method of any one of claims 53-57, wherein the T-cells are CD4⁺ T-cells or CD8⁺ T-cells.
60. The method of any of claims 53-59, wherein an amount of cytokine and/or T-cells in the subject with enhanced function after administration is greater than an amount of cytokine and/or T-cells in the subject with enhanced function prior to administration.
61. The method of claim 60, wherein an amount cytokine and/or T-cells in the blood, lymphoid tissue, or tumour of the subject after administration is greater than an amount of cytokine and/or T-cells in the blood, lymphoid tissue, or tumour of the subject prior to administration.
62. The method of claim 59, wherein an amount cytokine and/or T-cells in the blood of the subject after administration is greater than an amount of cytokine and/or T-cells in the blood of the subject prior to administration.

63. The method of any one of claims 53-62, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially.

64. The method of any one of claims 53-63, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered simultaneously.

65. The method of claim 64, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in the same dosage form.

66. The method of claim 64, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in separate dosage forms.

67. The method of any one of claims 53-63, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered sequentially.

68. The method of claim 67, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered prior to the immunotherapeutic agent.

69. The method of claim 67, wherein the immunotherapeutic agent is administered prior to (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-

[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof.

70. The method of any one of claims 53-69, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

71. The method of claim 70, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

72. The method of claim 71, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

73. The method of claim 70, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

74. The method of claim 70, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

75. The method of claim 70, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGHT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B7-H3, or any combination thereof.

76. The method of claim 75, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

77. The method of claim 76, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

78. The method of any one of claims 53-77, wherein the subject suffers from a cancer selected from is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

79. The method of any one of claims 53-77, wherein the subject suffers from a cancer selected from chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, and non-small cell lung cancer.

80. The method of claim 79, wherein the cancer is non-small cell lung cancer.

81. The method of any one of claims 53-80, wherein at least one of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, or intraperitoneally.

82. The method of any one of claims 53-81, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered in a sub-therapeutically effective amount.

83. The method of any one of claims 53-82, wherein the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

84. The method of any one of claims 53-83, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-

imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

85. The method of claim 84, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dosage of about 50 mg/kg.

86. The method of any one of claims 53-83, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

87. The method of claim 86, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

88. The method of claim 86, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 to about 150 mg/m² of body weight of a subject.

89. The method of claim 86, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/m² of body weight of a subject.

90. The method of claim 86, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/ m² of body weight of a subject.

91. The method of claim 86, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/ m² of body weight of a subject.

92. The method of claim 86, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/ m² of body weight of a subject.

93. A method of enhancing an immune response in a subject comprising administering to the subject having a therapeutically effective amount of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

94. The method of claim 93, wherein T-cell proliferation is increased in the subject.

95. The method of claim 93, wherein T-cell function is enhanced in the subject.

96. The method of claim 95, wherein the T-cell function enhancement is characterized as an increase in cytokine release and/or an increase in cytotoxic capacity.

97. The method of claim 96, wherein the cytokine is IFN γ .

98. The method of any one of claims 94-97, wherein the T-cells are CD3⁺ T-cells.

99. The method of claim 98, wherein the CD3⁺ T-cells are selected from CD4⁺ T-cells and CD8⁺ T-cells.

100. The method of any of claims 93-99, wherein an amount of cytokine and/or T-cells in the subject with enhanced function after administration is greater than an amount of cytokine and/or T-cells in the subject with enhanced function prior to administration.

101. The method of claim 100, wherein an amount cytokine and/or T-cells in the blood, lymphoid tissue, or tumour of the subject after administration is greater than an amount of cytokine and/or T-cells in the blood, lymphoid tissue, or tumour of the subject prior to administration.

102. The method of claim 101, wherein an amount cytokine and/or T-cells in the blood of the subject after administration is greater than an amount of cytokine and/or T-cells in the blood of the subject prior to administration.

103. The method of any one of claims 93-102, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially.

104. The method of claim any one of claims 93-103, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered simultaneously.

105. The method of any one of claims 93-104, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in the same dosage form.

106. The method of any one of claims 93-104, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in separate dosage forms.

107. The method of any one of claims 93-103, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered sequentially.

108. The method of claim 107, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered prior to the immunotherapeutic agent.

109. The method of claim 107, wherein the immunotherapeutic agent is administered prior to (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof.

110. The method of any one of claims 93-109, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, or vaccine, or a cellular therapy.

111. The method of claim 110, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

112. The method of claim 111, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

113. The method of claim 110, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

114. The method of claim 110, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

115. The method of claim 110, wherein the immune checkpoint inhibitor is an inhibitor of an immune checkpoint protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

116. The method of claim 115, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

117. The method of claim 116, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

118. The method of any one of claims 93-117, wherein the subject suffers from a cancer selected from bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

119. The method of any one of claims 93-117, wherein the subject suffers from a cancer selected from chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, and retinoblastoma, and non-small cell lung cancer.

120. The method of claim 119, wherein the cancer is non-small cell lung cancer.

121. The method of any one of claims 93-120, wherein at least one of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, or intraperitoneally.

122. The method of any one of claims 93-121, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered in a sub-therapeutically effective amount.

123. The method of any one of claims 93-122, wherein the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

124. The method of any one of claims 93-123, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

125. The method of claim 124, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dosage of about 50 mg/kg.

126. The method of any one of claims 93-125, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and the immunotherapeutic agent is

administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

127. The method of claim 126, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

128. The method of claim 126, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 to about 150 mg/m² of body weight of a subject.

129. The method of claim 126, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/m² of body weight of a subject.

130. The method of claim 126, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/m² of body weight of a subject.

131. The method of claim 126, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/m² of body weight of a subject.

132. The method of claim 126, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/m² of body weight of a subject.

133. A method of treating a disease ameliorated by inhibiting the binding of a human PD-1 ligand to human PD-1, comprising administering to a subject having the disease a therapeutically effective amount of (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

134. The method of claim 133, wherein (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially.

135. The method of claim any one of claims 133-134, wherein (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered simultaneously.

136. The method of any one of claims 133-135, wherein (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in the same dosage form.

137. The method of any one of claims 133-135, wherein (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in separate dosage forms.

138. The method of any one of claims 133-134, wherein (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered sequentially.

139. The method of claim 138, wherein (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered prior to the immunotherapeutic agent.

140. The method of claim 138, wherein the immunotherapeutic agent is administered prior to (2E)-4-{{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof.

141. The method of any one of claims 133-140, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

142. The method of any one of claims 141, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

143. The method of claim 142, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

144. The method of claim 141, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

145. The method of claim 141, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

146. The method of claim 141, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGHT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, and B7-H3, or any combination thereof.

147. The method of claim 146, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

148. The method of claim 147, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

149. The method of any one of claims 133-148, wherein the subject suffers from a cancer selected from bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

150. The method of any one of claims 133-149, wherein the disease is a cancer selected from chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, and retinoblastoma, and non-small cell lung cancer.

151. The method of claim 150, wherein the cancer is non-small cell lung cancer.

152. The method of any one of claims 133-151, wherein at least one of (2E)-4- {[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, or intraperitoneally.

153. The method of any one of claims 133-152, wherein (2E)-4- {[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-

imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered in a sub-therapeutically effective amount.

154. The method of any one of claims 133-153, wherein the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

155. The method of any one of claims 133-154, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

156. The method of claim 155, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dosage of about 50 mg/kg.

157. The method of any one of claims 133-154, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

158. The method of claim 157, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

159. The method of claim 157, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 to about 150 mg/ m² of body weight of a subject.

160. The method of claim 157, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/ m² of body weight of a subject.

161. The method of claim 157, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/ m² of body weight of a subject.

162. The method of claim 157, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/ m² of body weight of a subject.

163. The method of claim 157, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/ m² of body weight of a subject.

164. A method of inhibiting tumour cell growth comprising administering to the tumour a therapeutically effective amount of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.

165. The method of claim 164, wherein the tumour is a solid tumour.

166. The method of claim 165, wherein the solid tumour is selected from bladder carcinoma, squamous cell carcinoma, transitional cell carcinoma, basal cell carcinoma, renal cell carcinoma, ductal cell carcinoma, and adenocarcinoma.

167. The method of any one of claims 165-166, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially.

168. The method of claim any one of claims 164-167, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered simultaneously.

169. The method of any one of claims 164-168, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in the same dosage form.

170. The method of any one of claims 164-168, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in separate dosage forms.

171. The method of any one of claims 164-167, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered sequentially.

172. The method of claim 171, wherein (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered prior to the immunotherapeutic agent.

173. The method of claim 171, wherein the immunotherapeutic agent is administered prior to (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof.

174. The method of any one of claims 164-173, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, or vaccine, or a cellular therapy.

175. The method of claim 174, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

176. The method of claim 175, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

177. The method of claim 176, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

178. The method of claim 174, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

179. The method of claim 174, wherein the immune checkpoint inhibitor is an inhibitor of an immune checkpoint protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

180. The method of claim 179, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

181. The method of claim 180, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

182. The method of any one of claims 164-181, wherein at least one of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, or intraperitoneally.

183. The method of any one of claims 164-182, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered in a sub-therapeutically effective amount.

184. The method of any one of claims 164-183, wherein the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

185. The method of any one of claims 164-184, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

186. The method of claim 185, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dosage of about 50 mg/kg.

187. The method of any one of claims 164-184, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

188. The method of claim 187, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

189. The method of claim 187, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 to about 150 mg/m² of body weight of a subject.

190. The method of claim 187, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/m² of body weight of a subject.

191. The method of claim 187, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/m² of body weight of a subject.

192. The method of claim 187, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/m² of body weight of a subject.

193. The method of claim 187, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/ m² of body weight of a subject.

194. A compound which is (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment of cancer, wherein the treatment comprises administering the compound in combination with an immunotherapeutic agent.

195. The compound of claim 194, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered separately, simultaneously, or sequentially.

196. The compound of claims 194-195, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered simultaneously.

197. The compound of claim 196, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in the same dosage form.

198. The compound of claim 196, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered in separate dosage forms.

199. The compound of any one of claims 194-195, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and the immunotherapeutic agent are administered sequentially.

200. The compound of claim 199, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered prior to the immunotherapeutic agent.

201. The compound of claim 199, wherein the immunotherapeutic agent is administered prior to (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof.

202. The compound of claim 194-201, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

203. The compound of claim 202, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

204. The compound of claim 203, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

205. The compound of claim 202, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

206. The compound of claim 194, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

207. The compound of claim 194, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3,

TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

208. The compound of claim 207, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

209. The compound of claim 208, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

210. The compound of any one of claims 194-209, wherein the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

211. The compound of any one of claims 194-209, wherein the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer.

212. The compound of claim 211, wherein the cancer is non-small cell lung cancer.

213. The compound of any one of claims 194-212, wherein at least one of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically

acceptable salt or solvate thereof, and the immunotherapeutic agent is administered orally, parenterally, rectally, topically, intravenously, or intraperitoneally.

214. The compound of any one of claims 194-213, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered in a sub-therapeutically effective amount.

215. The compound of any one of claims 194-214, wherein the immunotherapeutic agent is administered in a sub-therapeutically effective amount.

216. The compound of any one of claims 194-215, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

217. The compound of claim 216, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dosage of about 50 mg/kg.

218. The compound of any one of claims 194-215, wherein (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and the immunotherapeutic agent is administered at a dose of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

219. The compound of claim 218, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

220. The compound of claim 218, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 to about 150 mg/m² of body weight of a subject.

221. The compound of claim 218, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/m² of body weight of a subject.

222. The compound of claim 218, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/m² of body weight of a subject.

223. The compound of claim 218, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/m² of body weight of a subject.

224. The compound of claim 218, wherein the dose of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/m² of body weight of a subject.

225. A pharmaceutical combination comprising (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-

imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for use in the treatment of cancer.

226. The pharmaceutical combination of claim 225, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

227. The pharmaceutical combination of claim 226, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

228. The pharmaceutical combination of claim 227, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

229. The pharmaceutical combination of claim 225, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

230. The pharmaceutical combination of claim 225, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

231. The pharmaceutical combination of claim 225, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

232. The pharmaceutical combination of claim 231, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

233. The pharmaceutical combination of claim 232, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

234. The pharmaceutical combination of any one of claims 225-233, wherein the combination is a synergistic combination.

235. The pharmaceutical combination of any one of claims 225-234, wherein the combination is for simultaneous, separate, or sequential administration.

236. The pharmaceutical combination of any one of claims 225-235, wherein the combination comprises:

a dosage of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

237. The pharmaceutical combination of claim 236, wherein the combination comprises: a dosage of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of about 50 mg/kg of body weight of a subject.

238. The pharmaceutical combination of any one of claims 225-235, wherein the combination comprises:

a dosage of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

239. The pharmaceutical combination of claim 238, wherein the combination comprises: a dosage of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-

dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

240. The pharmaceutical combination of claim 239, wherein the combination comprises: a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of about 150 mg/m² of body weight of a subject.

241. The pharmaceutical combination of claims 225-240, wherein the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

242. The pharmaceutical combination of any one of claims 224-240, wherein the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer.

243. The pharmaceutical combination of claim 242, wherein the cancer is non-small cell lung cancer.

244. Use of a combination comprising a therapeutically effective amount of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for the treatment of cancer in a subject.

245. The use of claim 244, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

246. The use of claim 244, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

247. The use of claim 246, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

248. The use of claim 244, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

249. The use of claim 244, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

250. The use of claim 244, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

251. The use of claim 250, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

252. The use of claim 251, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

253. The use of any one of claims 244-252, wherein the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the

small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

254. The use of any one of claims 244-252, wherein the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer.

255. The use of claim 254, wherein the cancer is non-small cell lung cancer.

256. Use of a combination comprising a therapeutically effective amount of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent in the manufacture of a medicament for the treatment of cancer in a subject.

257. The use of claim 256, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

258. The use of claim 257, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

259. The use of claim 258, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

260. The use of claim 258, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

261. The use of claim 258, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

262. The use of claim 258, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

263. The use of claim 262, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

264. The use of claim 263, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

265. The use of any one of claims 256-264, wherein the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

266. The use of any one of claims 256-264, wherein the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer.

267. The use of claim 264, wherein the cancer is non-small cell lung cancer.

268. A method of sensitizing a hypoxic tumour to a checkpoint inhibitor comprising administering to the hypoxic tumour a therapeutically effective amount of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent.
269. The method of claim 268, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.
270. The method of claim 269, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, and/or CTLA-4.
271. A pharmaceutical composition comprising:
- (a) (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof; or (E)-N-(4-((3-bromo-4-chlorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)-4-(dimethylamino)but-2-enamide, or a pharmaceutically acceptable salt or solvate thereof; and
 - (b) an immunotherapeutic agent.
272. The pharmaceutical composition of claim 271, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.
273. The pharmaceutical composition of claim 271, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.
274. The pharmaceutical composition of claim 273, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.
275. The pharmaceutical composition of claim 271, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

276. The pharmaceutical composition of claim 271, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

277. The pharmaceutical composition of claim 271, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

278. The pharmaceutical composition of claim 277, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

279. The pharmaceutical composition of claim 278, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

280. The pharmaceutical composition of any one of claims 271-279, wherein the combination is a synergistic combination.

281. The pharmaceutical composition of any one of claims 271-280, wherein the combination is for simultaneous, separate, or sequential administration.

282. The pharmaceutical composition of any one of claims 271-281, wherein the combination comprises:

a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

283. The pharmaceutical composition of claim 282, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 50 mg/kg of body weight of a subject.

284. The pharmaceutical composition of any one of claims 271-281, wherein the combination comprises:

a dosage of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

285. The pharmaceutical composition of claim 284, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

286. The pharmaceutical composition of claim 285, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is from about 75 mg/m² to about 150 mg/m² of body weight of a subject.

287. The pharmaceutical composition of claim 285, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 75 mg/m² of body weight of a subject.

288. The pharmaceutical composition of claim 285, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 80 mg/ m² of body weight of a subject.

289. The pharmaceutical composition of claim 285, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 100 mg/ m² of body weight of a subject.

290. The pharmaceutical composition of claim 285, wherein the dose of (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, is about 150 mg/ m² of body weight of a subject.

291. A pharmaceutical composition comprising the pharmaceutical combination of any of claims 271-290 and a pharmaceutically acceptable excipient.

292. A pharmaceutical composition comprising (2E)-4-{{4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl}amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, and an immunotherapeutic agent for use in the treatment of cancer.

293. The pharmaceutical composition of claim 292, wherein the immunotherapeutic agent is an immune checkpoint inhibitor, an anti-cancer antibody therapy, a vaccine, or a cellular therapy.

294. The pharmaceutical composition of claim 293, wherein the immunotherapeutic agent is an inhibitor of an immune checkpoint protein.

295. The pharmaceutical composition of claim 297, wherein the immune checkpoint inhibitor is an anti-immune-checkpoint inhibitor antibody.

296. The pharmaceutical composition of claim 292, wherein the immunotherapeutic agent is an immunomodulatory molecule that directly influences T-cell function.

297. The pharmaceutical composition of claim 292, wherein the immunotherapeutic agent is an immunostimulatory monoclonal antibody agonist of a co-stimulatory receptor.

298. The pharmaceutical composition of claim 292, wherein the immunotherapeutic agent targets a protein selected from Programmed Death 1, Programmed Death-Ligand 1, CTLA-4, PD-L2, LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137, CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS, KIR, LAIR1, LIGHT, MARCO, PS, OX-40, SLAM, TIGIT, VISTA, VTCN1, intrinsic checkpoint blockades, CISH, BMS-202, BMS-8, AUPM170/CA327, LAG3, TIM3, VISTA, B70H3, or any combination thereof.

299. The pharmaceutical composition of claim 298, wherein the immunotherapeutic agent is an inhibitor of PD-1, PD-L1, and/or CTLA-4.

300. The pharmaceutical composition of claim 299, wherein the immune checkpoint inhibitor is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, cemiplimab, and/or ipilimumab.

301. The pharmaceutical composition of any one of claims 292-300, wherein the combination is a synergistic combination.

302. The pharmaceutical composition of any one of claims 292-301, wherein the combination is for simultaneous, separate, or sequential administration.

303. The pharmaceutical composition of any one of claims 292-302, wherein the combination comprises:

a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/kg of body weight of a subject to about 200 mg/kg of body weight of a subject.

304. The pharmaceutical composition of claim 303, wherein the combination comprises: a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of about 50 mg/kg of body weight of a subject.

305. The pharmaceutical composition of any one of claims 292-302, wherein the combination comprises:

a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject; and

a dosage of the immunotherapeutic agent of from about 0.1 mg/m² of body weight of a subject to about 300 mg/m² of body weight of a subject.

306. The pharmaceutical composition of claim 305, wherein the combination comprises: a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of from about 0.1 mg/m² to about 150 mg/m² of body weight of a subject.

307. The pharmaceutical composition of claim 306, wherein the combination comprises: a dosage of (2E)-4-{[4-(3-bromo-4-chloroanilino)pyrido[3,4-d]pyrimidin-6-yl]amino}-N,N-dimethyl-N-[(1-methyl-4-nitro-1H-imidazol-5-yl)methyl]-4-oxo-2-buten-1-ammonium bromide, or a pharmaceutically acceptable salt or solvate thereof, of about 150 mg/m² of body weight of a subject.

308. The pharmaceutical composition of claims 292-307, wherein the cancer is bone cancer, lung cancer, breast cancer, cancer of the head and neck, prostate cancer, pancreatic cancer, skin cancer, uterine cancer, ovarian cancer, cancer of the urethra, cancer of the adrenal gland, cancer

of the small intestine, cancer of the kidney, cancer of the bladder, cancers of the brain, colorectal cancer, esophageal cancer, gastric cancer, anal cancer, liver cancer, thyroid cancer, ocular cancer, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland and testicular cancer.

309. The pharmaceutical composition of any one of claims 224-307, wherein the cancer is chronic or acute leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, carcinoma of the cervix, carcinoma of the vulva, carcinoma of the vagina, Hodgkin's Disease, brain stem glioma, melanoma, Merkel cell carcinoma, Urothelial carcinoma, lymphomas, gliomas, meningiomas, pituitary adenomas, nerve sheath tumours, retinoblastoma, or non-small cell lung cancer.

310. The pharmaceutical composition of claim 309, wherein the cancer is non-small cell lung cancer.

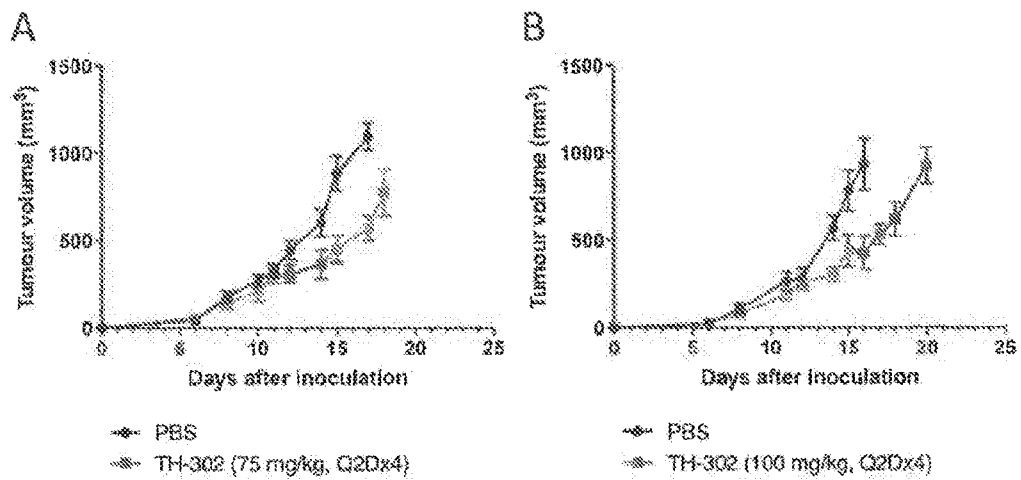


FIG. 1

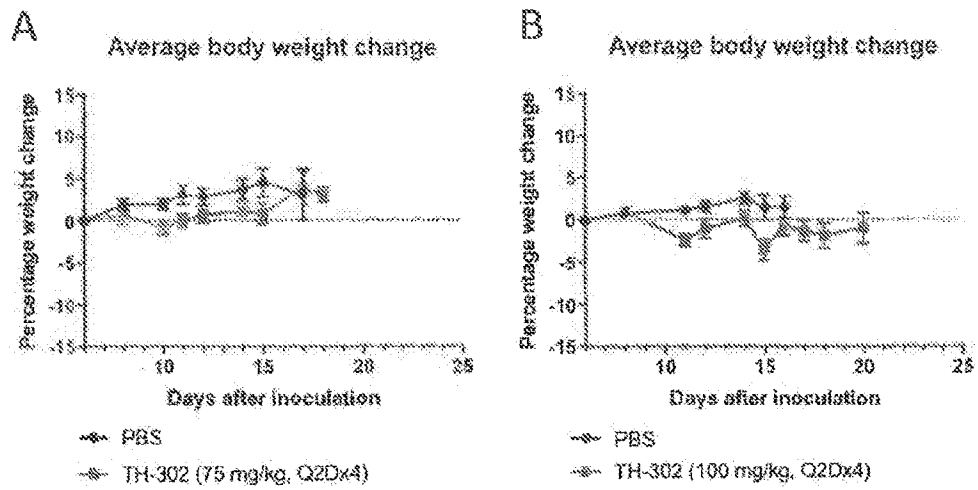


FIG. 2

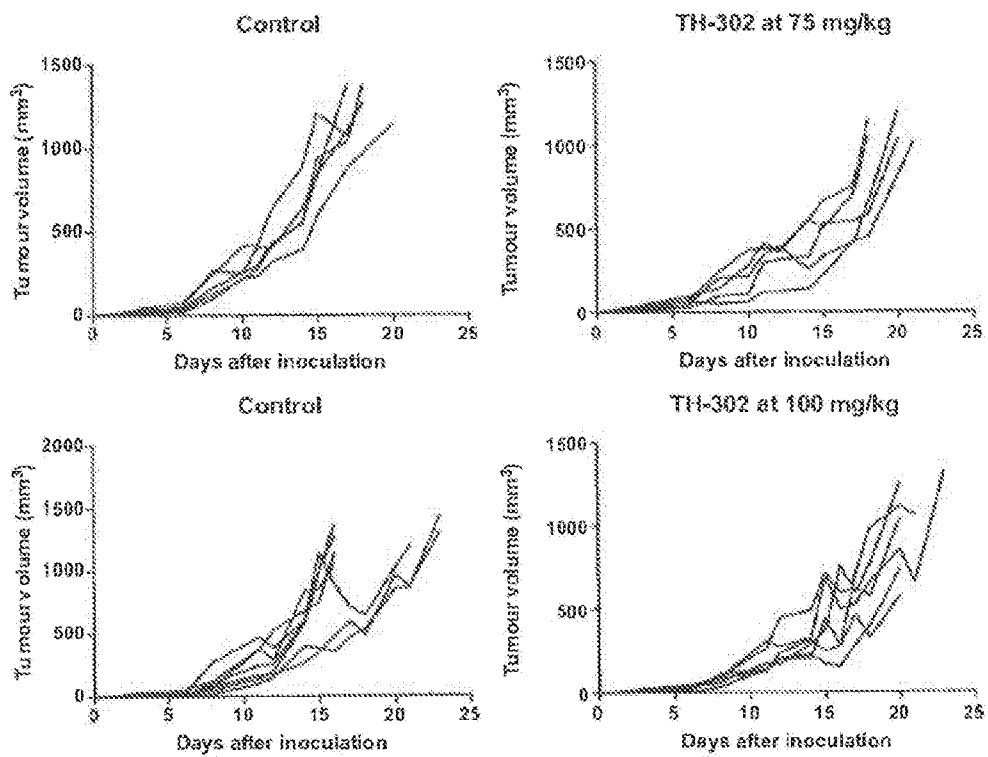


FIG. 3

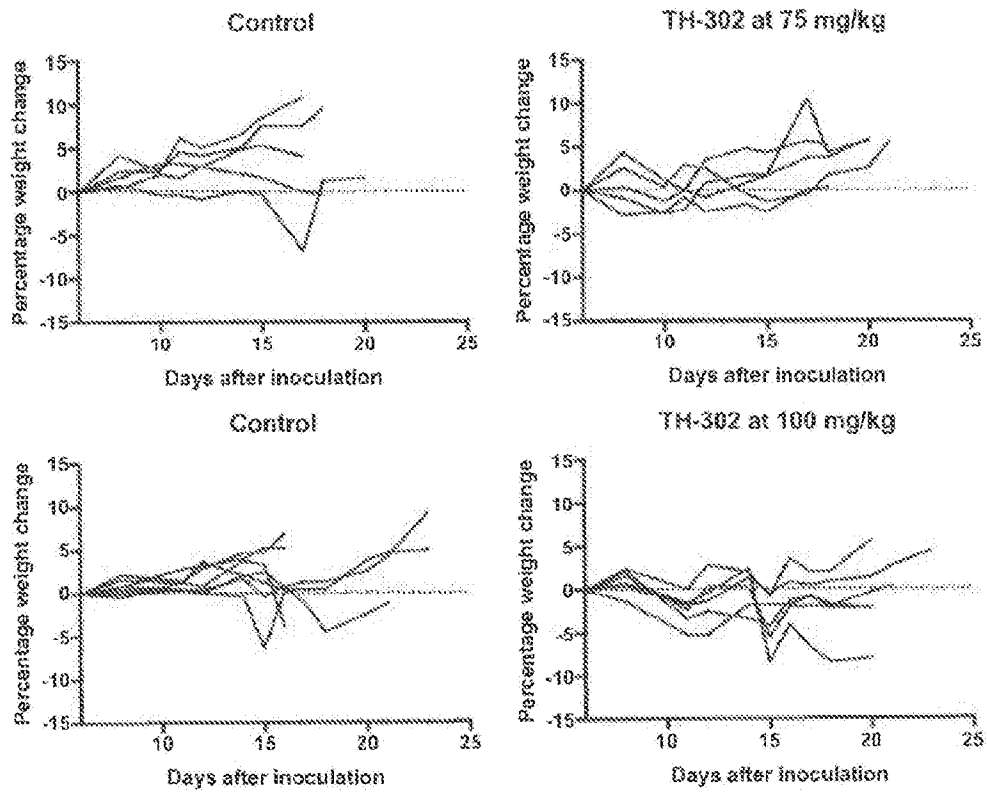


FIG. 4

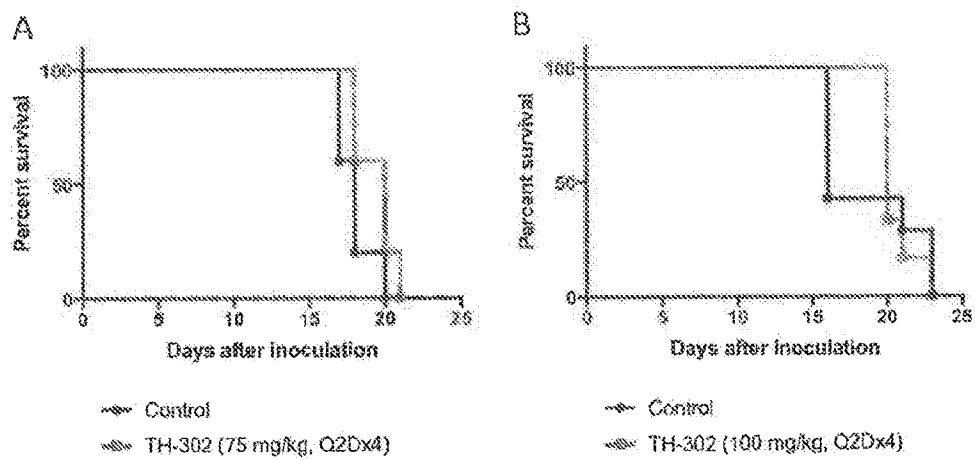


FIG. 5

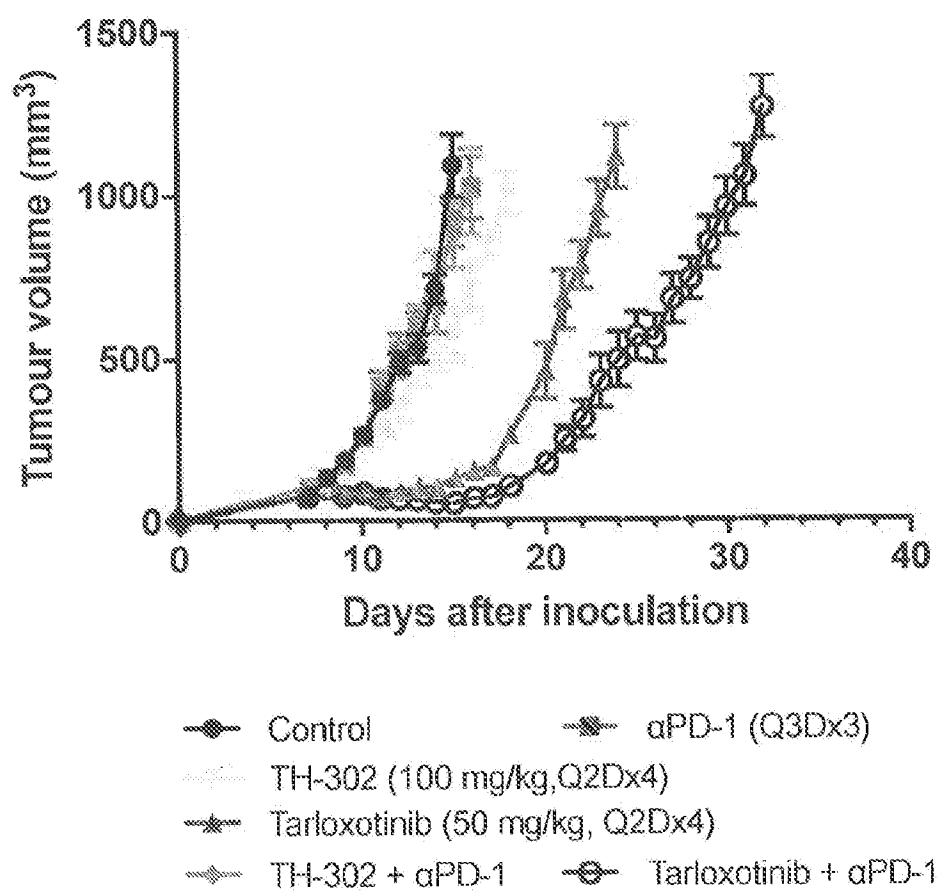


FIG. 6

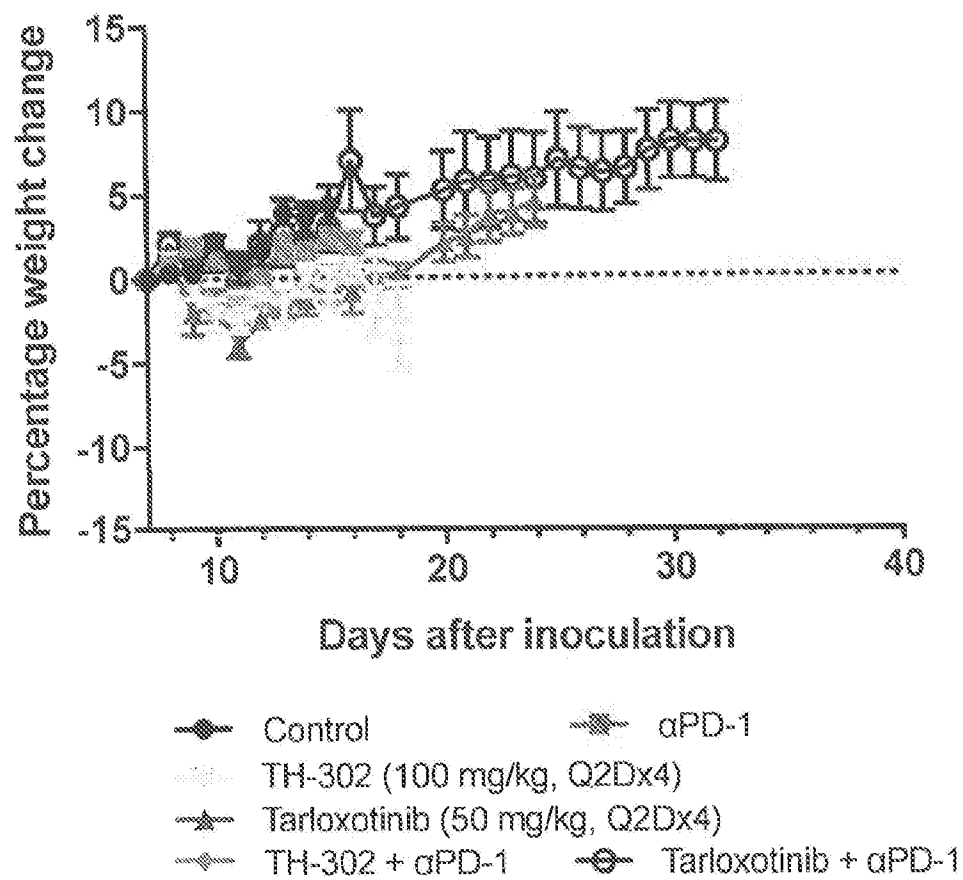


FIG. 7

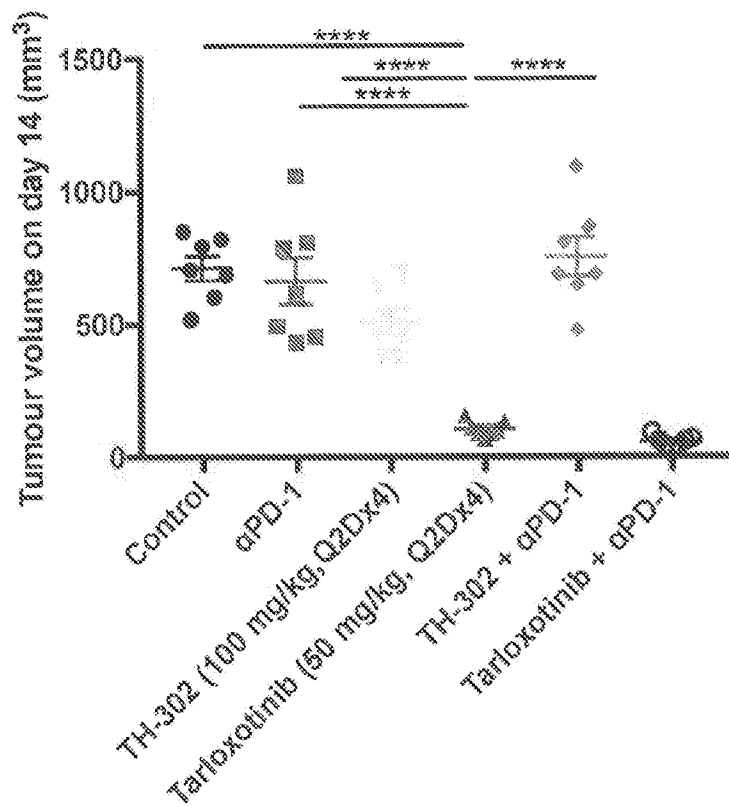


FIG. 8

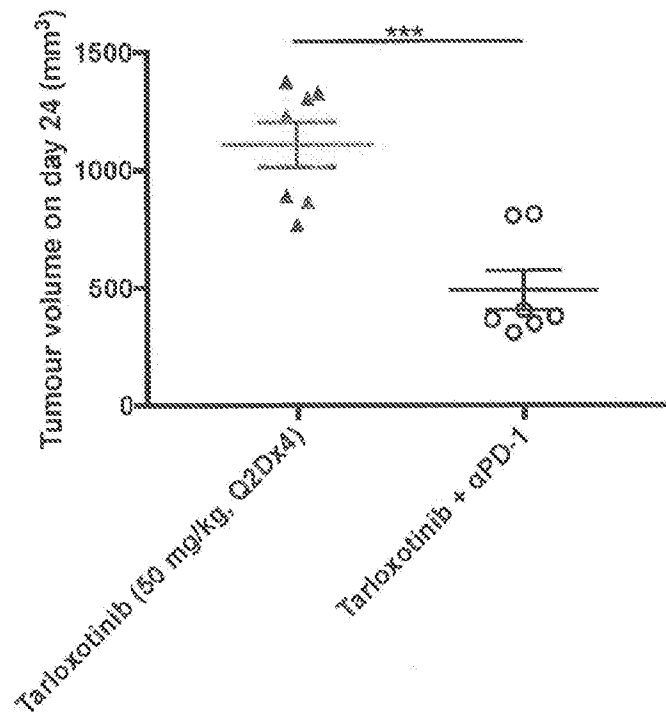


FIG. 9

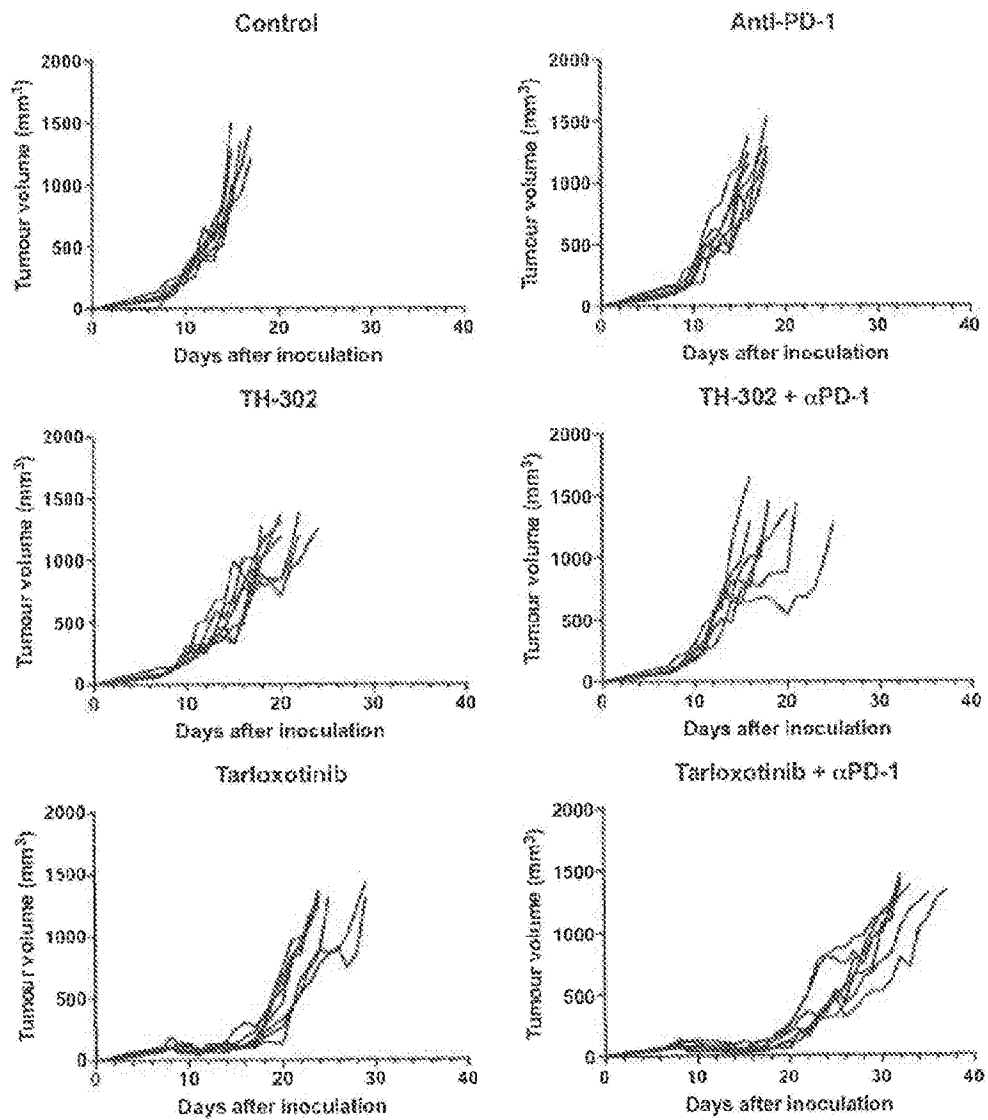


FIG. 10

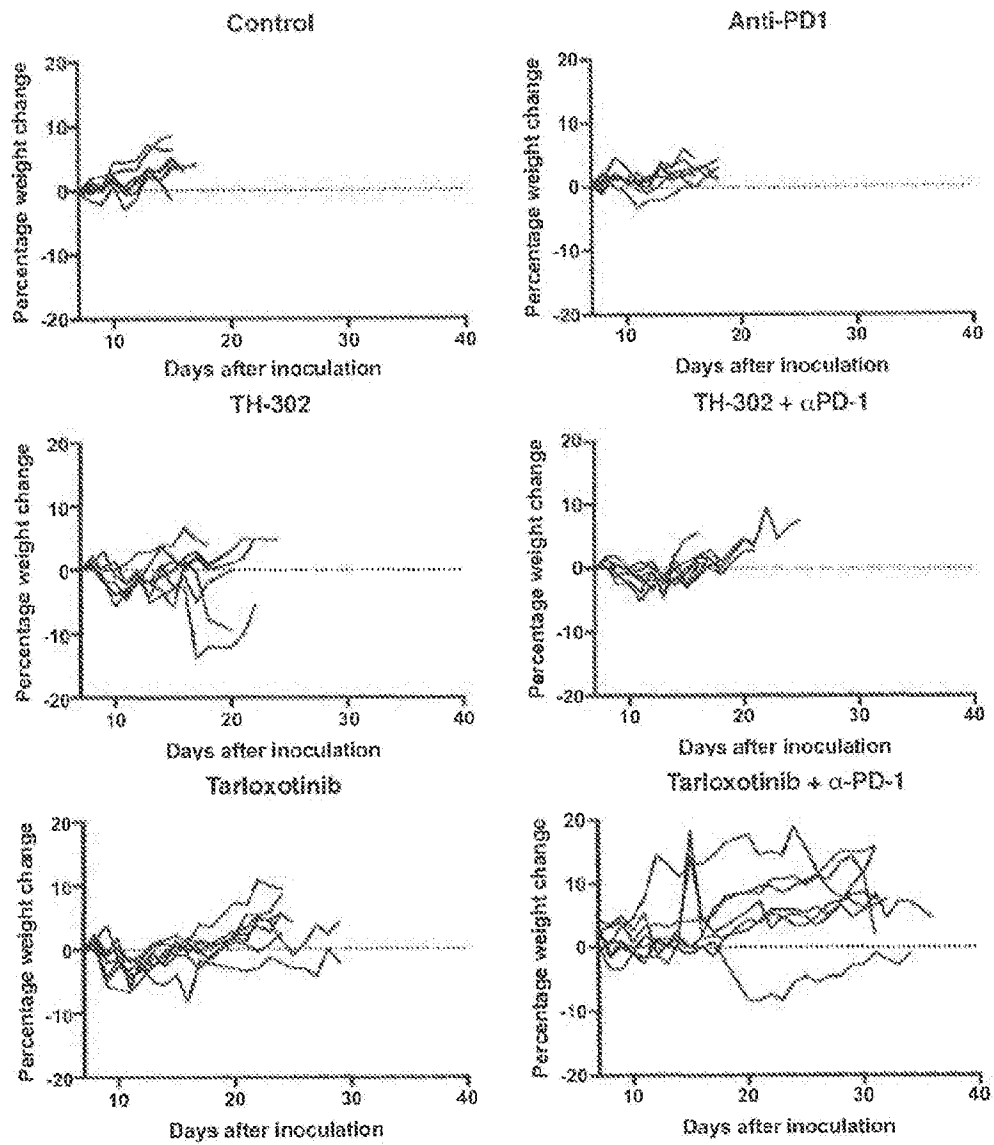


FIG. 11

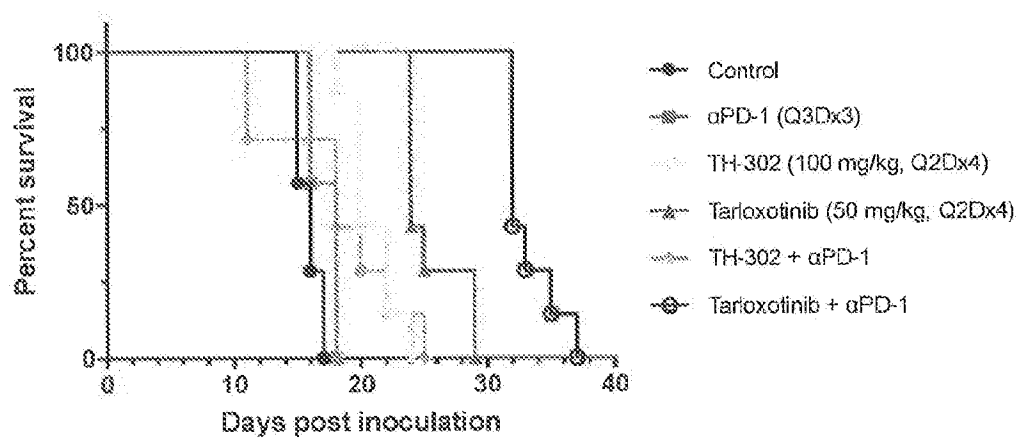


FIG. 12

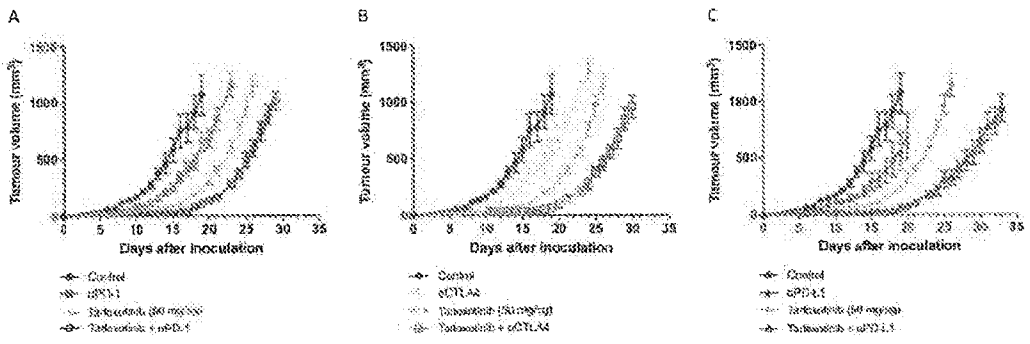


FIG. 13

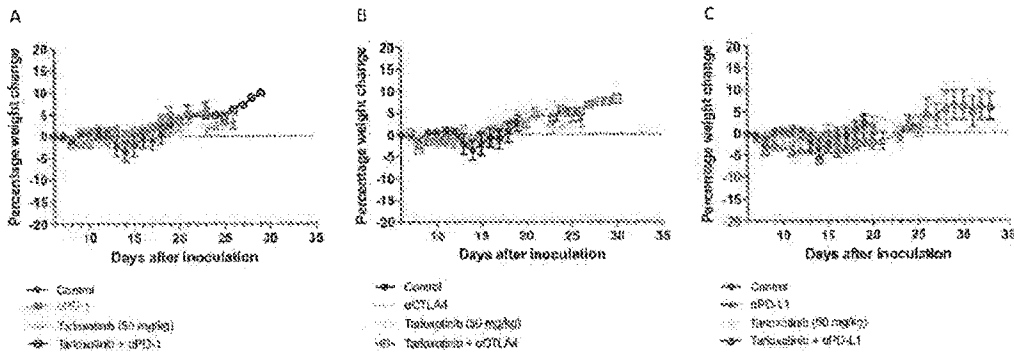


FIG. 14

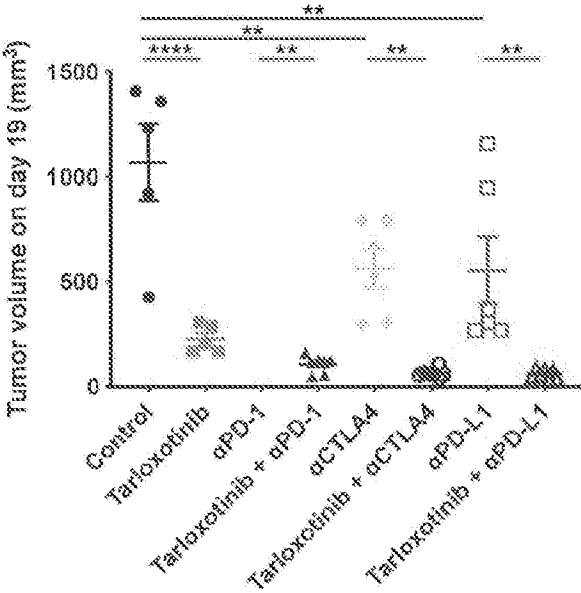


FIG. 15

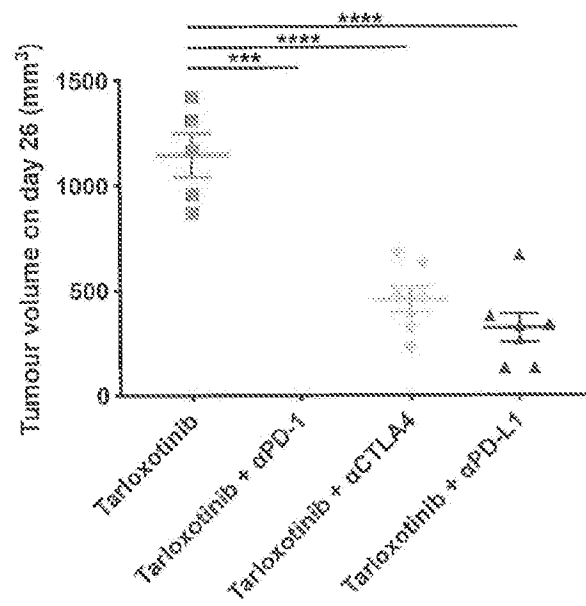


FIG. 16

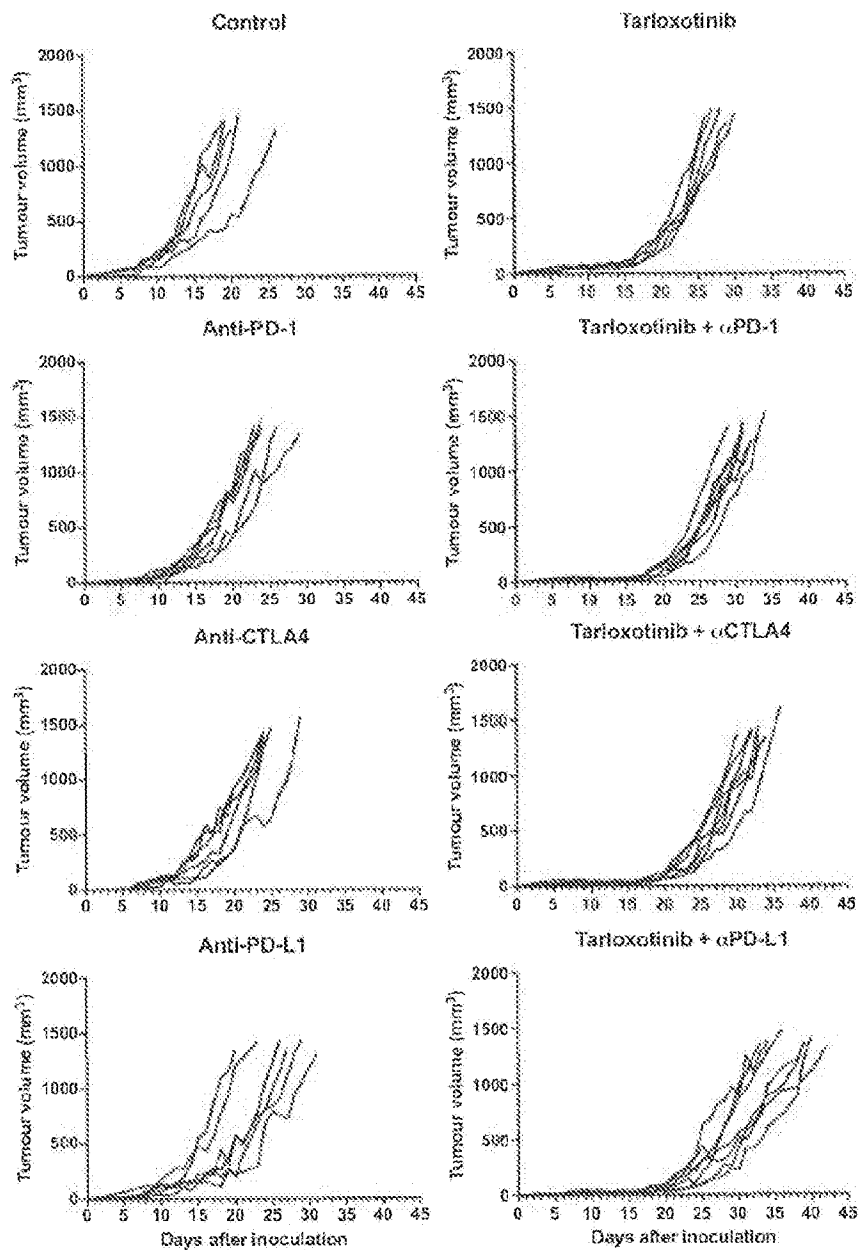


FIG. 17

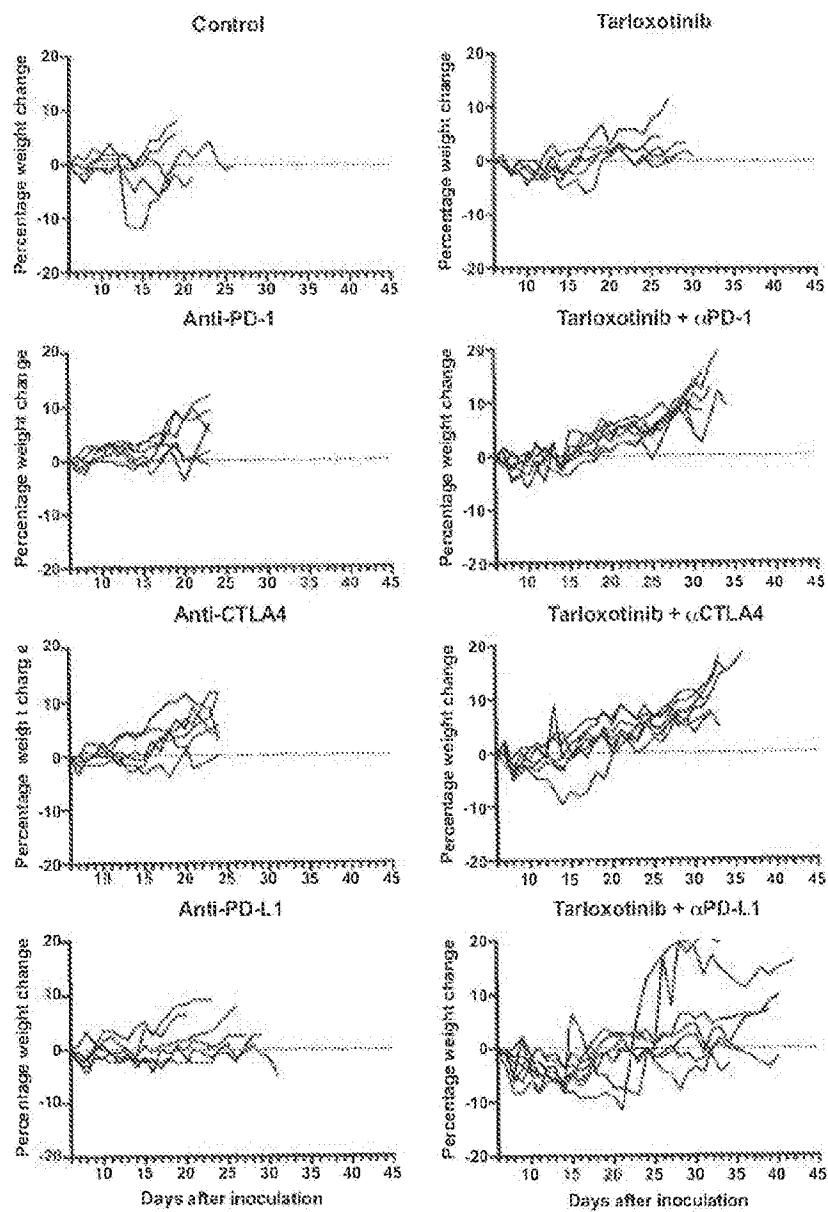


FIG. 18

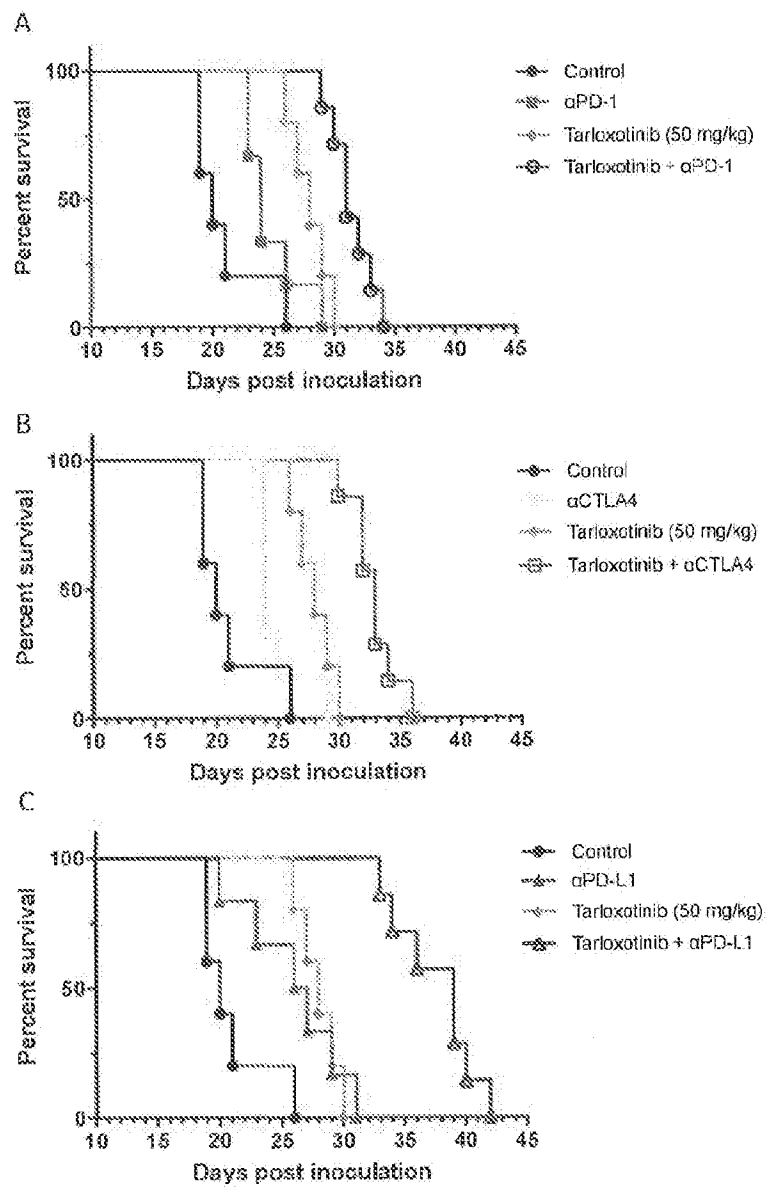


FIG. 19

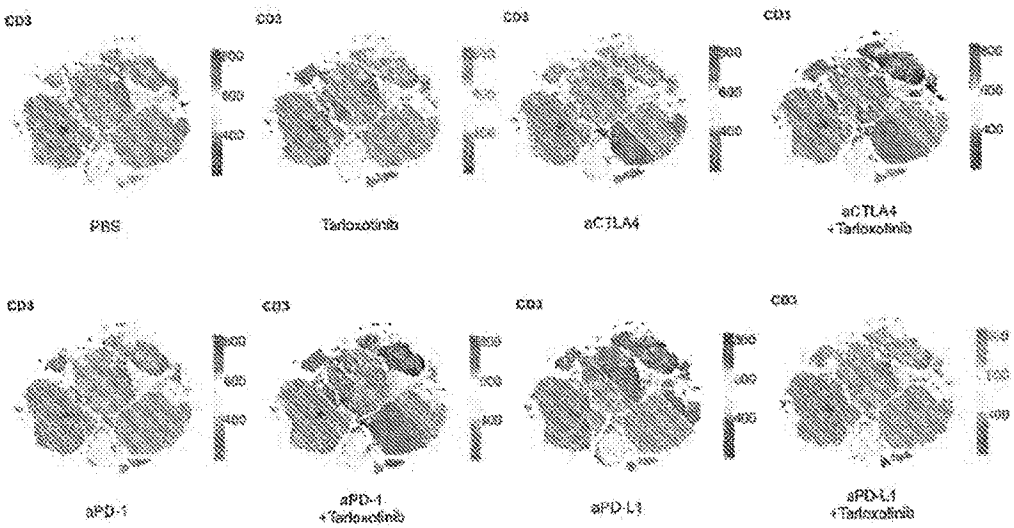


FIG. 20

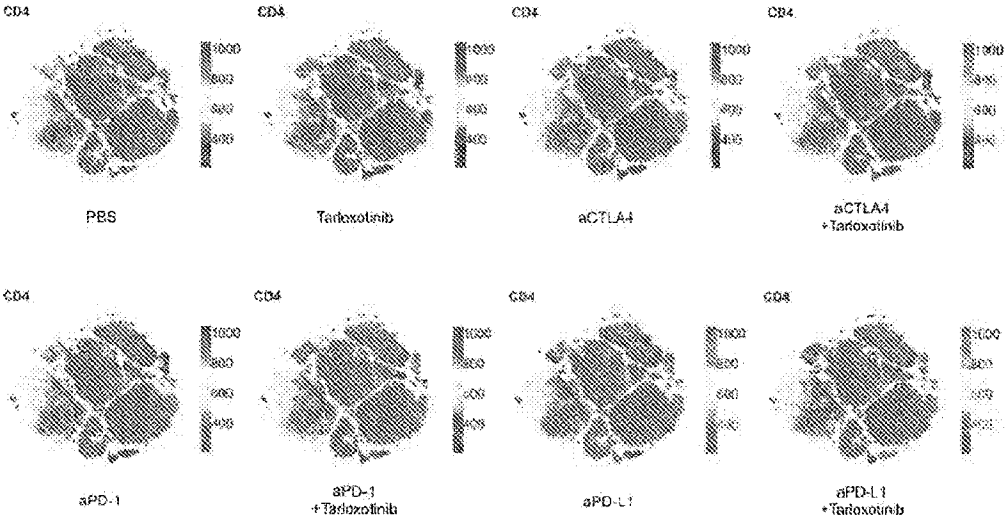


FIG 21

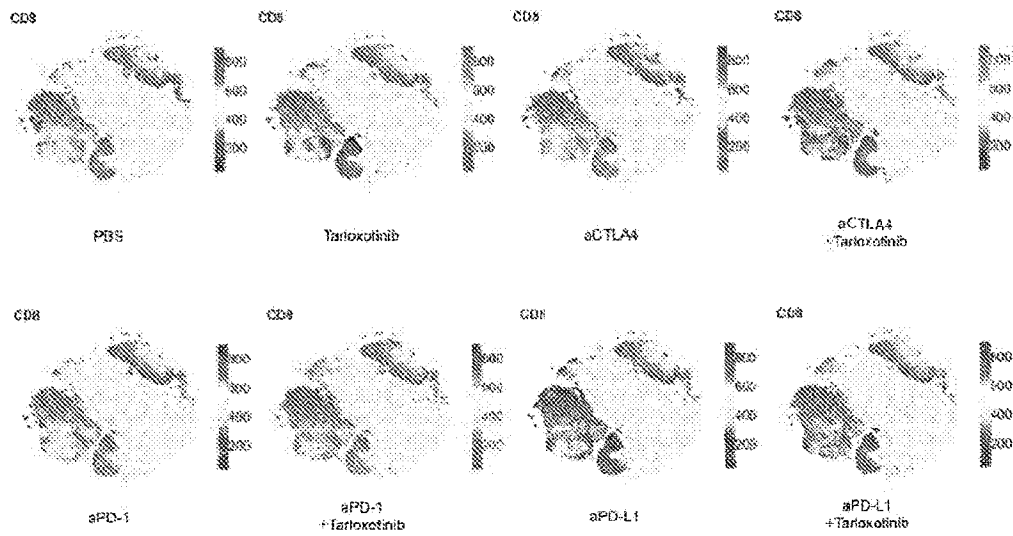


FIG. 22

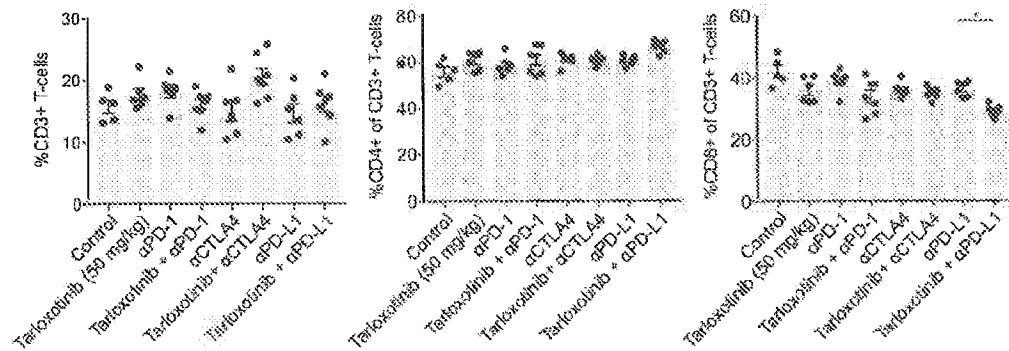


FIG. 23

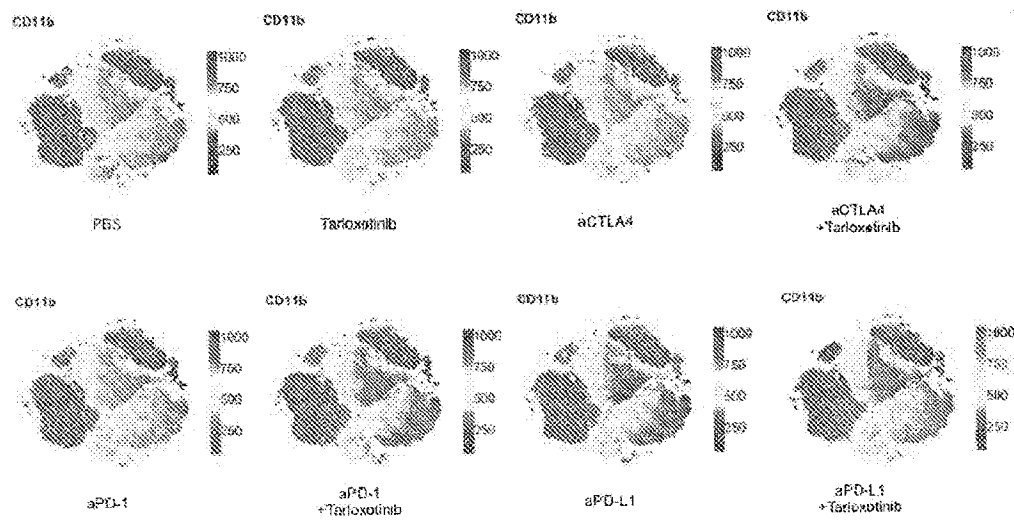


FIG. 24

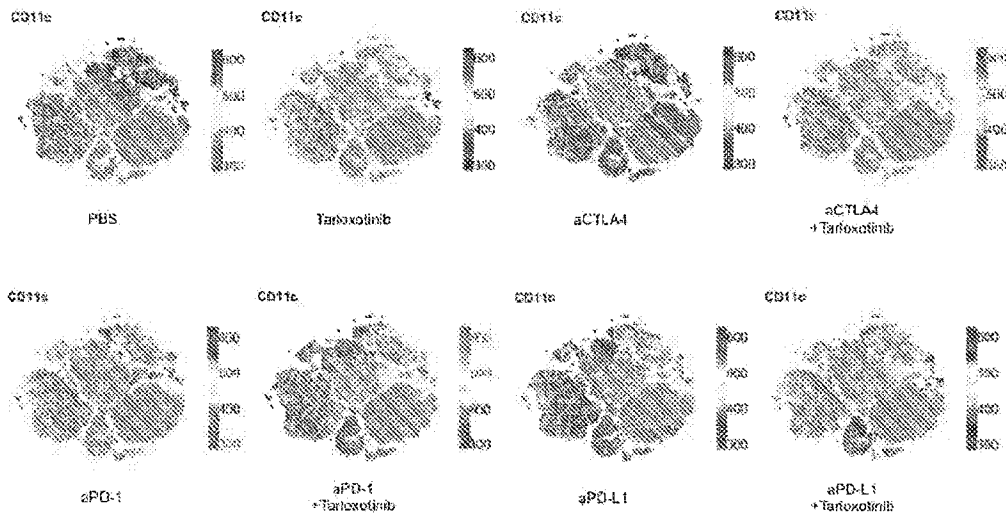


FIG. 25

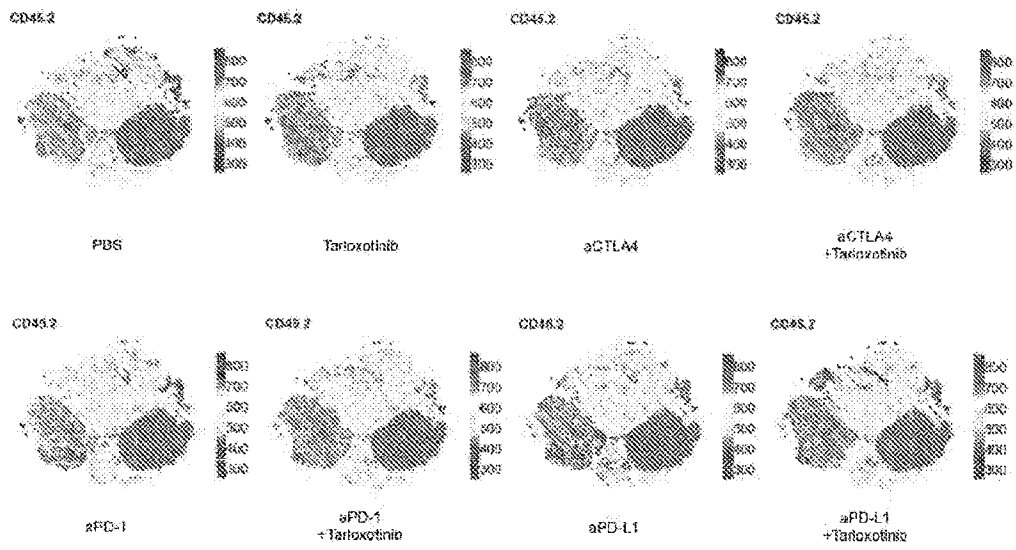


FIG. 26

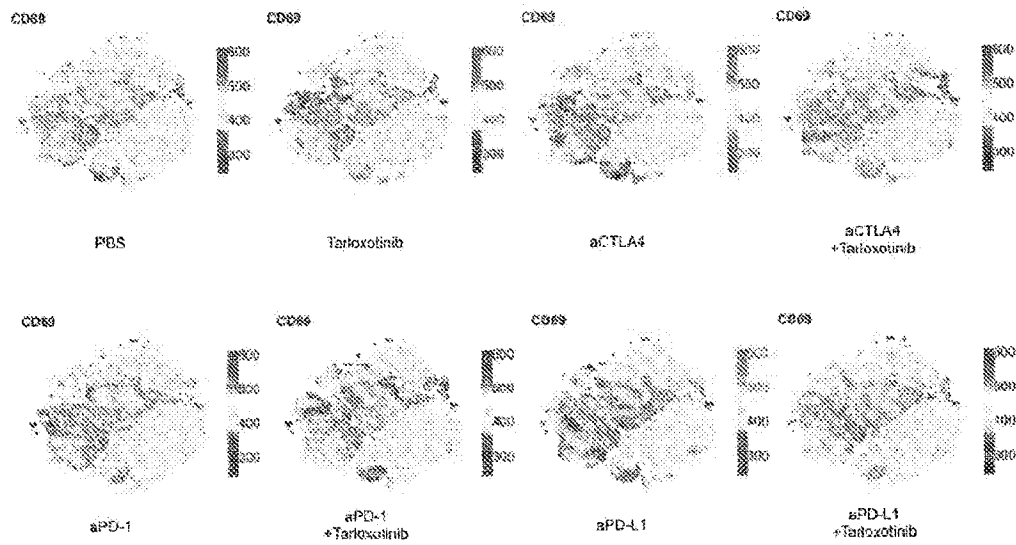


FIG. 27

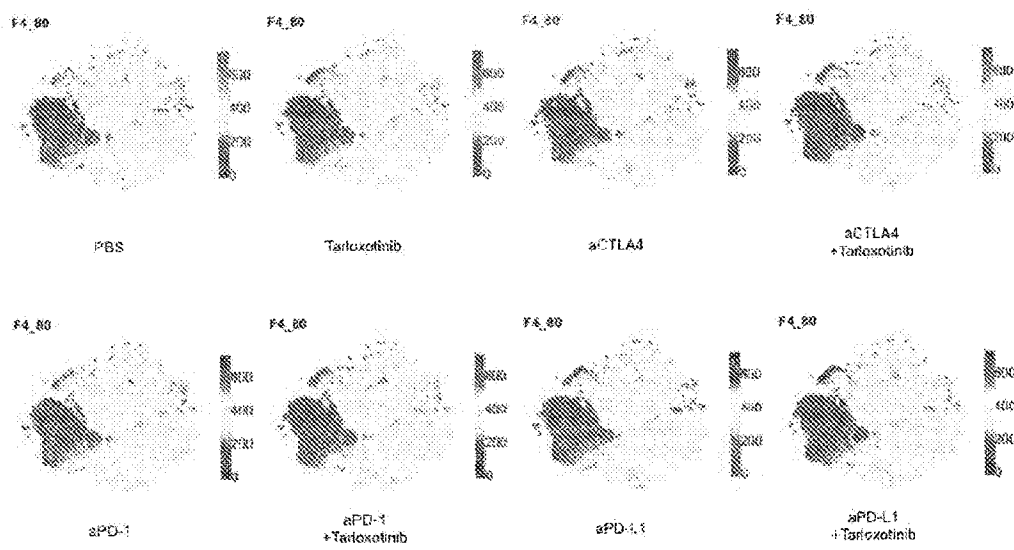


FIG. 28

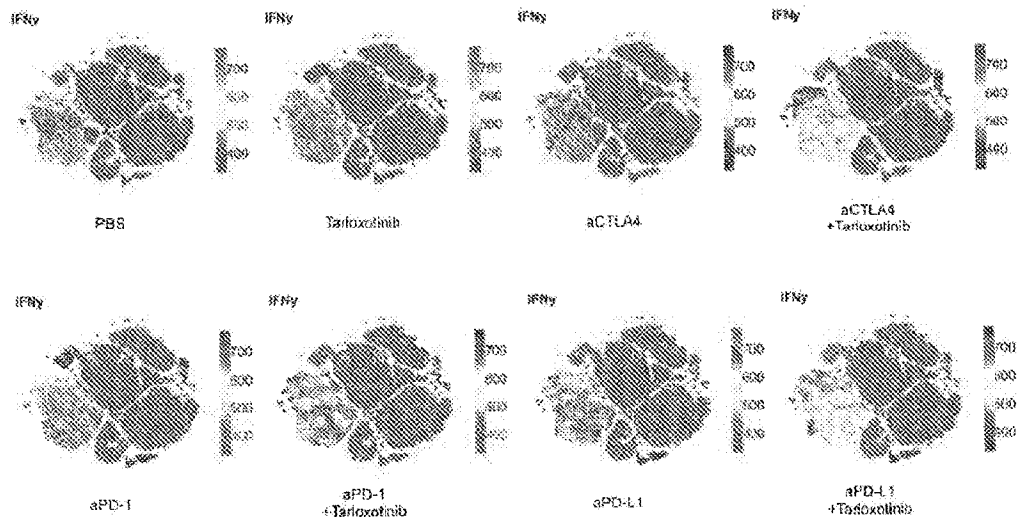


FIG. 29

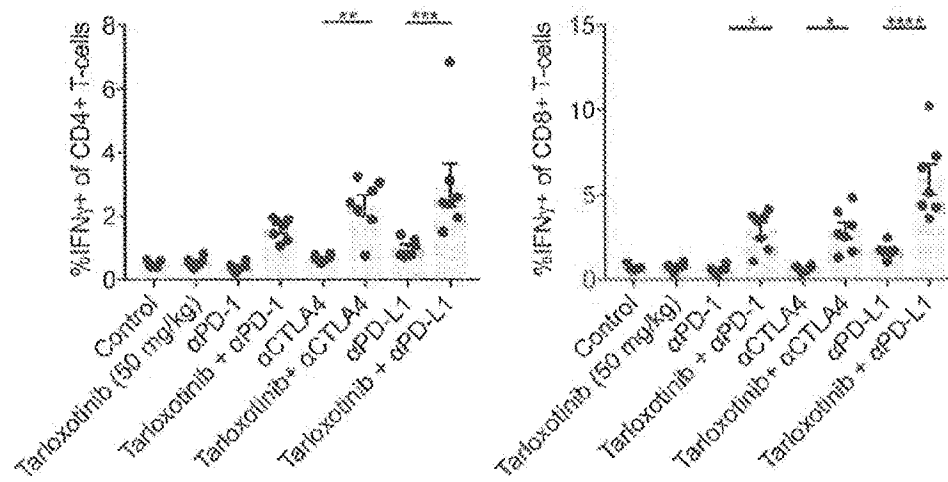


FIG. 30

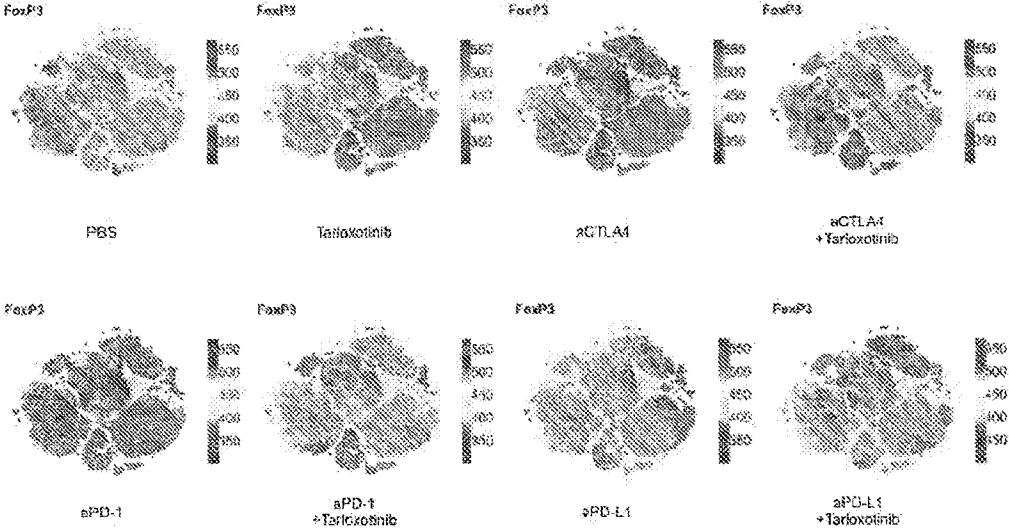


FIG. 31

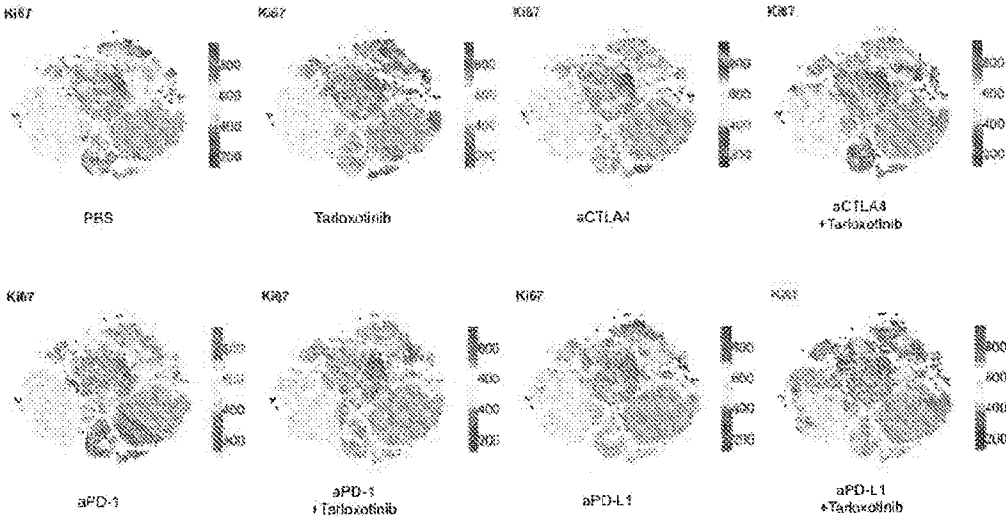


FIG. 32

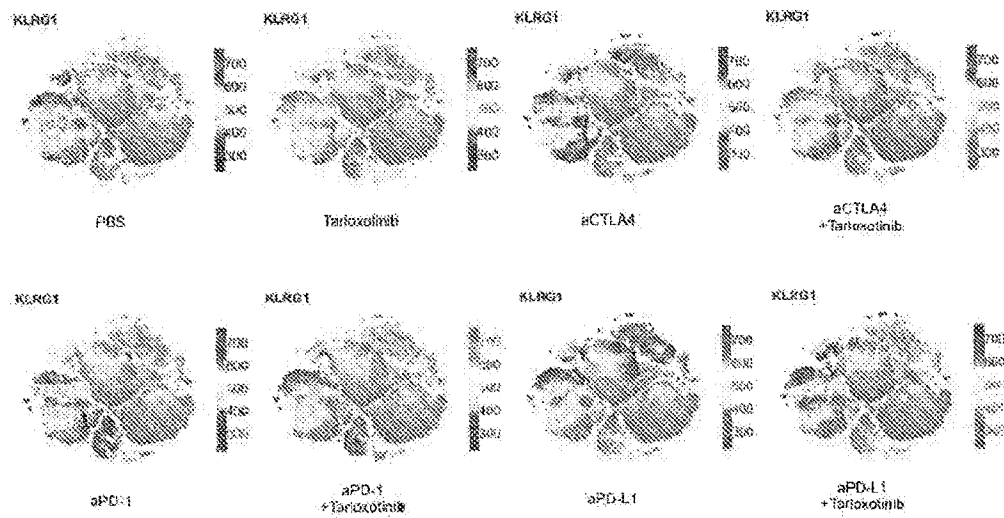


FIG. 33

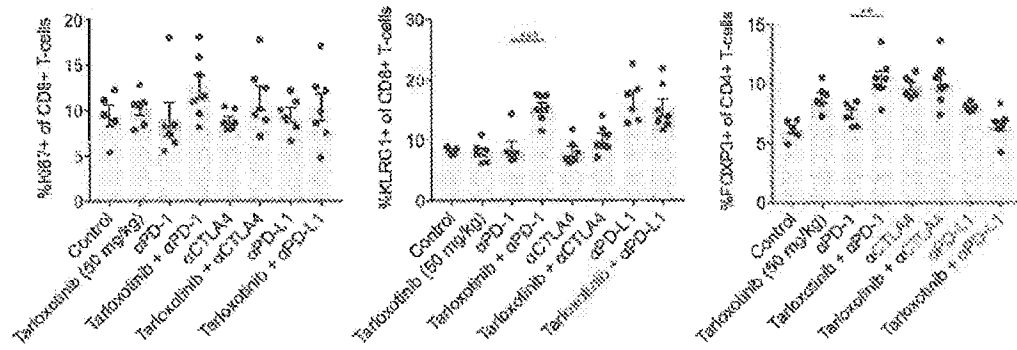


FIG. 34

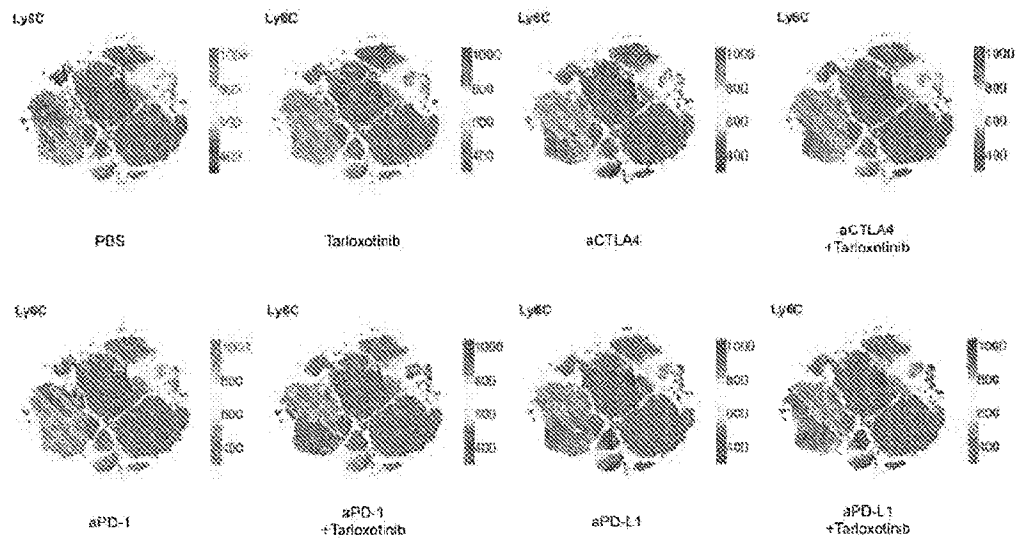


FIG. 35

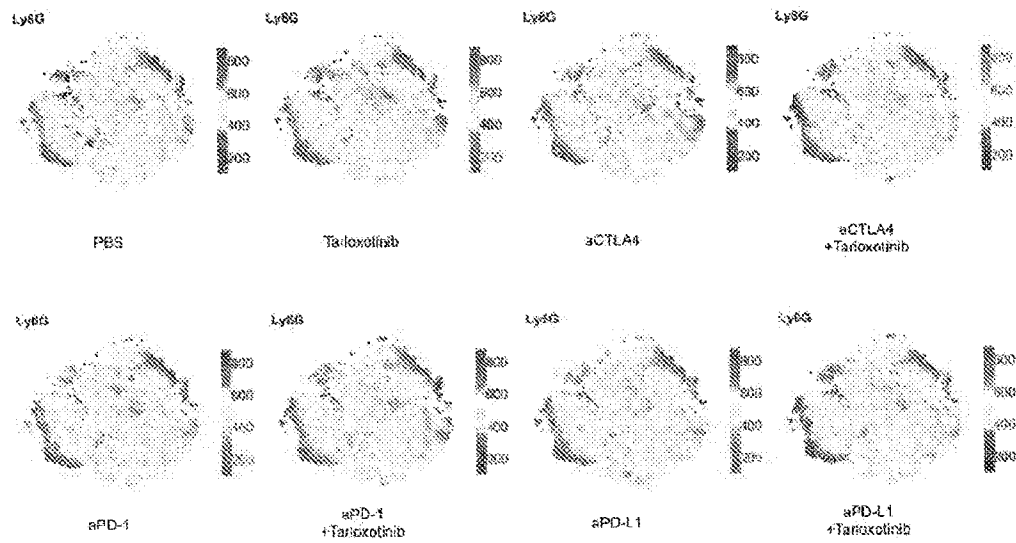


FIG. 36

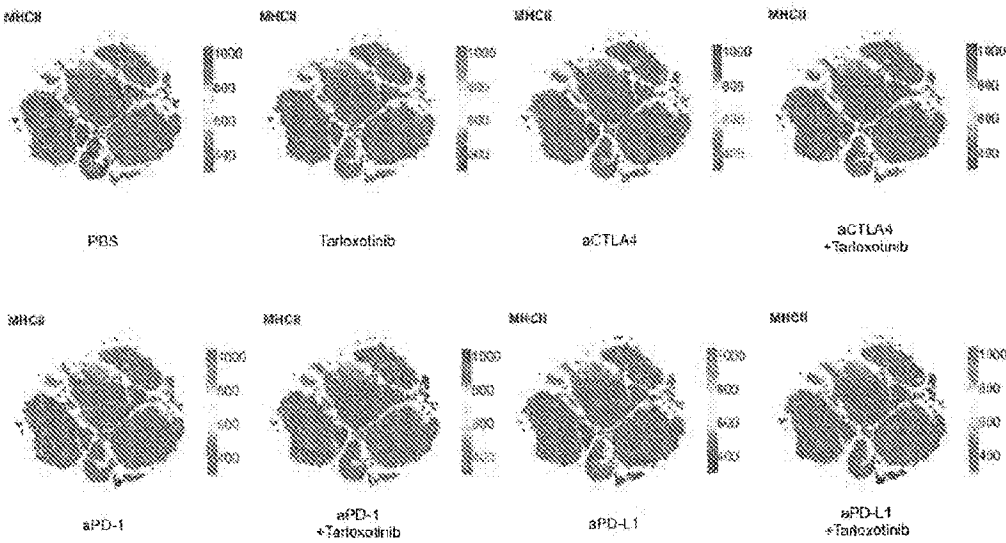


FIG. 37

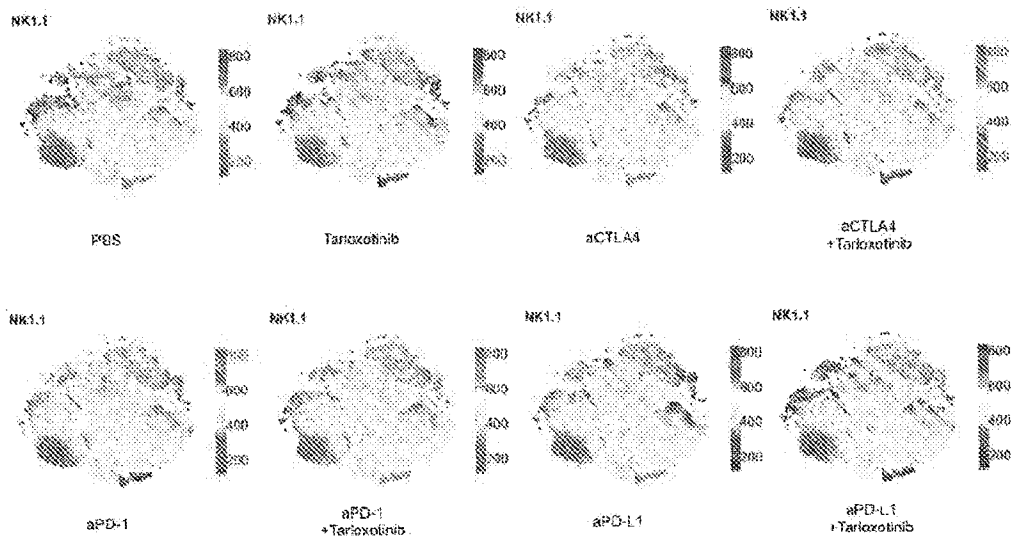


FIG. 38

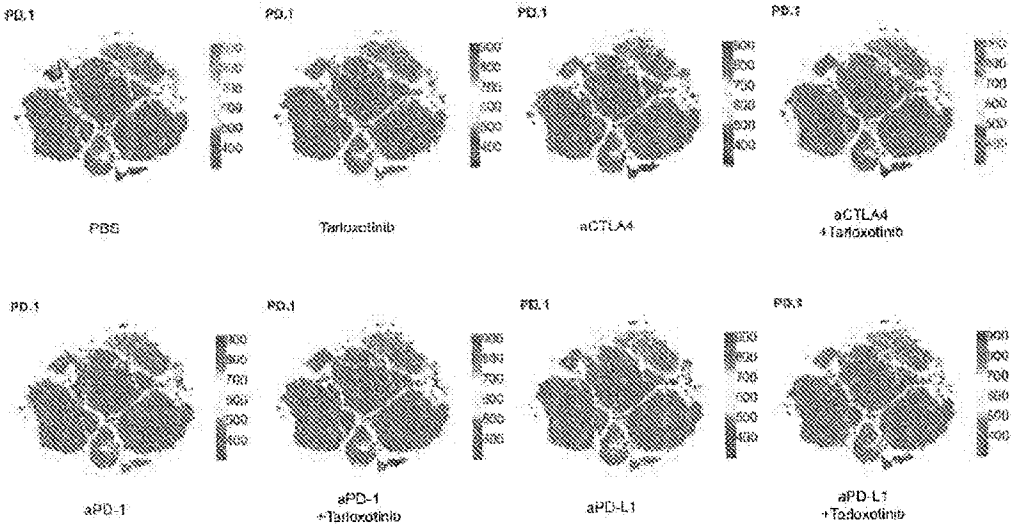


FIG. 39

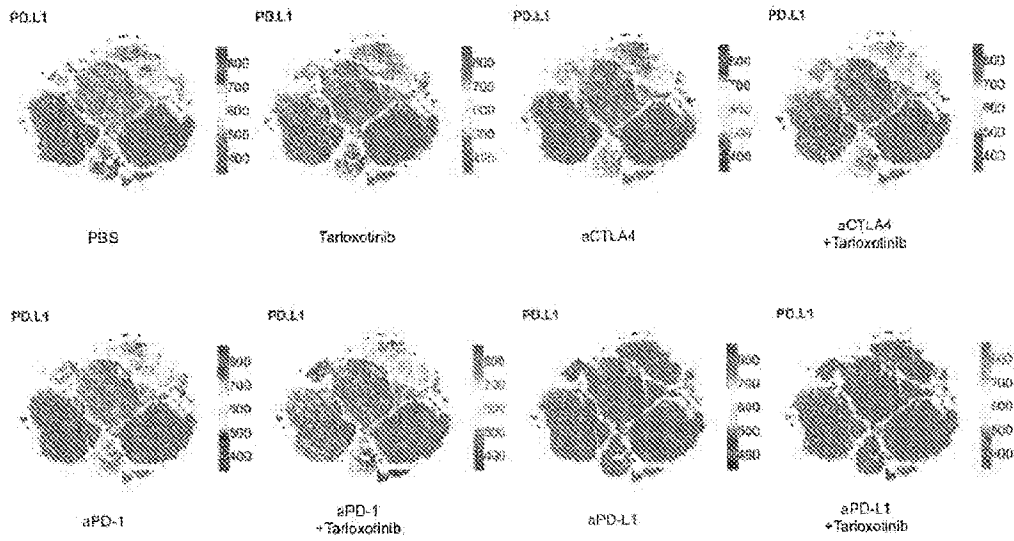


FIG. 40

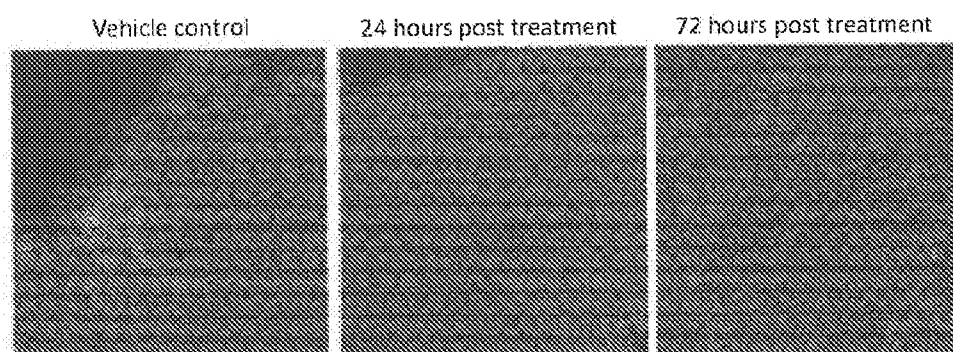


FIG. 41

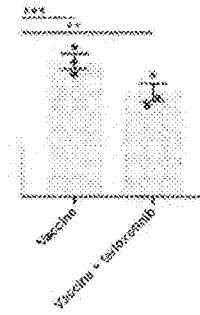


FIG. 42

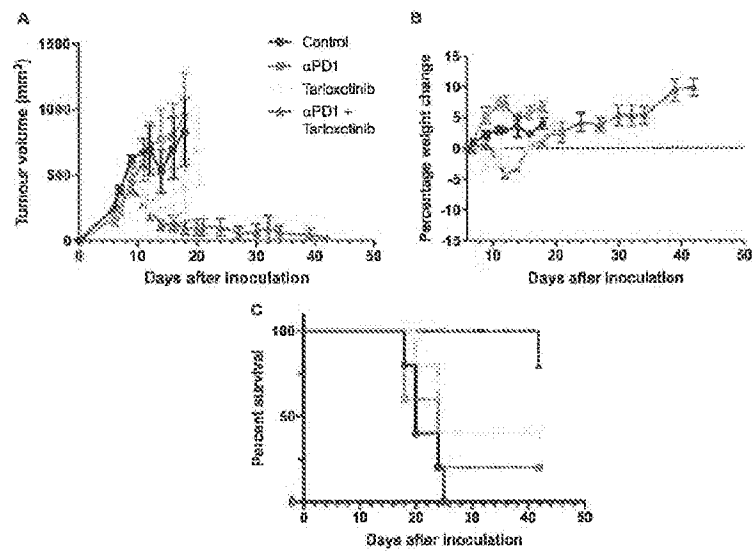


FIG. 43

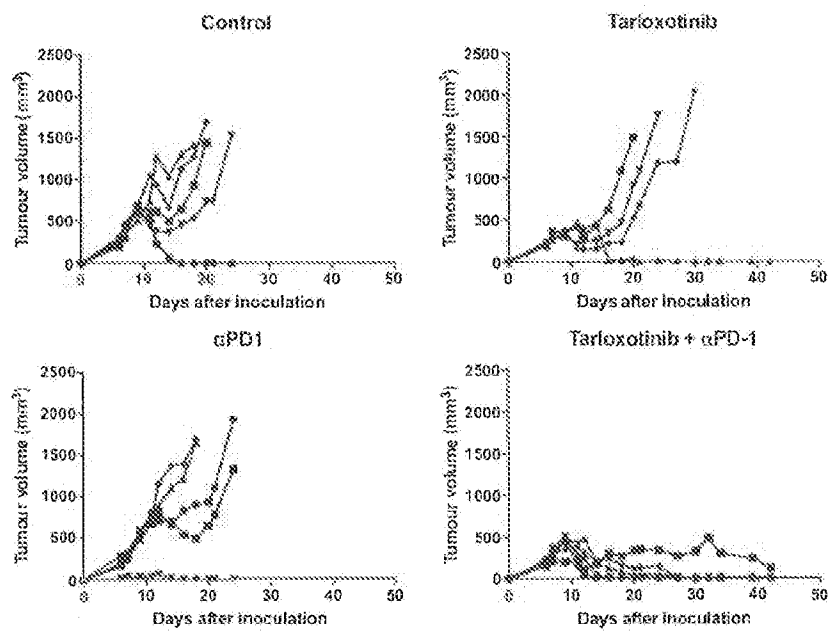


FIG. 44

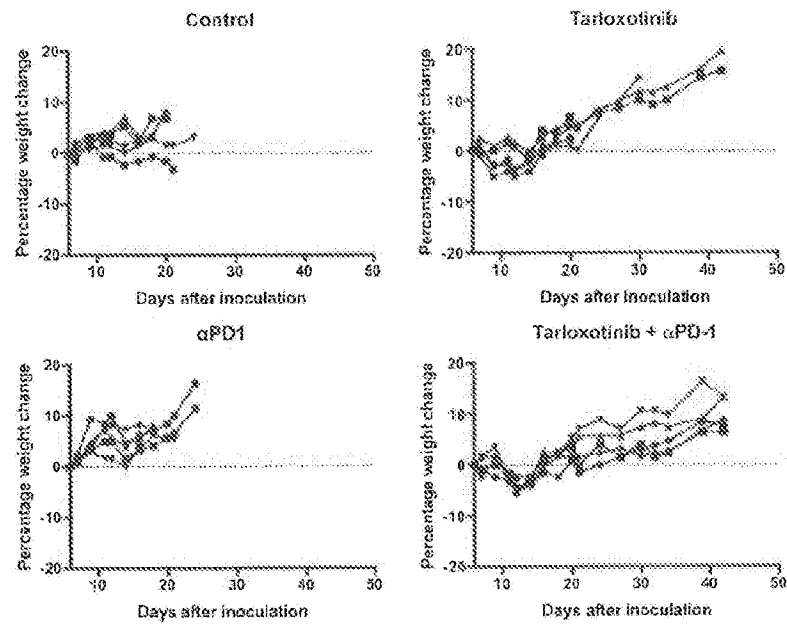


FIG. 45

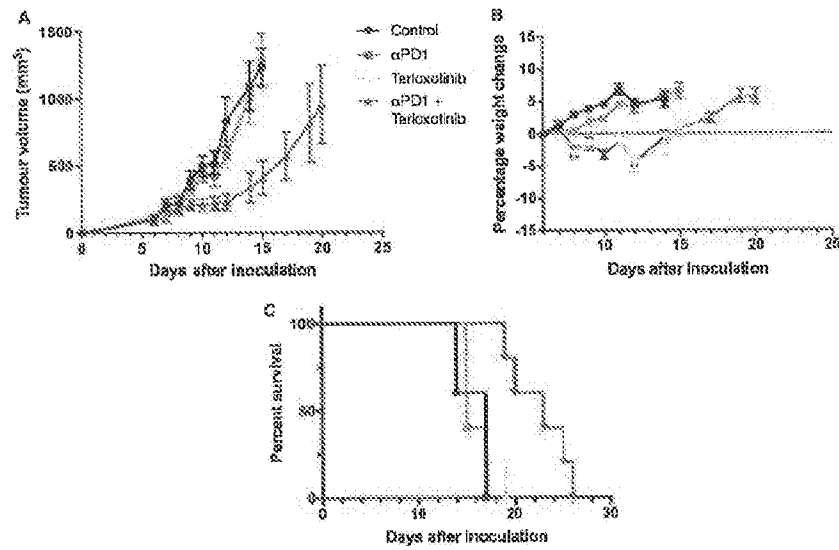


FIG. 46

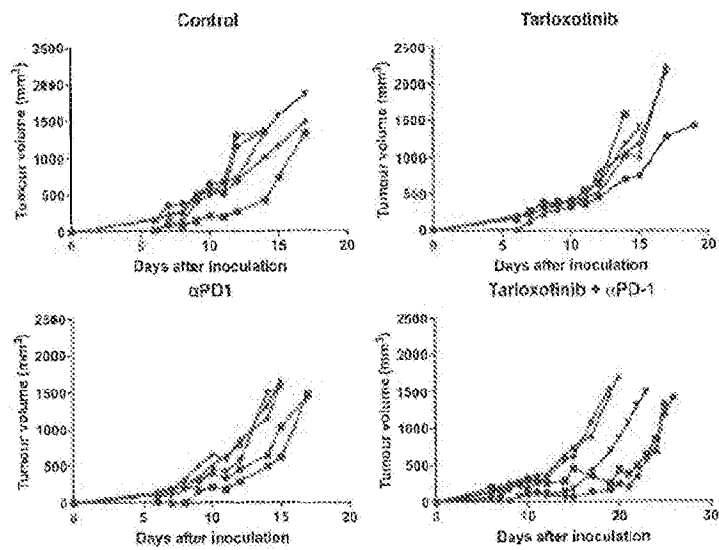


FIG. 47

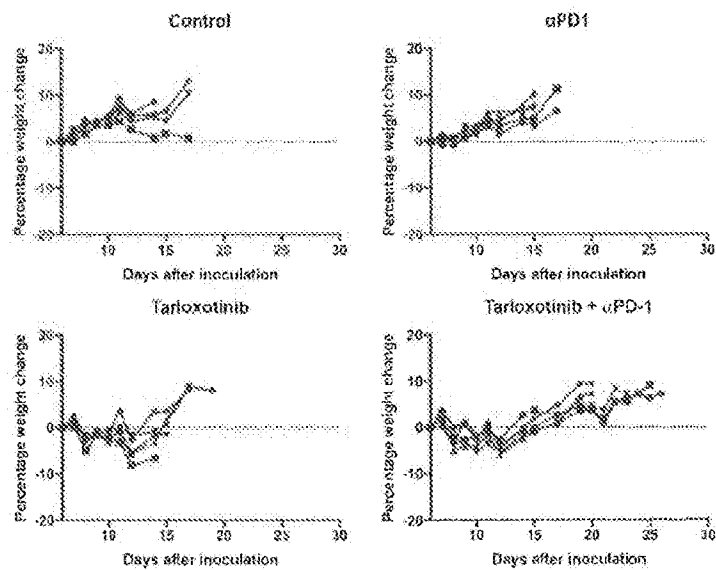


FIG. 48

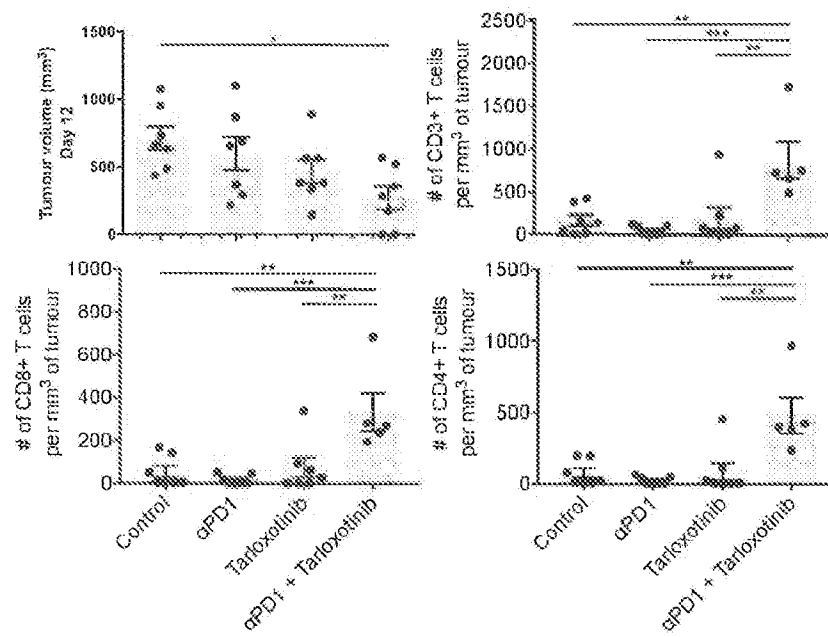


FIG. 49

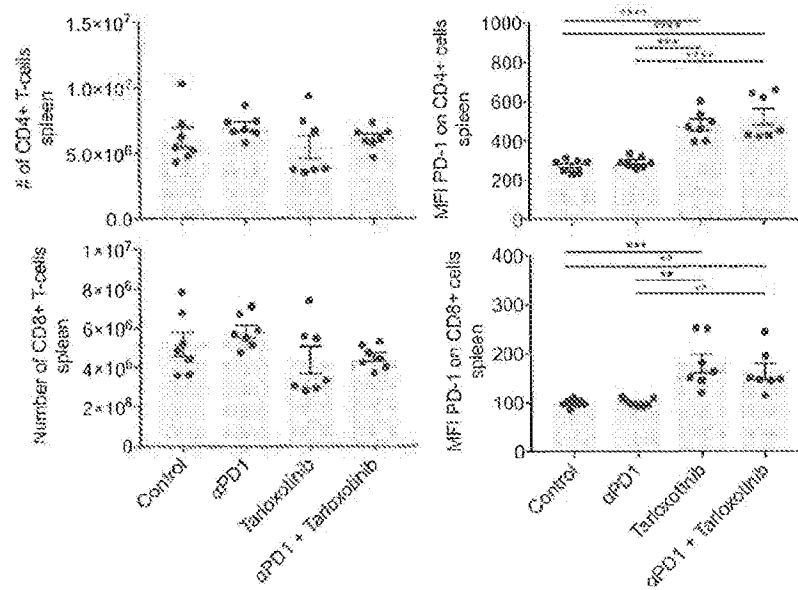


FIG. 30

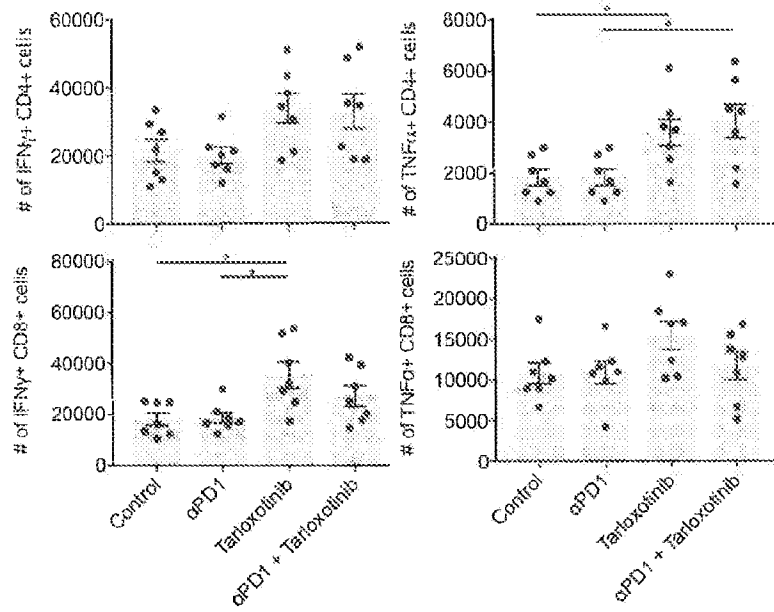


FIG. 31

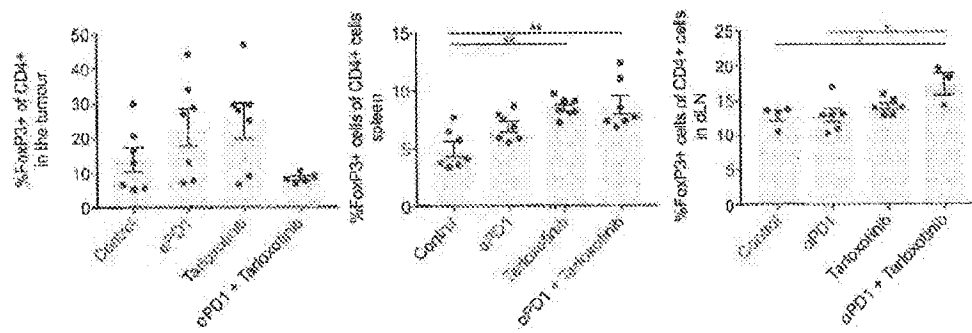


FIG. 52

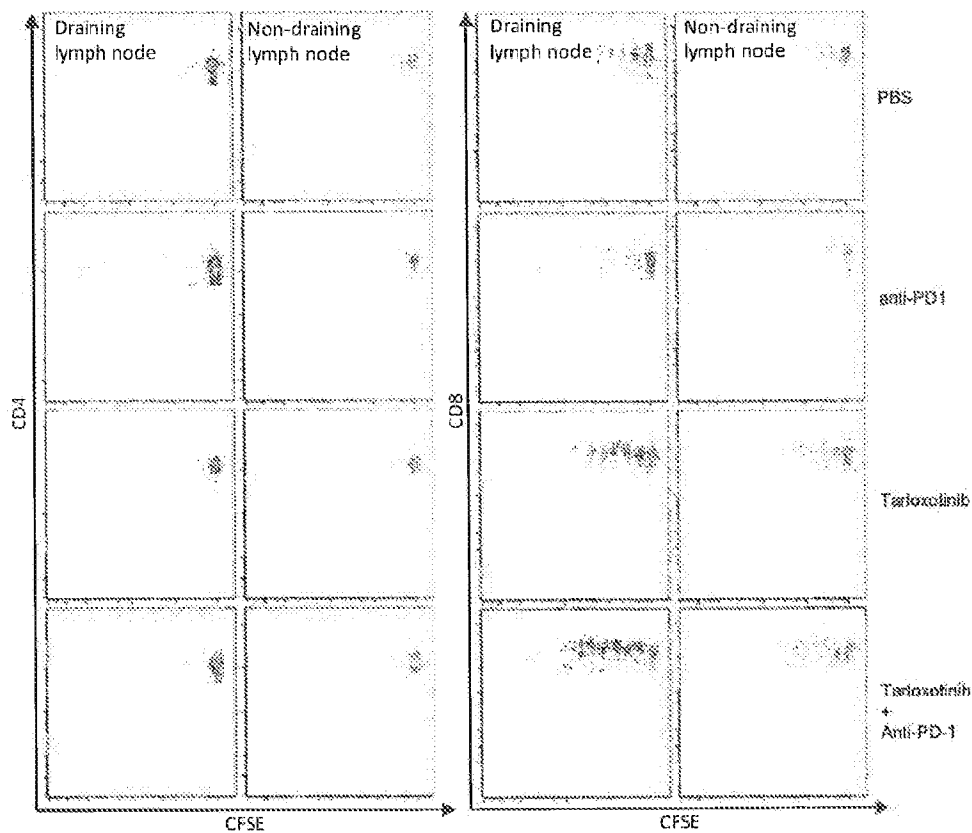


FIG. 53

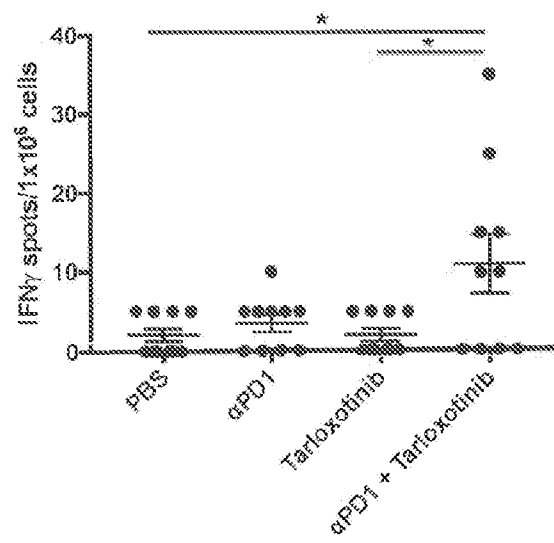


FIG. 54

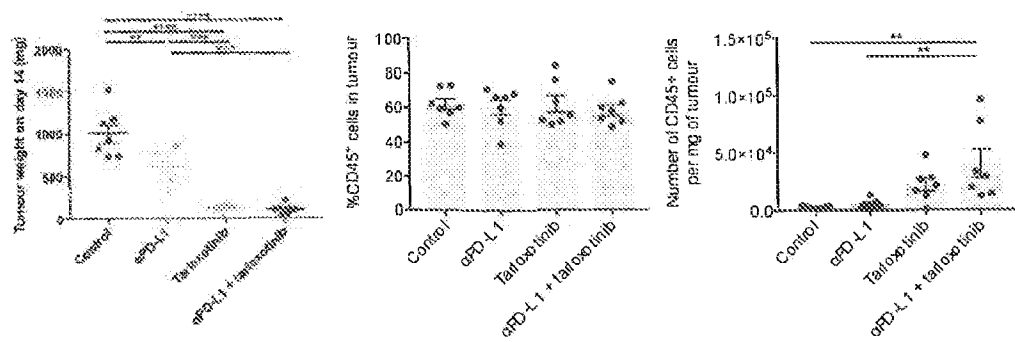


FIG. 55

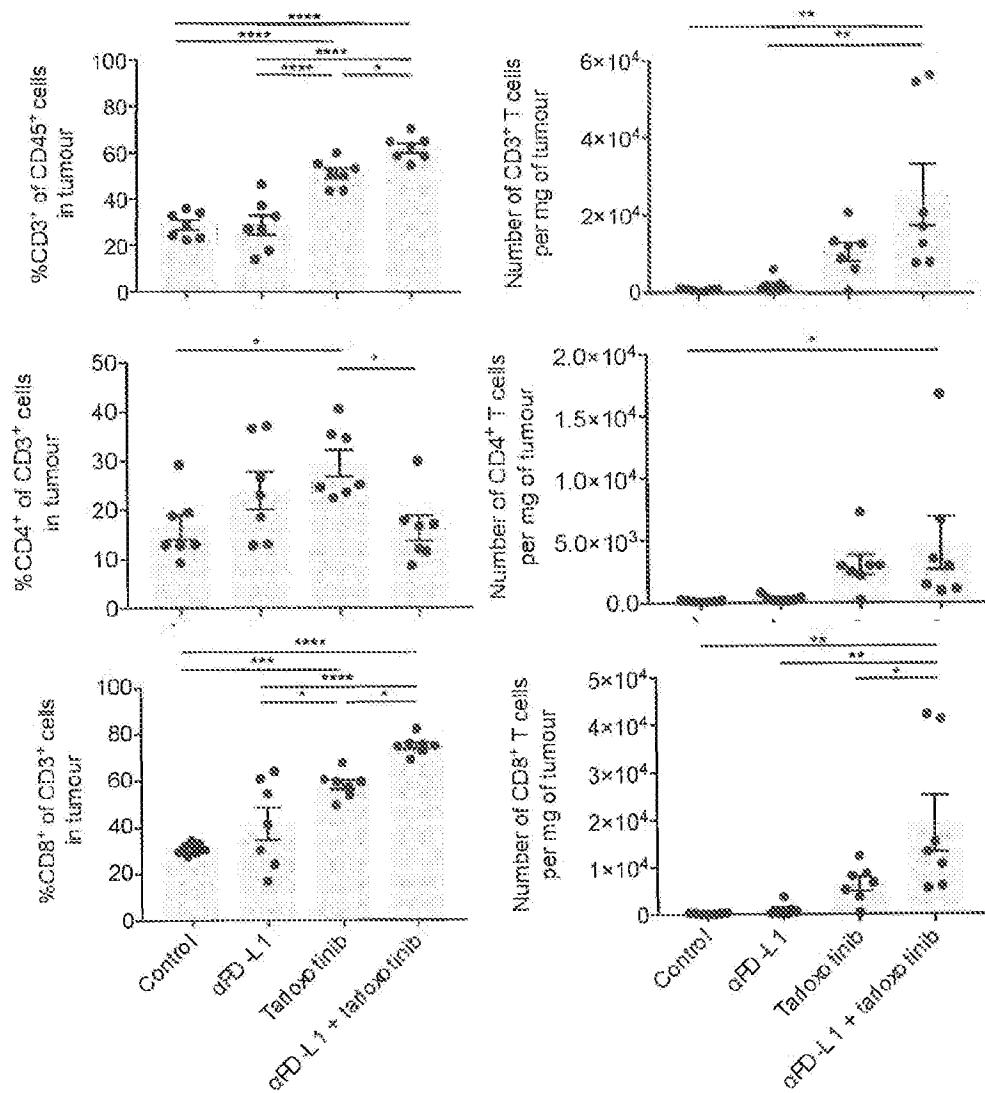


FIG. 56

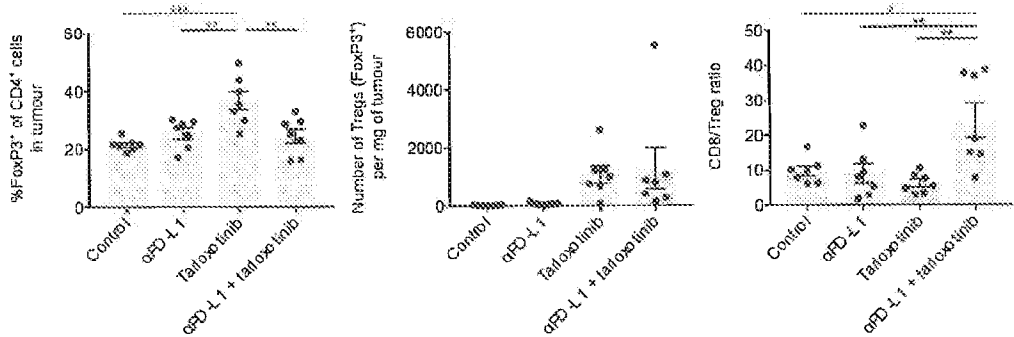


FIG. 57

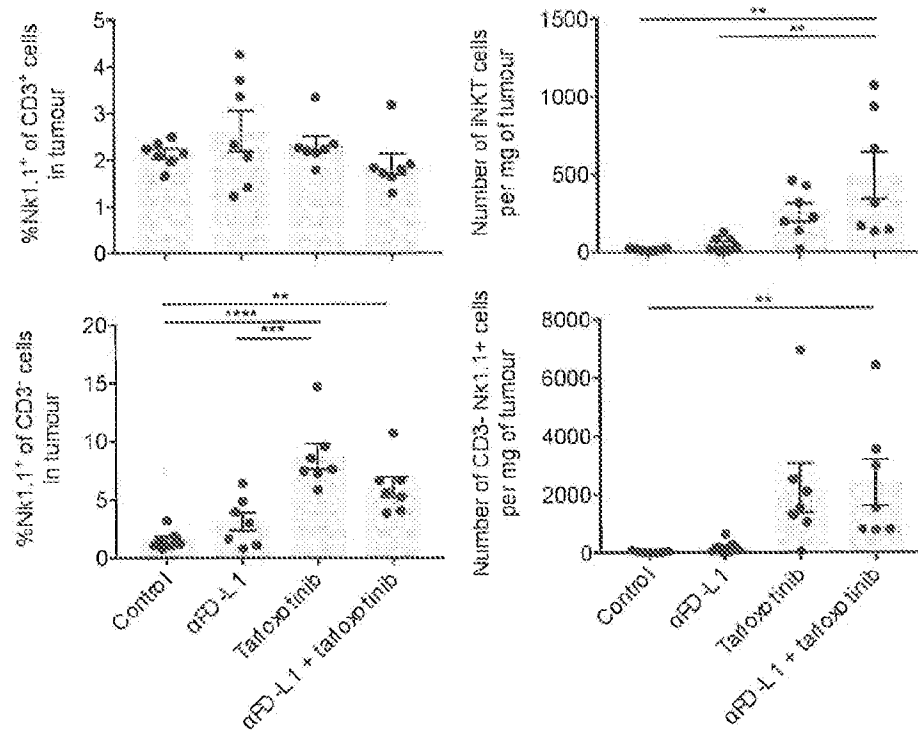


FIG. 58

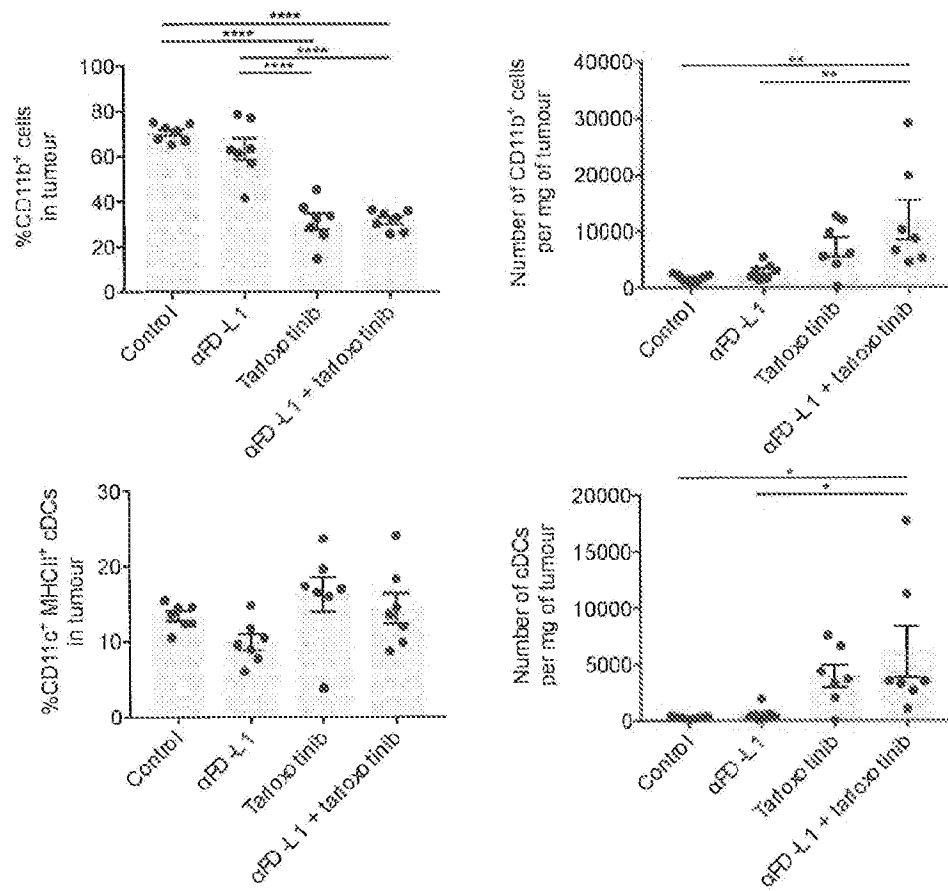


FIG. 59

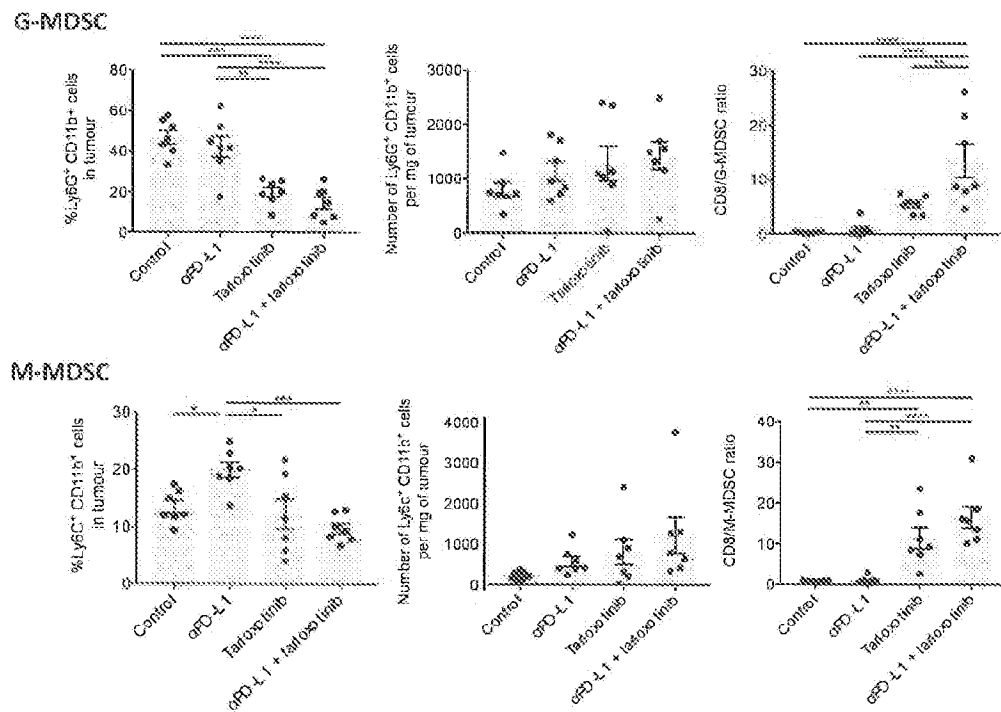


FIG. 60

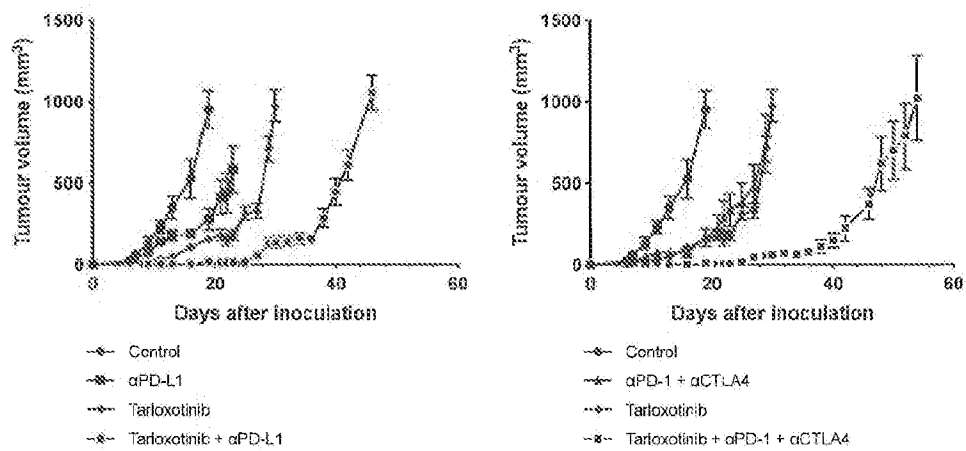


FIG. 61

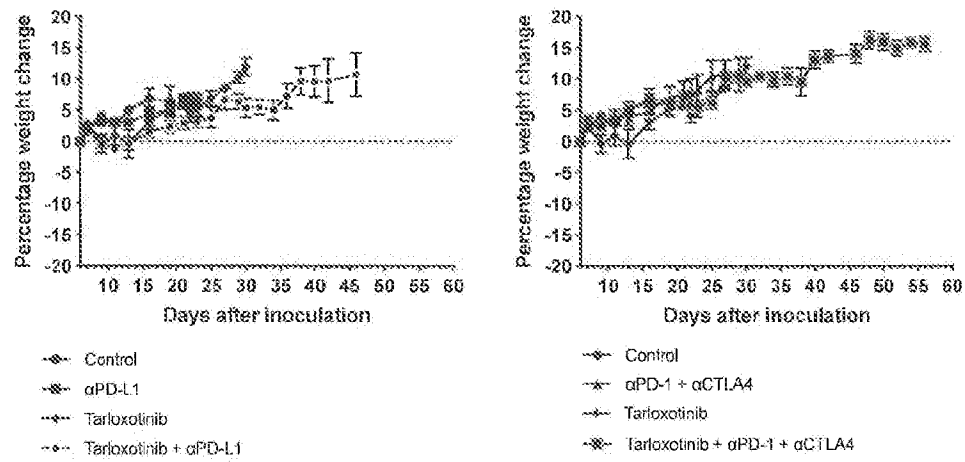


FIG. 62

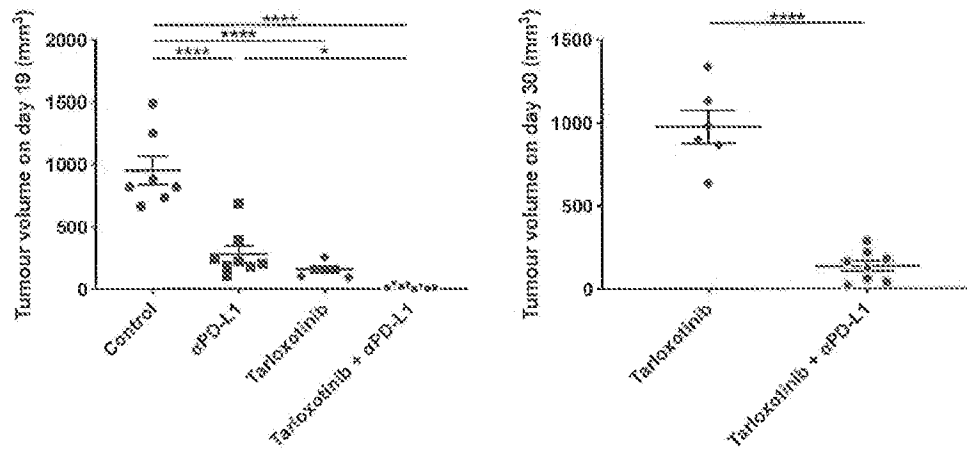


FIG. 63

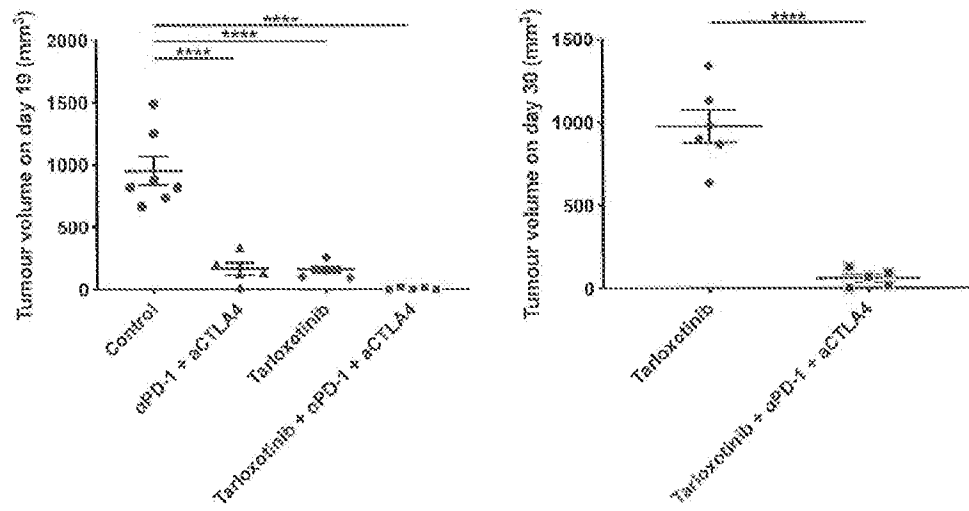


FIG. 64

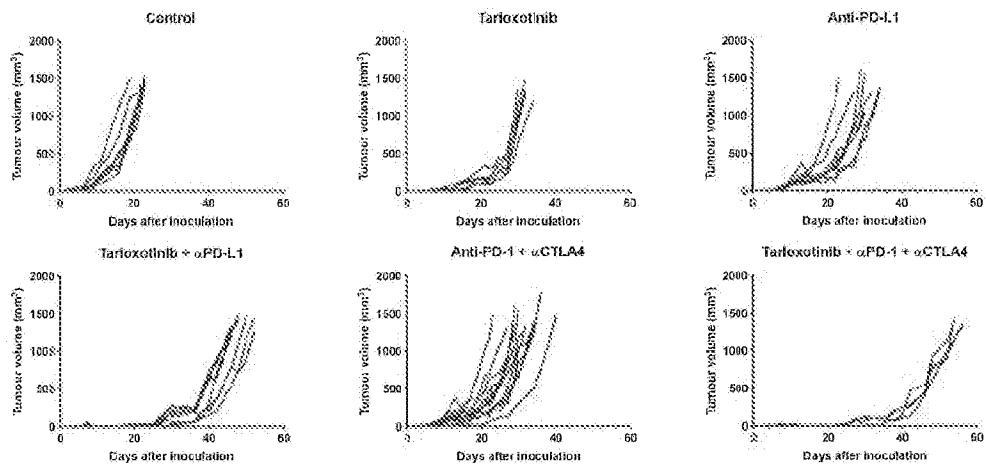


FIG. 65

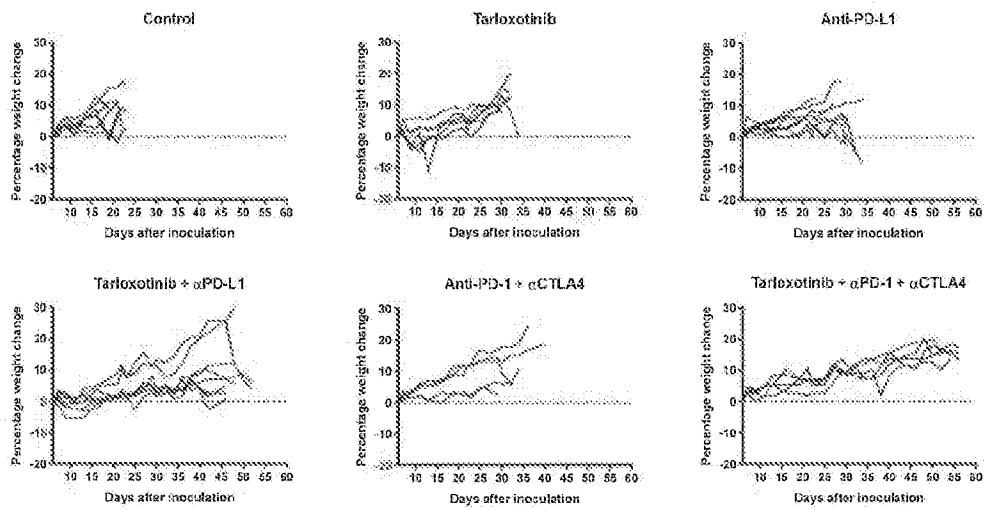


FIG. 66

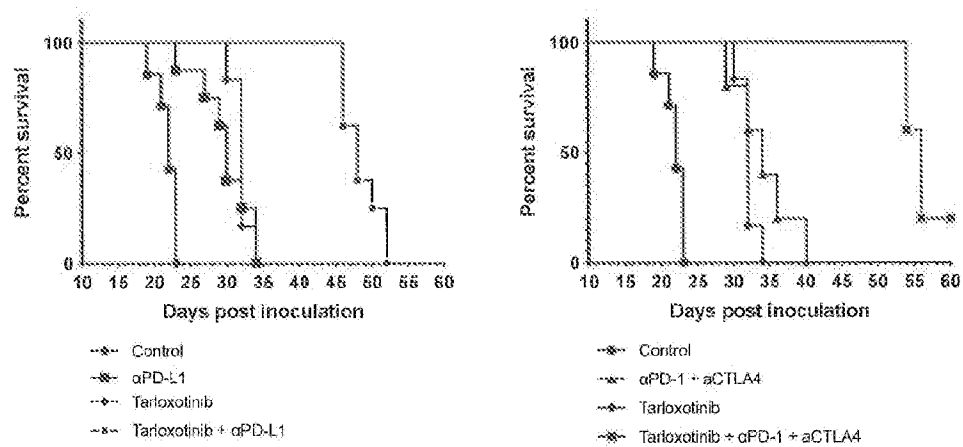


FIG. 67

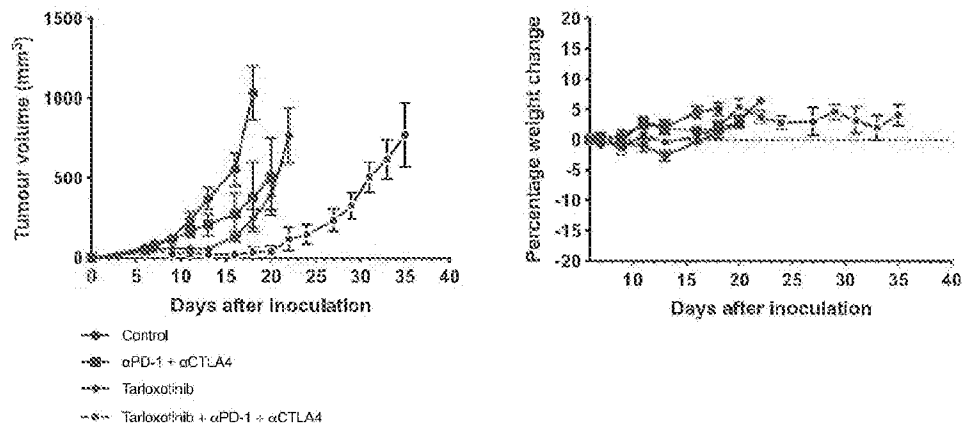


FIG. 68

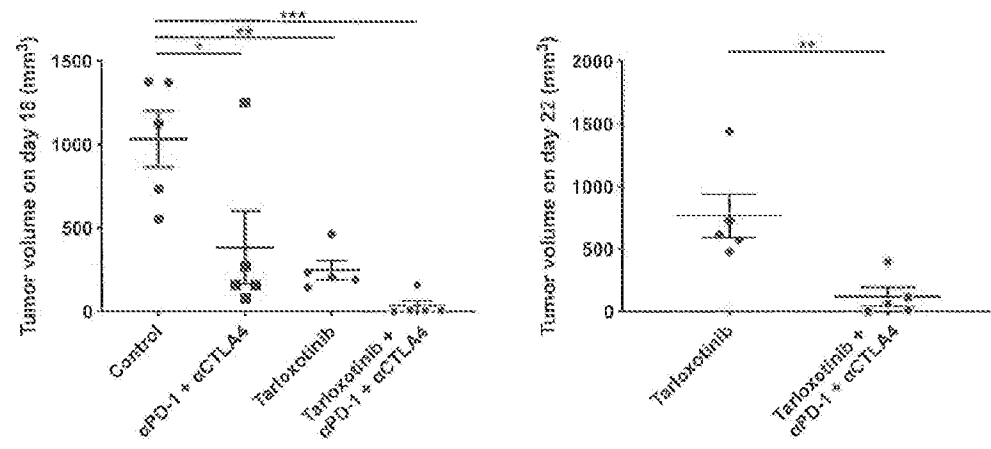


FIG. 69

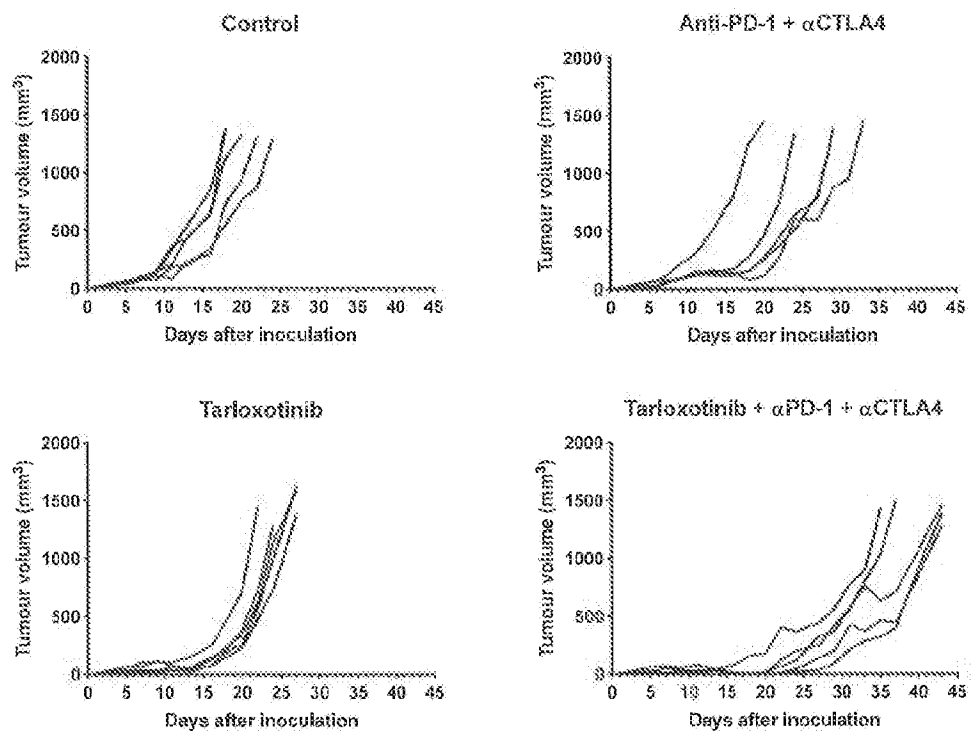


FIG. 70

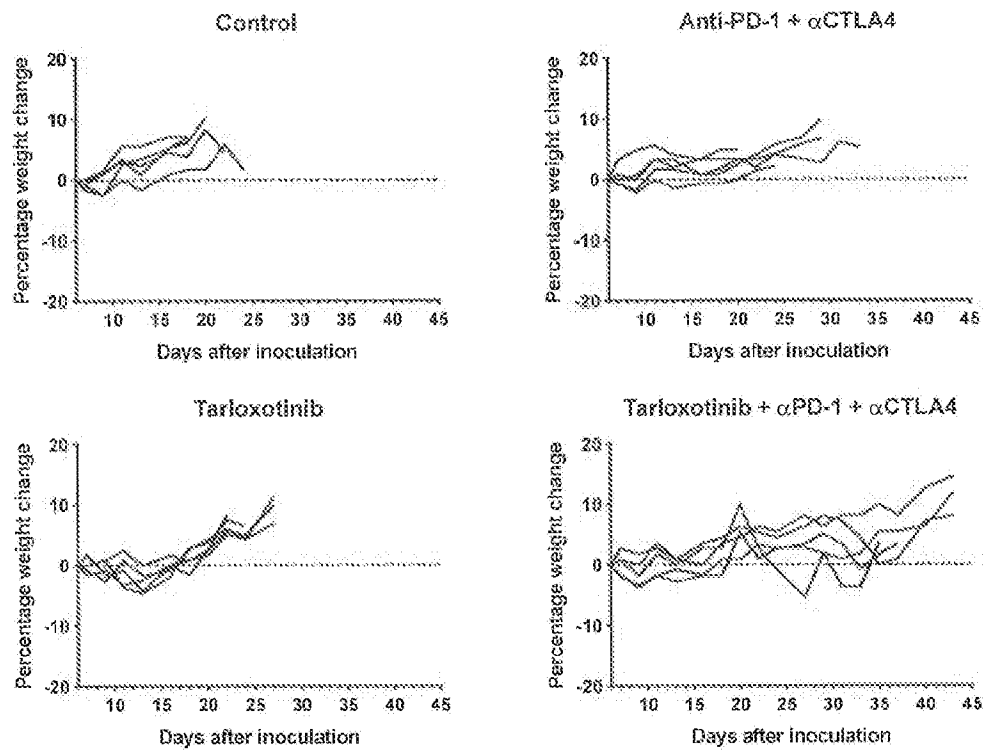


FIG. 71

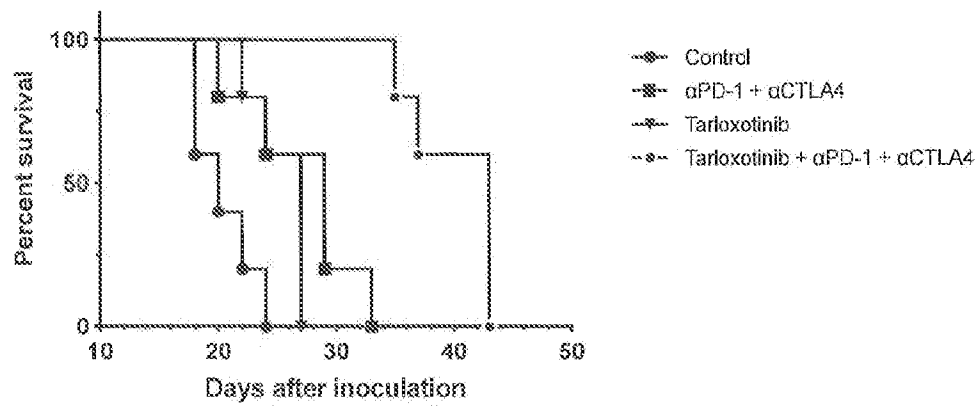


FIG. 72

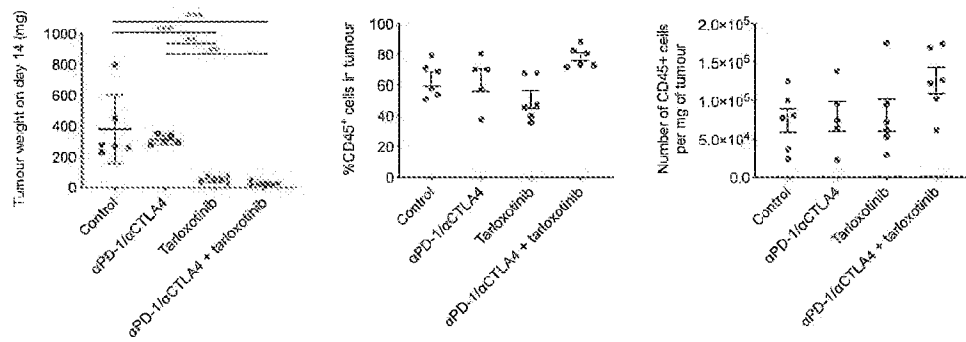


FIG. 73

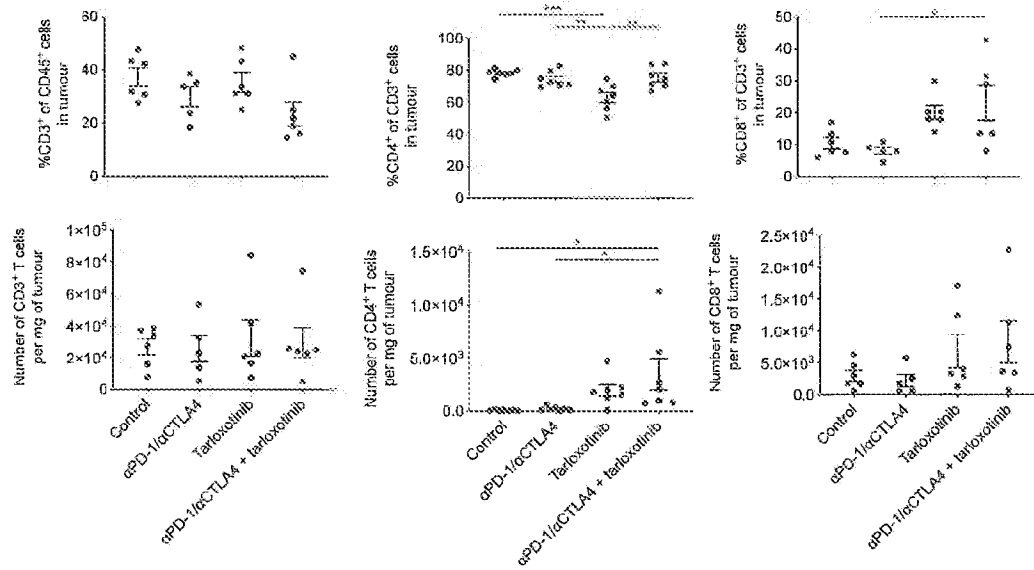


FIG. 74

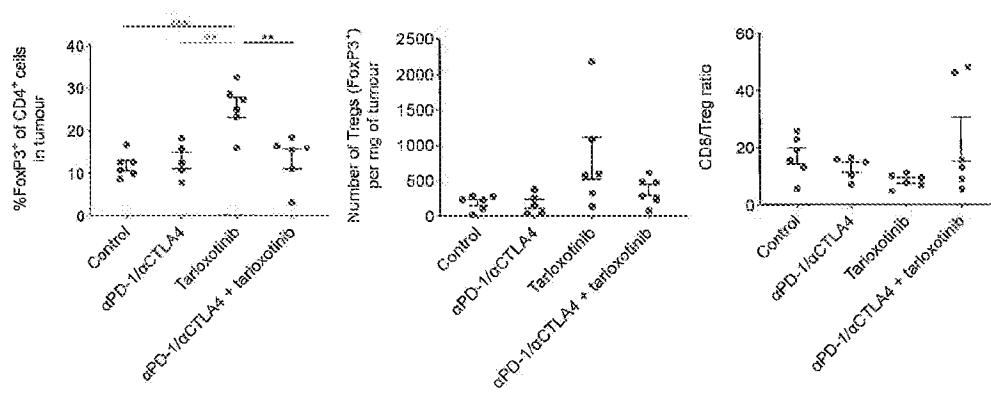


FIG. 75

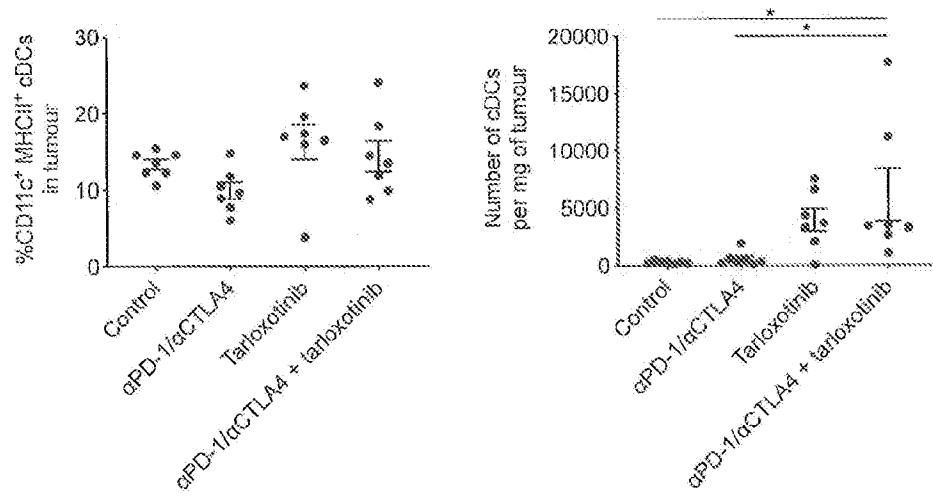
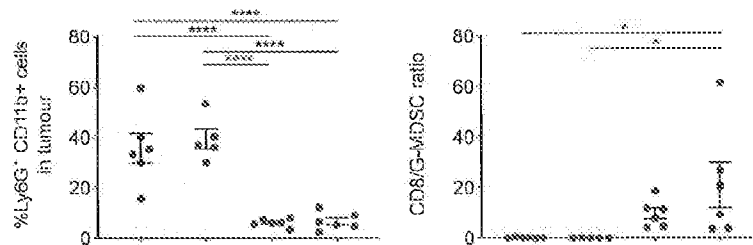


FIG. 76

G-MDSC



M-MDSC

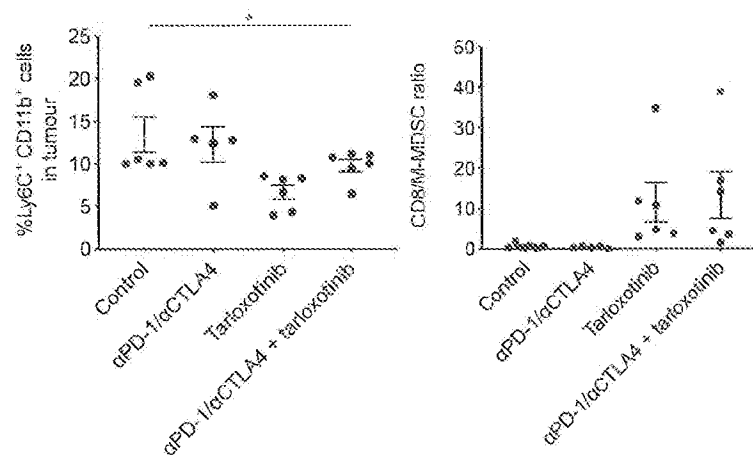


FIG. 77

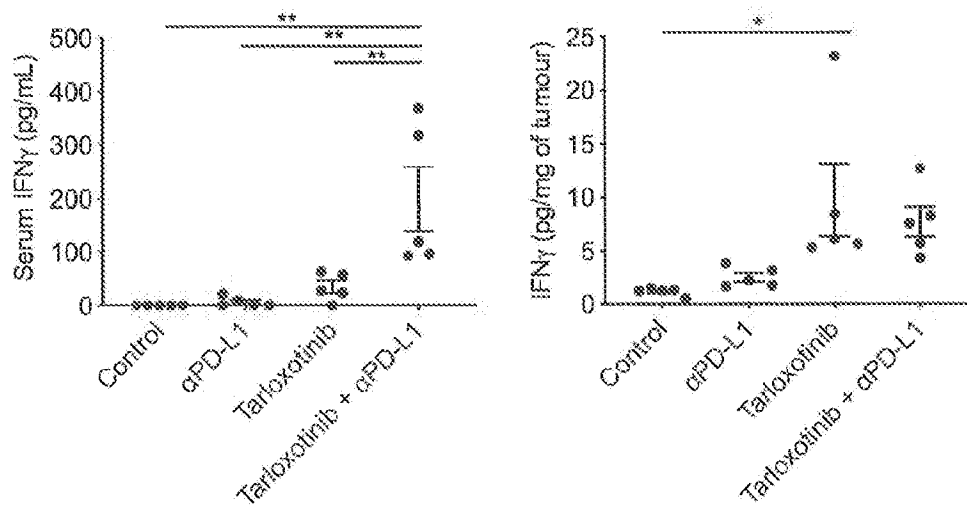


FIG. 78

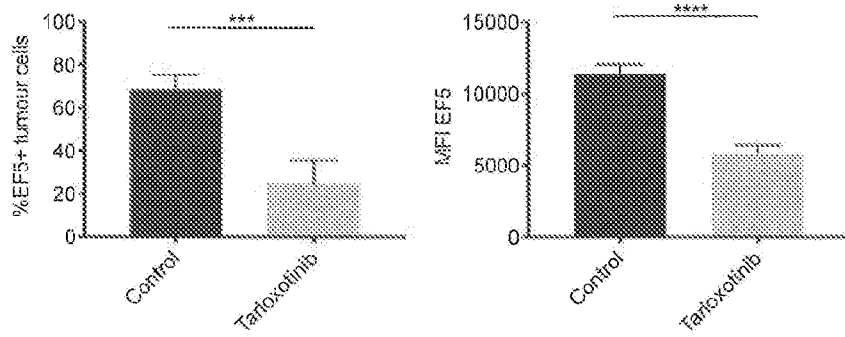


FIG. 79

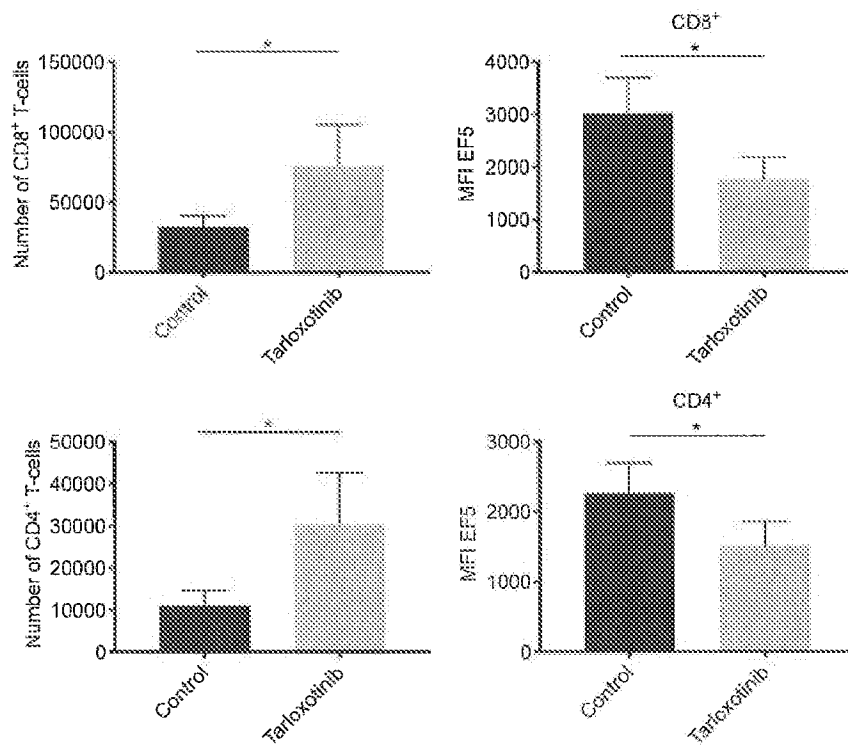


FIG. 80

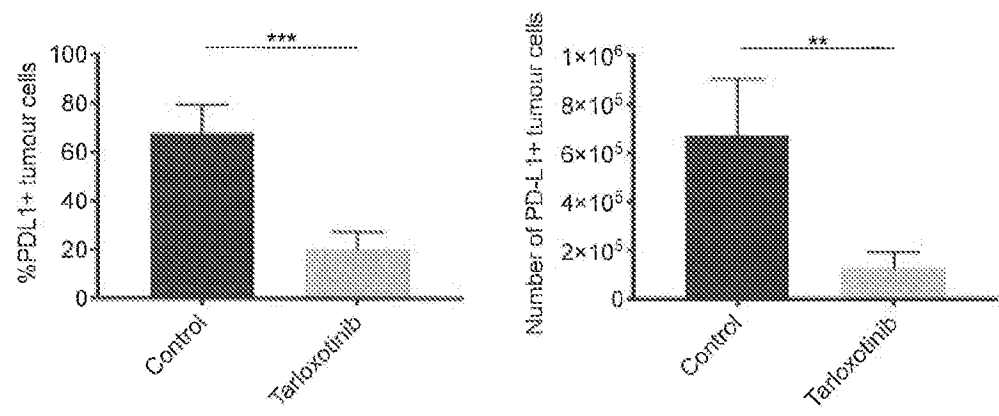


FIG. 81