

What is claimed is:

1. An *in vitro* method predictive of a positive or negative response to immune checkpoint inhibitor (ICI) therapies and/or combination therapies to alter the level, form, and localization of immune checkpoint proteins using a modulator in a biological sample or subject to produce a more robust response to immune checkpoint protein inhibitor (ICI) and/or to identify, monitor, prognose, and predict responsiveness to an ICI monotherapy regime and/or combination regimes comprising:

a) exposing a biological sample obtained from a ~~or~~ subject which—who would otherwise not optimally respond to immune checkpoint inhibitors to ~~the~~ a modulator to produce a ~~robust~~ response; and/or

b) measuring a static biomarker(s) and/or determining the difference between dynamic biomarker(s) in an unmodulated portion of the sample and after contacting one or more portions of the sample with one or more modulators ~~*ex vivo*, prior to, during, simultaneously with, throughout, or following the administration of ICI monotherapies and/or combination therapies;~~ wherein the difference in the basal and modulated biomarker(s) and the modulated portion(s) of the sample is expressed as a value which is predictive of a positive or negative response to ICI ~~monotherapies~~ and/or combination therapies.

2. The method of claim 1, wherein the sample is from:

(i) a subject with cancer;-

~~The method of claim 2, optionally~~ wherein the cancer is selected from the group consisting of colorectal, esophageal, stomach, lung, mesothelioma, prostate, uterine, breast, skin, endocrine, melanoma, urinary, pancreas, ovarian, cervical, head and neck, liver, bone, biliary tract, small intestine, hematopoietic/blood cancers (myeloma, leukemia, and lymphoma), vaginal, testicular, anal, kidney, brain, eye cancer, leukemia, lymphoma, soft tissue, melanoma, mixed types, and metastases thereof,

optionally wherein the tumor sample is from a solid tumor;- or

3. ~~—~~ (ii)

4. ~~The method of claim 1, wherein the sample is from single or multiple tumor tissues~~ of an unknown primary or any type of tumor.

5. ~~The method of claim 3, wherein the tumor sample is from a solid tumor.~~

6. ~~The method of claim 5, wherein the tumor sample is obtained by fine needle aspiration, core biopsy, collecting circulating tumor cells, surgical excision, or other tumor sample acquisition method.~~

~~7.3.~~ The method of claim 1 or 2, further comprising stratification of patients on their predicted responsiveness to a therapeutic agent or therapeutic regimen and providing a positive or negative treatment value which corresponds to positive or negative clinical outcome, respectively, and/or:

~~8.~~ ~~The method of claim 1, wherein the method is further used to identify, monitor, predict the existence of and characterize functional sub-populations of cancer cells, which can be used to predict a positive or negative response to ICI ~~mon~~otherapies and/or combination therapies.~~

~~9.~~ ~~The method of claim 8, optionally wherein the identification and characterization of functional sub-populations can be used to predict and identify the mechanisms of innate and acquired resistance to ICI ~~mon~~otherapies and/or combination therapies.~~

~~10.4.~~ The method according to any one of claims 1-3, wherein the biological sample is:

(i) a cancer cell or a cancer cell subpopulation, wherein the cancer cell or cancer cell subpopulation comprises a cancer stem cell that expresses one or more of CD133, CD44, ABCG2, and/or ALDH1A1 and/or that does not stain with Hoechst; or

~~11.~~ ~~The method of claim 1, wherein the biological sample is~~ (ii) an immune cell or an immune cell subpopulation, wherein the immune cell or immune cell subpopulation comprises T cells, CD8⁺ T cells, exhausted T cells, active T cells, B cells, NK cells, dendritic cells, myeloid derived suppressor cells, macrophages, granulocytes, and mast cells.

~~12.5.~~ The method according to any one of claims 1-4, wherein the modulator is a biologic agent, biosimilar, derivative, mutant, peptide, fragment, analog, or mimetic of the modulator; optionally wherein the modulator is:

~~13.~~ ~~The method of claim 12, wherein the modulator is~~ (i) an interferon (IFN) optionally selected from:

~~14.~~ ~~The method of claim 13, wherein the interferon is IFN- α , IFN- β , or IFN- γ .~~ or:

~~15.~~ ~~The method of claim 12, wherein modulator is~~ (ii) an interleukin (IL); optionally selected from

~~16.~~ ~~The method of claim 15, wherein the interleukin is IL-1, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, IL-22, IL-23, IL-27, or IL-35.~~ or

~~17.~~ ~~The method of claim 12, wherein the modulator is~~ (iii) fibroblast growth factor (FGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2), prostacyclin (PGI2), prostaglandin D2 (PGD2) and prostaglandin F2 α (PGF2 α), indoleamine-pyrrole 2,3-dioxygenase (IDO), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF α), tumor growth factor beta (TGF β), a derivative

thereof, a mutant thereof, a peptide thereof, a fragment thereof, an analog thereof or a mimetic thereof;:-

18. ~~The methods of claims 12, and optionally~~ wherein the biologic agent, biosimilar, derivative, mutant, peptide, fragment, analog or mimetic of the modulator enhances or decreases half-life and/or target binding, and has a similar effect as the intact modulator.

19. ~~The method of claim 1, wherein the biological sample/cancer subject is treated in a manner to alter conditions selected from the group consisting of abundance of proteins, carbohydrates, lipids, molecules found within fetal bovine serum, and growth factors, hypoxia, oxidative stress, and physical exercise/stress.~~

20. ~~The method of claim 1, wherein the sample is modulated using a patients' "milieu" from a tumor biopsy optionally consisting of various cell types selected from the group consisting of fibroblasts, inflammatory and immune cells, and soluble factors derived from tumor and associated cells.~~

21. ~~The method of claim 1, wherein the sample is modulated using the patients' own immune cells selected from the group consisting of those derived from tumors and peripheral blood immune cells, wherein the immune cells derived from tumors are selected from the group consisting of T cells, B cells, Natural Killer (NK) cells, macrophages and dendritic cells, myeloid derived suppressor cells and granulocytes, and wherein the immune cells derived from peripheral blood are selected from the group consisting of T cell, B cells, NK cells, monocytes, dendritic cells, tumor cells, and granulocytes.~~

22.6. The method according to any one of claims 1-5, wherein multiple modulators are added together, sequentially or alternately,:- and/or

23. ~~The method of claim 1, wherein the modulator is a reactive oxygen species, a free radical molecule, or radiation therapy selected from the group consisting of x-rays, gamma rays, and charged particles,:- or a phenothiazine, a chemotherapeutic agent, a molecularly targeted agent or an autophagy influencer; optionally wherein~~

24. ~~The method of claim 1, wherein the modulator is a phenothiazine.~~

25. ~~The method of claim 24, wherein (i) the phenothiazine is prochlorperazine, and/or:-~~

26. ~~The method of claim 1, wherein the modulator is a chemotherapeutic agent.~~

27. ~~The method of claim 26, wherein (ii) the chemotherapeutic agent is an alkylating agent:-~~

- ~~28. The method of claim 27, wherein the alkylating agent is such as a mustard gas derivative, selected from the group consisting of mechlorethamine, cyclophosphamide, chlorambucil, melphalan, and ifosfamide;:-~~
- ~~29. The method of claim 27, wherein the alkylating agent is an ethylenimine, selected from the group consisting of thiotepa and hexamethylmelamine; or:-~~
- ~~30. The method of claim 27, wherein the alkylating agent is an alkylsulfonate, selected from the group consisting of busulfan;:- or~~
- ~~31. The method of claim 27, wherein the alkylating agent is a hydrazine and/or triazine, selected from the group consisting of altretamine, procarbazine, dacarbazine and temozolomide;:- or~~
- ~~32. The method of claim 27, wherein the alkylating agent is a nitrosureas, selected from the group consisting of carmustine, lomustine and streptozocin;:- or~~
- ~~33. The method of claim 27, wherein the alkylating agent is a metal salt, selected from the group consisting of carboplatin, cisplatin, and oxaliplatin;:- or~~
- ~~34. The method of claim 26, wherein (iii) the chemotherapeutic agent is a plant alkaloid such as:-~~
- ~~35. The method of claim 34, wherein the plant alkaloid is a vinca alkaloid, selected from the group consisting of vincristine, vinblastine and vinorelbine;:- or~~
- ~~36. The method of claim 34, wherein the plant alkaloid is a taxane, selected from the group consisting of paclitaxel and docetaxel;:- or~~
- ~~37. The method of claim 34, wherein the plant alkaloid is a podophyllotoxin, selected from the group consisting of etoposide and teniposide;:- or~~
- ~~38. The method of claim 34, wherein the plant alkaloid is a camptothecin analog, selected from the group consisting of irinotecan and topotecan;:- or~~
- ~~39. The method of claim 26, (iv) wherein the chemotherapeutic agent is an antitumor antibiotic:-~~
- ~~40. The method of claim 39, wherein the antibiotic is such as an anthracycline, selected from the group consisting of doxorubicin, daunorubicin, epirubicin, mitoxantrone, and idarubicin;:- or~~
- ~~41. The method of claim 39, wherein the antibiotic is a chromomycin, selected from the group consisting of dactinomycin and plicamycin;:- or~~
- ~~42. The method of claim 39, wherein the antibiotic is mitomycin or bleomycin;:- or~~
- ~~43. The method of claim 26, wherein (v) the chemotherapeutic agent is an antimetabolite:-~~
- ~~44. The method of claim 43, wherein the antimetabolite is such as a folic acid antagonist, selected from the group consisting of methotrexate and pemetrexed;:- or~~

- 45.** ~~The method of claim 43, wherein the antimetabolite is a pyrimidine antagonist, selected from the group consisting of 5-fluorouracil, floxuridine, cytarabine, capecitabine, and gemcitabine-; or~~
- 46.** ~~The method of claim 43, wherein the antimetabolite is a purine antagonist, selected from the group consisting of 6-mercaptopurine and 6-thioguanine-; or~~
- 47.** ~~The method of claim 43, wherein the antimetabolite is an adenosine deaminase inhibitor, selected from the group consisting of cladribine, fludarabine, nelarabine and pentostatin-; or~~
- 48.** ~~The method of claim 26, wherein (vi) the chemotherapeutic agent is a topoisomerase inhibitor-~~
- 49.** ~~The method of claim 48, wherein the topoisomerase inhibitor is such as a topoisomerase I inhibitors selected from the group consisting of irinotecan and topotecan-; or~~
- 50.** ~~The method of claim 48, wherein the topoisomerase inhibitor is a topoisomerase II inhibitor selected from the group consisting of amsacrine, etoposide, etoposide phosphate, and teniposide-₂ and/or~~
- 51.** ~~The method of claim 1, wherein (vii) the modulator is a molecularly targeted agent-~~
- 52.** ~~The method of claim 51, wherein the molecularly targeted agent is such as an inhibitor of epidermal growth factor receptor (EGFR), selected from the group consisting of erlotinib, cetuximab, osimertinib, vandetanib, panitumumab, necitumumab, gefitinib and afatinib-; or~~
- 53.** ~~The method of claim 51, wherein the molecularly targeted agent is an inhibitor of ALK, selected from the group consisting of alectinib, brigatinib, ceritinib, and crizotinib-; or~~
- 54.** ~~The method of claim 51, wherein the molecularly targeted agent is an inhibitor of one or more members of the VEGF family selected from the group consisting of bevacizumab, pazopanib, ramucirumab, sorafenib, Ziv-aflibercept, lenvatinib, axitinib, vandetanib, cabozantinib, and regorafenib-; or~~
- 55.** ~~The method of claim 51, wherein the molecularly targeted agent is an inhibitor of KIT, selected from the group consisting of axitinib, cabozantinib, imatinib, pazopanib, regorafenib-; or~~
- 56.** ~~The method of claim 51, wherein the molecularly targeted agent is an inhibitor of human epidermal growth factor receptor 2 (HER2), selected from the group consisting of lapatinib, neratinib, pertuzumab, dacomitinib, trastuzumab and ado-trastuzumab emtansine-; or~~
- 57.** ~~The method of claim 51, wherein the molecularly targeted agent is an inhibitor of CDK4 and CDK6, selected from the group consisting of palbociclib and ribociclib-; or~~
- 58.** ~~The method of claim 51, wherein the molecularly targeted agent is an inhibitor of BRAF, selected from the group consisting of dabrafenib and vemurafenib-; or~~

- ~~59. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of poly-ADP-ribose polymerase (PARP), selected from the group consisting of niraparib, olaparib, and rucaparib;~~ or
- ~~60. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of one or more members of the Janus kinase (JAK) family, selected from the group consisting of ruxolitinib and tofacitinib;~~ or
- ~~61. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of mTOR, selected from the group consisting of everolimus and temsirolimus;~~ or
- ~~62. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of mitogen-activated protein kinase kinase (MEK), selected from the group consisting of cobimetinib and trametinib;~~ or
- ~~63. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of extracellular signal-regulated kinase (ERK), wherein the ERK inhibitor is ulixertinib;~~ or
- ~~64. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of platelet-derived growth factor receptors (PDGFR) or part thereof selected from the group consisting of axitinib, imatinib, olaratumab, pazopanib, regorafenib, and sorafenib;~~ or
- ~~65. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of RAF, selected from the group consisting of regorafenib and sorafenib;~~ or
- ~~66. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of RET, selected from the group consisting of regorafenib, vandetanib and cabozantinib;~~ or
- ~~67. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of MET, selected from the group consisting of cabozantinib and crizotinib;~~ or
- ~~68. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of ROS1, selected from the group consisting of crizotinib;~~ or
- ~~69. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of any one of PIGF, PTCH, Smoothened, RANKL, and B4GALNT1, and wherein the inhibitor is selected from the group consisting of Ziv-aflibercept, vismodegib, sonidegib, denosumab, and dinutuximab;~~ or
- ~~70. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of the Signal Transducer and Activator of Transcription (STAT) family selected from the group consisting of danvatirsen, AZD9150 and TTI-101;~~ or

- ~~71. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of the histone deacetylase (HDAC) family selected from the group consisting of nexturastat A, ricolinostat, trichostatin A, vorinostat, panobinostat, vaproic acid, belinostat, and entinostat; or~~
- ~~72. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of the bromodomain and extra-terminal (BET) family, selected from the group consisting of RG6146, ABBV-075, OTX015/MK-8628, GSK2820151/I-BET151, CC-90010, PLX51107, and LY294002, and wherein the BET family comprises BRD1 and BRD4; or~~
- ~~73. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of neurotrophic tyrosine kinase receptor (NTRK), selected from the group consisting of larotrectinib and entrectinib; or~~
- ~~74. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of Bcl-2, selected from the group consisting of venetoclax, navitoclax, and obatoclax; or~~
- ~~75. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of ATM, selected from the group consisting of AZD1390, AZD0156, and M3541; or~~
- ~~76. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of ATR, selected from the group consisting of AZD6738 and M6620; or~~
- ~~77. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of A2AR, selected from the group consisting of AZD4635 and CPI-444; or~~
- ~~78. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of WEE1, selected from the group consisting of adavosertib and ADZ1775; or~~
- ~~79. The method of claim 51, wherein the molecularly targeted agent is an inhibitor of FGFR, selected from the group consisting of erdafitinib, pemigatinib and TAS-120; and/or~~
- ~~80. The method of claim 1, wherein the modulator is an autophagy influencer.~~
- ~~81. The method of claim 80, wherein (viii) the autophagy influencer is metformin, melatonin, trehalose, spermidine, spermine, azithromycin, chloroquine, or chloramphenicol.~~

7. The method according to any one of claims 1-6, wherein the biological sample is treated in a manner to alter conditions selected from the group consisting of abundance of proteins, carbohydrates, lipids, molecules found within fetal bovine serum, and growth factors, hypoxia, oxidative stress, and physical exercise/stress.

~~82.8.~~ The method according to any one of claims 1-7, wherein the biomarker is measured on tumor cells, stromal cells, immune cells, or subpopulations thereof, wherein the

subpopulation of tumor cells comprises cancer stem cells that express one or more of CD133, CD44, ABCG2, and/or ALDH1A1, wherein the stromal cells are fibroblasts, and wherein the immune cells are selected from the group consisting of T cells, B cells, NK cells, dendritic cells, myeloid derived suppressor cells, macrophages, granulocytes, and mast cells; or

the biomarker being measured is the abundance of endosomes or exosomes, the contents of endosomes or exosomes including the presence of an Interleukin, FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, GM-CSF, TNF α , and TGF β , or the abundance and release of an interleukin, FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, GM-CSF, TNF α , and TGF β ; or the molecules being measured are an Interleukin, FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, GM-CSF, TNF α , and TGF β , which are generated by the tumor cells or cells found in the tumor microenvironment;

or the biomarker being measured is Toll-like receptor (TLR), NKG2D, B3NT3, NF κ Bp65, SWI/SNF complex, BRG1, BAF180, or CTCF;

or the biomarker is a miRNA optionally wherein the miRNA is miRNA-570, miRNA-513 or miRNA-155;

or the biomarker is the localization, level, and/or state of a molecule or molecules, optionally wherein

~~83. The method of claim 1, wherein the biomarker is the localization, level, and/or state of a molecule or molecules.~~

~~84. The method of claim 83, wherein (i) the molecule(s) being measured is a protein or a nucleic acid; or~~

~~85. The method of claim 83, wherein (ii) the state of the molecule(s) being measured is phosphorylation, acylation, alkylation, amidation, glypiation, glycation, glycosylation, ubiquitination, degradation product(s), truncation, mutation status, or binding of the molecule(s) to promoters that induce programmed cell death protein 1 (PD-1) ligand expression including Gamma Activated Sequence (GAS) promoter/enhancer regions and the promoter of PD-1 ligand genes, methylation status, chromatin modification of the promoter/enhancer region, and the status of the SWI/SNF complexes; or~~

~~86. The method of claim 83, wherein (iii) the localization of the molecule(s) being measured is extracellular or cellular, wherein cellular localization comprises intracellular, compartmentalized, nuclear or nucleoli, and membrane bound; wherein compartmentalized localization includes Golgi, endoplasmic reticulum, lysosomal, endosomal, exosomal, mitochondrial, vacuole, and~~

cytosolic localization; and wherein membrane bound includes plasma, nuclear and other organelle membranes; or

87. ~~The method of claim 83, wherein (iv) the molecule(s) being measured is PD-1, PD-1 ligand (PD-L1), PD-L2, AZAR, CD40, CD40L, galectin-9, IDO1, CTLA-4, KIRs, 4-1BB, GITR, T cell immunoglobulin mucin 3 (TIM-3), LAG-3 (CD223), Killer immunoglobulin-like receptors (KIRs), VISTA, Adenosine, MHC I and/or MHC II; or~~

88. ~~The method of claim 83, wherein (v) the molecule(s) being measured is in a pathway that influences PD-1 ligand expression including the immediate target or downstream target of an Interleukin; is FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, or GM-CSF; is in the TNF- α , the TGF- β , the IFN α , IFN β , or IFN γ response pathways, in the JAK/STAT pathway, in the EGFR response pathway, in the PI3K pathway, in the MAPK pathway, and/or in the mevalonate pathway;~~

89. ~~The method of claim 88, wherein the molecule(s) being measured is such as IFN-alpha, beta and/or gamma receptors; or~~

90. ~~The method of claim 88, wherein the molecule(s) being measured is from the a STAT family member;~~

91. ~~The method of claim 90, wherein the STAT family member is optionally selected from STAT-1, STAT-2, STAT-3 or STAT-4; or~~

92. ~~The method of claim 88, wherein the molecule(s) being measured is from the Janus Kinase (JAK) family; member optionally selected from~~

93. ~~The method of claim 92, wherein the JAK family member is JAK-1 or JAK-2; or~~

94. ~~The method of claim 88, wherein the wherein the molecule(s) being measured is from the an Interferon Regulatory Factors (IRF) family member;~~

95. ~~The method of claim 94, wherein the molecule measured is optionally selected from IRF-1 or IRF-9; or~~

96. ~~The method of claim 88, wherein the wherein the molecule(s) being measured is EGFR; or~~

97. ~~The method of claim 83, wherein the wherein the molecule(s) being measured is from the PI3K pathway;~~

98. ~~The method of claim 88, wherein the molecule measured is PTEN, PI3K, PDK1, AKT, mTOR, S6, S6-Kinase, CREB, GSK3B, mTORC1 or mTORC2; or~~

99. ~~The method of claim 88, wherein the wherein the molecule(s) being measured is from the MAPK pathway;~~

~~100. The method of claim 99, wherein the molecule measured is optionally selected from Src, Fak, Ras, Raf, Mek, Erk, CREB, Sos-1, SHC, NFkB, cMyc, ELK-1, Tristetraprolin (TTP), c-Fos or c-Jun, or -~~

~~101. The method of claim 88, wherein the molecule measured is a receptor tyrosine kinase (RTK), wherein the RTK is selected from the group consisting of RET, EGFR, MET, KIT, ALK, HER2, ROS1, JAK2, VEGFR, and PDGFR-, or~~

~~102. The method of claim 88, wherein the molecule being measured is hypoxia inducible factor1 α HIF-1 α or HIF-2 α -, or~~

~~103. The method of claim 88, wherein the molecule being measured is an interleukin receptor selected from the group consisting of GP130, IL1R, TNFR, and IL2Ra/b/g-, or~~

~~104. The method of claim 88, wherein the molecule being measured is a direct or indirect target of interferon, interleukin or TNF receptors, selected from the group consisting of TRAF2, TRAF-5, TRAF-6, I-Kappa B, MyD88, IRAK1, IRAK2, IRAK3, IRAK4, MKK, IKK, NFATc1, p38, p48, JNK, AP1, IRS, SHC, GRB2, and SOS-, or~~

~~105. The method of claim 88, wherein the molecule being measured is part of the mevalonate pathway optionally selected from-~~

~~106. The method of claim 105, wherein the molecule measured is ARF6 and/or AMAP1-, or~~

~~The method of claim 88, wherein the biomarkers are markers of autophagy selected from the group consisting of LC3I, LC3II, LC3/LC3II/LC3II ratios, mTOR, AMPK, p62/SQSTM1, Beclin1, ATM, UNC-51-like kinase-1, -2, and -3-, or~~

~~(vi) the molecule(s) being measured is from the PI3K pathway.~~

~~107.~~

~~108. The method of claim 1, wherein the biomarker being measured is the abundance of endosomes or exosomes, the contents of endosomes or exosomes including the presence of an Interleukin, FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, GM-CSF, TNF α , and TGF β , or the abundance and release of an interleukin, FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, GM-CSF, TNF α , and TGF β .~~

~~109.9. The method according to any one of claims 1-8, wherein the ICI response biomarkers are combined with:- (i) p53 mutation status-, or~~

~~110. The method of claim 1, wherein the ICI response biomarkers are combined with (ii) tumor mutation burden-, or~~

~~111. The method of claim 1, wherein the ICI response biomarkers are combined with (iii) the abundance, phenotypic and functional activity, including cell proliferation, survival, and~~

cytotoxicity, of tumor-infiltrating immune cells selected from the group consisting of myeloid dendritic cells, macrophages, granulocytes, mast cells and tumor-infiltrating lymphocytes (TILs); T helper cells, T cytotoxic cells, T regulatory cells, B cells and NK cells-, or

~~112. The method of claim 1, wherein the ICI response biomarkers are combined with (iv) PD-1 baseline level to predict a positive response to anti-PD-1 therapy for patients with PD-L1 negative tumors-, or~~

~~113. The method of claim 1, wherein the ICI response biomarkers are combined with (v) markers of immune senescence, including diminished cell proliferation, IFN γ , TNF α and IL-2 cytokine secretion, display of PD-1, Tim3 and LAG3, and lysosomal-associated membrane protein-1 (CD107a) indicative of T cell impairment and immune escape-, or~~

~~114. The method of claim 1, wherein the response biomarkers are combined with (vi) static levels of the biomarkers including PD-1, PD-1 ligand, AZAR, CD40, CD40L, galectin-9, IDO1, CTLA-4, KIRs, 4-1BB, GITR, T cell immunoglobulin mucin 3 (TIM-3), LAG-3 (CD223), Killer immunoglobulin-like receptors (KIRs), VISTA, Adenosine, MHC I and MHC II-, or~~

~~115. The method of claim 1, wherein the ICI response biomarkers are combined with (vii) the size of the MHC-bound peptides-, or~~

~~116. The method of claim 1, wherein the molecules being measured are an Interleukin, FGF, EGF, VEGF, PGE2, PGI2, PGD2, PGF2 α , IDO, GM-CSF, TNF α , and TGF β , which are generated by the tumor cells or cells found in the tumor microenvironment.~~

~~117. The method of claim 1, wherein the biomarker being measured is Toll like receptor (TLR), NKG2D, B3NT3, NFkB65, SWI/SNF complex, BRG1, BAF180, or CTCF.~~

~~118. The method of claim 1, wherein the biomarker is a miRNA.~~

~~119. The method of claim 1, wherein the miRNA is miRNA 570, miRNA 513 or miRNA 155.~~

~~120. The method of claim 1, wherein the response biomarkers are combined with (viii) the patient race, gender and age or age-related biomarkers selected from the group consisting of follicle-stimulating hormone, biopsy type, tumor stage, tumor type and histological categorization including cell cycle status, cell type and differentiation status of the sample.~~

~~121.10. The method according to any one of claims 1-9, wherein the expression level of a biomarker is normalized against a normalization biomarker selected from the group consisting of Na/K-ATPase, HSP90, actin, GAPDH, vinculin, tubulin, Na/K-ATPase, HSP90 and histone.~~

~~122.11. The method according to any one of claims 1-10, wherein the biomarker(s) are measured using immunoassays, multiplexed assays, PCR, transcription factor assays, DNA hypersensitivity assays, nucleic acid or sequencing/mutation testing-, optionally wherein~~

~~123.~~ The method of claim 122, wherein (i) the immunoassay is selected from the group consisting of a western blot, dot blot, ELISA, immunohistochemistry, immunocytochemistry, and immunofluorescence-, or

~~124.~~ The method of claim 122, wherein (ii) the multiplexed assay is selected from the group consisting of flow cytometry, microarrays, and bead-based including Luminex multiplex assays-, or

~~125.~~ The method of claim 122, wherein (iii) the PCR is selected from the group consisting of qPCR, RT-PCR, real-time PCR and endpoint PCR-, or

~~126.~~ The method of claim 122, wherein (iv) the transcription factor identification assay is selected from the group consisting of protein arrays, chromatin immunoprecipitation (CHIP) and CHIP-seq assays, DNA precipitation and DIP-seq assays, microsphere assays, DNase sensitivity and gel shift assays-, or

~~127.~~ The method of claim 122, wherein (v) the nucleic acid or sequencing mutation testing is selected from the group consisting of massively parallel signature sequencing (MPSS), 454 pyrosequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Ion Torrent semiconductor sequencing, Heliscope single molecule sequencing, single molecule real-time (SMRT) sequencing, sequencing by hybridization, and sequencing with mass spectrometry.

~~128.12.~~ The method according to any one of claims 1-11, wherein the biological sample is processed in part, or entirely, using one or more manual methods and/or automated systems.

~~129.13.~~ The method according to any one of claims 1-12, wherein the biomarker or panel of biomarkers selected from PD-1, PD-L1, PD-L2, AZAR, CD40, CD40L, galectin-9, IDO1, CTLA-4, KIRs, 4-1BB, GITR, TIM-3, LAG-3, lysosomal-associated membrane protein-1, KIRs, VISTA, Adenosine, MHC I, MHC II, STAT-1, STAT-2, STAT-3, STAT-4, JAK-1, JAK-2, IRF-1, IRF-9, EGFR, PTEN, PI3K, PDK1, AKT, mTOR, S6, S6-Kinase, CREB, GSK3B, mTORC1, mTORC2, Src, Fak, Ras, Raf, Mek, Erk, CREB, Sos-1, SHC, NFkB, cMyc, ELK-1, Tristetraprolin, c-Fos, c-Jun, RET, EGFR, MET, KIT, ALK, HER2, ROS1, JAK2, VEGFR, PDGFR, Hif-1 α , Hif-2 α , GP130, IL1R, TNFR, IL2Ra/b/g, TRAF2, TRAF-5, TRAF-6, I-Kappa B, MyD88, IRAK1, IRAK2, IRAK3, IRAK4, MKK, IKB, IKK, NFATc1, p38, p48, JNK, AP1, IRS, SHC, GRB2, SOS, Toll-like receptor (TLR), NKG2D, B3NT3, NFkBp65, SWI/SNF complex, BRG1, BAF180, CTCF, miRNA-570, miRNA-513, miRNA-155, IFN α , IFN β , or IFN γ , IL-1, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, IL-22, IL-23, IL-27, IL-35, FGF, EGF, VEGF, prostaglandin E2, prostacyclin, prostaglandin D2, prostaglandin F2 α , IDO, GM-CSF, TNF α , TGF β , tumor mutation burden, p53 mutation status, the abundance, phenotypic and functional

activity, including cell proliferation, survival, and cytotoxicity, of tumor-infiltrating immune cells selected from the group consisting of myeloid dendritic cells, macrophages, granulocytes, mast cells and tumor-infiltrating lymphocytes (TILs); T helper cells, T cytotoxic cells, T regulatory cells, B cells and NK cells, patient race, gender and age or age-related biomarkers selected from the group consisting of follicle-stimulating hormone, biopsy type, tumor stage, tumor type, histological categorization including cell cycle status, cell type and differentiation status of the sample, actin, GAPDH, vinculin, tubulin, and histone is used to predict the likelihood of response comprising of detecting the expression levels of biomarkers in a specimen as compared with a non-responsive group and a responsive group of samples;

~~130.~~ ~~The method of claim 129, optionally~~ wherein the predicting of likelihood of response is performed by a software classification algorithm; ~~and/or~~

~~131.~~ ~~The method of claim 129, wherein~~ the expression levels of the biomarkers are evaluated by applying a statistical method selected from the group consisting of receiver operating characteristic (ROC) curve cut point analysis, regression analysis, discriminant analysis, classification tree analysis, random forests, support vector machine, OneR, kNN and heuristic naive Bayes analysis, neural nets and variants thereof.

~~132,14.~~ ~~The method according to any one of claims 1-13, wherein the modulator comprises one or more therapeutic PD-1 or PD-L1 antibodies selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, and durvalumab;~~

~~133.~~ ~~The method of claim 132, optionally~~ wherein the therapeutic antibody is conjugated to a detection molecule selected from the group consisting of fluorescein isothiocyanate (FITC), Qdot 655, horseradish peroxidase (HRP), and alkaline phosphatase (AP); ~~and/or~~

~~134.~~ ~~The method of claim 132, wherein~~ multiple therapeutic PD-1 or PD-L1 antibodies are used as a modulator on a single sample;

~~135.~~ ~~The method of claim 134, optionally~~ wherein each therapeutic antibody is conjugated to a unique detection molecule selected from the group consisting of FITC, tetramethylrhodamine (TRITC), Qdot 655, HRP, AP, and 3,3'-Diaminobenzidine (DAB).

~~136,15.~~ ~~The method of claim 132,14, wherein the biomarker is the ability for PD-1 or PD-L1 protein to bind to PD-L1 or PD-1, respectively;~~

~~137.~~ ~~The method of claim 136, optionally~~ wherein a PD-1 or PD-L1 protein is conjugated to a single detection molecule selected from the group consisting of FITC, TRITC, Qdot 655, HRP, DAB, and AP;

138. ~~The method of claim 134,~~or wherein the biomarker is the intensity of detection molecules in the sample.