(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 August 2023 (17.08.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/154895 A1

(51) International Patent Classification:
C12Q 1/6886 (2018.01) *A61K 39/395* (2006.01)
C12Q 1/6827 (2018.01) *A61P 35/00* (2006.01)
C12Q 1/6869 (2018.01)

(21) International Application Number:
PCT/US2023/062434

(22) International Filing Date:
10 February 2023 (10.02.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/309,449 11 February 2022 (11.02.2022) US
63/335,079 26 April 2022 (26.04.2022) US

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

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

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*

WO 2023/154895 A1

(54) Title: USE OF TUMOR MUTATIONAL BURDEN AS A PREDICTIVE BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR VERSUS CHEMOTHERAPY EFFECTIVENESS IN CANCER TREATMENT

(57) Abstract: Disclosed herein are methods of treating an individual having a cancer, of treating or identifying an individual having cancer for a treatment, or stratifying individuals having a cancer for a treatment based on a tumor mutational burden (TMB) score or a TMB score and a microsatellite instability assessment.

USE OF TUMOR MUTATIONAL BURDEN AS A PREDICTIVE BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR VERSUS CHEMOTHERAPY EFFECTIVENESS IN CANCER TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/309,449, filed on February 11, 2022, and U.S. Provisional Application No. 63/335,079, filed on April 26, 2022, the contents of each of which are hereby incorporated by reference in their entirety.

FIELD

[0002] Provided herein are methods of selecting a treatment for an individual having a cancer, of treating or identifying an individual having a cancer for a treatment, or stratifying individuals having cancer for a treatment based on a tumor mutational burden (TMB) score.

BACKGROUND

[0003] Cancer can be caused by genomic mutations, and cancer cells may accumulate mutations during cancer development and progression. These mutations may be the consequence of intrinsic malfunction of DNA repair, replication, or modification mechanisms, or may be a consequence of exposure to external mutagens. Certain mutations confer growth advantages on cancer cells and are positively selected in the microenvironment of the tissue in which the cancer arises. Detection of these mutations in patient samples using next generation sequencing (NGS) or other genomic analysis techniques can provide valuable insights with respect to diagnosis, prognosis, and treatment of cancer.

[0004] Immune checkpoint inhibitor (ICPI) therapies have increasingly been used to treat metastatic cancers. However, despite successes, many clinical trials have failed to identify survival improvements in patients treated with ICPI versus chemotherapy. Thus, there is a need in the art to identify groups of patients who have comparable or superior outcomes on ICPI without chemotherapy, ICPI in the first line, and ICPI vs chemotherapy after a first line chemotherapy regimen.

BRIEF SUMMARY OF THE INVENTION

[0005] Provided herein are methods for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score is at least a threshold TMB score the individual is identified for treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0006] Further provided herein are methods of selecting a treatment for an individual having a cancer, the method comprising determining a tumor mutational burden (TMB) score for a tumor

biopsy sample obtained from the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0007] Further provided herein are methods of identifying one or more treatment options for an individual having a cancer, the method comprising: (a) determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and (b) generating a report comprising one or more treatment options identified for the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0008] Further provided herein are methods of stratifying an individual with a cancer for treatment with a therapy comprising determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and (a) if the TMB score is at least a threshold TMB score, identifying the individual as a candidate for receiving an immune checkpoint inhibitor therapy, or (b) if the TMB score is less than a threshold TMB score, identifying the individual as a candidate for receiving a chemotherapy regimen; wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0009] In some embodiments of the preceding methods, the method further comprises assessing microsatellite instability, wherein the identification is further based on the cancer being microsatellite instability-high (MSI-H). In some embodiments, microsatellite instability is assessed by next generation sequencing (NGS).

[0010] In some embodiments of the preceding methods, the individual is identified to have an increased survival as compared to treatment with a chemotherapy regimen.

[0011] Further provided herein are methods of predicting survival of an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC). Further provided herein are methods of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy

sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC). In some embodiments of the preceding methods, the method further the increased survival is increased overall survival (OS). In some embodiments of the preceding methods, the method further the increased survival is increased progression-free survival (PFS). Further provided herein are methods of predicting survival of an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to a patient with a TMB score that is less than the threshold TMB score, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC). Further provided herein are methods of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to as compared to a patient with a TMB score that is less than the threshold TMB score, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC). In some embodiments, the increased survival is increased overall survival (OS). In some embodiments, the increased survival is increased progression-free survival (PFS).

[0012] Further provided herein are methods of predicting a duration of therapeutic response for an individual having a cancer, comprising: acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and comparing the TMB score for the sample to a threshold TMB score, wherein if the TMB score is greater than or equal to the threshold TMB score, the individual is predicted to have a longer duration of therapeutic response to an immune checkpoint inhibitor; and wherein if the TMB score is less than the threshold TMB score, the subject is predicted to have a shorter duration of therapeutic response to an immune checkpoint inhibitor. In some embodiments, longer duration of therapeutic response is one or more of increase progression-free survival (PFS) and overall survival (OS), e.g., as compared to the PFS or OS of an individual with a threshold TMB score, and wherein shorter duration of therapeutic response is one or more of decreased PFS and decreased OS, e.g., as compared to the PFS or OS of an individual with a threshold TMB score.

[0013] Further provided herein are methods for treating an individual having a cancer, the method comprising: (a) determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and (b) treating the individual with an immune checkpoint inhibitor therapy if the TMB score is at least a threshold TMB score; wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC). In some embodiments, the method further comprises assessing microsatellite instability, wherein (b) is further based on the cancer being microsatellite instability-high (MSI-H). In some embodiments, microsatellite instability is assessed by next generation sequencing (NGS).

[0014] In some embodiments of the preceding methods, the method further comprises treating the individual with a chemotherapy if the TMB score is less than the threshold TMB score. In some embodiments, the chemotherapy comprises one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, aa dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2”-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside (“Ara-C”), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0015] In some embodiments of the preceding methods, the threshold TMB score is about 8 mutations/Mb, about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, or about 20 mutations/Mb. In some embodiments of the preceding methods, the threshold TMB score is about 10 mutations/Mb. In some embodiments of the preceding methods, the threshold TMB score is 10

mutations/Mb. In some embodiments of the preceding methods, the threshold TMB score is about 20 mutations/Mb. In some embodiments of the preceding methods, the threshold TMB score is 20 mutations/Mb. In some embodiments of the preceding methods, the TMB score is determined based on between about 100 kb to about 10 Mb of sequenced DNA. In some embodiments of the preceding methods, the TMB score is determined based on between about 0.8 Mb to about 1.1 Mb of sequenced DNA.

[0016] In some embodiments of the preceding methods, the method further comprises treating the individual with an immune checkpoint inhibitor if the TMB score is at least the threshold TMB score.

[0017] In some embodiments of the preceding methods, the cancer is prostate cancer that is metastatic castration-resistant prostate cancer.

[0018] In some embodiments of the preceding methods, the cancer is NSCLC, and the NSCLC is advanced NSCLC (aNSCLC).

[0019] In some embodiments of the preceding methods, the cancer is a metastatic urothelial carcinoma.

[0020] In some embodiments of the preceding methods, the cancer is a metastatic gastric adenocarcinoma.

[0021] In some embodiments of the preceding methods, the cancer is a metastatic endometrial cancer.

[0022] In some embodiments of the preceding methods, the immune checkpoint inhibitor comprises a small molecule inhibitor, an antibody, a nucleic acid, an antibody-drug conjugate, a recombinant protein, a fusion protein, a natural compound, a peptide, a PROteolysis-TArgeting Chimera (PROTAC), a cellular therapy, a treatment for cancer being tested in a clinical trial, an immunotherapy, or any combination thereof. In some embodiments, the immune checkpoint inhibitor is a PD-1 inhibitor. In some embodiments, the immune checkpoint inhibitor comprises one or more of nivolumab, pembrolizumab, cemiplimab, or dostarlimab. In some embodiments, the immune checkpoint inhibitor is a PD-L1 inhibitor. In some embodiments, the immune checkpoint inhibitor comprises one or more of atezolizumab, avelumab, or durvalumab. In some embodiments, the immune checkpoint inhibitor is a CTLA-4 inhibitor. In some embodiments, the CTLA-4 inhibitor comprises ipilimumab.

[0023] In some embodiments of the preceding methods, the individual previously received treatment with an anti-cancer therapy for the cancer. In some embodiments, the anti-cancer therapy is one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

[0024] In some embodiments of the preceding methods, the individual did not previously receive a regimen of chemotherapy for the cancer.

[0025] In some embodiments of the preceding methods, the individual previously received a regimen of chemotherapy for the cancer. In some embodiments, the previous regimen of chemotherapy comprised one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, aa dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2"-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside ("Ara-C"), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0026] In some embodiments of the preceding methods, the immune checkpoint inhibitor therapy is the only anti-cancer therapy indicated or administered for the cancer.

[0027] In some embodiments of the preceding methods, the immune checkpoint inhibitor therapy is a single-active-agent therapy.

[0028] In some embodiments of the preceding methods, the immune checkpoint inhibitor therapy comprises two or more active agents.

[0029] In some embodiments of the preceding methods, the immune checkpoint inhibitor therapy comprises a first round of an immune checkpoint inhibitor and a subsequent round of therapy with a different immune checkpoint inhibitor.

[0030] In some embodiments of the preceding methods, the immune checkpoint inhibitor therapy is the first line therapy for the cancer.

[0031] In some embodiments of the preceding methods, the immune checkpoint inhibitor therapy is the second line therapy for the cancer.

[0032] In some embodiments of the preceding methods, the method further comprises treating the individual with an additional anti-cancer therapy. In some embodiments, the additional anti-cancer

therapy comprises one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

[0033] In some embodiments of the preceding methods, the TMB score or microsatellite instability is determined by sequencing. In some embodiments, the sequencing comprises use of a massively parallel sequencing (MPS) technique, whole genome sequencing (WGS), whole exome sequencing (WES), targeted sequencing, direct sequencing, next-generation sequencing (NGS), or a Sanger sequencing technique. In some embodiments, the sequencing comprises: (a) providing a plurality of nucleic acid molecules obtained from the tumor biopsy sample, wherein the plurality of nucleic acid molecules comprise a mixture of tumor nucleic acid molecules and non-tumor nucleic acid molecules; (b) optionally, ligating one or more adapters onto one or more nucleic acid molecules from the plurality of nucleic acid molecules; (c) amplifying nucleic acid molecules from the plurality of nucleic acid molecules; (d) capturing nucleic acid molecules from the amplified nucleic acid molecules, wherein the captured nucleic acid molecules are captured from the amplified nucleic acid molecules by hybridization to one or more bait molecules; (e) sequencing, by a sequencer, at least a portion of the captured nucleic acid molecules to obtain a plurality of sequence reads corresponding to one or more genomic loci within a subgenomic interval in the sample. In some embodiments, the adapters comprise one or more of amplification primer sequences, flow cell adapter hybridization sequences, unique molecular identifier sequences, substrate adapter sequences, or sample index sequences. In some embodiments, amplifying nucleic acid molecules comprises performing a polymerase chain reaction (PCR) technique, a non-PCR amplification technique, or an isothermal amplification technique. In some embodiments, the one or more bait molecules comprise one or more nucleic acid molecules, each comprising a region that is complementary to a region of a captured nucleic acid molecule. In some embodiments, the one or more bait molecules each comprise a capture moiety. In some embodiments, the capture moiety is biotin.

[0034] In some embodiments of the preceding methods, the individual is a human.

[0035] In some embodiments of the preceding methods, if the TMB score is at least the threshold TMB score, the individual is predicted to have increased time to next treatment (TTNT) when treated with an immune checkpoint inhibitor, as compared to a chemotherapy.

[0036] Further provided herein is a kit comprising an immune checkpoint inhibitor and instructions for use according to any of the preceding methods.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] Various aspects of at least one example are discussed below with reference to the accompanying figures, which are not intended to be drawn to scale. The figures are included to provide an illustration and a further understanding of the various aspects and examples, and are

incorporated in and constitute a part of this specification, but are not intended as a definition of the limits of a particular example. The drawings, together with the remainder of the specification, serve to explain principles and operations of the described and claimed aspects and examples. In the figures, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every figure.

[0038] FIGs. 1A-1B show propensity weighting (pre-adjustment and post-adjustment balance) for urothelial carcinoma patients stratified by TMB less than 10 mutations/Mb (**FIG. 1A**) and TMB of at least 10 (**FIG. 1B**), showing bias towards chemotherapy (left-biased) or immune checkpoint inhibitory therapy (right-biased).

[0039] FIGs. 2A-2C are a series of Kaplan-Meier survival curves for urothelial carcinoma patients receiving 1st line single-agent immune checkpoint inhibitory therapy, without weighting or adjustment, stratified by tumor mutational burden, and as assessed by progression-free survival (PFS) (**FIG. 2A**), time to next treatment (TTNT) (**FIG. 2B**), and overall survival (OS) (**FIG. 2C**). X-axes are truncated at 36 months for PFS and TTNT and 48 months for OS. Overall survival estimates are left truncated with at-risk tables adjusted accordingly.

[0040] FIGs. 3A-3F are a series of Kaplan-Meier survival curves for urothelial carcinoma patients showing adjusted-for-imbalances (i.e., propensity weights applied) treatment outcomes of 1st line immune checkpoint inhibitor (ICPI) vs. chemotherapy, as assessed by PFS (**FIGs. 3A-3B**), TTNT (**FIGs. 3C-3D**), and OS (**FIGs. 3E and 3F**) and stratified by TMB less than 10 mutations/Mb and TMB of at least 10 mutations/Mb. X-axes are truncated at 36 months for PFS and TTNT and 48 months for OS. Overall survival estimates are left truncated with at-risk tables adjusted accordingly. ICPI curves reflect the observed, unadjusted PFS, TTNT, and OS, while chemotherapy arms are adjusted by propensity weights.

[0041] FIGs. 4A-4F are a series of Kaplan-Meier survival curves for urothelial carcinoma patients showing the unadjusted-for-imbalances (i.e., no propensity weights) treatment outcomes of 1st line ICPI vs. chemotherapy, as assessed by PFS (**FIGs. 4A-4B**), TTNT (**FIGs. 4C-4D**), and OS (**FIGs. 4E-4F**) and stratified by TMB less than 10 mutations/Mb and TMB of at least 10 mutations/Mb. X-axes are truncated at 36 months for PFS and TTNT and 48 months for OS. Overall survival estimates are left truncated. ICPI curves reflect the observed, unadjusted PFS, TTNT, and OS, while chemotherapy arms are adjusted by propensity weights.

[0042] FIGs. 5A-5C show assessment of ICPI vs. chemotherapy outcomes with TMB and PD-L1 as assessed by PFS (**FIG. 5A**), TTNT (**FIG. 5B**), and OS (**FIG. 5C**). 64.3% of the analysis cohort did not have evaluable PD-L1, and only those with evaluable PD-L1 are represented. With respect to the central vertical axis, left bias indicates ICPI favor and right bias indicates chemotherapy favor.

[0043] FIGs. 6A-6C show comparisons of the real-world analysis cohort with three randomized, controlled clinical trials (DANUBE, IMvigor130, and KEYNOTE-361). ECOG performance scores are shown in **FIG. 6A**. The real-world analysis cohort and the three clinical trials are stratified by

TMB (less than 10 mutations/Mb vs. at least 10 mutations/Mb) for patient outcomes, as assessed by PFS (**FIG. 6B**), and OS (**FIG. 6C**).

[0044] FIG. 7 shows a flowchart for analyses of ICPI vs. chemotherapy for metastatic gastric adenocarcinoma patients.

[0045] FIGs. 8A-8D are a series of Kaplan-Meier survival curves for metastatic gastric adenocarcinoma patients showing the adjusted-for-imbalances (i.e., propensity weights applied) treatment outcomes of 2nd line ICPI vs. chemotherapy for time to next treatment (TTNT) with TMB less than 10 mut/Mb (**FIG. 8A**), TTNT with TMB of at least 10 mut/Mb (**FIG. 8B**), OS with TMB less than 10 mut/Mb (**FIG. 8C**), and OS with TMB of at least 10 mut/Mb (**FIG. 8D**).

[0046] FIGs. 9A-9D are a series of Kaplan-Meier survival curves for metastatic gastric adenocarcinoma patients showing the unadjusted-for-imbalances (i.e., no propensity weights applied) treatment outcomes of 2nd line ICPI vs. chemotherapy for TTNT with TMB less than 10 mut/Mb (**FIG. 9A**), TTNT with TMB of at least 10 mut/Mb (**FIG. 9B**), OS for TMB less than 10 mut/Mb (**FIG. 9C**), and OS for TMB of at least 10 mut/Mb (**FIG. 9D**).

[0047] FIGs. 10A-10D are analyses of metastatic gastric adenocarcinoma patients who received 1st line chemotherapy followed by 2nd line ICPI. **FIG. 10A** shows TTNT for individual patients by stacked horizontal bar plots per patient in the Sequential Cohort with TMB less than 10 mut/Mb. **FIG. 10B** shows TTNT for individual patients by stacked horizontal bar plots per patient with TMB of at least 10 mut/Mb. MSI status is listed at right hand side of bars. **FIG. 10C** shows point estimates and confidence intervals from Cox models comparing intra-patient TTNT. **FIG. 10D** shows unadjusted overall survival from time of 1st line chemotherapy start by TMB.

[0048] FIGs. 11A-11C show point estimates and confidence intervals for biomarker-defined groups of metastatic gastric adenocarcinoma patients as assessed by TTNT in the 2nd line ICPI vs. chemotherapy cohort (**FIG. 11A**), OS in the 2nd line ICPI vs. chemotherapy cohort (**FIG. 11B**), and intra-patient TTNT (**FIG. 11C**).

[0049] FIGs. 12A-12B show KeyNote-061 and the real-world analysis cohort (2L comparative effectiveness cohort) compared with respect to their patient ECOG score distributions (**FIG. 12A**) and overall survival by TMB subgroups (**FIG. 12B**). KN-061 had less than 1% as ECOG 2, and the real-world analysis cohort had less than 1% ECOG 0, which are not labeled. High TMB for the real-world analysis cohort is TMB of at least 10 mut/Mb; for KN-061, a whole exome sequencing assay with "high" threshold chosen for concordance to TMB of at least 10 mut/Mb.

[0050] FIGs. 13A-13D are a series of Kaplan-Meier survival curves for metastatic gastric adenocarcinoma patients showing the adjusted-for-imbalances treatment outcomes of 1st line ICPI vs. chemotherapy for time to next treatment (TTNT) with TMB less than 10 mut/Mb (**FIG. 13A**), TTNT with TMB of at least 10 mut/Mb (**FIG. 13B**), OS with TMB less than 10 mut/Mb (**FIG. 13C**), and OS with TMB of at least 10 mut/Mb (**FIG. 13D**). X-axes are truncated at 36 months for TTNT and 48

months for OS. Overall survival estimates are left truncated with at-risk tables adjusted accordingly. Visualizations are adjusted by propensity weights.

[0051] FIGs. 14A-14D are a series of Kaplan-Meier survival curves for metastatic gastric adenocarcinoma patients showing the unadjusted-for-imbalances (i.e., propensity weights not applied) treatment outcomes of 1st line ICPI vs. chemotherapy for time to next treatment (TTNT) with TMB less than 10 mut/Mb (**FIG. 14A**), TTNT with TMB of at least 10 mut/Mb (**FIG. 14B**), OS with TMB less than 10 mut/Mb (**FIG. 14C**), and OS with TMB of at least 10 mut/Mb (**FIG. 14D**). X-axes are truncated at 36 months for TTNT and 48 months for OS. Overall survival estimates are left truncated with at-risk tables adjusted accordingly.

[0052] FIG. 15 shows treatment outcomes of metastatic castration resistant prostate cancer (mCRPC) patients as assessed by change in prostate-specific antigen (PSA) on single-agent taxane and stratified by TMB less than 10 and TMB of at least 10.

[0053] FIG. 16 shows treatment outcomes of mCRPC patients as assessed by change in PSA on single-agent anti-PD1 axis therapy and stratified by TMB less than 10 and TMB of at least 10.

[0054] FIGs. 17A-17D are a series of Kaplan-Meier survival curves for mCRPC patients showing the treatment outcomes of single-agent taxane vs. ICPI for time to next treatment (TTNT) with TMB less than 10 mutations/Mb (**FIG. 17A**), TTNT with TMB of at least 10 mutations/Mb (**FIG. 17B**), overall survival (OS) with TMB less than 10 mutations/Mb (**FIG. 17C**), and OS with TMB of at least 10 (**FIG. 17D**).

[0055] FIG. 18 provides a flowchart showing the cohort selection schema used in Example 4.

[0056] FIG. 19A provides an adjusted Kaplan-Meier plot of real-world progression free survival (rwPFS) for NCLC patients who were treated with ICPI monotherapy. **FIG. 19B** provides an adjusted Kaplan-Meier plot of rwPFS for NCLC patients who were treated with ICPI therapy + chemotherapy. **FIG. 19C** provides an adjusted Kaplan-Meier plot of real-world overall survival (rwOS) for NCLC patients who were treated with ICPI monotherapy. **FIG. 19D** provides an adjusted Kaplan-Meier plot of rwOS for NCLC patients who were treated with ICPI therapy + chemotherapy. Outcomes were stratified by TMB <10 and TMB >= 10.

[0057] FIG. 20A provides an adjusted Kaplan-Meier plot of real-world progression free survival (rwPFS) for NCLC patients who were treated with ICPI monotherapy. **FIG. 20B** provides an adjusted Kaplan-Meier plot of rwPFS for NCLC patients who were treated with ICPI therapy + chemotherapy. **FIG. 20C** provides an adjusted Kaplan-Meier plot of real-world overall survival (rwOS) for NCLC patients who were treated with ICPI monotherapy. **FIG. 20D** provides an adjusted Kaplan-Meier plot of rwOS for NCLC patients who were treated with ICPI therapy + chemotherapy. Outcomes were stratified by TMB <20 and TMB >= 20.

[0058] FIG. 21A provides a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<10 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<10 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=10

(i.e., PDL1- / TBM +), and PDL1>1% and TMB>=10 (i.e., PDL1+ / TMB+). **FIG. 21B** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwPFS. **FIG. 21C** provides a Kaplan-Meier plot of rwOS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<10 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<10 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=10 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=10 (i.e., PDL1+ / TMB+). **FIG. 21D** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwOS.

[0059] FIG. 22A provides a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<20 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<20 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=20 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=20 (i.e., PDL1+ / TMB+). **FIG. 22B** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwPFS. **FIG. 22C** provides a Kaplan-Meier plot of rwOS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<20 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<20 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=20 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=20 (i.e., PDL1+ / TMB+). **FIG. 22D** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwOS.

[0060] FIG. 23A shows the point estimation and 95% confidence interval of HR (hazard ratio) for biomarker and treatment interaction are shown for different TMB or PDL1 cutoffs for rwPFS. **FIG. 23B** shows the point estimation and 95% confidence interval of HR (hazard ratio) for biomarker and treatment interaction are shown for different TMB or PDL1 cutoffs for rwOS. **FIG. 23C** provides a Kaplan-Meier plot of rwPFS for patients with PD-L1 scores between 1-49% who were treated with ICPI monotherapy or ICPI therapy + chemotherapy. **FIG. 23D** provides a Kaplan-Meier plot of rwPFS for patients with PD-L1 scores >=50% who were treated with ICPI monotherapy or ICPI therapy + chemotherapy.

[0061] FIG. 24 shows the top 30 altered genes in the ICPI monotherapy cohort (-) and ICPI therapy + chemotherapy cohort (+). Multi: multiple alterations in the indicated gene; RE: rearrangement; CN: copy number change; SV: short variant mutation (base substitution or insertion/deletion).

[0062] FIG. 25A shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI monotherapy. **FIG. 25B** shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI therapy + chemotherapy. **FIG. 25C** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI monotherapy. **FIG. 25D** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI therapy + chemotherapy. Outcomes were stratified by PDL1 TPS < 1% and PDL1 TPS >= 1%.

[0063] FIG. 26A shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI monotherapy. **FIG. 26B** shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI therapy + chemotherapy. **FIG. 26C** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI monotherapy. **FIG. 26D** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI therapy + chemotherapy. Outcomes were stratified by PDL1 TC <50% and PDL1 TC >= 50%.

[0064] FIG. 27 provides box plots of TMB levels in different PDL1 subgroups to show association between PDL1 expression and TMB. Kruskal-Wallis test p = 0.007, effect size: 0.0046.

[0065] FIG. 28A shows a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI monotherapy. **FIG. 28B** shows a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI therapy + chemotherapy. **FIG. 28C** shows a Kaplan-Meier plot of rwOS for patients who were treated with ICPI monotherapy. **FIG. 28D** shows a Kaplan-Meier plot of rwOS for patients who were treated with ICPI therapy + chemotherapy.

[0066] FIGs. 29A-B shows a pan-tumor assessment of the clinical validity of TMB to prognosticate outcome on single-agent ICPI. Stratified Cox proportional hazards (CoxPH models, labeled "estimate") were utilized to assess the pan-tumor associations. **FIG. 29A** shows the association of the observed features on time to next treatment. **FIG. 29B** shows the association of the observed features on overall survival.

[0067] FIGs. 30A-B shows the per tumor type assessment of the clinical validity of TMB to prognosticate outcome on single-agent ICPI. Cox models adjusted for ECOG (Eastern Cooperative Oncology Group score), line of therapy, sex, age, and opioid use are shown per disease type for (**FIG. 30A**) time to next treatment and (**FIG. 30B**) overall survival.

[0068] FIGs. 31A-B show the per tumor type assessment of the clinical validity of TMB to prognosticate outcome on single-agent ICPI for patients with tumor having MSS status. For MSS groups, outcomes of patients with MSS (microsatellite stable) & TMB $\geq$ 10 tumors compared to those with TMB < 10. Disease areas are shown for those with minimum 5 patients per group. Cox models adjusted for ECOG, line of therapy, sex, age, and opioid use are shown per disease type for time to next treatment (**FIG. 31A**) and overall survival (**FIG. 31B**).

[0069] FIGs. 32A-B show the pan-tumor assessment of the clinical validity of TMB to prognosticate outcome on ICPI combination therapy. Stratified CoxPH models were utilized to assess the pan-tumor associations of observed features. "Estimate" indicates the Cox proportional hazard. **FIG. 32A** shows the association of the observed features on time to next treatment. **FIG. 32B** shows the association of the observed features on overall survival.

DETAILED DESCRIPTION

[0070] Described herein are methods comprising determining a tumor mutational burden (TMB) score for a sample obtained from an individual having a cancer and comparing the determined TMB score

with a threshold TMB score. It has been discovered that comparison of the determined TMB score with a threshold TMB score can help guide treatment decisions, including selecting between a chemotherapy regimen and an immune checkpoint inhibitor (ICPI) therapy.

[0071] Thus, described herein are methods for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising determining a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein if the TMB score is at least a threshold TMB score the individual is identified for treatment with an immune checkpoint inhibitor therapy. Further described herein are methods of selecting a treatment for an individual having a cancer, the method comprising determining a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy. Further described herein are methods of identifying one or more treatment options for an individual having a cancer, the method comprising: (a) determining a tumor mutational burden (TMB) score for a sample obtained from the individual; and (b) generating a report comprising one or more treatment options identified for the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy. Further described herein are methods of stratifying an individual with a cancer for treatment with a therapy comprising determining a tumor mutational burden (TMB) score for a sample obtained from the individual; and (a) if the TMB score is at least a threshold TMB score, identifying the individual as a candidate for receiving an immune checkpoint inhibitor therapy, or (b) if the TMB score is less than a threshold TMB score, identifying the individual as a candidate for receiving a chemotherapy regimen. Further described herein are methods of predicting survival of an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen. Further described herein are methods of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein if the TMB score for the sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen. Further described herein are methods for treating an individual having a cancer, the method comprising: (a) determining a tumor mutational burden (TMB) score for a sample obtained from the individual; and (b) treating the individual with an immune checkpoint inhibitor therapy if the TMB score is at least a threshold TMB score. In any of the provided methods, in addition to determining a TMB score, the methods may further comprise assessing microsatellite instability. It has been discovered that assessment of microsatellite instability as being microsatellite

instability-high (MSI-H) or not microsatellite instability-high (such as MSI low (MSI-L) or stable microsatellite (MSS)), combined with TMB being below a threshold TMB score or at least the threshold TMB score, can further guide treatment decisions, including selecting between a chemotherapy regimen and an immune checkpoint inhibitor (ICPI) therapy for an individual having a cancer. In some embodiments of these methods, the sample is a tumor biopsy sample. In some embodiments of these methods, the sample is a blood sample.

[0072] Further described herein are methods comprising assessing a microsatellite instability for a tumor biopsy obtained from an individual having a cancer. Assessment of the microsatellite instability as high (i.e., MSI-H) or not MSI-H (such as MSI-L or MSS) can help guide treatment decisions, including selecting between a chemotherapy regimen and an immune checkpoint inhibitor (ICPI) therapy.

[0073] Thus, described herein are methods for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising assessing microsatellite instability for a sample obtained from the individual, wherein if the microsatellite instability is MSI-H the individual is identified for treatment with an immune checkpoint inhibitor therapy. Further described herein are methods of selecting a treatment for an individual having a cancer, the method comprising assessing microsatellite instability for a sample obtained from the individual, wherein microsatellite instability that is MSI-H identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy. Further described herein are methods of identifying one or more treatment options for an individual having a metastatic cancer, the method comprising: (a) assessing microsatellite instability for a sample obtained from the individual; and (b) generating a report comprising one or more treatment options identified for the individual, wherein a microsatellite instability that is MSI-H identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy. Further described herein are methods of stratifying an individual with a cancer for treatment with a therapy comprising assessing microsatellite instability for a sample obtained from the individual; and (a) if the microsatellite instability is MSI-H, identifying the individual as a candidate for receiving an immune checkpoint inhibitor therapy, or (b) if the microsatellite instability is not MSI-H, identifying the individual as a candidate for receiving a chemotherapy regimen. Further described herein are methods of predicting survival of an individual having a cancer, comprising acquiring knowledge of microsatellite instability for a sample obtained from the individual, wherein if the microsatellite instability is MSI-H for the sample obtained from the individual, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen. Further described herein are methods of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of microsatellite instability for a sample obtained from the individual, wherein if the microsatellite instability is MSI-H for the sample obtained from the individual, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared

to treatment with a chemotherapy regimen. Further described herein are methods for treating an individual having a cancer, the method comprising: (a) assessing microsatellite instability for a sample obtained from the individual; and (b) treating the individual with an immune checkpoint inhibitor therapy if the microsatellite instability is assessed as MSI-H. In some embodiments of these methods, the sample is a tumor biopsy sample. In some embodiments of these methods, the sample is a blood sample.

I. General Techniques

[0074] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

II. Definitions

[0075] Certain terms are defined. Additional terms are defined throughout the specification.

[0076] As used herein, the articles "a" and "an" refer to one or to more than one (*e.g.*, to at least one) of the grammatical object of the article.

[0077] "About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are

within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

[0078] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0079] The terms "cancer" and "tumor" are used interchangeably herein. These terms refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells can exist alone within an animal, or can be a non-tumorigenic cancer cell, such as a leukemia cell. These terms include a solid tumor, a soft tissue tumor, or a metastatic lesion. As used herein, the term "cancer" includes premalignant, as well as malignant cancers.

[0080] "Polynucleotide," "nucleic acid," or "nucleic acid molecule" as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. In addition, the term "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. The term "polynucleotide" specifically includes cDNAs.

[0081] A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally-occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, and the like) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, and the like), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, and the like), those with intercalators (e.g., acridine, psoralen, and the like), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, and the like), those containing alkylators, those with modified linkages (e.g., alpha anomeric

nucleic acids), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-0-methyl-, 2'-0-allyl-, 2'-fluoro-, or 2'-azido-ribose, carbocyclic sugar analogs, a-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(0)S ("thioate"), P(S)S ("dithioate"), "(0)NR_2 ("amidate"), P(0)R, P(0)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1 -20 C) optionally containing an ether (-0-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. A polynucleotide can contain one or more different types of modifications as described herein and/or multiple modifications of the same type. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0082] The term “detection” includes any means of detecting, including direct and indirect detection. The term “biomarker” as used herein refers to an indicator, e.g., predictive, diagnostic, and/or prognostic, which can be detected in a sample. The biomarker may serve as an indicator of a particular subtype of a disease or disorder (e.g., cancer) characterized by certain, molecular, pathological, histological, and/or clinical features (e.g., responsiveness to therapy, e.g., a checkpoint inhibitor). In some embodiments, a biomarker is a collection of genes or a collective number of mutations/alterations (e.g., somatic mutations) in a collection of genes. Biomarkers include, but are not limited to, polynucleotides (e.g., DNA and/or RNA), polynucleotide alterations (e.g., polynucleotide copy number alterations, e.g., DNA copy number alterations, or other mutations or alterations), polypeptides, polypeptide and polynucleotide modifications (e.g., post-translational modifications), carbohydrates, and/or glycolipid-based molecular markers.

[0083] “Amplification,” as used herein generally refers to the process of producing multiple copies of a desired sequence. “Multiple copies” means at least two copies. A “copy” does not necessarily mean perfect sequence complementarity or identity to the template sequence. For example, copies can include nucleotide analogs such as deoxyinosine, intentional sequence alterations (such as sequence alterations introduced through a primer comprising a sequence that is hybridizable, but not complementary, to the template), and/or sequence errors that occur during amplification.

[0084] The technique of "polymerase chain reaction" or "PCR" as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described, for example, in U.S. Pat. No. 4,683,195. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage, or plasmid sequences, etc. See generally Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263 (1987) and Erlich, ed., PCR Technology (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

[0085] "Individual response" or "response" can be assessed using any endpoint indicating a benefit to the individual, including, without limitation, (1) inhibition, to some extent, of disease progression (e.g., cancer progression), including slowing down or complete arrest; (2) a reduction in tumor size; (3) inhibition (i.e., reduction, slowing down, or complete stopping) of cancer cell infiltration into adjacent peripheral organs and/or tissues; (4) inhibition (i.e. reduction, slowing down, or complete stopping) of metastasis; (5) relief, to some extent, of one or more symptoms associated with the disease or disorder (e.g., cancer); (6) increase or extension in the length of survival, including overall survival and progression free survival; and/or (7) decreased mortality at a given point of time following treatment.

[0086] An "effective response" of a patient or a patient's "responsiveness" to treatment with a medicament and similar wording refers to the clinical or therapeutic benefit imparted to a patient at risk for, or suffering from, a disease or disorder, such as cancer. In one embodiment, such benefit includes any one or more of: extending survival (including overall survival and/or progression-free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer.

[0087] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

[0088] As used herein, the terms "individual," "patient," or "subject" are used interchangeably and refer to any single animal, *e.g.*, a mammal (including such non-human animals as, for example, dogs, cats, horses, rabbits, zoo animals, cows, pigs, sheep, and non-human primates) for which treatment is desired. In particular embodiments, the patient herein is a human.

[0089] As used herein, "administering" is meant a method of giving a dosage of an agent or a pharmaceutical composition (e.g., a pharmaceutical composition including the agent) to a subject (e.g., a patient). Administering can be by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include, for example, intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0090] The terms "concurrently" or "in combination" are used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time. Accordingly, concurrent administration includes a dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s).

[0091] "Acquire" or "acquiring" as the terms are used herein, refer to obtaining possession of a physical entity, or a value, *e.g.*, a numerical value, by "directly acquiring" or "indirectly acquiring" the physical entity or value. "Directly acquiring" means performing a process (*e.g.*, performing a synthetic or analytical method) to obtain the physical entity or value. "Indirectly acquiring" refers to receiving the physical entity or value from another party or source (*e.g.*, a third-party laboratory that directly acquired the physical entity or value). Directly acquiring a physical entity includes performing a process that includes a physical change in a physical substance, *e.g.*, a starting material. Exemplary changes include making a physical entity from two or more starting materials, shearing or fragmenting a substance, separating or purifying a substance, combining two or more separate entities into a mixture, performing a chemical reaction that includes breaking or forming a covalent or non-covalent bond. Directly acquiring a value includes performing a process that includes a physical change in a sample or another substance, *e.g.*, performing an analytical process which includes a physical change in a substance, *e.g.*, a sample, analyte, or reagent (sometimes referred to herein as "physical analysis"), performing an analytical method, *e.g.*, a method which includes one or more of the following: separating or purifying a substance, *e.g.*, an analyte, or a fragment or other derivative thereof, from another substance; combining an analyte, or fragment or other derivative thereof, with another substance, *e.g.*, a buffer, solvent, or reactant; or changing the structure of an analyte, or a fragment or other derivative thereof, *e.g.*, by breaking or forming a covalent or non-covalent bond, between a first and a second atom of the analyte; or by changing the structure of a reagent, or a

fragment or other derivative thereof, *e.g.*, by breaking or forming a covalent or non-covalent bond, between a first and a second atom of the reagent.

[0092] "Acquiring a sequence" or "acquiring a read" as the term is used herein, refers to obtaining possession of a nucleotide sequence or amino acid sequence, by "directly acquiring" or "indirectly acquiring" the sequence or read. "Directly acquiring" a sequence or read means performing a process (*e.g.*, performing a synthetic or analytical method) to obtain the sequence, such as performing a sequencing method (*e.g.*, a Next-generation Sequencing (NGS) method). "Indirectly acquiring" a sequence or read refers to receiving information or knowledge of, or receiving, the sequence from another party or source (*e.g.*, a third-party laboratory that directly acquired the sequence). The sequence or read acquired need not be a full sequence, *e.g.*, sequencing of at least one nucleotide, or obtaining information or knowledge, that identifies one or more of the alterations disclosed herein as being present in a sample, biopsy or subject constitutes acquiring a sequence.

[0093] Directly acquiring a sequence or read includes performing a process that includes a physical change in a physical substance, *e.g.*, a starting material, such as a sample described herein. Exemplary changes include making a physical entity from two or more starting materials, shearing or fragmenting a substance, such as a genomic DNA fragment; separating or purifying a substance (*e.g.*, isolating a nucleic acid sample from a tissue); combining two or more separate entities into a mixture, performing a chemical reaction that includes breaking or forming a covalent or non-covalent bond. Directly acquiring a value includes performing a process that includes a physical change in a sample or another substance as described above. The size of the fragment (*e.g.*, the average size of the fragments) can be 2500 bp or less, 2000 bp or less, 1500 bp or less, 1000 bp or less, 800 bp or less, 600 bp or less, 400 bp or less, or 200 bp or less. In some embodiments, the size of the fragment (*e.g.*, cfDNA) is between about 150 bp and about 200 bp (e.g., between about 160 bp and about 170 bp). In some embodiments, the size of the fragment (*e.g.*, DNA fragments from liquid biopsy samples) is between about 150 bp and about 250 bp. In some embodiments, the size of the fragment (*e.g.*, cDNA fragments obtained from RNA in liquid biopsy samples) is between about 100 bp and about 150 bp.

[0094] "Alteration" or "altered structure" as used herein, of a gene or gene product (*e.g.*, a marker gene or gene product) refers to the presence of a mutation or mutations within the gene or gene product, *e.g.,* a mutation, which affects integrity, sequence, structure, amount or activity of the gene or gene product, as compared to the normal or wild-type gene. The alteration can be in amount, structure, and/or activity in a cancer tissue or cancer cell, as compared to its amount, structure, and/or activity, in a normal or healthy tissue or cell (*e.g.,* a control), and is associated with a disease state, such as cancer. For example, an alteration which is associated with cancer, or predictive of responsiveness to anti-cancer therapeutics, can have an altered nucleotide sequence (*e.g.*, a mutation), amino acid sequence, chromosomal translocation, intra-chromosomal inversion, copy number, expression level, protein level, protein activity, epigenetic modification (*e.g.*, methylation or acetylation status, or post-translational modification, in a cancer tissue or cancer cell, as compared to

a normal, healthy tissue or cell. Exemplary mutations include, but are not limited to, point mutations (*e.g.*, silent, missense, or nonsense), deletions, insertions, inversions, duplications, amplification, translocations, inter- and intra-chromosomal rearrangements. Mutations can be present in the coding or non-coding region of the gene. In certain embodiments, the alteration(s) is detected as a rearrangement, *e.g.*, a genomic rearrangement comprising one or more introns or fragments thereof (*e.g.*, one or more rearrangements in the 5'- and/or 3'-UTR). In certain embodiments, the alterations are associated (or not associated) with a phenotype, *e.g.*, a cancerous phenotype (*e.g.*, one or more of cancer risk, cancer progression, cancer treatment or resistance to cancer treatment). In one embodiment, the alteration (or tumor mutational burden) is associated with one or more of: a genetic risk factor for cancer, a positive treatment response predictor, a negative treatment response predictor, a positive prognostic factor, a negative prognostic factor, or a diagnostic factor.

[0095] As used herein, the term "indel" refers to an insertion, a deletion, or both, of one or more nucleotides in a nucleic acid of a cell. In certain embodiments, an indel includes both an insertion and a deletion of one or more nucleotides, where both the insertion and the deletion are nearby on the nucleic acid. In certain embodiments, the indel results in a net change in the total number of nucleotides. In certain embodiments, the indel results in a net change of about 1 to about 50 nucleotides.

[0096] "Subgenomic interval" as that term is used herein, refers to a portion of genomic sequence. In an embodiment, a subgenomic interval can be a single nucleotide position, *e.g.*, a variant at the position is associated (positively or negatively) with a tumor phenotype. In an embodiment, a subgenomic interval comprises more than one nucleotide position. Such embodiments include sequences of at least 2, 5, 10, 50, 100, 150, or 250 nucleotide positions in length. Subgenomic intervals can comprise an entire gene, or a portion thereof, *e.*g., the coding region (or portions thereof), an intron (or portion thereof) or exon (or portion thereof). A subgenomic interval can comprise all or a part of a fragment of a naturally occurring, *e.g.*, genomic DNA, nucleic acid. *E.g.*, a subgenomic interval can correspond to a fragment of genomic DNA which is subjected to a sequencing reaction. In an embodiment, a subgenomic interval is continuous sequence from a genomic source. In an embodiment, a subgenomic interval includes sequences that are not contiguous in the genome, *e.g.*, subgenomic intervals in cDNA can include exon-exon junctions formed as a result of splicing. In an embodiment, the subgenomic interval comprises a tumor nucleic acid molecule. In an embodiment, the subgenomic interval comprises a non-tumor nucleic acid molecule.

[0097] In an embodiment, a subgenomic interval comprises or consists of: a single nucleotide position; an intragenic region or an intergenic region; an exon or an intron, or a fragment thereof, typically an exon sequence or a fragment thereof; a coding region or a non-coding region, *e.g.*, a promoter, an enhancer, a 5' untranslated region (5' UTR), or a 3' untranslated region (3' UTR), or a fragment thereof; a cDNA or a fragment thereof; an SNP; a somatic mutation, a germline mutation or both; an alteration, *e.g.*, a point or a single mutation; a deletion mutation (*e.g.*, an in-frame deletion, an

intragenic deletion, a full gene deletion); an insertion mutation (*e.g.*, intragenic insertion); an inversion mutation (*e.g.*, an intra-chromosomal inversion); an inverted duplication mutation; a tandem duplication (*e.g.*, an intrachromosomal tandem duplication); a translocation (*e.g.,* a chromosomal translocation, a non-reciprocal translocation); a rearrangement (*e.g.*, a genomic rearrangement (*e.g.*, a rearrangement of one or more introns, a rearrangement of one or more exons, or a combination and/or a fragment thereof; a rearranged intron can include a 5'- and/or 3'- UTR)); a change in gene copy number; a change in gene expression; a change in RNA levels; or a combination thereof. The "copy number of a gene" refers to the number of DNA sequences in a cell encoding a particular gene product. Generally, for a given gene, a mammal has two copies of each gene. The copy number can be increased, *e.g.*, by gene amplification or duplication, or reduced by deletion.

[0098] "Subject interval", as that term is used herein, refers to a subgenomic interval or an expressed subgenomic interval. In an embodiment, a subgenomic interval and an expressed subgenomic interval correspond, meaning that the expressed subgenomic interval comprises sequence expressed from the corresponding subgenomic interval. In an embodiment, a subgenomic interval and an expressed subgenomic interval are non-corresponding, meaning that the expressed subgenomic interval does not comprise sequence expressed from the non-corresponding subgenomic interval, but rather corresponds to a different subgenomic interval. In an embodiment, a subgenomic interval and an expressed subgenomic interval partially correspond, meaning that the expressed subgenomic interval comprises sequence expressed from the corresponding subgenomic interval and sequence expressed from a different corresponding subgenomic interval.

[0099] As used herein, the term "library" refers to a collection of nucleic acid molecules. In one embodiment, the library includes a collection of nucleic acid nucleic acid molecules, *e.g.*, a collection of whole genomic, subgenomic fragments, cDNA, cDNA fragments, RNA, *e.g.,* mRNA, RNA fragments, or a combination thereof. Typically, a nucleic acid molecule is a DNA molecule, *e.g.*, genomic DNA or cDNA. A nucleic acid molecule can be fragmented, *e.g.*, sheared or enzymatically prepared, genomic DNA. Nucleic acid molecules comprise sequence from a subject and can also comprise sequence not derived from the subject, *e.g.*, an adapter sequence, a primer sequence, or other sequences that allow for identification, *e.g.*, "barcode" sequences. In one embodiment, a portion or all of the library nucleic acid molecules comprises an adapter sequence. The adapter sequence can be located at one or both ends. The adapter sequence can be useful, *e.g.*, for a sequencing method (*e.g.,* an NGS method), for amplification, for reverse transcription, or for cloning into a vector. The library can comprise a collection of nucleic acid molecules, *e.g.*, a target nucleic acid molecule (*e.g.*, a tumor nucleic acid molecule, a reference nucleic acid molecule, or a combination thereof). The nucleic acid molecules of the library can be from a single individual. In embodiments, a library can comprise nucleic acid molecules from more than one subject (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30 or more subjects), *e.g.*, two or more libraries from different subjects can be combined to form a library

comprising nucleic acid molecules from more than one subject. In one embodiment, the subject is a human having, or at risk of having, a cancer or tumor.

[0100] "Complementary" refers to sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. It is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds ("base pairing") with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. In certain embodiments, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. In other embodiments, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

[0101] "Likely to" or "increased likelihood," as used herein, refers to an increased probability that an item, object, thing or person will occur. Thus, in one example, a subject that is likely to respond to treatment has an increased probability of responding to treatment relative to a reference subject or group of subjects.

[0102] "Unlikely to" refers to a decreased probability that an event, item, object, thing or person will occur with respect to a reference. Thus, a subject that is unlikely to respond to treatment has a decreased probability of responding to treatment relative to a reference subject or group of subjects.

[0103] "Next-generation sequencing" or "NGS" or "NG sequencing" as used herein, refers to any sequencing method that determines the nucleotide sequence of either individual nucleic acid molecules (*e.g.*, in single molecule sequencing) or clonally expanded proxies for individual nucleic acid molecules in a high throughput fashion (*e.g.*, greater than 10^3, 10^4, 10^5 or more molecules are sequenced simultaneously). In one embodiment, the relative abundance of the nucleic acid species in the library can be estimated by counting the relative number of occurrences of their cognate sequences in the data generated by the sequencing experiment. Next-generation sequencing methods are known in the art, and are described, *e.g.*, in Metzker, M. (2010) *Nature Biotechnology Reviews* 11:31-46, incorporated herein by reference. Next-generation sequencing can detect a variant present in less than 5% or less than 1% of the nucleic acids in a sample.

[0104] "Nucleotide value" as referred herein, represents the identity of the nucleotide(s) occupying or assigned to a nucleotide position. Typical nucleotide values include: missing (*e.g.*, deleted); additional (*e.g.*, an insertion of one or more nucleotides, the identity of which may or may not be

included); or present (occupied); A; T; C; or G. Other values can be, *e.g.,* not Y, wherein Y is A, T, G, or C; A or X, wherein X is one or two of T, G, or C; T or X, wherein X is one or two of A, G, or C; G or X, wherein X is one or two of T, A, or C; C or X, wherein X is one or two of T, G, or A; a pyrimidine nucleotide; or a purine nucleotide. A nucleotide value can be a frequency for 1 or more, *e.g.*, 2, 3, or 4, bases (or other value described herein, *e.g.*, missing or additional) at a nucleotide position. *E.g.*, a nucleotide value can comprise a frequency for A, and a frequency for G, at a nucleotide position.

[0105] "Or" is used herein to mean, and is used interchangeably with, the term "and/or", unless context clearly indicates otherwise. The use of the term "and/or" in some places herein does not mean that uses of the term "or" are not interchangeable with the term "and/or" unless the context clearly indicates otherwise.

[0106] A "control nucleic acid" or "reference nucleic acid" as used herein, refers to nucleic acid molecules from a control or reference sample. Typically, it is DNA, *e.g.*, genomic DNA, or cDNA derived from RNA, not containing the alteration or variation in the gene or gene product. In certain embodiments, the reference or control nucleic acid sample is a wild-type or a non-mutated sequence. In certain embodiments, the reference nucleic acid sample is purified or isolated (*e.g.*, it is removed from its natural state). In other embodiments, the reference nucleic acid sample is from a blood control, a normal adjacent tissue (NAT), or any other non-cancerous sample from the same or a different subject. In some embodiments, the reference nucleic acid sample comprises normal DNA mixtures. In some embodiments, the normal DNA mixture is a process matched control. In some embodiments, the reference nucleic acid sample has germline variants. In some embodiments, the reference nucleic acid sample does not have somatic alterations, e.g., serves as a negative control.

[0107] "Threshold value," as used herein, is a value that is a function of the number of reads required to be present to assign a nucleotide value to a subject interval (*e.g.,* a subgenomic interval or an expressed subgenomic interval). *E.g.*, it is a function of the number of reads having a specific nucleotide value, *e.g.*, "A," at a nucleotide position, required to assign that nucleotide value to that nucleotide position in the subgenomic interval. The threshold value can, *e.g.*, be expressed as (or as a function of) a number of reads, *e.g.*, an integer, or as a proportion of reads having the value. By way of example, if the threshold value is X, and X+1 reads having the nucleotide value of "A" are present, then the value of "A" is assigned to the position in the subject interval (*e.g.,* subgenomic interval or expressed subgenomic interval). The threshold value can also be expressed as a function of a mutation or variant expectation, mutation frequency, or of Bayesian prior. In an embodiment, a mutation frequency would require a number or proportion of reads having a nucleotide value, *e.g.*, A or G, at a position, to call that nucleotide value. In embodiments the threshold value can be a function of mutation expectation, *e.g.*, mutation frequency, and tumor type. *E.g.*, a variant at a nucleotide position could have a first threshold value if the patient has a first tumor type and a second threshold value if the patient has a second tumor type.

[0108] As used herein, "target nucleic acid molecule" refers to a nucleic acid molecule that one desires to isolate from the nucleic acid library. In one embodiment, the target nucleic acid molecules can be a tumor nucleic acid molecule, a reference nucleic acid molecule, or a control nucleic acid molecule, as described herein.

[0109] "Tumor nucleic acid molecule," or other similar term (*e.g.*, a "tumor or cancer-associated nucleic acid molecule"), as used herein refers to a nucleic acid molecule having sequence from a tumor cell. The terms "tumor nucleic acid molecule" and "tumor nucleic acid" may sometimes be used interchangeably herein. In one embodiment, the tumor nucleic acid molecule includes a subject interval having a sequence (*e.g.*, a nucleotide sequence) that has an alteration (*e.g.*, a mutation) associated with a cancerous phenotype. In other embodiments, the tumor nucleic acid molecule includes a subject interval having a wild-type sequence (*e.g.*, a wild-type nucleotide sequence). For example, a subject interval from a heterozygous or homozygous wild-type allele present in a cancer cell. A tumor nucleic acid molecule can include a reference nucleic acid molecule. Typically, it is DNA, *e.g.*, genomic DNA, or cDNA derived from RNA, from a sample. In certain embodiments, the sample is purified or isolated (*e.g.*, it is removed from its natural state). In some embodiments, the tumor nucleic acid molecule is a cfDNA. In some embodiments, the tumor nucleic acid molecule is a ctDNA. In some embodiments, the tumor nucleic acid molecule is DNA from a CTC.

[0110] "Variant," as used herein, refers to a structure that can be present at a subgenomic interval that can have more than one structure, *e.g.*, an allele at a polymorphic locus.

[0111] An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. In certain embodiments, an "isolated" nucleic acid molecule is free of sequences (such as protein-encoding sequences) which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kB, less than about 4 kB, less than about 3 kB, less than about 2 kB, less than about 1 kB, less than about 0.5 kB or less than about 0.1 kB of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as an RNA molecule or a cDNA molecule, can be substantially free of other cellular material or culture medium, *e.g.,* when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals, *e.g.,* when chemically synthesized.

III. Individuals to be treated, assessed, and identified

[0112] The methods of the present disclosure pertain, in certain embodiments, to individuals having a cancer and/or samples (such as tumor biopsy samples and/or blood samples) obtained from an individual having a cancer. In some embodiments, the methods provide improved treatments for the individuals, based on determining a TMB score and/or assessing a microsatellite instability in a

sample obtained from the individual. In some embodiments, the methods provide improved methods of selecting a treatment, identifying one or more treatment options, stratifying the individual for treatment with a therapy, predicting survival of the individual, and/or monitoring, evaluating, or screening the individual, each based in part on determining a TMB score and/or a microsatellite instability of a sample obtained from the individual.

[0113] In some embodiments, the individual has a cancer. In some embodiments, the individual has been, or is being treated, for cancer. In some embodiments, the individual is in need of being monitored for cancer progression or regression, e.g., after being treated with a cancer therapy. In some embodiments, the individual is in need of being monitored for relapse of cancer. In some embodiments, the individual is at risk of having a cancer. In some embodiments, the individual is suspected of having cancer. In some embodiments, the individual is being tested for cancer. In some embodiments, the individual has a genetic predisposition to a cancer (e.g., having a mutation that increases his or her baseline risk for developing a cancer). In some embodiments, the individual has been exposed to an environment (e.g., radiation or chemical) that increases his or her risk for developing a cancer. In some embodiments, the individual is in need of being monitored for development of a cancer. In some embodiments, the individual is in need of a first line treatment for the cancer. In some embodiments, the individual is in need of a second line treatment for the cancer.

[0114] In certain embodiments, the sample is from an individual having a cancer. Exemplary cancers include, but are not limited to, B cell cancer, *e.g.,* multiple myeloma, melanomas, breast cancer, lung cancer (such as non-small cell lung carcinoma or NSCLC), bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, adenocarcinomas, inflammatory myofibroblastic tumors, gastrointestinal stromal tumor (GIST), colon cancer, multiple myeloma (MM), myelodysplastic syndrome (MDS), myeloproliferative disorder (MPD), acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), polycythemia Vera, Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), soft-tissue sarcoma, fibrosarcoma, myxosarcoma, liposarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma,

hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, hepatocellular carcinoma, thyroid cancer, gastric cancer, head and neck cancer, small cell cancers, essential thrombocythemia, agnogenic myeloid metaplasia, hypereosinophilic syndrome, systemic mastocytosis, familiar hypereosinophilia, chronic eosinophilic leukemia, neuroendocrine cancers, carcinoid tumors, and the like. In some embodiments, the cancer is a NSCLC (such as advanced NSCLCL or "aNSCLC," colorectal cancer, cholangiocarcinoma, breast cancer, stomach cancer, melanoma, pancreatic cancer, prostate cancer, ovarian cancer, esophageal cancer, or a cancer of unknown primary. In some embodiments, the cancer is metastatic urothelial carcinoma. In some embodiments, the cancer is metastatic gastric adenocarcinoma. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is a metastatic endometrial cancer. In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is castration resistant prostate cancer. In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the NSCLC is advanced NSCLC (aNSCLC).

[0115] In certain embodiments, the sample is from an individual having a solid tumor, a hematological cancer, or a metastatic form thereof. In certain embodiments, the sample is obtained from a subject having a cancer, or at risk of having a cancer. In certain embodiments, the sample is obtained from an individual who has not received a therapy to treat a cancer, is receiving a therapy to treat a cancer, or has received a therapy to treat a cancer, as described herein.

[0116] In some embodiments, the cancer is a hematologic malignancy (or premaligancy). As used herein, a hematologic malignancy refers to a tumor of the hematopoietic or lymphoid tissues, *e.g.,* a tumor that affects blood, bone marrow, or lymph nodes. Exemplary hematologic malignancies include, but are not limited to, leukemia (*e.g.,* acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, acute monocytic leukemia (AMoL), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), or large granular lymphocytic leukemia), lymphoma (*e.g.,* AIDS-related lymphoma, cutaneous T-cell lymphoma, Hodgkin lymphoma (*e.g.,* classical Hodgkin lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma), mycosis fungoides, non-Hodgkin lymphoma (*e.g.,* B-cell non-Hodgkin lymphoma (*e.g.,* Burkitt lymphoma, small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, or mantle cell lymphoma) or T-cell non-Hodgkin lymphoma (mycosis fungoides, anaplastic large cell lymphoma, or precursor T-lymphoblastic lymphoma)), primary central nervous system lymphoma, Sézary syndrome, Waldenström macroglobulinemia), chronic myeloproliferative neoplasm, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, or

myelodysplastic/myeloproliferative neoplasm. Premaligancy, as used herein, refers to a tissue that is not yet malignant but is poised to become malignant.

[0117] In some embodiments, the individual has been previously treated with an anti-cancer therapy, e.g., one or more anti-cancer therapies (e.g. any of the anti-cancer therapies of the disclosure). For example, the sample may be from an individual that has been treated with an anti-cancer therapy comprising one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, or a cytotoxic agent. In some embodiments, the individual has previously been treated with a chemotherapy or an immune-oncology therapy. In some embodiments, for a patient who has been previously treated with an anti-cancer therapy, a post-anti-cancer therapy sample, e.g., specimen is obtained, e.g., collected. In some embodiments, the post-anti-cancer therapy sample is a sample obtained, e.g., collected, after the completion of the targeted therapy.

[0118] In some embodiments, the individual has not previously received, or is not currently receiving, a treatment for the cancer. In some embodiments, the individual has not previously received a regimen of chemotherapy. In some embodiments, the individual has not received a chemotherapy for the cancer.

[0119] In some embodiments, the individual has previously received, or is currently receiving, a treatment for the cancer. In some embodiments, the individual has previously received a regimen of chemotherapy. In some embodiments, the individually has previously received a chemotherapy for the cancer.

[0120] In some embodiments, the individual is in need of a first line therapy for the cancer. In some embodiments, the individual is in need of a second line therapy for the cancer.

[0121] In some embodiments, the individual is a human. In some embodiments, the individual is a non-human mammal.

IV. Samples and processing

[0122] The methods of the present disclosure involve, in certain embodiments, determining or assessing a feature (such as a TMB score and/or a microsatellite instability) of a sample obtained from an individual having a cancer. In some embodiments, the sample is associated with the cancer to be treated or assessed. In some embodiments, the sample is from a solid tumor (e.g., a tumor biopsy sample). In some embodiments, the sample is from a liquid sample (e.g., a liquid biopsy sample). In some embodiments, the sample is from a blood sample.

[0123] In some embodiments, the sample is associated with a cancer that is a B cell cancer, *e.g.*, multiple myeloma, melanomas, breast cancer, lung cancer (such as non-small cell lung carcinoma or NSCLC, including, e.g., advanced NSCLC), bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous

system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, adenocarcinomas, inflammatory myofibroblastic tumors, gastrointestinal stromal tumor (GIST), colon cancer, multiple myeloma (MM), myelodysplastic syndrome (MDS), myeloproliferative disorder (MPD), acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), polycythemia Vera, Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), soft-tissue sarcoma, fibrosarcoma, myxosarcoma, liposarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, hepatocellular carcinoma, thyroid cancer, gastric cancer, head and neck cancer, small cell cancers, essential thrombocythemia, agnogenic myeloid metaplasia, hypereosinophilic syndrome, systemic mastocytosis, familiar hypereosinophilia, chronic eosinophilic leukemia, neuroendocrine cancers, carcinoid tumors, and the like. In some embodiments, the cancer is a NSCLC (e.g., advanced NSCLC), colorectal cancer, cholangiocarcinoma, breast cancer, stomach cancer, melanoma, pancreatic cancer, prostate cancer, ovarian cancer, esophageal cancer, or a cancer of unknown primary. In some embodiments, the tumor biopsy sample is associated with a metastatic urothelial carcinoma. In some embodiments, the sample is associated with a gastric adenocarcinoma. In some embodiments, the sample is associated with a breast cancer. In some embodiments, the sample is associated with a metastatic endometrial cancer. In some embodiments, the sample is associated with a prostate cancer. In some embodiments, the sample is associated with a metastatic castration resistant prostate cancer. In some embodiments, the sample is associated with a colorectal cancer. In some embodiments, the sample is associated with a lung cancer. In some embodiments, the lung cancer is NSCLC. In some embodiments, the NSCLC is advanced NSCLC. In some embodiments, the sample is associated with a melanoma.

[0124] In some embodiments, the sample comprises a nucleic acid, *e.g.*, DNA, RNA, or both. In certain embodiments, the sample comprises one or more nucleic acids from a cancer. In certain embodiments, the sample further comprises one or more non-nucleic acid components from the

tumor, *e.g.*, a cell, protein, carbohydrate, or lipid. In certain embodiments, the sample further comprises one or more nucleic acids from a non-tumor cell or tissue.

[0125] In some embodiments, the sample comprises one or more nucleic acids, *e.g.*, DNA, RNA, or both, from a premalignant or malignant cell, a cell from a solid tumor, a soft tissue tumor or a metastatic lesion, a cell from a hematological cancer, a histologically normal cell, a circulating tumor cells (CTCs), or a combination thereof. In some embodiments, the sample comprises one or more cells chosen from a premalignant or malignant cell, a cell from a solid tumor, a soft tissue tumor or a metastatic lesion, a cell from a hematological cancer, a histologically normal cell, a circulating tumor cell (CTC), or a combination thereof.

[0126] In some embodiments, the sample comprises RNA (e.g., mRNA), DNA, circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), or cell-free RNA (cfRNA) from the cancer. In some embodiments, the sample comprises cell-free DNA (cfDNA). In some embodiments, cfDNA comprises DNA from healthy tissue, e.g., non-diseased cells, or tumor tissue, e.g., tumor cells. In some embodiments cfDNA from tumor tissue comprises circulating tumor DNA (ctDNA). In some embodiments, the sample further comprises a non-nucleic acid component, *e.g.*, a cell, protein, carbohydrate, or lipid, *e.g.*, from the tumor.

[0127] In some embodiments, the sample is a liquid sample that comprises blood, plasma, serum, cerebrospinal fluid, sputum, stool, urine, or saliva. In some embodiments, the sample comprises blood, plasma or serum. In certain embodiments, the sample comprises cerebral spinal fluid (CSF). In certain embodiments, the sample comprises pleural effusion. In certain embodiments, the sample comprises ascites. In certain embodiments, the sample comprises urine.

[0128] In some embodiments, the sample comprises or is from a blood sample, e.g., is or is from a peripheral whole blood sample. In some embodiments, the peripheral whole blood sample is collected in, e.g., two tubes, e.g., with about 8.5 ml blood per tube. In some embodiments, the peripheral whole blood sample is collected by venipuncture, e.g., according to CLSI H3-A6. In some embodiments, the blood is immediately mixed after collecting, e.g., by gentle inversion, for, e.g., about 8-10 times. In some embodiments, inversion is performed by a complete, e.g., full, 180° turn, e.g., of the wrist. In some embodiments, the blood sample is shipped, e.g., at ambient temperature, e.g., 43-99°F or 6-37°C on the same day as collection. In some embodiments, the blood sample is not frozen or refrigerated. In some embodiments, the collected blood sample is kept, e.g., stored, at 43-99°F or 6-37°C.

[0129] In some embodiments of the methods described herein, the method further comprise isolating nucleic acids from a sample described herein. In some embodiments of the methods described herein, the method includes isolating nucleic acids from a sample to provide an isolated nucleic acid sample. In an embodiment, the method includes isolating nucleic acids from a control to provide an isolated control nucleic acid sample. In an embodiment, a method further comprises rejecting a sample with no detectable nucleic acid.

[0130] In some embodiments of the methods described herein, the method further comprises acquiring a value for nucleic acid yield in said sample and comparing the acquired value to a reference criterion, *e.g.*, wherein if said acquired value is less than said reference criterion, then amplifying the nucleic acid prior to library construction. In an embodiment, a method further comprises acquiring a value for the size of nucleic acid fragments in said sample and comparing the acquired value to a reference criterion, *e.g.*, a size, *e.g.,* average size, of at least 300, 600, or 900 bps. A parameter described herein can be adjusted or selected in response to this determination.

[0131] In some embodiments, the nucleic acids are isolated when they are partially purified or substantially purified. In some embodiments, a nucleic acid is isolated when purified away from other cellular components (e.g. proteins, carbohydrates, or lipids) or other contaminants by standard techniques.

[0132] Protocols for DNA isolation from a sample are known in the art, *e.g.*, as provided in Example 1 of International Patent Application Publication No. WO 2012/092426. Additional methods to isolate nucleic acids (*e.g.*, DNA) from formaldehyde- or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissues are disclosed, *e.g.*, in Cronin M. *et al.*, (2004) *Am J Pathol.* 164(1):35–42; Masuda N. *et al.*, (1999) *Nucleic Acids Res.* 27(22):4436–4443; Specht K. *et al.*, (2001) *Am J Pathol.* 158(2):419–429, Ambion RecoverAll™ Total Nucleic Acid Isolation Protocol (Ambion, Cat. No. AM1975, September 2008), Maxwell® 16 FFPE Plus LEV DNA Purification Kit Technical Manual (Promega Literature #TM349, February 2011), E.Z.N.A.® FFPE DNA Kit Handbook (OMEGA bio-tek, Norcross, GA, product numbers D3399-00, D3399-01, and D3399-02; June 2009), and QIAamp® DNA FFPE Tissue Handbook (Qiagen, Cat. No. 37625, October 2007). RecoverAll™ Total Nucleic Acid Isolation Kit uses xylene at elevated temperatures to solubilize paraffin-embedded samples and a glass-fiber filter to capture nucleic acids. Maxwell® 16 FFPE Plus LEV DNA Purification Kit is used with the Maxwell® 16 Instrument for purification of genomic DNA from 1 to 10 µm sections of FFPE tissue. DNA is purified using silica-clad paramagnetic particles (PMPs), and eluted in low elution volume. The E.Z.N.A.® FFPE DNA Kit uses a spin column and buffer system for isolation of genomic DNA. QIAamp® DNA FFPE Tissue Kit uses QIAamp® DNA Micro technology for purification of genomic and mitochondrial DNA. Protocols for DNA isolation from blood are disclosed, *e.g.,* in the Maxwell® 16 LEV Blood DNA Kit and Maxwell 16 Buccal Swab LEV DNA Purification Kit Technical Manual (Promega Literature #TM333, January 1, 2011).

[0133] Protocols for RNA isolation are disclosed, *e.g.,* in the Maxwell® 16 Total RNA Purification Kit Technical Bulletin (Promega Literature #TB351, August 2009).

[0134] The isolated nucleic acids (*e.g.*, genomic DNA) can be fragmented or sheared by practicing routine techniques. For example, genomic DNA can be fragmented by physical shearing methods, enzymatic cleavage methods, chemical cleavage methods, and other methods well known to those skilled in the art. The nucleic acid library can contain all or substantially all of the complexity of the genome. The term "substantially all" in this context refers to the possibility that there can in practice

be some unwanted loss of genome complexity during the initial steps of the procedure. The methods described herein also are useful in cases where the nucleic acid library is a portion of the genome, *e.g.*, where the complexity of the genome is reduced by design. In some embodiments, any selected portion of the genome can be used with a method described herein. In certain embodiments, the entire exome or a subset thereof is isolated.

[0135] In certain embodiments, the method further includes isolating nucleic acids from the sample to provide a library (*e.g.*, a nucleic acid library as described herein). In certain embodiments, the sample includes whole genomic, subgenomic fragments, or both. The isolated nucleic acids can be used to prepare nucleic acid libraries. Protocols for isolating and preparing libraries from whole genomic or subgenomic fragments are known in the art (*e.g.*, Illumina's genomic DNA sample preparation kit). In certain embodiments, the genomic or subgenomic DNA fragment is isolated from a subject's sample (*e.g.*, a sample described herein).

[0136] In still other embodiments, the nucleic acids used to generate the library include RNA or cDNA derived from RNA. In some embodiments, the RNA includes total cellular RNA. In other embodiments, certain abundant RNA sequences (*e.g.*, ribosomal RNAs) have been depleted. In some embodiments, the poly(A)-tailed mRNA fraction in the total RNA preparation has been enriched. In some embodiments, the cDNA is produced by random-primed cDNA synthesis methods. In other embodiments, the cDNA synthesis is initiated at the poly(A) tail of mature mRNAs by priming by oligo(dT)-containing oligonucleotides. Methods for depletion, poly(A) enrichment, and cDNA synthesis are well known to those skilled in the art.

[0137] In other embodiments, the nucleic acids are fragmented or sheared by a physical or enzymatic method, and optionally, ligated to synthetic adapters, size-selected (*e.g.*, by preparative gel electrophoresis) and amplified (*e.g.*, by PCR). Alternative methods for DNA shearing are known in the art, *e.g.*, as described in Example 4 in International Patent Application Publication No. WO 2012/092426. For example, alternative DNA shearing methods can be more automatable and/or more efficient (*e.g.*, with degraded FFPE samples). Alternatives to DNA shearing methods can also be used to avoid a ligation step during library preparation.

[0138] In other embodiments, the isolated DNA (*e.g.*, the genomic DNA) is fragmented or sheared. In some embodiments, the library includes less than 50% of genomic DNA, such as a subfraction of genomic DNA that is a reduced representation or a defined portion of a genome, *e.g.*, that has been subfractionated by other means. In other embodiments, the library includes all or substantially all genomic DNA.

[0139] In other embodiments, the fragmented and adapter-ligated group of nucleic acids is used without explicit size selection or amplification prior to hybrid selection. In some embodiments, the nucleic acid is amplified by a specific or non-specific nucleic acid amplification method that is well known to those skilled in the art. In some embodiments, the nucleic acid is amplified, *e.g.*, by a whole-genome amplification method such as random-primed strand-displacement amplification.

[0140] The methods described herein can be performed using a small amount of nucleic acids, *e.g.*, when the amount of source DNA or RNA is limiting (*e.g.*, even after whole-genome amplification). In one embodiment, the nucleic acid comprises less than about 5 μg, 4 μg, 3 μg, 2 μg, 1 μg, 0.8 μg, 0.7 μg, 0.6 μg, 0.5 μg, or 400 ng, 300 ng, 200 ng, 100 ng, 50 ng, 10 ng, 5 ng, 1 ng, or less of nucleic acid sample. For example, one can typically begin with 50-100 ng of genomic DNA. One can start with less, however, if one amplifies the genomic DNA (*e.g.*, using PCR) before the hybridization step, *e.g.*, solution hybridization. Thus it is possible, but not essential, to amplify the genomic DNA before hybridization, *e.g.*, solution hybridization.

[0141] In some embodiments, sequencing comprises providing a plurality of nucleic acid molecules obtained from the sample; amplifying nucleic acid molecules from the plurality of nucleic acid molecules; capturing nucleic acid molecules from the amplified nucleic acid molecules; and sequencing, by a sequencer, the captured nucleic acid molecules to obtain a plurality of sequence reads corresponding to one or more genomic loci within a subgenomic interval in the sample. In some embodiments, the plurality of nucleic acid molecules comprises a mixture of tumor nucleic acid molecules and non-tumor nucleic acid molecules.

[0142] In some embodiments, amplification of the nucleic acid molecules is performed by a polymerase chain reaction (PCR) technique, a non-PCR amplification technique, or an isothermal amplification technique.

[0143] In some embodiments, sequencing further comprises ligating one or more adapters onto one or more nucleic acid molecules from the plurality of nucleic acid molecules. In some embodiments, the adapters comprise one or more of amplification primer sequences, flow cell adapter hybridization sequences, unique molecular identifier sequences, substrate adapter sequences, or sample index sequences.

[0144] In some embodiments, nucleic acid molecules from a library are isolated, *e.g.*, using solution hybridization, thereby providing a library catch. The library catch, or a subgroup thereof, can be sequenced. Accordingly, the methods described herein can further include analyzing the library catch. In some embodiments, the library catch is analyzed by a sequencing method, *e.g.*, a next-generation sequencing method as described herein. In some embodiments, the method includes isolating a library catch by solution hybridization, and subjecting the library catch to nucleic acid sequencing. In certain embodiments, the library catch is re-sequenced.

[0145] In some embodiments, the captured nucleic acid molecules are captured from the amplified nucleic acid molecules by hybridization to one or more bait molecules. In some embodiments, the one or more bait molecules comprise one or more nucleic acid molecules, each comprising a region that is complementary to a region of a captured nucleic acid molecule. In some embodiments, the one or more bait molecules each comprise a capture moiety. In some embodiments, the capture moiety is biotin.

[0146] Any method of sequencing known in the art can be used. Sequencing of nucleic acids, e.g., isolated by solution hybridization, are typically carried out using next-generation sequencing (NGS). Sequencing methods suitable for use herein are described in the art, *e.g.,* as described in International Patent Application Publication No. WO 2012/092426. In some embodiments, sequencing is performed using a massively parallel sequencing (MPS) technique, whole genome sequencing (WGS), whole exome sequencing (WES), targeted sequencing, direct sequencing, next-generation sequencing (NGS), or a Sanger sequencing technique.

[0147] In some embodiments, sequencing comprises detecting alterations present in the genome, whole exome or transcriptome of an individual. In some embodiments, sequencing comprises DNA and/or RNA sequencing, *e.g.*, targeted DNA and/or RNA sequencing. In some embodiments, the sequencing comprises detection of a change (*e.g.*, an increase or decrease) in the level of a gene or gene product, *e.g.*, a change in expression of a gene or gene product described herein.

[0148] Sequencing can, optionally, include a step of enriching a sample for a target RNA. In other embodiments, sequencing includes a step of depleting the sample of certain high abundance RNAs, *e.g.*, ribosomal or globin RNAs. The RNA sequencing methods can be used, alone or in combination with the DNA sequencing methods described herein. In one embodiment, sequencing includes a DNA sequencing step and an RNA sequencing step. The methods can be performed in any order. For example, the method can include confirming by RNA sequencing the expression of an alteration described herein, *e.g.*, confirming expression of a mutation or a fusion detected by the DNA sequencing methods of the invention. In other embodiments, sequencing includes performing an RNA sequencing step, followed by a DNA sequencing step.

[0149] In some embodiments, the sample is associated with a cancer that has not been previously treated with an anti-cancer therapy. In some embodiments, the sample is associated with a cancer that has not been previously treated with a chemotherapy. In some embodiments, the sample is associated with a cancer that has been previously treated an anti-cancer therapy. In some embodiments, the sample is associated with a cancer that has been previously treated with a chemotherapy.

[0150] In some embodiments, the sample is a mammalian sample. In some embodiments, the sample is a human sample. In some embodiments, the sample is a non-human mammalian sample.

V. Methods for determining tumor mutational burden

[0151] Tumor mutational burden (TMB) is, broadly, the number of somatic mutations per megabase of genomic region. It has been discovered that a TMB score (which can be expressed, for example, as mutations per megabase, i.e., mutations/Mb) can inform treatment decisions, including, in some embodiments, whether to administer an immune checkpoint inhibitor therapy to a patient if a TMB score is at least a threshold TMB score or administer a chemotherapy regimen if a TMB score is below a threshold TMB score. The TMB score can be determined using a variety of techniques including next-generation sequencing (NGS) techniques.

[0152] In some embodiments, the methods of the disclosure comprise determining a tumor mutational burden (TMB) score in a sample, such as a tumor sample. In some embodiments, the TMB score is a blood TMB (bTMB) score. In some embodiments, the TMB score is a tissue TMB score (tTMB score).

[0153] In some embodiments, the TMB score is determined by sequencing. In some embodiments, the TMB score is determined by sequencing using a high-throughput sequencing technique, such as next-generation sequencing (NGS), an NGS-based method, or an NGS-derived method. In some embodiments, the NGS method is selected from whole genome sequencing (WGS), whole exome sequencing (WES) or a comprehensive genomic profiling (CGP). In some embodiments, the sequencing comprises sequencing a panel of cancer genes. In some embodiments, the TMB score reflects the number of nonsynonymous mutations, such as missense mutations or nonsense mutations, in a sequence. In some embodiments, the TMB score is determined by normalizing a matched tumor biopsy sample sequence with germline sequences to exclude inherited germline mutations.

[0154] The "threshold TMB score" described herein refers to a predetermined TMB score which a measured TMB score (i.e., the TMB score determined in a sample from an individual having a cancer) is compared with. Comparison of the determined TMB score with the threshold TMB score is used, in certain embodiments, to inform treatment decisions or identify treatment options for an individual. In some embodiments, the threshold TMB score is at least 8 mutations/Mb, such as at least any of about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, about 20 mutations/Mb, about 21 mutations/Mb, about 22 mutations/Mb, about 23 mutations/Mb, about 24 mutations/Mb, about 25 mutations/Mb, about 26 mutations/Mb, about 27 mutations/Mb, about 28 mutations/Mb, about 29 mutations/Mb, about 30 mutations/Mb, about 31 mutations/Mb, about 32 mutations/Mb, about 33 mutations/Mb, about 34 mutations/Mb, about 35 mutations/Mb, about 36 mutations/Mb, about 37 mutations/Mb, about 38 mutations/Mb, about 39 mutations/Mb, about 40 mutations/Mb, about 41 mutations/Mb, about 42 mutations/Mb, about 43 mutations/Mb, about 44 mutations/Mb, about 45 mutations/Mb, about 46 mutations/Mb, about 47 mutations/Mb, about 48 mutations/Mb, about 49 mutations/Mb, or about 50 mutations/Mb. In some embodiments, the threshold TMB score is about 8 mutations/Mb, such as at least any of about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, about 20 mutations/Mb, about 21 mutations/Mb, about 22 mutations/Mb, about 23 mutations/Mb, about 24 mutations/Mb, about 25 mutations/Mb, about 26 mutations/Mb, about 27 mutations/Mb, about 28 mutations/Mb, about 29 mutations/Mb, about 30 mutations/Mb, about 31 mutations/Mb, about 32 mutations/Mb, about 33 mutations/Mb, about 34 mutations/Mb, about 35 mutations/Mb, about 36 mutations/Mb, about 37 mutations/Mb, about 38 mutations/Mb, about 39

mutations/Mb, about 40 mutations/Mb, about 41 mutations/Mb, about 42 mutations/Mb, about 43 mutations/Mb, about 44 mutations/Mb, about 45 mutations/Mb, about 46 mutations/Mb, about 47 mutations/Mb, about 48 mutations/Mb, about 49 mutations/Mb, or about 50 mutations/Mb. In some embodiments, the threshold TMB score is about 10 mutations/Mb. In some embodiments, the threshold TMB score is 10 mutations/Mb. In some embodiments, the threshold TMB score is a "high tumor mutational burden score," e.g., a TMB score of at least about 10 mutations/Mb or more, such as any of at least about 15 mutations/Mb, about 20 mutations/Mb, about 25 mutations/Mb, about 30 mutations/Mb, about 35 mutations/Mb, about 40 mutations/Mb, about 45 mutations/Mb, about 50 mutations/Mb, or more.

[0155] As used herein, the terms "solid tumor TMB score," "tissue TMB score," and "tTMB score," are used interchangeably and refer to a numerical value that reflects the number of somatic mutations detected in a tumor biopsy sample (e.g., a solid tumor biopsy sample) obtained from an individual (e.g., an individual at risk of or having a cancer). The tTMB score can be measured, for example, on a whole genome or exome basis, or on the basis of a subset of the genome or exome (e.g., a predetermined set of genes). In some embodiments, the tTMB score can be measured based on intergenic sequences. In some embodiments, the tTMB score measured on the basis of a subset of genome or exome can be extrapolated to determine a whole genome or exome tTMB score. In certain embodiments, the predetermined set of genes does not comprise the entire genome or the entire exome. In other embodiments, the set of subgenomic intervals does not comprise the entire genome or the entire exome. In some embodiments, the predetermined set of genes comprise a plurality of genes, which in mutant form, are associated with an effect on cell division, growth or survival, or are associated with cancer. In some embodiments, the predetermined set of genes comprise at least about 50 or more, about 100 or more, about 150 or more, about 200 or more, about 250 or more, about 300 or more, about 350 or more, about 400 or more, about 450 or more, or about 500 or more genes. In some embodiments, the pre-determined set of genes covers about 1 Mb (e.g., about 1.1 Mb, e.g., about 1.125 Mb). In some embodiments, the tTMB score is determined from measuring the number of somatic mutations in cell-free DNA (cfDNA) in a sample. In some embodiments, the tTMB score is determined from measuring the number of somatic mutations in circulating tumor DNA (ctDNA) in a sample. In some embodiments, the number of somatic mutations is the number of single nucleotide variants (SNVs) counted or a sum of the number of SNVs and the number of indel mutations counted. In some embodiments, the tTMB score refers to the number of accumulated somatic mutations in a tumor.

[0156] As used herein, the terms "blood tumor mutational burden score," "blood tumor mutation burden score," and "bTMB score," each of which may be used interchangeably, refer to a numerical value that reflects the number of somatic mutations detected in a blood sample (e.g., a whole blood sample, a plasma sample, a serum sample, or a combination thereof) obtained from an individual (e.g., an individual at risk of or having a cancer). The bTMB score can be measured, for example, on a

whole genome or exome basis, or on the basis of a subset of the genome or exome (e.g., a predetermined set of genes). In certain embodiments, a bTMB score can be measured based on intergenic sequences. In some embodiments, the bTMB score measured on the basis of a subset of genome or exome can be extrapolated to determine a whole genome or exome bTMB score. In certain embodiments, the predetermined set of genes does not comprise the entire genome or the entire exome. In other embodiments, the set of subgenomic intervals does not comprise the entire genome or the entire exome. In some embodiments, the predetermined set of genes comprise a plurality of genes, which in mutant form, are associated with an effect on cell division, growth or survival, or are associated with cancer. In some embodiments, the predetermined set of genes comprise at least about 50 or more, about 100 or more, about 150 or more, about 200 or more, about 250 or more, about 300 or more, about 350 or more, about 400 or more, about 450 or more, or about 500 or more genes. In some embodiments, the pre-determined set of genes covers about 1 Mb (e.g., about 1.1 Mb, e.g., about 1.125 Mb). In some embodiments, the bTMB score is determined from measuring the number of somatic mutations in cell-free DNA (cfDNA) in a sample. In some embodiments, the bTMB score is determined from measuring the number of somatic mutations in circulating tumor DNA (ctDNA) in a sample. In some embodiments, the number of somatic mutations is the number of single nucleotide variants (SNVs) counted or a sum of the number of SNVs and the number of indel mutations counted. In some embodiments, the bTMB score refers to the number of accumulated somatic mutations in a tumor.

[0157] In some embodiments, tumor mutational burden (e.g. bTMB or solid tumor TMB) is measured using any suitable method known in the art. For example, tumor mutational burden may be measured using whole-exome sequencing (WES), next-generation sequencing, whole genome sequencing, gene-targeted sequencing, or sequencing of a panel of genes, e.g., panels including cancer-related genes. See, e.g., Melendez et al., Transl Lung Cancer Res (2018) 7(6):661-667. In some embodiments, tumor mutational burden is measured using gene-targeted sequencing, e.g., using a nucleic acid hybridization-capture method, e.g., coupled with sequencing. See, e.g., Fancello et al., J Immunother Cancer (2019) 7:183.

[0158] In some embodiments, tumor mutational burden is measured according to the methods provided in WO2017151524A1, which is hereby incorporated by reference in its entirety. In some embodiments, tumor mutational burden is measured according to the methods described in Montesion, M., et al., Cancer Discovery (2021) 11(2):282-92. In some embodiments, tumor mutational burden is measured according to the methods described in Chalmers et al., "Analysis of 100,00 human cancer genomes reveals the landscape of tumor mutational burden," *Genome Med.* 2017;9(1):34). In some embodiments, the tumor mutational burden is measured according to the methods described in Huang, R., et al., "Durable responders in advanced NSCLC with elevated TMB and treated with 1L immune checkpoint inhibitor: a real-world outcomes analysis," J Immunother Cancer (2023) 11(1):e005801. In some embodiments, the tumor mutational burden is measured

according to the methods described in Quintanilha, J., et al., "Comparative Effectiveness of Immune Checkpoint Inhibitors vs Chemotherapy in Patients With Metastatic Colorectal Cancer With Measures of Microsatellite Instability, Mismatch Repair, or Tumor Mutational Burden," JAMA Netw Open. (2023) 6(1):e2252244.

[0159] In some embodiments, tumor mutational burden is assessed based on the number of non-driver somatic coding mutations/megabase (mut/Mb) of genome sequenced.

[0160] In some embodiments, tumor mutational burden is measured in the sample using next-generation sequencing. In some embodiments, tumor mutational burden is measured in the sample by whole exome sequencing. In some embodiments, tumor mutational burden is measured in the sample using whole genome sequencing. In some embodiments, tumor mutational burden is measured in the sample by gene-targeted sequencing. In some embodiments, tumor mutational burden is measured on between about 0.8 Mb and about 1.3 Mb of sequenced DNA. In some embodiments, tumor mutational burden is measured on any of about 0.8 Mb, about 0.81 Mb, about 0.82 Mb, about 0.83 Mb, about 0.84 Mb, about 0.85 Mb, about 0.86 Mb, about 0.87 Mb, about 0.88 Mb, about 0.89 Mb, about 0.9 Mb, about 0.91 Mb, about 0.92 Mb, about 0.93 Mb, about 0.94 Mb, about 0.95 Mb, about 0.96 Mb, about 0.97 Mb, about 0.98 Mb, about 0.99 Mb, about 1 Mb, about 1.01 Mb, about 1.02 Mb, about 1.03 Mb, about 1.04 Mb, about 1.05 Mb, about 1.06 Mb, about 1.07 Mb, about 1.08 Mb, about 1.09 Mb, about 1.1 Mb, about 1.2 Mb, or about 1.3 Mb of sequenced DNA. In some embodiments, tumor mutational burden is measured on about 0.8 Mb of sequenced DNA. In some embodiments, tumor mutational burden is measured on between about 0.83 Mb and about 1.14 Mb of sequenced DNA. In some embodiments, tumor mutational burden is measured on up to about 1.24 Mb of sequenced DNA. In some embodiments, tumor mutational burden is measured on up to about 1.1 Mb of sequenced DNA. In some embodiments, tumor mutational burden is measured on about 0.79 Mb of sequenced DNA.

[0161] In some embodiments, the TMB score is less than about 10 mutations/Mb. In some embodiments, the TMB score is more than about 10 mutations/Mb. In some embodiments, the TMB score is at least 10 mutations/Mb. In some embodiments, the TMB score is a high tumor mutational burden score, e.g., of at least about 10 mut/Mb. In some embodiments, the TMB score is at least about 10 mut/Mb. In some embodiments, the TMB score is at least about 20 mut/Mb. In some embodiments, the TMB score is between about 10 mut/Mb and about 15 mut/Mb, between about 15 mut/Mb and about 20 mut/Mb, between about 20 mut/Mb and about 25 mut/Mb, between about 25 mut/Mb and about 30 mut/Mb, between about 30 mut/Mb and about 35 mut/Mb, between about 35 mut/Mb and about 40 mut/Mb, between about 40 mut/Mb and about 45 mut/Mb, between about 45 mut/Mb and about 50 mut/Mb, between about 50 mut/Mb and about 55 mut/Mb, between about 55 mut/Mb and about 60 mut/Mb, between about 60 mut/Mb and about 65 mut/Mb, between about 65 mut/Mb and about 70 mut/Mb, between about 70 mut/Mb and about 75 mut/Mb, between about 75 mut/Mb and about 80 mut/Mb, between about 80 mut/Mb and about 85 mut/Mb, between about 85

mut/Mb and about 90 mut/Mb, between about 90 mut/Mb and about 95 mut/Mb, or between about 95 mut/Mb and about 100 mut/Mb. In some embodiments, the TMB score is between about 100 mut/Mb and about 110 mut/Mb, between about 110 mut/Mb and about 120 mut/Mb, between about 120 mut/Mb and about 130 mut/Mb, between about 130 mut/Mb and about 140 mut/Mb, between about 140 mut/Mb and about 150 mut/Mb, between about 150 mut/Mb and about 160 mut/Mb, between about 160 mut/Mb and about 170 mut/Mb, between about 170 mut/Mb and about 180 mut/Mb, between about 180 mut/Mb and about 190 mut/Mb, between about 190 mut/Mb and about 200 mut/Mb, between about 210 mut/Mb and about 220 mut/Mb, between about 220 mut/Mb and about 230 mut/Mb, between about 230 mut/Mb and about 240 mut/Mb, between about 240 mut/Mb and about 250 mut/Mb, between about 250 mut/Mb and about 260 mut/Mb, between about 260 mut/Mb and about 270 mut/Mb, between about 270 mut/Mb and about 280 mut/Mb, between about 280 mut/Mb and about 290 mut/Mb, between about 290 mut/Mb and about 300 mut/Mb, between about 300 mut/Mb and about 310 mut/Mb, between about 310 mut/Mb and about 320 mut/Mb, between about 320 mut/Mb and about 330 mut/Mb, between about 330 mut/Mb and about 340 mut/Mb, between about 340 mut/Mb and about 350 mut/Mb, between about 350 mut/Mb and about 360 mut/Mb, between about 360 mut/Mb and about 370 mut/Mb, between about 370 mut/Mb and about 380 mut/Mb, between about 380 mut/Mb and about 390 mut/Mb, between about 390 mut/Mb and about 400 mut/Mb, or more than 400 mut/Mb. In some embodiments, the TMB score is at least about 100 mut/Mb, at least about 110 mut/Mb, at least about 120 mut/Mb, at least about 130 mut/Mb, at least about 140 mut/Mb, at least about 150 mut/Mb, or more. In some embodiments, the TMB score is determined based on between about 0.8 Mb to about 1.1 Mb.

VI. Methods for determining microsatellite instability

[0162] Some aspects of the disclosure provide for further analysis of a microsatellite instability (MSI) status. It has been discovered that assessment of microsatellite instability can inform treatment decisions, including, in some embodiments, whether to administer an immune checkpoint inhibitor therapy to a patient if MSI is assessed to be MSI-H in a sample obtained from an individual having a cancer, or whether to administer a chemotherapy to a patient if MSI is assessed to be not MSI-H (such as MSI-L or MSS) in the sample obtained from the individual having the cancer. Microsatellites are sequences of 1-6 nucleotides typically repeated 5-50 times within the genome. Microsatellite instability can be classified into categories of degree, such as high (MSI-H), low (MSI-L), or stable (MSS). MSS refers to microsatellite status that does not display somatic changes in the number of the repeated nucleotide sequences. MSI-L refers to a microsatellite status that has an intermediate phenotype between MSS and MSI-H.

[0163] Microsatellite instability may be assessed using any suitable method known in the art. For example, microsatellite instability may be measured using next generation sequencing (see, e.g., Hempelmann et al., J Immunother Cancer (2018) 6(1):29), Fluorescent multiplex PCR and capillary

electrophoresis (see, e.g., Arulananda et al., J Thorac Oncol (2018) 13(10):1588–94), immunohistochemistry (see, e.g., Cheah et al., Malays J Pathol (2019) 41(2):91–100), or single-molecule molecular inversion probes (smMIPs, see, e.g., Waalkes et al., Clin Chem (2018) 64(6):950–8). In some embodiments, microsatellite instability is assessed based on DNA sequencing (e.g., next generation sequencing) of up to about 114 loci. In some embodiments, microsatellite instability is assessed based on DNA sequencing (e.g., next generation sequencing) of intronic homopolymer repeat loci for length variability. In some embodiments, microsatellite instability is assessed based on DNA sequencing (e.g., next generation sequencing) about 114 intronic homopolymer repeat loci for length variability. In some embodiments, microsatellite instability status (e.g., microsatellite instability high) is defined as described in Trabucco et al., J Mol Diagn. 2019 Nov;21(6):1053-1066.

VII. Immune checkpoint inhibitors

[0164] The methods described herein pertain, in certain embodiments, to means of predicting the efficacy of an immune checkpoint inhibitor (ICPI) therapy and/or administering an ICPI to an individual having a cancer. In some embodiments, the efficacy of the ICPI therapy is predicted as a first line treatment. In some embodiments, the ICPI therapy is administered as a first line therapy for a cancer. In some embodiments, the ICPI therapy is the only treatment administered or indicated. In some embodiments, the ICPI therapy consists of a single active agent, such as a single immune checkpoint inhibitor. In some embodiments, the efficacy of the ICPI therapy is predicted as a second line treatment. In some embodiments, the ICPI therapy is administered as a second line therapy for a cancer. In some embodiments, the ICPI therapy is administered or indicated to be administered with another treatment, such as a non-ICPI therapy. In some embodiments, the ICPI therapy comprises a combination ICPI therapy comprising two or more ICPIs, that is, two or more different active agents which target immune checkpoints. In some embodiments, the two or more different active agents each target a different immune checkpoint protein.

[0165] As is known in the art, a checkpoint inhibitor targets at least one immune checkpoint protein to alter the regulation of an immune response. Immune checkpoint proteins include, e.g., CTLA4, PD-L1, PD-1, PD-L2, VISTA, B7-H2, B7-H3, B7-H4, B7-H6, 2B4, ICOS, HVEM, CEACAM, LAIR1, CD80, CD86, CD276, VTCN1, MHC class I, MHC class II, GALS, adenosine, TGFR, CSF1R, MICA/B, arginase, CD160, gp49B, PIR-B, KIR family receptors, TIM-1 , TIM-3, TIM-4, LAG-3, BTLA, SIRPalpha (CD47), CD48, 2B4 (CD244), B7.1, B7.2, ILT-2, ILT-4, TIGIT, LAG-3, BTLA, IDO, OX40, and A2aR. In some embodiments, molecules involved in regulating immune checkpoints include, but are not limited to: PD-1 (CD279), PD-L1 (B7-H1, CD274), PD-L2 (B7-CD, CD273), CTLA-4 (CD152), HVEM, BTLA (CD272), a killer-cell immunoglobulin-like receptor (KIR), LAG-3 (CD223), TIM-3 (HAVCR2), CEACAM, CEACAM-1, CEACAM-3, CEACAM-5, GAL9, VISTA (PD-1H), TIGIT, LAIR1, CD160, 2B4, TGFRbeta, A2AR, GITR (CD357), CD80

(B7-1), CD86 (B7-2), CD276 (B7-H3), VTCNI (B7-H4), MHC class I, MHC class II, GALS, adenosine, TGFR, B7-H1, OX40 (CD134), CD94 (KLRD1), CD137 (4-1BB), CD137L (4-1BBL), CD40, IDO, CSF1R, CD40L, CD47, CD70 (CD27L), CD226, HHLA2, ICOS (CD278), ICOSL (CD275), LIGHT (TNFSF14, CD258), NKG2a, NKG2d, OX40L (CD134L), PVR (NECL5, CD155), SIRPa, MICA/B, and/or arginase. In some embodiments, an immune checkpoint inhibitor (i.e., a checkpoint inhibitor) decreases the activity of a checkpoint protein that negatively regulates immune cell function, e.g., in order to enhance T cell activation and/or an anti-cancer immune response. In other embodiments, a checkpoint inhibitor increases the activity of a checkpoint protein that positively regulates immune cell function, e.g., in order to enhance T cell activation and/or an anti-cancer immune response. In some embodiments, the checkpoint inhibitor is an antibody. Examples of checkpoint inhibitors include, without limitation, a PD-1 axis binding antagonist, a PD-L1 axis binding antagonist (e.g., an anti-PD-L1 antibody, e.g., atezolizumab (MPDL3280A)), an antagonist directed against a co-inhibitory molecule (e.g., a CTLA4 antagonist (e.g., an anti-CTLA4 antibody), a TIM-3 antagonist (e.g., an anti-TIM-3 antibody), or a LAG-3 antagonist (e.g., an anti-LAG-3 antibody)), or any combination thereof. In some embodiments, the immune checkpoint inhibitors comprise drugs such as small molecules, recombinant forms of ligand or receptors, or antibodies, such as human antibodies (see, e.g., International Patent Publication W02015016718; Pardoll, Nat Rev Cancer, 12(4): 252-64, 2012; both incorporated herein by reference). In some embodiments, known inhibitors of immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized or human forms of antibodies may be used.

[0166] In some embodiments according to any of the embodiments described herein, the immune checkpoint inhibitor comprises a PD-1 antagonist/inhibitor or a PD-L1 antagonist/inhibitor.

[0167] In some embodiments, the checkpoint inhibitor is a PD-L1 axis binding antagonist, e.g., a PD-1 binding antagonist, a PD-L1 binding antagonist, or a PD-L2 binding antagonist. PD-1 (programmed death 1) is also referred to in the art as "programmed cell death 1," "PDCD1," "CD279," and "SLEB2." An exemplary human PD-1 is shown in UniProtKB/Swiss-Prot Accession No. Q15116. PD-L1 (programmed death ligand 1) is also referred to in the art as "programmed cell death 1 ligand 1," "PDCD1 LG1," "CD274," "B7-H," and "PDL1." An exemplary human PD-L1 is shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1. PD-L2 (programmed death ligand 2) is also referred to in the art as "programmed cell death 1 ligand 2," "PDCD1 LG2," "CD273," "B7-DC," "Btdc," and "PDL2." An exemplary human PD-L2 is shown in UniProtKB/Swiss-Prot Accession No. Q9BQ51. In some instances, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2.

[0168] In some instances, the PD-1 binding antagonist/inhibitor is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific embodiment, the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another instance, a PD-L1 binding antagonist/inhibitor is a molecule that inhibits the binding of PD-L1 to its binding ligands. In a specific embodiment, PD-L1 binding partners are PD-1 and/or B7-1. In another instance, the PD-L2 binding antagonist is a

molecule that inhibits the binding of PD-L2 to its ligand binding partners. In a specific embodiment, the PD-L2 binding ligand partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or an oligopeptide. In some embodiments, the PD-1 binding antagonist is a small molecule, a nucleic acid, a polypeptide (e.g., antibody), a carbohydrate, a lipid, a metal, or a toxin.

[0169] In some instances, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody), for example, as described below. In some instances, the anti-PD-1 antibody is MDX-1 106 (nivolumab), MK-3475 (pembrolizumab, Keytruda®), cemiplimab, dostarlimab, MEDI-0680 (AMP-514), PDR001, REGN2810, MGA-012, JNJ-63723283, BI 754091, or BGB-108. In other instances, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence)). In some instances, the PD-1 binding antagonist is AMP-224. Other examples of anti-PD-1 antibodies include, but are not limited to, MEDI-0680 (AMP-514; AstraZeneca), PDR001 (CAS Registry No. 1859072-53-9; Novartis), REGN2810 (LIBTAYO® or cemiplimab-rwlc; Regeneron), BGB-108 (BeiGene), BGB-A317 (BeiGene), BI 754091, JS-001 (Shanghai Junshi), STI-A1110 (Sorrento), INCSHR-1210 (Incyte), PF-06801591 (Pfizer), TSR-042 (also known as ANB011; Tesaro/AnaptysBio), AM0001 (ARMO Biosciences), ENUM 244C8 (Enumeral Biomedical Holdings), or ENUM 388D4 (Enumeral Biomedical Holdings). In some embodiments, the PD-1 axis binding antagonist comprises tislelizumab (BGB-A317), BGB-108, STI-A1110, AM0001, BI 754091, sintilimab (IBI308), cetrelimab (JNJ-63723283), toripalimab (JS-001), camrelizumab (SHR-1210, INCSHR-1210, HR-301210), MEDI-0680 (AMP-514), MGA-012 (INCMGA 0012), nivolumab (BMS-936558, MDX1106, ONO-4538), spartalizumab (PDR00l), pembrolizumab (MK-3475, SCH 900475, Keytruda®), PF-06801591, cemiplimab (REGN-2810, REGEN2810), dostarlimab (TSR-042, ANB011), FITC-YT-16 (PD-1 binding peptide), APL-501 or CBT-501 or genolimzumab (GB-226), AB-122, AK105, AMG 404, BCD-100, F520, HLX10, HX008, JTX-4014, LZM009, Sym021, PSB205, AMP-224 (fusion protein targeting PD-1), CX-188 (PD-1 probody), AGEN-2034, GLS-010, budigalimab (ABBV-181), AK-103, BAT-1306, CS-1003, AM-0001, TILT-123, BH-2922, BH-2941, BH-2950, ENUM-244C8, ENUM-388D4, HAB-21, H EISCOI 11-003, IKT-202, MCLA-134, MT-17000, PEGMP-7, PRS-332, RXI-762, STI-1110, VXM-10, XmAb-23104, AK-112, HLX-20, SSI-361, AT-16201, SNA-01, AB122, PD1-PIK, PF-06936308, RG-7769, CAB PD-1 Abs, AK-123, MEDI-3387, MEDI-5771, 4H1128Z-E27, REMD-288, SG-001, BY-24.3, CB-201, IBI-319, ONCR-177, Max-1, CS-4100, JBI-426, CCC-0701, or CCX- 4503, or derivatives thereof.

[0170] In some embodiments, the PD-L1 binding antagonist is a small molecule that inhibits PD-1. In some embodiments, the PD-L1 binding antagonist is a small molecule that inhibits PD-L1. In some embodiments, the PD-L1 binding antagonist is a small molecule that inhibits PD-L1 and VISTA or PD-L1 and TIM3. In some embodiments, the PD-L1 binding antagonist is CA-170 (also known as

AUPM-170). In some embodiments, the PD-L1 binding antagonist is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody can bind to a human PD-L1, for example a human PD-L1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof. In some embodiments, the PD-L1 binding antagonist is a small molecule, a nucleic acid, a polypeptide (e.g., antibody), a carbohydrate, a lipid, a metal, or a toxin.

[0171] In some instances, the PD-L1 binding antagonist is an anti-PD-L1 antibody, for example, as described below. In some instances, the anti-PD-L1 antibody is capable of inhibiting the binding between PD-L1 and PD-1, and/or between PD-L1 and B7-1. In some instances, the anti-PD-L1 antibody is a monoclonal antibody. In some instances, the anti-PD-L1 antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or (Fab')2 fragment. In some instances, the anti-PD-L1 antibody is a humanized antibody. In some instances, the anti-PD-L1 antibody is a human antibody. In some instances, the anti-PD-L1 antibody is selected from YW243.55.S70, MPDL3280A (atezolizumab), MDX-1 105, MEDI4736 (durvalumab), or MSB0010718C (avelumab). In some embodiments, the PD-L1 axis binding antagonist comprises atezolizumab, avelumab, durvalumab (imfinzi), BGB-A333, SHR-1316 (HTI-1088), CK-301, BMS-936559, envafolimab (KN035, ASC22), CS1001, MDX-1105 (BMS-936559), LY3300054, STI-A1014, FAZ053, CX-072, INCB086550, GNS-1480, CA-170, CK-301, M-7824, HTI-1088 (HTI-131 , SHR-1316), MSB-2311, AK- 106, AVA-004, BBI-801, CA-327, CBA-0710, CBT-502, FPT-155, IKT-201, IKT-703, 10-103, JS-003, KD-033, KY-1003, MCLA-145, MT-5050, SNA-02, BCD-135, APL-502 (CBT-402 or TQB2450), IMC-001, KD-045, INBRX-105, KN-046, IMC-2102, IMC-2101, KD-005, IMM-2502, 89Zr-CX-072, 89Zr-DFO-6E11, KY-1055, MEDI-1109, MT-5594, SL-279252, DSP-106, Gensci-047, REMD-290, N-809, PRS-344, FS-222, GEN-1046, BH-29xx, or FS-118, or a derivative thereof.

[0172] In some embodiments, the checkpoint inhibitor is an antagonist/inhibitor of CTLA4. In some embodiments, the checkpoint inhibitor is a small molecule antagonist of CTLA4. In some embodiments, the checkpoint inhibitor is an anti-CTLA4 antibody. CTLA4 is part of the CD28-B7 immunoglobulin superfamily of immune checkpoint molecules that acts to negatively regulate T cell activation, particularly CD28-dependent T cell responses. CTLA4 competes for binding to common ligands with CD28, such as CD80 (B7-1) and CD86 (B7-2), and binds to these ligands with higher affinity than CD28. Blocking CTLA4 activity (e.g., using an anti-CTLA4 antibody) is thought to enhance CD28-mediated costimulation (leading to increased T cell activation/priming), affect T cell development, and/or deplete Tregs (such as intratumoral Tregs). In some embodiments, the CTLA4 antagonist is a small molecule, a nucleic acid, a polypeptide (e.g., antibody), a carbohydrate, a lipid, a metal, or a toxin. In some embodiments, the CTLA-4 inhibitor comprises ipilimumab (IBI310, BMS-734016, MDX010, MDX-CTLA4, MEDI4736), tremelimumab (CP-675, CP-675,206), APL-509, AGEN1884, CS1002, AGEN1181, Abatacept (Orencia, BMS-188667, RG2077), BCD-145, ONC-392, ADU-1604, REGN4659, ADG116, KN044, KN046, or a derivative thereof.

[0173] In some embodiments, the anti-PD-1 antibody or antibody fragment is MDX-1106 (nivolumab), MK-3475 (pembrolizumab, Keytruda®), cemiplimab, dostarlimab, MEDI-0680 (AMP-514), PDR001, REGN2810, MGA-012, JNJ-63723283, BI 754091, BGB-108, BGB-A317, JS-001, STI-A1110, INCSHR-1210, PF-06801591, TSR-042, AM0001, ENUM 244C8, or ENUM 388D4. In some embodiments, the PD-1 binding antagonist is an anti-PD-1 immunoadhesin. In some embodiments, the anti-PD-1 immunoadhesin is AMP-224. In some embodiments, the anti-PD-L1 antibody or antibody fragment is YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), MSB0010718C (avelumab), LY3300054, STI-A1014, KN035, FAZ053, or CX-072.

[0174] In some embodiments, the immune checkpoint inhibitor comprises a LAG-3 inhibitor (e.g., an antibody, an antibody conjugate, or an antigen-binding fragment thereof). In some embodiments, the LAG-3 inhibitor comprises a small molecule, a nucleic acid, a polypeptide (e.g., an antibody), a carbohydrate, a lipid, a metal, or a toxin. In some embodiments, the LAG-3 inhibitor comprises a small molecule. In some embodiments, the LAG-3 inhibitor comprises a LAG-3 binding agent. In some embodiments, the LAG-3 inhibitor comprises an antibody, an antibody conjugate, or an antigen-binding fragment thereof. In some embodiments, the LAG-3 inhibitor comprises eftilagimod alpha (IMP321, IMP-321, EDDP-202, EOC-202), relatlimab (BMS-986016), GSK2831781 (IMP-731), LAG525 (IMP701), TSR-033, EVIP321 (soluble LAG-3 protein), BI 754111, IMP761, REGN3767, MK-4280, MGD-013, XmAb22841, INCAGN-2385, ENUM-006, AVA-017, AM-0003, iOnctura anti-LAG-3 antibody, Arcus Biosciences LAG-3 antibody, Sym022, a derivative thereof, or an antibody that competes with any of the preceding.

[0175] In some embodiments, the immune checkpoint inhibitor is monovalent and/or monospecific. In some embodiments, the immune checkpoint inhibitor is multivalent and/or multispecific.

[0176] In some embodiments, the immune checkpoint inhibitor may be administered in combination with an immunoregulatory molecule or a cytokine. An immunoregulatory profile is required to trigger an efficient immune response and balance the immunity in a subject. Examples of suitable immunoregulatory cytokines include, but are not limited to, interferons (e.g., IFNα, IFNβ and IFNγ), interleukins (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 and IL-20), tumor necrosis factors (e.g., TNFα and TNFβ), erythropoietin (EPO), FLT-3 ligand, gIp10, TCA-3, MCP-1, MIF, MIP-1α, MIP-1β, Rantes, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), or granulocyte-macrophage colony stimulating factor (GM-CSF), as well as functional fragments thereof. In some embodiments, any immunomodulatory chemokine that binds to a chemokine receptor, i.e., a CXC, CC, C, or CX3C chemokine receptor, can be used in the context of the present disclosure. Examples of chemokines include, but are not limited to, MIP-3α (Lax), MIP-3β, Hcc-1, MPIF-1, MPIF-2, MCP-2, MCP-3, MCP-4, MCP-5, Eotaxin, Tarc, Elc, I309, IL-8, GCP-2 Groα, Gro-β, Nap-2, Ena-78, Ip-10, MIG, I-Tac, SDF-1, or BCA-1 (Blc), as well as functional

fragments thereof. In some embodiments, the immunoregulatory molecule is included with any of the treatments provided herein.

[0177] In some embodiments, the immune checkpoint inhibitor is a first line immune checkpoint inhibitor (e.g., it is a first line therapy for the cancer). In some embodiments, the immune checkpoint inhibitor is a second line immune checkpoint inhibitor (e.g., it is a second line therapy for the cancer). In some embodiments, an immune checkpoint inhibitor is administered in combination with one or more additional anti-cancer therapies or treatments.

VIII. Chemotherapies

[0178] The methods described herein pertain, in certain embodiments, to means of predicting the efficacy of a chemotherapy regimen and/or administering a chemotherapy regimen to an individual having a cancer. For example, in some embodiments, when a TMB score of a tumor is determined to be below a threshold level, such as below 10 mut/megabase or below 20 mut/megabase, then the tumor is not indicated as a suitable candidate for an ICPI therapy and is instead indicad for therapy with a chemotherapy regimen.

[0179] In some embodiments, the methods provided herein comprise administering to an individual a chemotherapy. Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclosphosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlomaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics, such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6- diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin,

puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, e.g., paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP- 16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (e.g., CPT-l l); topoisomerase inhibitor RFS 2000; difluorometlhylomithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

[0180] Some non-limiting examples of chemotherapeutic drugs of the present disclosure are carboplatin (Paraplatin), cisplatin (Platinol, Platinol-AQ), cyclophosphamide (Cytoxan, Neosar), docetaxel (Taxotere), doxorubicin (Adriamycin), erlotinib (Tarceva), etoposide (VePesid), fluorouracil (5-FU), gemcitabine (Gemzar), imatinib mesylate (Gleevec), irinotecan (Camptosar), methotrexate (Folex, Mexate, Amethopterin), paclitaxel (Taxol, Abraxane), sorafinib (Nexavar), sunitinib (Sutent), topotecan (Hycamtin), vincristine (Oncovin, Vincasar PFS), and vinblastine (Velban).

IX. Additional anti-cancer agents

[0181] In some embodiments, an additional anti-cancer agent is administered in addition to the treatments otherwise described (e.g., in addition to a chemotherapy regimen as described in Section VIII or in addition to an ICPI as described in Section VII). It is understood that if a tumor is determined to be a suitable candidate for an ICPI therapy using the methods described herein, such as if the tumor is found to have a TMB score of at least 10 mut/megabase or at least 20 mut/megabase,

the subject having the tumor may, in some embodiments, further benefit from treatment with an additional anti-cancer agent in addition to the ICPI therapy. Likewise, if a tumor is determined to be a suitable candidate for a chemotherapy regimen, such as if the tumor is found to have a TMBV score less than 10 mut/megabase, the subject having the tumor may, in some embodiments, further benefit from treatment with an additional anti-cancer agent in addition to the chemotherapy regimen.

[0182] In some embodiments, the additional anti-cancer therapy comprises a kinase inhibitor. In some embodiments, the methods provided herein comprise administering to the individual a kinase inhibitor, e.g., in combination with another therapy such as an immune checkpoint inhibitor. Examples of kinase inhibitors include those that target one or more receptor tyrosine kinases, e.g., BCR-ABL, B-Raf, EGFR, HER-2/ErbB2, IGF-IR, PDGFR-a, PDGFR- β, cKit, Flt-4, Flt3, FGFR1, FGFR3, FGFR4, CSF1R, c-Met, RON, c-Ret, or ALK; one or more cytoplasmic tyrosine kinases, e.g., c-SRC, c-YES, Abl, or JAK-2; one or more serine/threonine kinases, e.g., ATM, Aurora A & B, CDKs, mTOR, PKCi, PLKs, b-Raf, S6K, or STK11/LKB1; or one or more lipid kinases, e.g., PI3K or SKI. Small molecule kinase inhibitors include PHA-739358, nilotinib, dasatinib, PD166326, NSC 743411, lapatinib (GW-572016), canertinib (CI-1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sutent (SU1 1248), sorafenib (BAY 43-9006), or leflunomide (SU101). Additional non-limiting examples of tyrosine kinase inhibitors include imatinib (Gleevec/Glivec) and gefitinib (Iressa).

[0183] In some embodiments, the additional anti-cancer therapy comprises an anti-angiogenic agent. In some embodiments, the methods provided herein comprise administering to the individual an anti-angiogenic agent, e.g., in combination with another therapy such as an immune checkpoint inhibitor. Angiogenesis inhibitors prevent the extensive growth of blood vessels (angiogenesis) that tumors require to survive. Non-limiting examples of angiogenesis-mediating molecules or angiogenesis inhibitors which may be used in the methods of the present disclosure include soluble VEGF (for example: VEGF isoforms, e.g., VEGF121 and VEGF165; VEGF receptors, e.g., VEGFR1, VEGFR2; and co-receptors, e.g., Neuropilin-1 and Neuropilin-2), NRP-1, angiopoietin 2, TSP-1 and TSP-2, angiostatin and related molecules, endostatin, vasostatin, calreticulin, platelet factor-4, TIMP and CDAI, Meth-1 and Meth-2, IFNα, IFN-β and IFN-γ, CXCL10, IL-4, IL-12 and IL-18, prothrombin (kringle domain-2), antithrombin III fragment, prolactin, VEGI, SPARC, osteopontin, maspin, canstatin, proliferin-related protein, restin and drugs such as bevacizumab, itraconazole, carboxyamidotriazole, TNP-470, CM101, IFN-a platelet factor-4, suramin, SU5416, thrombospondin, VEGFR antagonists, angiostatic steroids and heparin, cartilage-derived angiogenesis inhibitory factor, matrix metalloproteinase inhibitors, 2-methoxyestradiol, tecogalan, tetrathiomolybdate, thalidomide, thrombospondin, prolactina v β3 inhibitors, linomide, or tasquinimod. In some embodiments, known therapeutic candidates that may be used according to the methods of the disclosure include naturally occurring angiogenic inhibitors, including without limitation, angiostatin, endostatin, or platelet factor-4. In another embodiment, therapeutic candidates that may be used according to the methods of

the disclosure include, without limitation, specific inhibitors of endothelial cell growth, such as TNP-470, thalidomide, and interleukin-12. Still other anti-angiogenic agents that may be used according to the methods of the disclosure include those that neutralize angiogenic molecules, including without limitation, antibodies to fibroblast growth factor, antibodies to vascular endothelial growth factor, antibodies to platelet derived growth factor, or antibodies or other types of inhibitors of the receptors of EGF, VEGF or PDGF. In some embodiments, anti-angiogenic agents that may be used according to the methods of the disclosure include, without limitation, suramin and its analogs, and tecogalan. In other embodiments, anti-angiogenic agents that may be used according to the methods of the disclosure include, without limitation, agents that neutralize receptors for angiogenic factors or agents that interfere with vascular basement membrane and extracellular matrix, including, without limitation, metalloprotease inhibitors and angiostatic steroids. Another group of anti-angiogenic compounds that may be used according to the methods of the disclosure includes, without limitation, anti-adhesion molecules, such as antibodies to integrin alpha v beta 3. Still other anti-angiogenic compounds or compositions that may be used according to the methods of the disclosure include, without limitation, kinase inhibitors, thalidomide, itraconazole, carboxyamidotriazole, CM101, IFN-α, IL-12, SU5416, thrombospondin, cartilage-derived angiogenesis inhibitory factor, 2-methoxyestradiol, tetrathiomolybdate, thrombospondin, prolactin, and linomide. In one particular embodiment, the anti-angiogenic compound that may be used according to the methods of the disclosure is an antibody to VEGF, such as Avastin®/bevacizumab (Genentech).

[0184] In some embodiments, the additional anti-cancer therapy comprises an anti-DNA repair therapy. In some embodiments, the methods provided herein comprise administering to the individual an anti-DNA repair therapy, e.g., in combination with another therapy such as an immune checkpoint inhibitor. In some embodiments, the anti-DNA repair therapy is a PARP inhibitor (e.g., talazoparib, rucaparib, olaparib), a RAD51 inhibitor (e.g., RI-1), or an inhibitor of a DNA damage response kinase, e.g., CHCK1 (e.g., AZD7762), ATM (e.g., KU-55933, KU-60019, NU7026, or VE-821), and ATR (e.g., NU7026).

[0185] In some embodiments, the additional anti-cancer therapy comprises a radiosensitizer. In some embodiments, the methods provided herein comprise administering to the individual a radiosensitizer, e.g., in combination with another therapy such as an immune checkpoint inhibitor. Exemplary radiosensitizers include hypoxia radiosensitizers such as misonidazole, metronidazole, and trans-sodium crocetinate, a compound that helps to increase the diffusion of oxygen into hypoxic tumor tissue. The radiosensitizer can also be a DNA damage response inhibitor interfering with base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), recombinational repair comprising homologous recombination (HR) and non-homologous end-joining (NHEJ), and direct repair mechanisms. Single strand break (SSB) repair mechanisms include BER, NER, or MMR pathways, while double stranded break (DSB) repair mechanisms consist of HR and NHEJ pathways. Radiation causes DNA breaks that, if not repaired, are lethal. SSBs are repaired through a

combination of BER, NER and MMR mechanisms using the intact DNA strand as a template. The predominant pathway of SSB repair is BER, utilizing a family of related enzymes termed poly-(ADP-ribose) polymerases (PARP). Thus, the radiosensitizer can include DNA damage response inhibitors such as PARP inhibitors.

[0186] In some embodiments, the additional anti-cancer therapy comprises an anti-inflammatory agent. In some embodiments, the methods provided herein comprise administering to the individual an anti-inflammatory agent, e.g., in combination with another therapy such as an immune checkpoint inhibitor. In some embodiments, the anti-inflammatory agent is an agent that blocks, inhibits, or reduces inflammation or signaling from an inflammatory signaling pathway In some embodiments, the anti-inflammatory agent inhibits or reduces the activity of one or more of any of the following: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-23; interferons (IFNs), e.g., IFNα, IFNβ, IFNγ, IFN-γ inducing factor (IGIF); transforming growth factor-β (TGF-β); transforming growth factor-α (TGF-α); tumor necrosis factors, e.g., TNF-α, TNF-β, TNF-RI, TNF-RII; CD23; CD30; CD40L; EGF; G-CSF; GDNF; PDGF-BB; RANTES/CCL5; IKK; NF-κB; TLR2; TLR3; TLR4; TL5; TLR6; TLR7; TLR8; TLR8; TLR9; and/or any cognate receptors thereof. In some embodiments, the anti-inflammatory agent is an IL-1 or IL-1 receptor antagonist, such as anakinra (Kineret®), rilonacept, or canakinumab. In some embodiments, the anti-inflammatory agent is an IL-6 or IL-6 receptor antagonist, e.g., an anti-IL-6 antibody or an anti-IL-6 receptor antibody, such as tocilizumab (ACTEMRA®), olokizumab, clazakizumab, sarilumab, sirukumab, siltuximab, or ALX-0061. In some embodiments, the anti-inflammatory agent is a TNF-α antagonist, e.g., an anti-TNFα antibody, such as infliximab (Remicade®), golimumab (Simponi®), adalimumab (Humira®), certolizumab pegol (Cimzia®) or etanercept. In some embodiments, the anti-inflammatory agent is a corticosteroid. Exemplary corticosteroids include, but are not limited to, cortisone (hydrocortisone, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, Ala-Cort®, Hydrocort Acetate®, hydrocortone phosphate Lanacort®, Solu-Cortef®), decadron (dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, Dexasone®, Diodex®, Hexadrol®, Maxidex®), methylprednisolone (6-methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, Duralone®, Medralone®, Medrol®, M-Prednisol®, Solu-Medrol®), prednisolone (Delta-Cortef®, ORAPRED®, Pediapred®, Prezone®), and prednisone (Deltasone®, Liquid Pred®, Meticorten®, Orasone®), and bisphosphonates (e.g., pamidronate (Aredia®), and zoledronic acid (Zometac®).

[0187] In some embodiments, the additional anti-cancer therapy comprises an anti-hormonal agent. In some embodiments, the methods provided herein comprise administering to the individual an anti-hormonal agent, e.g., in combination with another therapy such as an immune checkpoint inhibitor. Anti-hormonal agents are agents that act to regulate or inhibit hormone action on tumors. Examples of anti-hormonal agents include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-

hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGACE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® (anastrozole); anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0188] In some embodiments, the anti-cancer therapy comprises an antimetabolite chemotherapeutic agent. In some embodiments, the methods provided herein comprise administering to the individual an antimetabolite chemotherapeutic agent, e.g., in combination with another therapy such as an immune checkpoint inhibitor. Antimetabolite chemotherapeutic agents are agents that are structurally similar to a metabolite, but cannot be used by the body in a productive manner. Many antimetabolite chemotherapeutic agents interfere with the production of RNA or DNA. Examples of antimetabolite chemotherapeutic agents include gemcitabine (GEMZAR®), 5-fluorouracil (5-FU), capecitabine (XELODA™), 6-mercaptopurine, methotrexate, 6-thioguanine, pemetrexed, raltitrexed, arabinosylcytosine ARA-C cytarabine (CYTOSAR-U®), dacarbazine (DTIC-DOMED), azocytosine, deoxycytosine, pyridmidene, fludarabine (FLUDARA®), cladrabine, and 2-deoxy-D-glucose. In some embodiments, an antimetabolite chemotherapeutic agent is gemcitabine. Gemcitabine HCl is sold by Eli Lilly under the trademark GEMZAR®.

[0189] In some embodiments, the additional anti-cancer therapy comprises a platinum-based chemotherapeutic agent. In some embodiments, the methods provided herein comprise administering to the individual a platinum-based chemotherapeutic agent, e.g., in combination with another therapy such as an immune checkpoint inhibitor. Platinum-based chemotherapeutic agents are chemotherapeutic agents that comprise an organic compound containing platinum as an integral part of the molecule. In some embodiments, a chemotherapeutic agent is a platinum agent. In some such embodiments, the platinum agent is selected from cisplatin, carboplatin, oxaliplatin, nedaplatin, triplatin tetranitrate, phenanthriplatin, picoplatin, or satraplatin.

[0190] In some embodiments, the additional anti-cancer therapy comprises a cancer immunotherapy, such as a cancer vaccine, cell-based therapy, T cell receptor (TCR)-based therapy, adjuvant immunotherapy, cytokine immunotherapy, and oncolytic virus therapy. In some embodiments, the methods provided herein comprise administering to the individual a cancer immunotherapy, such as a cancer vaccine, cell-based therapy, T cell receptor (TCR)-based therapy, adjuvant immunotherapy,

cytokine immunotherapy, and oncolytic virus therapy, e.g., in combination with another therapy such as an immune checkpoint inhibitor. In some embodiments, the cancer immunotherapy comprises a small molecule, nucleic acid, polypeptide, carbohydrate, toxin, cell-based agent, or cell- binding agent. Examples of cancer immunotherapies are described in greater detail herein but are not intended to be limiting. In some embodiments, the cancer immunotherapy activates one or more aspects of the immune system to attack a cell (e.g., a tumor cell) that expresses a neoantigen, e.g., a neoantigen expressed by a cancer of the disclosure. The cancer immunotherapies of the present disclosure are contemplated for use as monotherapies, or in combination approaches comprising two or more in any combination or number, subject to medical judgement. Any of the cancer immunotherapies (optionally as monotherapies or in combination with another cancer immunotherapy or other therapeutic agent described herein) may find use in any of the methods described herein.

[0191] In some embodiments, the cancer immunotherapy comprises a cancer vaccine. A range of cancer vaccines have been tested that employ different approaches to promoting an immune response against a cancer (see, e.g., Emens L A, Expert Opin Emerg Drugs 13(2): 295-308 (2008) and US20190367613). Approaches have been designed to enhance the response of B cells, T cells, or professional antigen-presenting cells against tumors. Exemplary types of cancer vaccines include, but are not limited to, DNA-based vaccines, RNA-based vaccines, virus transduced vaccines, peptide-based vaccines, dendritic cell vaccines, oncolytic viruses, whole tumor cell vaccines, tumor antigen vaccines, etc. In some embodiments, the cancer vaccine can be prophylactic or therapeutic. In some embodiments, the cancer vaccine is formulated as a peptide-based vaccine, a nucleic acid-based vaccine, an antibody based vaccine, or a cell based vaccine. For example, a vaccine composition can include naked cDNA in cationic lipid formulations; lipopeptides (e.g., Vitiello, A. et ah, J. Clin. Invest. 95:341, 1995), naked cDNA or peptides, encapsulated e.g., in poly(DL-lactide-co-glycolide) ("PLG") microspheres (see, e.g., Eldridge, et ah, Molec. Immunol. 28:287-294, 1991: Alonso et al, Vaccine 12:299- 306, 1994; Jones et al, Vaccine 13:675-681, 1995); peptide composition contained in immune stimulating complexes (ISCOMS) (e.g., Takahashi et al, Nature 344:873-875, 1990; Hu et al, Clin. Exp. Immunol. 113:235-243, 1998); or multiple antigen peptide systems (MAPs) (see e.g., Tam, J. P., Proc. Natl Acad. Sci. U.S.A. 85:5409-5413, 1988; Tam, J.P., J. Immunol. Methods 196: 17-32, 1996). In some embodiments, a cancer vaccine is formulated as a peptide-based vaccine, or nucleic acid based vaccine in which the nucleic acid encodes the polypeptides. In some embodiments, a cancer vaccine is formulated as an antibody-based vaccine. In some embodiments, a cancer vaccine is formulated as a cell based vaccine. In some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine. In some embodiments, the cancer vaccine is a multivalent long peptide, a multiple peptide, a peptide mixture, a hybrid peptide, or a peptide pulsed dendritic cell vaccine (see, e.g., Yamada et al, Cancer Sci, 104: 14-21) , 2013). In some embodiments, such cancer vaccines augment the anti-cancer response.

[0192] In some embodiments, the cancer vaccine comprises a polynucleotide that encodes a neoantigen, e.g., a neoantigen expressed by a cancer of the disclosure. In some embodiments, the cancer vaccine comprises DNA or RNA that encodes a neoantigen. In some embodiments, the cancer vaccine comprises a polynucleotide that encodes a neoantigen. In some embodiments, the cancer vaccine further comprises one or more additional antigens, neoantigens, or other sequences that promote antigen presentation and/or an immune response. In some embodiments, the polynucleotide is complexed with one or more additional agents, such as a liposome or lipoplex. In some embodiments, the polynucleotide(s) are taken up and translated by antigen presenting cells (APCs), which then present the neoantigen(s) via MHC class I on the APC cell surface.

[0193] In some embodiments, the cancer vaccine is selected from sipuleucel-T (Provenge®, Dendreon/Valeant Pharmaceuticals), which has been approved for treatment of asymptomatic, or minimally symptomatic metastatic castrate-resistant (hormone-refractory) prostate cancer; and talimogene laherparepvec (Imlygic®, BioVex/Amgen, previously known as T-VEC), a genetically modified oncolytic viral therapy approved for treatment of unresectable cutaneous, subcutaneous and nodal lesions in melanoma. In some embodiments, the cancer vaccine is selected from an oncolytic viral therapy such as pexastimogene devacirepvec (PexaVec/JX-594, SillaJen/formerly Jennerex Biotherapeutics), a thymidine kinase- (TK-) deficient vaccinia virus engineered to express GM-CSF, for hepatocellular carcinoma (NCT02562755) and melanoma (NCT00429312); pelareorep (Reolysin®, Oncolytics Biotech), a variant of respiratory enteric orphan virus (reovirus) which does not replicate in cells that are not RAS-activated, in numerous cancers, including colorectal cancer (NCT01622543), prostate cancer (NCT01619813), head and neck squamous cell cancer (NCT01166542), pancreatic adenocarcinoma (NCT00998322), and non-small cell lung cancer (NSCLC) (NCT 00861627); enadenotucirev (NG-348, PsiOxus, formerly known as ColoAdl), an adenovirus engineered to express a full length CD80 and an antibody fragment specific for the T-cell receptor CD3 protein, in ovarian cancer (NCT02028117), metastatic or advanced epithelial tumors such as in colorectal cancer, bladder cancer, head and neck squamous cell carcinoma and salivary gland cancer (NCT02636036); ONCOS-102 (Targovax/formerly Oncos), an adenovirus engineered to express GM-CSF, in melanoma (NCT03003676), and peritoneal disease, colorectal cancer or ovarian cancer (NCT02963831); GL-ONC1 (GLV-1h68/GLV-1h153, Genelux GmbH), vaccinia viruses engineered to express beta-galactosidase (beta-gal)/beta-glucoronidase or beta-gal/human sodium iodide symporter (hNIS), respectively, were studied in peritoneal carcinomatosis (NCT01443260), fallopian tube cancer, ovarian cancer (NCT 02759588); or CG0070 (Cold Genesys), an adenovirus engineered to express GM-CSF in bladder cancer (NCT02365818); anti-gp100; STINGVAX; GVAX; DCVaxL; and DNX-2401. In some embodiments, the cancer vaccine is selected from JX-929 (SillaJen/formerly Jennerex Biotherapeutics), a TK- and vaccinia growth factor-deficient vaccinia virus engineered to express cytosine deaminase, which is able to convert the prodrug 5-fluorocytosine to the cytotoxic drug 5-fluorouracil; TGO1 and TG02 (Targovax/formerly Oncos), peptide-based

immunotherapy agents targeted for difficult-to-treat RAS mutations; and TILT-123 (TILT Biotherapeutics), an engineered adenovirus designated: Ad5/3-E2F-delta24-hTNFα-IRES-hIL20; and VSV-GP (ViraTherapeutics) a vesicular stomatitis virus (VSV) engineered to express the glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV), which can be further engineered to express antigens designed to raise an antigen-specific CD8+ T cell response. In some embodiments, the cancer vaccine comprises a vector-based tumor antigen vaccine. Vector-based tumor antigen vaccines can be used as a way to provide a steady supply of antigens to stimulate an anti-tumor immune response. In some embodiments, vectors encoding for tumor antigens are injected into an individual (possibly with pro-inflammatory or other attractants such as GM-CSF), taken up by cells in vivo to make the specific antigens, which then provoke the desired immune response. In some embodiments, vectors may be used to deliver more than one tumor antigen at a time, to increase the immune response. In addition, recombinant virus, bacteria or yeast vectors can trigger their own immune responses, which may also enhance the overall immune response.

[0194] In some embodiments, the cancer vaccine comprises a DNA-based vaccine. In some embodiments, DNA-based vaccines can be employed to stimulate an anti-tumor response. The ability of directly injected DNA that encodes an antigenic protein, to elicit a protective immune response has been demonstrated in numerous experimental systems. Vaccination through directly injecting DNA that encodes an antigenic protein, to elicit a protective immune response often produces both cell-mediated and humoral responses. Moreover, reproducible immune responses to DNA encoding various antigens have been reported in mice that last essentially for the lifetime of the animal (see, e.g., Yankauckas et al. (1993) DNA Cell Biol., 12: 771-776). In some embodiments, plasmid (or other vector) DNA that includes a sequence encoding a protein operably linked to regulatory elements required for gene expression is administered to individuals (e.g. human patients, non-human mammals, etc.). In some embodiments, the cells of the individual take up the administered DNA and the coding sequence is expressed. In some embodiments, the antigen so produced becomes a target against which an immune response is directed.

[0195] In some embodiments, the cancer vaccine comprises an RNA-based vaccine. In some embodiments, RNA-based vaccines can be employed to stimulate an anti-tumor response. In some embodiments, RNA-based vaccines comprise a self-replicating RNA molecule. In some embodiments, the self-replicating RNA molecule may be an alphavirus-derived RNA replicon. Self-replicating RNA (or "SAM") molecules are well known in the art and can be produced by using replication elements derived from, e.g., alphaviruses, and substituting the structural viral proteins with a nucleotide sequence encoding a protein of interest. A self-replicating RNA molecule is typically a +-strand molecule which can be directly translated after delivery to a cell, and this translation provides a RNA-dependent RNA polymerase which then produces both antisense and sense transcripts from the delivered RNA. Thus, the delivered RNA leads to the production of multiple daughter RNAs. These daughter RNAs, as well as collinear subgenomic transcripts, may be translated

themselves to provide in situ expression of an encoded polypeptide, or may be transcribed to provide further transcripts with the same sense as the delivered RNA which are translated to provide in situ expression of the antigen.

[0196] In some embodiments, the cancer immunotherapy comprises a cell-based therapy. In some embodiments, the cancer immunotherapy comprises a T cell-based therapy. In some embodiments, the cancer immunotherapy comprises an adoptive therapy, e.g., an adoptive T cell-based therapy. In some embodiments, the T cells are autologous or allogeneic to the recipient. In some embodiments, the T cells are CD8+ T cells. In some embodiments, the T cells are CD4+ T cells. Adoptive immunotherapy refers to a therapeutic approach for treating cancer or infectious diseases in which immune cells are administered to a host with the aim that the cells mediate either directly or indirectly specific immunity to (i.e., mount an immune response directed against) cancer cells. In some embodiments, the immune response results in inhibition of tumor and/or metastatic cell growth and/or proliferation, and in related embodiments, results in neoplastic cell death and/or resorption. The immune cells can be derived from a different organism/host (exogenous immune cells) or can be cells obtained from the subject organism (autologous immune cells). In some embodiments, the immune cells (e.g., autologous or allogeneic T cells (e.g., regulatory T cells, CD4+ T cells, CD8+ T cells, or gamma-delta T cells), NK cells, invariant NK cells, or NKT cells) can be genetically engineered to express antigen receptors such as engineered TCRs and/or chimeric antigen receptors (CARs). For example, the host cells (e.g., autologous or allogeneic T-cells) are modified to express a T cell receptor (TCR) having antigenic specificity for a cancer antigen. In some embodiments, NK cells are engineered to express a TCR. The NK cells may be further engineered to express a CAR. Multiple CARs and/or TCRs, such as to different antigens, may be added to a single cell type, such as T cells or NK cells. In some embodiments, the cells comprise one or more nucleic acids/expression constructs/vectors introduced via genetic engineering that encode one or more antigen receptors, and genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature (e.g. chimeric). In some embodiments, a population of immune cells can be obtained from a subject in need of therapy or suffering from a disease associated with reduced immune cell activity. Thus, the cells will be autologous to the subject in need of therapy. In some embodiments, a population of immune cells can be obtained from a donor, such as a histocompatibility-matched donor. In some embodiments, the immune cell population can be harvested from the peripheral blood, cord blood, bone marrow, spleen, or any other organ/tissue in which immune cells reside in said subject or donor. In some embodiments, the immune cells can be isolated from a pool of subjects and/or donors, such as from pooled cord blood. In some embodiments, when the population of immune cells is obtained from a

donor distinct from the subject, the donor may be allogeneic, provided the cells obtained are subject-compatible, in that they can be introduced into the subject. In some embodiments, allogeneic donor cells may or may not be human-leukocyte-antigen (HLA)-compatible. In some embodiments, to be rendered subject-compatible, allogeneic cells can be treated to reduce immunogenicity.

[0197] In some embodiments, the cell-based therapy comprises a T cell-based therapy, such as autologous cells, e.g., tumor-infiltrating lymphocytes (TILs); T cells activated ex-vivo using autologous DCs, lymphocytes, artificial antigen-presenting cells (APCs) or beads coated with T cell ligands and activating antibodies, or cells isolated by virtue of capturing target cell membrane; allogeneic cells naturally expressing anti-host tumor T cell receptor (TCR); and non-tumor-specific autologous or allogeneic cells genetically reprogrammed or "redirected" to express tumor-reactive TCR or chimeric TCR molecules displaying antibody-like tumor recognition capacity known as "T-bodies". Several approaches for the isolation, derivation, engineering or modification, activation, and expansion of functional anti-tumor effector cells have been described in the last two decades and may be used according to any of the methods provided herein. In some embodiments, the T cells are derived from the blood, bone marrow, lymph, umbilical cord, or lymphoid organs. In some embodiments, the cells are human cells. In some embodiments, the cells are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen- specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. In some embodiments, the cells may be allogeneic and/or autologous. In some embodiments, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs).

[0198] In some embodiments, the T cell-based therapy comprises a chimeric antigen receptor (CAR)-T cell-based therapy. This approach involves engineering a CAR that specifically binds to an antigen of interest and comprises one or more intracellular signaling domains for T cell activation. The CAR is then expressed on the surface of engineered T cells (CAR-T) and administered to a patient, leading to a T-cell-specific immune response against cancer cells expressing the antigen.

[0199] In some embodiments, the T cell-based therapy comprises T cells expressing a recombinant T cell receptor (TCR). This approach involves identifying a TCR that specifically binds to an antigen of interest, which is then used to replace the endogenous or native TCR on the surface of engineered T cells that are administered to a patient, leading to a T-cell-specific immune response against cancer cells expressing the antigen.

[0200] In some embodiments, the T cell-based therapy comprises tumor-infiltrating lymphocytes (TILs). For example, TILs can be isolated from a tumor or cancer of the present disclosure, then

isolated and expanded in vitro. Some or all of these TILs may specifically recognize an antigen expressed by the tumor or cancer of the present disclosure. In some embodiments, the TILs are exposed to one or more neoantigens, e.g., a neoantigen, in vitro after isolation. TILs are then administered to the patient (optionally in combination with one or more cytokines or other immune-stimulating substances).

[0201] In some embodiments, the cell-based therapy comprises a natural killer (NK) cell-based therapy. Natural killer (NK) cells are a subpopulation of lymphocytes that have spontaneous cytotoxicity against a variety of tumor cells, virus-infected cells, and some normal cells in the bone marrow and thymus. NK cells are critical effectors of the early innate immune response toward transformed and virus-infected cells. NK cells can be detected by specific surface markers, such as CD16, CD56, and CD8 in humans. NK cells do not express T-cell antigen receptors, the pan T marker CD3, or surface immunoglobulin B cell receptors. In some embodiments, NK cells are derived from human peripheral blood mononuclear cells (PBMC), unstimulated leukapheresis products (PBSC), human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), bone marrow, or umbilical cord blood by methods well known in the art.

[0202] In some embodiments, the cell-based therapy comprises a dendritic cell (DC)-based therapy, e.g., a dendritic cell vaccine. In some embodiments, the DC vaccine comprises antigen-presenting cells that are able to induce specific T cell immunity, which are harvested from the patient or from a donor. In some embodiments, the DC vaccine can then be exposed in vitro to a peptide antigen, for which T cells are to be generated in the patient. In some embodiments, dendritic cells loaded with the antigen are then injected back into the patient. In some embodiments, immunization may be repeated multiple times if desired. Methods for harvesting, expanding, and administering dendritic cells are known in the art; see, e.g., WO2019178081. Dendritic cell vaccines (such as Sipuleucel-T, also known as APC8015 and PROVENGE®) are vaccines that involve administration of dendritic cells that act as APCs to present one or more cancer-specific antigens to the patient's immune system. In some embodiments, the dendritic cells are autologous or allogeneic to the recipient.

[0203] In some embodiments, the cancer immunotherapy comprises a TCR-based therapy. In some embodiments, the cancer immunotherapy comprises administration of one or more TCRs or TCR-based therapeutics that specifically bind an antigen expressed by a cancer of the present disclosure. In some embodiments, the TCR-based therapeutic may further include a moiety that binds an immune cell (e.g., a T cell), such as an antibody or antibody fragment that specifically binds a T cell surface protein or receptor (e.g., an anti-CD3 antibody or antibody fragment).

[0204] In some embodiments, the immunotherapy comprises adjuvant immunotherapy. Adjuvant immunotherapy comprises the use of one or more agents that activate components of the innate immune system, e.g., HILTONOL® (imiquimod), which targets the TLR7 pathway.

[0205] In some embodiments, the immunotherapy comprises cytokine immunotherapy. Cytokine immunotherapy comprises the use of one or more cytokines that activate components of the immune

system. Examples include, but are not limited to, aldesleukin (PROLEUKIN®; interleukin-2), interferon alfa-2a (ROFERON®-A), interferon alfa-2b (INTRON®-A), and peginterferon alfa-2b (PEGINTRON®).

[0206] In some embodiments, the immunotherapy comprises oncolytic virus therapy. Oncolytic virus therapy uses genetically modified viruses to replicate in and kill cancer cells, leading to the release of antigens that stimulate an immune response. In some embodiments, replication-competent oncolytic viruses expressing a tumor antigen comprise any naturally occurring (e.g., from a "field source") or modified replication-competent oncolytic virus. In some embodiments, the oncolytic virus, in addition to expressing a tumor antigen, may be modified to increase selectivity of the virus for cancer cells. In some embodiments, replication-competent oncolytic viruses include, but are not limited to, oncolytic viruses that are a member in the family of myoviridae, siphoviridae, podpviridae, teciviridae, corticoviridae, plasmaviridae, lipothrixviridae, fuselloviridae, poxyiridae, iridoviridae, phycodnaviridae, baculoviridae, herpesviridae, adnoviridae, papovaviridae, polydnaviridae, inoviridae, microviridae, geminiviridae, circoviridae, parvoviridae, hcpadnaviridae, retroviridae, cyctoviridae, reoviridae, birnaviridae, paramyxoviridae, rhabdoviridae, filoviridae, orthomyxoviridae, bunyaviridae, arenaviridae, Leviviridae, picornaviridae, sequiviridae, comoviridae, potyviridae, caliciviridae, astroviridae, nodaviridae, tetraviridae, tombusviridae, coronaviridae, glaviviridae, togaviridae, and barnaviridae. In some embodiments, replication-competent oncolytic viruses include adenovirus, retrovirus, reovirus, rhabdovirus, Newcastle Disease virus (NDV), polyoma virus, vaccinia virus (VacV), herpes simplex virus, picornavirus, coxsackie virus and parvovirus. In some embodiments, a replicative oncolytic vaccinia virus expressing a tumor antigen may be engineered to lack one or more functional genes in order to increase the cancer selectivity of the virus. In some embodiments, an oncolytic vaccinia virus is engineered to lack thymidine kinase (TK) activity. In some embodiments, the oncolytic vaccinia virus may be engineered to lack vaccinia virus growth factor (VGF). In some embodiments, an oncolytic vaccinia virus may be engineered to lack both VGF and TK activity. In some embodiments, an oncolytic vaccinia virus may be engineered to lack one or more genes involved in evading host interferon (IFN) response such as E3L, K3L, B18R, or B8R. In some embodiments, a replicative oncolytic vaccinia virus is a Western Reserve, Copenhagen, Lister or Wyeth strain and lacks a functional TK gene. In some embodiments, the oncolytic vaccinia virus is a Western Reserve, Copenhagen, Lister or Wyeth strain lacking a functional B18R and/or B8R gene. In some embodiments, a replicative oncolytic vaccinia virus expressing a tumor antigen may be locally or systemically administered to a subject, e.g. via intratumoral, intraperitoneal, intravenous, intra-arterial, intramuscular, intradermal, intracranial, subcutaneous, or intranasal administration.

[0207] In some embodiments, the anti-cancer therapy comprises a nucleic acid molecule, such as a dsRNA, an siRNA, or an shRNA. In some embodiments, the methods provided herein comprise administering to the individual a nucleic acid molecule, such as a dsRNA, an siRNA, or an shRNA, e.g., in combination with another anti-cancer therapy. As is known in the art, dsRNAs having a duplex

structure are effective at inducing RNA interference (RNAi). In some embodiments, the anti-cancer therapy comprises a small interfering RNA molecule (siRNA). dsRNAs and siRNAs can be used to silence gene expression in mammalian cells (e.g., human cells). In some embodiments, a dsRNA of the disclosure comprises any of between about 5 and about 10 base pairs, between about 10 and about 12 base pairs, between about 12 and about 15 base pairs, between about 15 and about 20 base pairs, between about 20 and 23 base pairs, between about 23 and about 25 base pairs, between about 25 and about 27 base pairs, or between about 27 and about 30 base pairs. As is known in the art, siRNAs are small dsRNAs that optionally include overhangs. In some embodiments, the duplex region of an siRNA is between about 18 and 25 nucleotides, e.g., any of 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides. siRNAs may also include short hairpin RNAs (shRNAs), e.g., with approximately 29-base-pair stems and 2-nucleotide 3' overhangs. Methods for designing, optimizing, producing, and using dsRNAs, siRNAs, or shRNAs, are known in the art.

X. Treatments and treatment effects

[0208] The methods described herein provide improved therapies and/or therapeutic effects. The improved therapies and/or therapeutic effects are based, in part, on the stratification of individuals with cancer having a TMB score below a threshold TMB score and individuals with cancer having a TMB score at least a threshold TMB score, the stratification of individuals with cancers that are MSI-H and individuals with cancers that are not MSI-H (such as MSI-L or MSS), or both. Once a TMB score is determined and/or a microsatellite instability status is assessed, the individuals can receive appropriate therapies, leading to improved clinical outcomes including improved survival (such as improved progression-free survival and/or improved overall survival) and/or increased time to next treatment (TTNT). The individuals may be any of the individuals described in Section III, above.

[0209] Accordingly, in some embodiments, the methods comprise administering an immune checkpoint inhibitor (ICPI, such as an ICPI described in Section VII) if a TMB score in a sample from an individual having a cancer is at least a threshold TMB score. In some embodiments, the methods comprise administering a chemotherapy (such as a chemotherapy described in Section VIII) if a TMB score is below a threshold TMB score. In some embodiments, the methods comprise administering an ICPI (such as an ICPI described in Section VII) if a sample from the individual is assessed to be MSI-H. In some embodiments, the methods comprise administering a chemotherapy (such as a chemotherapy described in Section VIII) if a sample from the individual is assessed to not be MSI-H (such as MSS or MSI-L).

[0210] In some embodiments, the methods of treatment described herein provide a clinical benefit and/or an improved clinical benefit for individuals having a cancer. In some embodiments, the methods provide improved clinical benefit when compared to an alternative therapy. For example, in some embodiments, the methods comprise administering an ICPI, wherein the individual will, or is expected to, benefit from the ICPI therapy as compared to treatment with a chemotherapy regimen. In

some embodiments, the methods comprise administering a chemotherapy regimen, where the individual will, or is expected to, benefit from the chemotherapy regimen as compared to treatment with an ICPI therapy.

[0211] In some embodiments, the clinical benefit is improved survival (such as improved PFS and/or improved OS). In some embodiments, the treatment improves PFS by at least one month, such as any of at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 18 months, at least about 2 years, at least about 3 years, at least about 4 years, or more, after administration. In some embodiments, the treatment improves OS by at least one month, such as any of at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 18 months, at least about 2 years, at least about 3 years, at least about 4 years, or more, after administration. In some embodiments, the treatment methods provide improved objective response rate of at least 20%, such as any of about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

XI. Cancers to be assessed or treated

[0212] The methods described herein pertain to individuals having a cancer and assessment of the cancers (by assessment of a sample, such as a blood sample or a tumor biopsy sample) to identify suitable treatments for the individual. Exemplary cancers to be treated or assessed include, but are not limited to, a B cell cancer, *e.g.,* multiple myeloma, melanomas, breast cancer, lung cancer (such as non-small cell lung carcinoma or NSCLC, including advanced NSCLC), bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, adenocarcinomas, inflammatory myofibroblastic tumors, gastrointestinal stromal tumor (GIST), colon cancer, multiple myeloma (MM), myelodysplastic syndrome (MDS), myeloproliferative disorder (MPD), acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), polycythemia Vera, Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), soft-tissue sarcoma, fibrosarcoma, myxosarcoma, liposarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor,

leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, hepatocellular carcinoma, thyroid cancer, gastric cancer, head and neck cancer, small cell cancers, essential thrombocythemia, agnogenic myeloid metaplasia, hypereosinophilic syndrome, systemic mastocytosis, familiar hypereosinophilia, chronic eosinophilic leukemia, neuroendocrine cancers, carcinoid tumors, and the like. In some embodiments, the cancer is a NSCLC, colorectal cancer, cholangiocarcinoma, breast cancer, stomach cancer, melanoma, pancreatic cancer, prostate cancer, ovarian cancer, esophageal cancer, or a cancer of unknown primary. In some embodiments, the cancer is metastatic urothelial carcinoma. In some embodiments, the cancer is metastatic gastric adenocarcinoma. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is metastatic endometrial cancer. In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is castration resistant prostate cancer. In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the lung cancer is non-small cell lung cancer (NSCLC). In some embodiments, the NSCLC is advanced NSCLC (aNSCLC). In some embodiments, the cancer is melanoma. In some embodiments, the cancer is a hematologic malignancy (or premaligancy). As used herein, a hematologic malignancy refers to a tumor of the hematopoietic or lymphoid tissues, *e.g.,* a tumor that affects blood, bone marrow, or lymph nodes. Exemplary hematologic malignancies include, but are not limited to, leukemia (*e.g.,* acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, acute monocytic leukemia (AMoL), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), or large granular lymphocytic leukemia), lymphoma (*e.g.,* AIDS-related lymphoma, cutaneous T-cell lymphoma, Hodgkin lymphoma (*e.g.,* classical Hodgkin lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma), mycosis fungoides, non-Hodgkin lymphoma (*e.g.,* B-cell non-Hodgkin lymphoma (*e.g.,* Burkitt lymphoma, small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, or mantle cell lymphoma) or T-cell non-Hodgkin lymphoma (mycosis fungoides, anaplastic large cell lymphoma, or precursor T-lymphoblastic lymphoma)), primary central nervous system lymphoma, Sézary syndrome, Waldenström macroglobulinemia), chronic myeloproliferative neoplasm, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, or myelodysplastic/myeloproliferative

neoplasm. Premaligancy, as used herein, refers to a tissue that is not yet malignant but is poised to become malignant.

[0213] In some embodiments, the cancer to be treated or assessed has never been treated with an anti-cancer therapy. In some embodiments, the cancer to be treated or assessed has never been treated, or is not currently being treated, with a chemotherapy regimen. In some embodiments, the cancer to be treated or assessed has previously been treated with an anti-cancer therapy. In some embodiments, the cancer to be treated or assessed has previously been treated with a chemotherapy regimen.

[0214] In some embodiments, the cancer to be treated or assessed has a TMB score of at least 8 mutations/Mb, such as any of about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, about 20 mutations/Mb, or more. In some embodiments, the cancer to be treated or assessed has a TMB score of at least 10 mutations/Mb. In some embodiments, the cancer to be treated or assessed has a TMB score of less than 12 mutations/Mb, such as any of less than about 11 mutations/Mb, about 10 mutations/Mb, about 9 mutations/Mb, about 8 mutations/Mb, about 7 mutations/Mb, about 6 mutations/Mb, or less. In some embodiments, the cancer to be treated or assessed has a TMB score of less than 10 mutations/Mb.

[0215] In some embodiments, the cancer to be treated or assessed is MSI-H. In some embodiments, the cancer to be treated or assessed is MSI-L. In some embodiments, the cancer to be treated or assessed is MSS.

[0216] In some embodiments, the cancer to be treated or assessed has a TMB score of at least 10 mutations/Mb and is MSI-H. In some embodiments, the cancer to be treated or assessed has a TMB score of at least 10 mutations/Mb and is MSI-L or MSS. In some embodiments, the cancer to be treated or assessed has a TMB score of less than 10 mutations/Mb and is MSI-H. In some embodiments, the cancer to be treated or assessed has a TMB score of less than 10 mutations/Mb and is MSI-L or MSS.

XII. Exemplary Embodiments

[0217] The following embodiments are exemplary and are not intended to limit the scope of the invention.

[0218] Embodiment 1. A method for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising determining a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein if the TMB score is at least a threshold TMB score the individual is identified for treatment with an immune checkpoint inhibitor therapy.

[0219] Embodiment 2. A method of selecting a treatment for an individual having a cancer, the method comprising determining a tumor mutational burden (TMB) score for a sample obtained from

the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy.

[0220] Embodiment 3. A method of identifying one or more treatment options for an individual having a cancer, the method comprising: (a) determining a tumor mutational burden (TMB) score for a sample obtained from the individual; and (b) generating a report comprising one or more treatment options identified for the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy.

[0221] Embodiment 4. A method of stratifying an individual with a cancer for treatment with a therapy comprising determining a tumor mutational burden (TMB) score for a sample obtained from the individual; and (a) if the TMB score is at least a threshold TMB score, identifying the individual as a candidate for receiving an immune checkpoint inhibitor therapy, or (b) if the TMB score is less than a threshold TMB score, identifying the individual as a candidate for receiving a chemotherapy regimen.

[0222] Embodiment 5. The method of any one of embodiments 1-4, further comprising assessing microsatellite instability, wherein the identification is further based on the cancer being microsatellite instability-high (MSI-H).

[0223] Embodiment 6. The method of any one of embodiments 1-5, wherein the individual is identified to have an increased survival as compared to treatment with a chemotherapy regimen.

[0224] Embodiment 7. A method of predicting survival of an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein if the TMB score for the sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen.

[0225] Embodiment 8. A method of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a sample obtained from the individual, wherein if the TMB score for the sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen.

[0226] Embodiment 9. The method of any one of embodiments 6-8, wherein the increased survival is increased overall survival (OS).

[0227] Embodiment 10. The method of any one of embodiments 6-8, wherein the increased survival is increased progression-free survival (PFS).

[0228] Embodiment 11. A method for treating an individual having a cancer, the method comprising: (a) determining a tumor mutational burden (TMB) score for a sample obtained from the individual; and (b) treating the individual with an immune checkpoint inhibitor therapy if the TMB score is at least a threshold TMB score.

[0229] Embodiment 12. The method of embodiment 11, further comprising assessing microsatellite instability, wherein (b) is further based on the cancer being microsatellite instability-high (MSI-H).

[0230] Embodiment 13. The method of any one of embodiments 1-12, further comprising treating the individual with a chemotherapy if the TMB score is less than the threshold TMB score.

[0231] Embodiment 14. The method of embodiment 13, wherein the chemotherapy comprises one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, aa dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2”-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside (“Ara-C”), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0232] Embodiment 15. The method of any one of embodiments 1-14, wherein the threshold TMB score is about 8 mutations/Mb, about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, or about 20 mutations/Mb.

[0233] Embodiment 16. The method of any one of embodiments 1-15, wherein the threshold TMB score is about 10 mutations/Mb.

[0234] Embodiment 17. The method of any one of embodiments 1-16, wherein the threshold TMB score is 10 mutations/Mb.

[0235] Embodiment 18. The method of any one of embodiments 1-17, wherein the TMB score is determined based on between about 100 kb to about 10 MB of sequenced DNA.

[0236] Embodiment 19. The method of any one of embodiments 1-18, wherein the TMB score is determined based on between about 0.8 Mb to about 1.1 MB of sequenced DNA.

[0237] Embodiment 20. A method for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising assessing microsatellite instability for a sample obtained from the individual, wherein if the microsatellite instability is MSI-H the individual is identified for treatment with an immune checkpoint inhibitor therapy.

[0238] Embodiment 21. A method of selecting a treatment for an individual having a cancer, the method comprising assessing microsatellite instability for a sample obtained from the individual, wherein microsatellite instability that is MSI-H identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy.

[0239] Embodiment 22. A method of identifying one or more treatment options for an individual having a metastatic cancer, the method comprising: (a) assessing microsatellite instability for a sample obtained from the individual; and (b) generating a report comprising one or more treatment options identified for the individual, wherein a microsatellite instability that is MSI-H identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy.

[0240] Embodiment 23. A method of stratifying an individual with a cancer for treatment with a therapy comprising assessing microsatellite instability for a sample obtained from the individual; and (a) if the microsatellite instability is MSI-H, identifying the individual as a candidate for receiving an immune checkpoint inhibitor therapy, or (b) if the microsatellite instability is not MSI-H, identifying the individual as a candidate for receiving a chemotherapy regimen.

[0241] Embodiment 24. The method of any one of embodiments 20-23, wherein the individual is identified to have an increased survival as compared to treatment with a chemotherapy regimen.

[0242] Embodiment 25. A method of predicting survival of an individual having a cancer, comprising acquiring knowledge of microsatellite instability for a sample obtained from the individual, wherein if the microsatellite instability is MSI-H for the sample obtained from the individual, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen.

[0243] Embodiment 26. A method of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of microsatellite instability for a sample obtained from the individual, wherein if the microsatellite instability is MSI-H for the sample obtained from the individual, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen.

[0244] Embodiment 27. The method of any one of embodiments 20-26, wherein the increased survival is increased overall survival (OS).

[0245] Embodiment 28. The method of any one of embodiments 20-26, wherein the increased survival is increased progression-free survival (PFS).

[0246] Embodiment 29. The method of any one of embodiments 1-28, further comprising treating the individual with an immune checkpoint inhibitor.

[0247] Embodiment 30. A method for treating an individual having a cancer, the method comprising: (a) assessing microsatellite instability for a sample obtained from the individual; and (b) treating the individual with an immune checkpoint inhibitor therapy if the microsatellite instability is assessed as MSI-H.

[0248] Embodiment 31. The method of any one of embodiments 20-30, wherein microsatellite instability is assessed by NGS.

[0249] Embodiment 32. The method of any one of embodiments 20-31, further comprising treating the individual with a chemotherapy if the microsatellite instability is not assessed as MSI-H.

[0250] Embodiment 33. The method of embodiment 32, wherein the chemotherapy comprises one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, aa dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2”-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside (“Ara-C”), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0251] Embodiment 34. The method of any one of embodiments 1-33, wherein the cancer is a metastatic cancer.

[0252] Embodiment 35. The method of any one of embodiments 1-34, wherein the cancer is a B cell cancer, a melanoma, breast cancer, lung cancer, bronchus cancer, colorectal cancer or carcinoma, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain cancer, central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine cancer, endometrial cancer, cancer of an oral cavity, cancer of a pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel cancer, appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, a cancer of

hematological tissue, an adenocarcinoma, an inflammatory myofibroblastic tumor, a gastrointestinal stromal tumor (GIST), colon cancer, multiple myeloma (MM), myelodysplastic syndrome (MDS), myeloproliferative disorder (MPD), acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), polycythemia Vera, Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), soft-tissue sarcoma, fibrosarcoma, myxosarcoma, liposarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, follicular lymphoma, diffuse large B-cell lymphoma, mantle cell lymphoma, hepatocellular carcinoma, thyroid cancer, gastric cancer or carcinoma, lung non-small cell lung carcinoma (NSCLC), head and neck cancer, small cell cancer, essential thrombocythemia, agnogenic myeloid metaplasia, hypereosinophilic syndrome, systemic mastocytosis, familiar hypereosinophilia, chronic eosinophilic leukemia, neuroendocrine cancers, or a carcinoid tumor.

[0253] Embodiment 36. The method of any one of embodiments 1-35, wherein the cancer is a metastatic urothelial carcinoma.

[0254] Embodiment 37. The method of any one of embodiments 1-35, wherein the cancer is a metastatic gastric adenocarcinoma.

[0255] Embodiment 38. The method of any one of embodiments 1-35, wherein the cancer is breast cancer.

[0256] Embodiment 39. The method of any one of embodiments 1-35, wherein the cancer is prostate cancer.

[0257] Embodiment 40. The method of embodiment 39, wherein the prostate cancer is metastatic castration resistant prostate cancer.

[0258] Embodiment 41. The method of any one of embodiments 1-35, wherein the cancer is colorectal cancer.

[0259] Embodiment 42. The method of any one of embodiments 1-35, wherein the cancer is lung cancer.

[0260] Embodiment 43. The method of embodiment 42, wherein the lung cancer is non-small cell lung cancer (NSCLC).

[0261] Embodiment 44. The method of claim 43, wheren the NSCLC is advanced NSCLC (aNSCLC).

[0262] Embodiment 45. The method of any one of embodiments 1-35, wherein the cancer is endometrial cancer.

[0263] Embodiment 46. The method of any one of embodiments 1-35, wherein the cancer is melanoma.

[0264] Embodiment 47. The method of any one of embodiments 1-46, wherein the immune checkpoint inhibitor comprises a small molecule inhibitor, an antibody, a nucleic acid, an antibody-drug conjugate, a recombinant protein, a fusion protein, a natural compound, a peptide, a PROteolysis-TArgeting Chimera (PROTAC), a cellular therapy, a treatment for cancer being tested in a clinical trial, an immunotherapy, or any combination thereof.

[0265] Embodiment 48. The method of embodiment 47, wherein the immune checkpoint inhibitor is a PD-1 inhibitor.

[0266] Embodiment 49. The method of embodiment 47, wherein the immune checkpoint inhibitor comprises one or more of nivolumab, pembrolizumab, cemiplimab, or dostarlimab.

[0267] Embodiment 50. The method of embodiment 47, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.

[0268] Embodiment 51. The method of embodiment 47, wherein the immune checkpoint inhibitor comprises one or more of atezolizumab, avelumab, or durvalumab.

[0269] Embodiment 52. The method of embodiment 47, wherein the immune checkpoint inhibitor is a CTLA-4 inhibitor.

[0270] Embodiment 53. The method of embodiment 52, wherein the CTLA-4 inhibitor comprises ipilimumab.

[0271] Embodiment 54. The method of any one of embodiments 1-53, wherein the individual did not previously receive a regimen of chemotherapy for the cancer.

[0272] Embodiment 55. The method of any one of embodiments 1-53, wherein the individual previously received a regimen of chemotherapy for the cancer.

[0273] Embodiment 56. The method of embodiment 55, wherein the previous regimen of chemotherapy comprised one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, a dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK

polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2"-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside ("Ara-C"), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0274] Embodiment 57. The method of any one of embodiments 1-56, wherein the immune checkpoint inhibitor therapy is the only anti-cancer therapy indicated or administered for the cancer.

[0275] Embodiment 58. The method of any one of embodiments 1-56, wherein the immune checkpoint inhibitor therapy is a single-active-agent therapy.

[0276] Embodiment 59. The method of any one of embodiments 1-56, wherein the immune checkpoint inhibitor comprises therapy two or more active agents.

[0277] Embodiment 60. The method of any one of embodiments 1-56, wherein the immune checkpoint inhibitor therapy comprises a first round of an immune checkpoint inhibitor and a subsequent round of therapy with a different immune checkpoint inhibitor.

[0278] Embodiment 61. The method of any one of embodiments 1-60, wherein the immune checkpoint inhibitor therapy is the first line therapy for the cancer.

[0279] Embodiment 62. The method of any one of embodiments 1-56, further comprising treating the individual with an additional anti-cancer therapy.

[0280] Embodiment 63. The method of embodiment 62, wherein the additional anti-cancer therapy comprises one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

[0281] Embodiment 64. The method of any one of embodiments 1-63, wherein the sample is a solid tumor biopsy sample obtained from the individual.

[0282] Embodiment 65. The method of any one of embodiments 1-63, wherein the sample is a liquid biopsy sample obtained from the individual.

[0283] Embodiment 66. The method of embodiment 65, wherein the liquid biopsy sample comprises blood, plasma, serum, cerebrospinal fluid, sputum, stool, urine, or saliva.

[0284] Embodiment 67. The method of embodiment 65 or embodiment 66, wherein the liquid biopsy sample comprises mRNA, DNA, circulating tumor DNA (ctDNA), cell-free DNA, or cell-free RNA from the cancer.

[0285] Embodiment 68. The method of any one of embodiments 1-67, wherein the TMB score or microsatellite instability is determined by sequencing.

[0286] Embodiment 69. The method of embodiment 68, wherein the sequencing comprises use of a massively parallel sequencing (MPS) technique, whole genome sequencing (WGS), whole exome sequencing, targeted sequencing, direct sequencing, next-generation sequencing (NGS), or a Sanger sequencing technique.

[0287] Embodiment 70. The method of embodiment 68 or embodiment 69, wherein the sequencing comprises: (a) providing a plurality of nucleic acid molecules obtained from the sample, wherein the plurality of nucleic acid molecules comprise a mixture of tumor nucleic acid molecules and non-tumor nucleic acid molecules; (b) optionally, ligating one or more adapters onto one or more nucleic acid molecules from the plurality of nucleic acid molecules; (c) amplifying nucleic acid molecules from the plurality of nucleic acid molecules; (d) capturing nucleic acid molecules from the amplified nucleic acid molecules, wherein the captured nucleic acid molecules are captured from the amplified nucleic acid molecules by hybridization to one or more bait molecules; (e) sequencing, by a sequencer, the captured nucleic acid molecules to obtain a plurality of sequence reads corresponding to one or more genomic loci within a subgenomic interval in the sample.

[0288] Embodiment 71. The method of embodiment 70, wherein the adapters comprise one or more of amplification primer sequences, flow cell adapter hybridization sequences, unique molecular identifier sequences, substrate adapter sequences, or sample index sequences.

[0289] Embodiment 72. The method of embodiment 70 or embodiment 71, wherein amplifying nucleic acid molecules comprises performing a polymerase chain reaction (PCR) technique, a non-PCR amplification technique, or an isothermal amplification technique.

[0290] Embodiment 73. The method of any one of embodiments 70-72, wherein the one or more bait molecules comprise one or more nucleic acid molecules, each comprising a region that is complementary to a region of a captured nucleic acid molecule.

[0291] Embodiment 74. The method of embodiment 73, wherein the one or more bait molecules each comprise a capture moiety.

[0292] Embodiment 75. The method of embodiment 74, wherein the capture moiety is biotin.

[0293] Embodiment 76. The method of any of embodiments 1-75, wherein the individual is a human.

[0294] Embodiment 77. A kit comprising an immune checkpoint inhibitor and instructions for use according to the method of any one of embodiments 1-76.

[0295] Embodiment 1A. A method for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score is at least a threshold TMB score the individual is identified for treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0296] Embodiment 2A. A method of selecting a treatment for an individual having a cancer, the method comprising determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0297] Embodiment 3A. A method of identifying one or more treatment options for an individual having a cancer, the method comprising:

determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and

generating a report comprising one or more treatment options identified for the individual, wherein a TMB score that is at least a threshold TMB score identifies the individual as one who may benefit from treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0298] Embodiment 4A. A method of stratifying an individual with a cancer for treatment with a therapy comprising determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and

if the TMB score is at least a threshold TMB score, identifying the individual as a candidate for receiving an immune checkpoint inhibitor therapy, or

if the TMB score is less than a threshold TMB score, identifying the individual as a candidate for receiving a chemotherapy regimen;

wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0299] Embodiment 5A. The method of any one of Embodiments 1A-4A, further comprising assessing microsatellite instability, wherein the identification is further based on the cancer being microsatellite instability-high (MSI-H).

[0300] Embodiment 6A. The method of Embodiment 5A, wherein microsatellite instability is assessed by next generation sequencing (NGS).

[0301] Embodiment 7A. The method of any one of Embodiments 1A-6A, wherein the individual is identified to have an increased survival as compared to treatment with a chemotherapy regimen.

[0302] Embodiment 8A. A method of predicting survival of an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased

survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0303] Embodiment 9A. A method of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to treatment with a chemotherapy regimen, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0304] Embodiment 10A. A method of predicting survival of an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to a patient with a TMB score that is less than the threshold TMB score, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0305] Embodiment 11A. A method of monitoring, evaluating, or screening an individual having a cancer, comprising acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score for the tumor biopsy sample obtained from the individual is at least a threshold TMB score, the individual is predicted to have increased survival when treated with an immune checkpoint inhibitor, as compared to as compared to a patient with a TMB score that is less than the threshold TMB score, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0306] Embodiment 12A. The method of any one of Embodiments 7A-11A, wherein the increased survival is increased overall survival (OS).

[0307] Embodiment 13A. The method of any one of Embodiments 7A-11A, wherein the increased survival is increased progression-free survival (PFS).

[0308] Embodiment 14A. A method of predicting a duration of therapeutic response for an individual having a cancer, comprising: acquiring knowledge of a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and comparing the TMB score for the sample to a threshold TMB score, wherein if the TMB score is greater than or equal to the threshold TMB score, the individual is predicted to have a longer duration of therapeutic response to an immune checkpoint

inhibitor; and wherein if the TMB score is less than the threshold TMB score, the subject is predicted to have a shorter duration of therapeutic response to an immune checkpoint inhibitor.

[0309] Embodiment 15A. The method of Embodiment 14A, wherein longer duration of therapeutic response is one or more of increase progression-free survival (PFS) and overall survival (OS), and wherein shorter duration of therapeutic response is one or more of decreased PFS and decreased OS.

[0310] Embodiment 16A. A method for treating an individual having a cancer, the method comprising:

determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and

treating the individual with an immune checkpoint inhibitor therapy if the TMB score is at least a threshold TMB score;

wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

[0311] Embodiment 17A. The method of Embodiment 16A, further comprising assessing microsatellite instability, wherein (b) is further based on the cancer being microsatellite instability-high (MSI-H).

[0312] Embodiment 18A. The method of Embodiment 17A, wherein microsatellite instability is assessed by next generation sequencing (NGS).

[0313] Embodiment 19A. The method of any one of Embodiments 1A-18A, further comprising treating the individual with a chemotherapy if the TMB score is less than the threshold TMB score.

[0314] Embodiment 20A. The method of Embodiment 19A, wherein the chemotherapy comprises one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, aa dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2”-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside (“Ara-C”), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate,

daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0315] Embodiment 21A. The method of any one of Embodiments 1A-20A, wherein the threshold TMB score is about 8 mutations/Mb, about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, or about 20 mutations/Mb.

[0316] Embodiment 22A. The method of any one of Embodiments 1A-21A, wherein the threshold TMB score is about 10 mutations/Mb.

[0317] Embodiment 23A. The method of any one of Embodiments 1A-22A, wherein the threshold TMB score is 10 mutations/Mb.

[0318] Embodiment 24A. The method of any one of Embodiments 1A-22A, wherein the threshold TMB score is 20 mutations/Mb. Embodiment 25A. The method of any one of Embodiments 1A-24A, wherein the TMB score is determined based on between about 100 kb to about 10 Mb of sequenced DNA.

[0319] Embodiment 26A. The method of any one of Embodiments 1A-25A, wherein the TMB score is determined based on between about 0.8 Mb to about 1.1 Mb of sequenced DNA.

[0320] Embodiment 27A. The method of any one of Embodiments 1A-26A, further comprising treating the individual with an immune checkpoint inhibitor if the TMB score is at least the threshold TMB score.

[0321] Embodiment 28A. The method of any one of Embodiments 1A-27A, wherein the cancer is prostate cancer that is metastatic castration-resistant prostate cancer.

[0322] Embodiment 29A. The method of any one of Embodiments 1A-27A, wherein the cancer is a metastatic urothelial carcinoma.

[0323] Embodiment 30A. The method of any one of Embodiments 1A-27A, wherein the cancer is a metastatic gastric adenocarcinoma.

[0324] Embodiment 31A. The method of any one of Embodiments 1A-27A, wherein the cancer is a metastatic endometrial cancer.

[0325] Embodiment 32A. The method of any one of Embodiments 1A-27A, wherein the cancer is NSCLC or advanced NSCLC (aNSCLC).

[0326] Embodiment 33A. The method of any one of Embodiments 1A-32A, wherein the immune checkpoint inhibitor comprises a small molecule inhibitor, an antibody, a nucleic acid, an antibody-drug conjugate, a recombinant protein, a fusion protein, a natural compound, a peptide, a PROteolysis-TArgeting Chimera (PROTAC), a cellular therapy, a treatment for cancer being tested in a clinical trial, an immunotherapy, or any combination thereof.

[0327] Embodiment 34A. The method of Embodiment 33A, wherein the immune checkpoint inhibitor is a PD-1 inhibitor.

[0328] Embodiment 35A. The method of Embodiment 33A, wherein the immune checkpoint inhibitor comprises one or more of nivolumab, pembrolizumab, cemiplimab, or dostarlimab.

[0329] Embodiment 36A. The method of Embodiment 33A, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.

[0330] Embodiment 37A. The method of Embodiment 33A, wherein the immune checkpoint inhibitor comprises one or more of atezolizumab, avelumab, or durvalumab.

[0331] Embodiment 38A. The method of Embodiment 33A, wherein the immune checkpoint inhibitor is a CTLA-4 inhibitor.

[0332] Embodiment 39A. The method of Embodiment 38A, wherein the CTLA-4 inhibitor comprises ipilimumab.

[0333] Embodiment 40A. The method of any one of Embodiments 1A-39A, wherein the individual previously received treatment with an anti-cancer therapy for the cancer.

[0334] Embodiment 41A. The method of Embodiment 40A, wherein the anti-cancer therapy is one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

[0335] Embodiment 42A. The method of any one of Embodiments 1A-41A, wherein the individual did not previously receive a regimen of chemotherapy for the cancer.

[0336] Embodiment 43A. The method of any one of Embodiments 1A-41A, wherein the individual previously received a regimen of chemotherapy for the cancer.

[0337] Embodiment 44A. The method of Embodiment 43A, wherein the previous regimen of chemotherapy comprised one or more of an alkylating agent, an alkyl sulfonates aziridine, an ethylenimine, a methylamelamine, an acetogenin, a camptothecin, a bryostatin, a callystatin, CC-1065, a cryptophycin, aa dolastatin, a duocarmycin, a eleutherobin, a pancratistatin, a sarcodictyin, a spongistatin, a nitrogen mustard, a nitrosureas, an antibiotic, a dynemicin, a bisphosphonate, an esperamicina a neocarzinostatin chromophore or a related chromoprotein enediyne antiobiotic chromophore, an anti-metabolite, a folic acid analogue, a purine analog, a pyrimidine analog, an androgens, an anti-adrenal, a folic acid replenisher, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, a PSK polysaccharide complex, razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2,2',2”-trichlorotriethylamine, a trichothecene, urethan, vindesine, dacarbazine, mannomustine,

mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside ("Ara-C"), cyclophosphamide, a taxoid, 6-thioguanine, mercaptopurine, a platinum coordination complex, vinblastine, platinum, etoposide (VP- 16), ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, xeloda, ibandronate, irinotecan, topoisomerase inhibitor RFS 2000, difluorometlhylomithine (DMFO), a retinoid, capecitabine, carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, a farnesyl-protein transferase inhibitor, transplatinum, or any combination thereof.

[0338] Embodiment 45A. The method of any one of Embodiments 1A-44A, wherein the immune checkpoint inhibitor therapy is the only anti-cancer therapy indicated or administered for the cancer.

[0339] Embodiment 46A. The method of any one of Embodiments 1A-45A, wherein the immune checkpoint inhibitor therapy is a single-active-agent therapy.

[0340] Embodiment 47A. The method of any one of Embodiments 1A-45A, wherein the immune checkpoint inhibitor therapy comprises two or more active agents.

[0341] Embodiment 48A. The method of any one of Embodiments 1A-47A, wherein the immune checkpoint inhibitor therapy comprises a first round of an immune checkpoint inhibitor and a subsequent round of therapy with a different immune checkpoint inhibitor.

[0342] Embodiment 49A. The method of any one of Embodiments 1A-48A, wherein the immune checkpoint inhibitor therapy is the first line therapy for the cancer.

[0343] Embodiment 50A. The method of any one of Embodiments 1A-48A, wherein the immune checkpoint inhibitor therapy is the second line therapy for the cancer.

[0344] Embodiment 51A. The method of any one of Embodiments 1A-50A, further comprising treating the individual with an additional anti-cancer therapy.

[0345] Embodiment 52A. The method of Embodiment 51A, wherein the additional anti-cancer therapy comprises one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

[0346] Embodiment 53A. The method of any one of Embodiments 1A-52A, wherein the TMB score or microsatellite instability is determined by sequencing.

[0347] Embodiment 54A. The method of Embodiment 53A, wherein the sequencing comprises use of a massively parallel sequencing (MPS) technique, whole genome sequencing (WGS), whole exome sequencing (WES), targeted sequencing, direct sequencing, next-generation sequencing (NGS), or a Sanger sequencing technique.

[0348] Embodiment 55A. The method of Embodiment 53A or Embodiment 54A, wherein the sequencing comprises:

providing a plurality of nucleic acid molecules obtained from the tumor biopsy sample, wherein the plurality of nucleic acid molecules comprise a mixture of tumor nucleic acid molecules and non-tumor nucleic acid molecules;

optionally, ligating one or more adapters onto one or more nucleic acid molecules from the plurality of nucleic acid molecules;

amplifying nucleic acid molecules from the plurality of nucleic acid molecules;

capturing nucleic acid molecules from the amplified nucleic acid molecules, wherein the captured nucleic acid molecules are captured from the amplified nucleic acid molecules by hybridization to one or more bait molecules;

sequencing, by a sequencer, at least a portion of the captured nucleic acid molecules to obtain a plurality of sequence reads corresponding to one or more genomic loci within a subgenomic interval in the sample.

[0349] Embodiment 56A. The method of Embodiment 55A, wherein the adapters comprise one or more of amplification primer sequences, flow cell adapter hybridization sequences, unique molecular identifier sequences, substrate adapter sequences, or sample index sequences.

[0350] Embodiment 57A. The method of Embodiment 55A or Embodiment 56A, wherein amplifying nucleic acid molecules comprises performing a polymerase chain reaction (PCR) technique, a non-PCR amplification technique, or an isothermal amplification technique.

[0351] Embodiment 58A. The method of any one of Embodiments 55A-57A, wherein the one or more bait molecules comprise one or more nucleic acid molecules, each comprising a region that is complementary to a region of a captured nucleic acid molecule.

[0352] Embodiment 59A. The method of Embodiment 58A, wherein the one or more bait molecules each comprise a capture moiety.

[0353] Embodiment 60A. The method of Embodiment 59A, wherein the capture moiety is biotin.

[0354] Embodiment 61A. The method of any of Embodiments 1A-60A, wherein the individual is a human.

[0355] Embodiment 62A. The method of any one of Embodiments 1A-61A, wherein if the TMB score is at least the threshold TMB score, the individual is predicted to have increased time to next treatment (TTNT) when treated with an immune checkpoint inhibitor, as compared to a chemotherapy.

[0356] Embodiment 63A. A kit comprising an immune checkpoint inhibitor and instructions for use according to the method of any one of Embodiments 1A-62A.

EXAMPLES

EXAMPLE 1: TUMOR MUTATIONAL BURDEN AS A PREDICTIVE BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR VS. CHEMOTHERAPY BENEFIT IN 1ST LINE METASTATIC UROTHELIAL CARCINOMA.

[0357] This example shows a comparison of the outcomes of real-world patients on immune checkpoint inhibitor (ICPI) compared with patients on chemotherapy in relation to tumor mutational burden (TMB), using outcomes such as PFS and OS.

[0358] Study design and patient selection. The cohort comprised patients with confirmed diagnosis of metastatic urothelial carcinoma (mUC) included in a de-identified clinico-genomic database. All patients underwent genomic testing using genomic profiling (CGP) assays.

[0359] De-identified clinical data originated from approximately 280 US cancer clinics (~800 sites of care). Retrospective longitudinal clinical data were derived from electronic health records (EHR), comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports, which were linked to genomic data derived from testing by de-identified, deterministic matching (see Singal et al., "Association of Patient Characteristics and Tumor Genomics with Clinical Outcomes Among Patients with Non-Small Cell Lung Cancer using a Clinicogenomic Database," *JAMA* 2019;321(14):1391-9). Clinical data included demographics, clinical and laboratory features, timing of treatment exposure, treatment progressions, and survival.

[0360] Patients were included in this study if they received a first line single-agent anti-PD1 axis therapy (pembrolizumab, atezolizumab, nivolumab, durvalumab, or avelumab) or carboplatin-based chemotherapy regimen and had TMB assessed via tissue biopsy. Patients who received both ICPI and chemotherapy in combination were not included. To reduce immortal time in analyses, patients for whom CGP report was received after cessation of 1st line therapy were excluded.

[0361] Comprehensive Genomic Profiling. Hybrid capture-based next-generation sequencing (NGS) assays were performed on patient tumor specimens in Clinical Laboratory Improvement Amendments (CLIA)–certified, College of American Pathologists (CAP)-accredited laboratory. Assays interrogated all exons from minimum 324 cancer related genes, plus select introns from minimum 28 genes for rearrangement detection. Samples were evaluated for alterations as previously described (see Frampton et al., "Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing," *Nat. Biotechnol.* 2013;31(11):1023-31). Tumor mutational burden (TMB) was determined on up to 1.1 Mb of sequenced DNA (see Chalmers et al., "Analysis of 100,00 human cancer genomes reveals the landscape of tumor mutational burden," *Genome Med.* 2017;9(1):34). MSI was determined on 95-114 loci, as previously described (see Trabucco et al., "A Novel Next-Generation Sequencing Approach to Detecting Microsatellite Instability and Pan-Tumor

Characterization of 1000 Microsatellite Instability-High Cases in 67,000 Patient Samples," *J. Mol. Diagn.* 2019;21(6):1053-66).

[0362] Outcomes. PFS was calculated from start of 1st line treatment to progression event (radiographic, clinical, or pathologic) or death. Patients without observed records of progression event or mortality were right-censored at their last clinic visit date. TTNT was calculated from treatment start date until the start of next treatment line (due to any cause), or death. Patients not yet reaching next treatment line or death were right censored at date of last clinical visit or structured activity. OS was calculated from start of 1st line treatment to death from any cause, and patients with no record of mortality were right censored at the date of last clinic visit. Because patients cannot enter the database until a CGP report is delivered, OS risk intervals were left truncated to the date of CGP report to further account for immortal time. Truncation independence with censoring was evaluated with Kendall's tau for both ICPI and chemotherapy groups separately, and in combination, with $p < 0.05$ considered acceptable. Database mortality information is a composite derived from 3 sources: documents within the EHR, Social Security Death Index, and a commercial death dataset mining data from obituaries and funeral homes, with validations reported in comparison to the National Death Index (see Zhang et al., "Validation analysis of a composite real-world mortality endpoint for patients with cancer in the United States," Health Services Research).

[0363] Statistical Analyses. Differences in time-to-event outcomes were assessed with the log-rank test and Cox proportional hazard (PH) models. Chi-square tests and Wilcoxon rank sum tests were used to assess differences between groups of categorical and continuous variables, respectively. Multiple comparison adjustments were not performed; p-values are reported to quantify the strength of association for biomarker and each outcome, not for null hypothesis significance testing, and interpretations adopted broadly considering consistency of multiple outcome measures in concert (PFS, TTNT, OS) with no outcome measure standing on its own.

[0364] Missing values were handled by simple imputation with expected values determined based using random forests with the R package 'missForest.' In subsequent analyses, imputed values were treated identically to measured values.

[0365] Propensity analyses made use of the full matching technique (R package 'MatchIt'), resulting in no patient exclusions but chemotherapy treatments receiving weights. Weights were capped at 10 equivalents to limit influence per observation. Among patients receiving chemotherapy, those with characteristics most similar to the ICPI patient population were weighted more, and those less like the ICPI patients weighted less. These weights were included in all Kaplan-Meier visualizations and Cox PH models, unless otherwise noted. Features included for adjustment in propensity model: Age, ECOG performance score, eGFR, stage at diagnosis, and TMB. Standardized mean difference (SMD) was utilized to assess balance, and within 10% considered acceptable (see Austin and Stuart, "Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the

propensity score to estimate causal treatment effects in observational studies," *Stat. Med.* 2015;34(28):3661-79).

[0366] For a 10 mut/Mb (10 mutations per megabase) threshold, propensity weights were created separately for TMB ≥ 10 group and TMB < 10 group for best possible within-group balance. Predictive biomarker associations (see Ballman, "Biomarker:Predictive or Prognostic?" *J. Clin. Oncol.* 2015;33(33):3968-71) made use of propensity weighted multivariable Cox proportional hazards regression models containing the following variables: drug class (ICPI or taxane), TMB (high vs. low) and the interaction term between drug class and biomarker. Hazard ratios for subgroup analyses were generated from Cox models stratified by group (i.e. TMB high vs. low). R version 3.6.3 software was used for all statistical analyses.

[0367] Results—Characteristics of Analysis Cohort. After selection, the cohort consisted of 401 unique patients treated in the 1st line setting, with 245 patients receiving ICPI, and 156 patients receiving carboplatin-based chemotherapy (see Table 1, below). Evaluating differences in patients treated with ICPI vs. carboplatin-based chemotherapy, strong imbalances did not exist between sex, TMB, eGFR, practice setting, primary cancer anatomical site, smoking status, race, or PD-L1 staining (however, only 30% of the cohort had available PD-L1 staining). Patients receiving ICPI were older (median 73, IQR 66-80 vs. median 69, IQR 63-76, p < 0.001), less likely to be stage IV at diagnosis (p < 0.001), have higher ECOG scores (p < 0.001). Patients receiving chemotherapy had very different subsequent treatments (p < 0.001) including much more frequent ICPI use (41.7% vs. 3.7%). 122 of 401 (30.4%) patients had TMB ≥ 10, and this subgroup showed similar imbalances (see Table 2, below).

Table 1. Patient characteristics.

	Chemo (N=156)	**ICPI (N=245)**	**Total (N=401)**	**p value**
Age				< 0.001
Median (Q1, Q3)	69.0 (62.8, 76.0)	73.0 (66.0, 80.0)	71.0 (64.0, 78.0)	
Sex				0.883
F	45 (28.8%)	69 (28.2%)	114 (28.4%)	
M	111 (71.2%)	176 (71.8%)	287 (71.6%)	
TMB				0.031
Median (Q1, Q3)	6.3 (3.5, 9.7)	7.5 (3.8, 12.5)	6.3 (3.8, 11.3)	
Stage at Diagnosis				< 0.001
Stage I-III	24 (15.4%)	80 (32.7%)	104 (25.9%)	
Stage IV	71 (45.5%)	59 (24.1%)	130 (32.4%)	
Unknown/not documented	61 (39.1%)	106 (43.3%)	167 (41.6%)	
ECOG				0.036
0	0 (0.0%)	1 (0.5%)	1 (0.3%)	
1	44 (31.7%)	75 (34.1%)	119 (33.1%)	
2	77 (55.4%)	93 (42.3%)	170 (47.4%)	
3+	18 (12.9%)	51 (23.2%)	69 (19.2%)	
Missing Observations	17	25	42	
GFR				0.82
60+	47 (30.1%)	64 (26.1%)	111 (27.7%)	
30 - 60	69 (44.2%)	113 (46.1%)	182 (45.4%)	
< 30	11 (7.1%)	21 (8.6%)	32 (8.0%)	
unknown	29 (18.6%)	47 (19.2%)	76 (19.0%)	

Cisplatin Ineligible				0.992
No	13 (8.3%)	20 (8.2%)	33 (8.2%)	
Yes	99 (63.5%)	157 (64.1%)	256 (63.8%)	
unknown	44 (28.2%)	68 (27.8%)	112 (27.9%)	
Practice Setting				0.261
Academic	18 (11.5%)	20 (8.2%)	38 (9.5%)	
Community	138 (88.5%)	225 (91.8%)	363 (90.5%)	
Race				0.674
Asian	3 (1.9%)	5 (2.0%)	8 (2.0%)	
Black or African American	8 (5.1%)	6 (2.4%)	14 (3.5%)	
Other Race	26 (16.7%)	44 (18.0%)	70 (17.5%)	
unknown	10 (6.4%)	13 (5.3%)	23 (5.7%)	
White	109 (69.9%)	177 (72.2%)	286 (71.3%)	
Primary Site				0.65
Bladder	115 (73.7%)	184 (75.1%)	299 (74.6%)	
Renal Pelvis	21 (13.5%)	28 (11.4%)	49 (12.2%)	
Ureter	19 (12.2%)	28 (11.4%)	47 (11.7%)	
Urethra	1 (0.6%)	5 (2.0%)	6 (1.5%)	
Smoking Status				0.297
History of smoking	117 (75.0%)	172 (70.2%)	289 (72.1%)	
No history of smoking	39 (25.0%)	73 (29.8%)	112 (27.9%)	
PDL1				0.171
CPS 0	12 (7.7%)	10 (4.1%)	22 (5.5%)	
CPS 1-4	9 (5.8%)	22 (9.0%)	31 (7.7%)	
CPS 20+	17 (10.9%)	38 (15.5%)	55 (13.7%)	
CPS 5-19	17 (10.9%)	18 (7.3%)	35 (8.7%)	
unknown	101 (64.7%)	157 (64.1%)	258 (64.3%)	
Subsequent Treatment				< 0.001
Chemo	17 (10.9%)	34 (13.9%)	51 (12.7%)	
ICPI	65 (41.7%)	9 (3.7%)	74 (18.5%)	
none	45 (28.8%)	92 (37.6%)	137 (34.2%)	
not reached	19 (12.2%)	69 (28.2%)	88 (21.9%)	
other	10 (6.4%)	41 (16.7%)	51 (12.7%)	

Table 2. Patient characteristics.

	Chemo (N=39)	**ICPI (N=83)**	**Total (N=122)**	**p value**
Age				0.032
Median (Q1, Q3)	68.0 (64.5, 76.5)	74.0 (68.5, 81.0)	73.0 (66.0, 78.0)	
Sex				0.734
F	11 (28.2%)	21 (25.3%)	32 (26.2%)	
M	28 (71.8%)	62 (74.7%)	90 (73.8%)	
TMB				0.084
Median (Q1, Q3)	15.0 (11.3, 20.0)	18.8 (12.5, 22.5)	17.5 (12.5, 21.3)	
Stage at Diagnosis				0.086
Stage I-III	7 (17.9%)	23 (27.7%)	30 (24.6%)	
Stage IV	17 (43.6%)	20 (24.1%)	37 (30.3%)	
Unknown/not documented	15 (38.5%)	40 (48.2%)	55 (45.1%)	
ECOG				0.794
1	14 (42.4%)	27 (37.0%)	41 (38.7%)	
2	15 (45.5%)	34 (46.6%)	49 (46.2%)	
3+	4 (12.1%)	12 (16.4%)	16 (15.1%)	
Missing Observations	6	10	16	
GFR				0.432
60+	12 (30.8%)	30 (36.1%)	42 (34.4%)	
30 - 60	20 (51.3%)	34 (41.0%)	54 (44.3%)	

< 30	1 (2.6%)	8 (9.6%)	9 (7.4%)	
unknown	6 (15.4%)	11 (13.3%)	17 (13.9%)	
Cisplatin Ineligible				0.807
No	2 (5.1%)	7 (8.4%)	9 (7.4%)	
Yes	27 (69.2%)	55 (66.3%)	82 (67.2%)	
unknown	10 (25.6%)	21 (25.3%)	31 (25.4%)	
Practice Setting				0.007
Academic	8 (20.5%)	4 (4.8%)	12 (9.8%)	
Community	31 (79.5%)	79 (95.2%)	110 (90.2%)	
Race				0.176
Asian	1 (2.6%)	0 (0.0%)	1 (0.8%)	
Black or African American	1 (2.6%)	0 (0.0%)	1 (0.8%)	
Other Race	5 (12.8%)	20 (24.1%)	25 (20.5%)	
unknown	3 (7.7%)	4 (4.8%)	7 (5.7%)	
White	29 (74.4%)	59 (71.1%)	88 (72.1%)	
Primary Site				0.647
Bladder	32 (82.1%)	61 (73.5%)	93 (76.2%)	
Renal Pelvis	3 (7.7%)	9 (10.8%)	12 (9.8%)	
Ureter	4 (10.3%)	11 (13.3%)	15 (12.3%)	
Urethra	0 (0.0%)	2 (2.4%)	2 (1.6%)	
Smoking Status				0.44
History of smoking	29 (74.4%)	56 (67.5%)	85 (69.7%)	
No history of smoking	10 (25.6%)	27 (32.5%)	37 (30.3%)	
PDL1				0.577
CPS 0	2 (5.1%)	4 (4.8%)	6 (4.9%)	
CPS 1-4	2 (5.1%)	8 (9.6%)	10 (8.2%)	
CPS 20+	3 (7.7%)	13 (15.7%)	16 (13.1%)	
CPS 5-19	6 (15.4%)	8 (9.6%)	14 (11.5%)	
unknown	26 (66.7%)	50 (60.2%)	76 (62.3%)	
Subsequent Treatment				< 0.001
Chemo	5 (12.8%)	9 (10.8%)	14 (11.5%)	
ICPI	19 (48.7%)	6 (7.2%)	25 (20.5%)	
none	8 (20.5%)	23 (27.7%)	31 (25.4%)	
not reached	5 (12.8%)	33 (39.8%)	38 (31.1%)	
other	2 (5.1%)	12 (14.5%)	14 (11.5%)	

[0368] Propensity weighting. After propensity weighting in the TMB < 10 group, no features had > 10% SMD. After propensity weighting in the TMB ≥ 10 group, residual imbalances > 10% SMD existed such that patients receiving ICPI vs. chemotherapy were more likely to be stage I-III at initial diagnosis, and more likely to have an ECOG score of 3 or greater (**FIG. 1A-1B**).

[0369] Patients with TMB of at least 10 had more favorable PFS, TTNT, and OS on 1st line single-agent ICPI. PFS, TTNT, and OS by on ICPI was stratified by whether patients had TMB < 10 or TMB ≥ 10. Compared to patients with TMB < 10, patients with TMB ≥ 10 had more favorable PFS (HR: 0.59, 95%CI: 0.41 – 0.85, p = 0.0048), TTNT (HR: 0.59, 95%CI: 0.43 – 0.83, p = 0.0020), and OS (HR: 0.47, 95%CI: 0.32 – 0.68, p = 0.0001) (**FIGs. 2A-2C**).

[0370] Comparing outcomes of patients who received 1st line ICPI vs. carboplatin-based chemotherapy, adjusting for known imbalances in treatment assignment with propensity weights (**FIGs. 1A-1B**), patients with TMB ≥ 10 had more favorable estimates for PFS (HR: 0.51, 95%CI: 0.32 – 0.82, p = 0.0058), TTNT (HR: 0.56, 95%CI: 0.35 – 0.91, p = 0.0197) and OS (HR: 0.56,

95%CI: 0.29 – 1.08, p = 0.084), whereas patients with TMB < 10 had comparable or less favorable PFS (HR: 1.26, 95%CI: 0.89 – 1.80, p = 0.20), TTNT (HR: 0.89, 95%CI: 0.68 – 1.18, p = 0.42), and OS (HR: 1.11; 95%CI: 0.79– 1.59, p = 0.54) (**FIG. 3A-3F**). ICPI vs. chemotherapy assessments unadjusted for treatment assignment imbalances show similar associations in aggregate (**FIGs. 4A-4F**).

[0371] Additionally, the outcomes of patient subgroups identified by TMB or PD-L1 were compared. PD-L1 staining was only available for 35.7% of the cohort. While the confidence intervals were unexpectedly wider for PD-L1 than TMB groups, the main effect estimate for PD-L1 CPS ≥ 10 was weaker than TMB ≥ 10 for PFS, TTNT, and OS (**FIGs. 5A-5C**).

[0372] The Analysis Cohort was compared to Randomized Controlled Trial Populations. Using ECOG scores as a proxy for patient frailty, we compared patient characteristics 1st line patients in the analysis cohort to 1st line patients in randomized controlled trials (FIG. 6A). The DANUBE, IMvigor130, and KEYNOTE-361 studies respectively reported 53.2%, 52.2%, and 44.8% of patients with ECOG score of 0, while 0.3% of the real-world analysis cohort had ECOG score of 0. The DANUBE, IMvigor130, and KEYNOTE-361 studies respectively reported 0%, 10.8%, and 6.9% of patients with ECOG score of 2, while 42.3% of the real-world analysis cohort had ECOG score of 2. None of the 1st line phase III trials included patients with an ECOG score of 3 or greater, while 19.2% of the real-world analysis cohort had an ECOG score of 3 or greater.

[0373] The aggregate of real-world and trial outcomes of ICPI vs. carboplatin-based chemotherapy by TMB was next evaluated. Across both randomized controlled trials (RCTs) and the real-world analysis, there was a consistent enrichment for benefit of ICPI vs. carboplatin-based chemotherapy in the TMB ≥ 10 subgroup (**FIGs. 6B-6C**).

[0374] Conclusions. A TMB-high cutoff (such as 10 mut/Mb) has clinical validity in the 1st line setting for the identification of patients with mUC likely to have improved outcomes on single-agent ICPI compared to chemotherapy (such as non-cisplatin chemotherapy).

EXAMPLE 2: REAL-WORLD VALIDATION OF TUMOR MUTATIONAL BURDEN AS A PREDICTIVE BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR VS. CHEMOTHERAPY EFFECTIVENESS IN METASTATIC GASTRIC ADENOCARCINOMA IN DIVERSE PATIENTS AND CLINICAL PRACTICES.

[0375] This example shows a real-world comparison of the outcomes of patients on immune checkpoint inhibitors (ICPI) vs. chemotherapy stratified by biomarkers including tumor mutational burden (TMB) score.

[0376] Real-world analysis design. To assess the validity of biomarkers and effectiveness of drugs using observational data, two complementary techniques were used: propensity analyses and crossover analyses, with interpretations resting on consistency of observations across different cohorts and methods of evaluation, similar to prior real-world analyses in metastatic prostate cancer (see, e.g., Graf et al., "Predictive Genomic Biomarkers of Hormonal Therapy Versus Chemotherapy Benefit in

Metastatic Castration-resistant Prostate Cancer," *European Urology*, 2021). ICPI vs. chemotherapy effectiveness was first evaluated between patients in 2nd line settings, stratified by TMB, then evaluated if patients with TMB ≥ 10 mut/Mb had enhanced relative effectiveness of 2nd line ICPI when used after 1st line chemotherapy within the same patients (flowchart provided in **FIG. 7**). Lastly, the outcomes of patients who received 1st line ICPI in comparison to 1st line chemotherapy was evaluated (**FIG. 7**).

[0377] Patient selection. The study comprised patients with confirmed diagnoses of gastric adenocarcinoma. All patients underwent genomic testing using comprehensive genomic profiling (CGP) assays. De-identified clinical data originated from approximately 280 US cancer clinics (~800 sites of care). Retrospective longitudinal clinical data were derived from electronic health records (EHR), comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports, which were linked to genomic data by de-identified, deterministic matching (Singal et al., "Association of Patient Characteristics and Tmor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database," *JAMA* 321:1391-1399, 2019). Clinical data included demographics, clinical and laboratory features, timing of treatment exposure, and survival.

[0378] Patient records were included in this study if they received a first or second line single-agent anti-PD1 axis therapy or standard chemotherapy (platinum regimens for 1st line, non-platinum regimens for 2nd line) and had TMB assessed via tissue specimen. Patients who received both ICPI and chemotherapy in combination at the same time were not included. Patients must have additionally tested negative for *ERBB2* amplification and not have received an anti-HER2 agent. Analyses were conducted in 3 cohorts after aforementioned exclusions:

[0379] 2L Comparative Effectiveness Cohort: Patients who received platinum chemotherapy in 1st line, who made it to 2nd line, and received a single-agent ICPI or non-platinum chemotherapy in 2nd line.

[0380] Sequential Cohort: Patients who received platinum chemotherapy in 1st line, who made it to 2nd line, and received a single-agent ICPI in 2nd line.

[0381] 1L Comparative Effectiveness Cohort: Patients who received either a single-agent ICPI, or a platinum-containing chemotherapy regiment in the 1st line.

[0382] Comprehensive Genomic Profiling: Hybrid capture-based next-generation sequencing (NGS) assays were performed on patient tumor specimens in Clinical Laboratory Improvement Amendments (CLIA)–certified, College of American Pathologists (CAP)-accredited laboratory. The assays interrogated all exons from minimum 324 cancer related genes, plus select introns from minimum 28 genes for rearrangement detection. Samples were evaluated for alterations as previously described (Frampton et al.," Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing," *Nat Biotechnol* 31:1023-31, 2013). Tumor mutational burden (TMB) was determined on up to 1.1 Mb of sequenced DNA (see Chalmers et al., "Analysis of

100,000 human cancer genomes reveals the landscape of tumor mutational burden," *Genome Med* 9:34, 2017). MSI was determined on 95-114 loci, as previously described (Trabucco et al., "A Novel Next-Generation Sequencing Approach to Detecting Microsatellite Instability and Pan-Tumor Characterization of 1000 Microsatellite Instability-High Cases in 67,000 Patient Samples," *J Mol Diagn* 21:1053-1066, 2019).

[0383] Outcomes: Time to next treatment (TTNT), like PFS, is a time-to-event proxy for drug clinical effectiveness (Khozin et al., "Real-world progression, treatment, and survival outcomes during rapid adoption of immunotherapy for advanced non-small cell lung cancer," *Cancer* 125:4019-4032, 2019). TTNT was calculated from treatment start date until the start of next treatment line (due to any cause), or death. Patients not yet reaching next treatment line or death were right censored at date of last clinical visit or structured activity. Overall survival (OS) was calculated from start of treatment to death from any cause, and patients with no record of mortality were right censored at the date of last clinic visit or structured activity. Because patients cannot enter the database until a CGP report is delivered, OS risk intervals were left truncated to the date of CGP report to account for immortal time (*see* McGough et al., "Penalized regression for left-truncated and right-censored survival data," *Stat Med*, 2021; *see, also*, Brown et al., "Implications of Selection Bias Due to Delayed Study Entry in Clinical Genomic Studies," *JAMA Oncology*, 2021). Flatiron Health database mortality information is a composite derived from 3 sources: documents within the EHR, Social Security Death Index, and a commercial death dataset mining data from obituaries and funeral homes. This mortality information has been externally validated in comparison to the National Death Index (Zhang et al., "Validation analysis of a composite real-world mortality endpoint for patients with cancer in the United States," Health Services Research).

[0384] Statistical analyses: Differences in time-to-event outcomes were assessed with the log-rank test and Cox proportional hazard (PH) models. Chi-square tests and Wilcoxon rank sum tests were used to assess differences between groups of categorical and continuous variables, respectively.Multiple comparison adjustments were not performed; p-values are reported to quantify the strength of association for biomarker and each outcome, not for null hypothesis significance testing, and interpretations adopted broadly considering consistency of multiple outcome measures in concert (TTNT, OS) across defined cohorts (inter-patient vs. intra-patient) with no outcome measure or cohort standing on its own. The default interpretation is that a biomarker correlating with OS but not TTNT within a cohort is likely a confounding artifact, and a biomarker correlating with TTNT but not OS is likely not remarkable. Additionally, while the effect size estimates may vary by cohort, the default assumption is that a biomarker effect should not be specific to any of the cohorts evaluated.

[0385] Missing values were handled by simple imputation with expected values determined using random forests with the R package "missForest." In subsequent analyses, imputed values were treated identically to measured values.

[0386] Propensity analyses made use of the full matching technique (R package, "MatchIt"), resulting in no patient exclusions but chemotherapy treatments receiving weights. Weights were capped at 10 equivalents to limit influence per observation. Among patients receiving chemotherapy, those with characteristics most similar to the ICPI patient population were weighted more, and those less like the ICPI patients weighted less. These weights were included in all Kaplan-Meier visualizations and Cox PH models, unless otherwise noted. Available features related to treatment assignment of ICPI vs. chemotherapy included for adjustment in propensity models: ECOG (0-2 vs. 3+), abnormal labs (bilirubin above ULN and/or albumin below ULN), PD-L1 (CPS5 vs. not), stage at diagnosis (stage IV vs. not), surgery (yes vs. no) and TMB (continuous). Standardized mean difference (SMD) was utilized to assess balance, and within 10% considered acceptable (see Austin and Stuart, "Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies," *Stat Med* 34:3661-79, 2015).

[0387] Due to focus on the 10 muts/Mb threshold, propensity weights were created separately for TMB $\geq$ 10 group and TMB < 10 group for best possible within-group balance. Predictive biomarker associations (see Ballman, "Biomarker: Predictive or Prognostic?" J Clin Oncol 33:3968-71, 2015) made use of propensity weighted multivariable Cox proportional hazards regression models containing the following variables: drug class (ICPI or taxane), TMB (high vs. low) and the interaction term between drug class and biomarker. Models evaluating intra-patient treatment interactions in the Sequential Cohort were additionally clustered on the individual patient, making use of robust variances calculated by generalized estimating equations within a working independence structure. Hazard ratios were then generated from adjusted Cox models stratified by group (i.e. TMB high vs. low). R version 3.6.3 software was used for all statistical analyses.

[0388] Results. *2L Comparative Effectiveness Cohort*: After selection, 263 patients received 2nd line non-platinum chemotherapy after platinum chemotherapy in 1st line, and 99 patients received 2nd line ICPI after platinum chemotherapy in 1st line. Differences of $p < 0.05$ were not observed by treatment group for age, sex, stage at diagnosis, smoking status, prior surgery, ECOG, albumin, or bilirubin. However, patients receiving ICPI had higher TMB ($p < 0.001$), PD-L1 CPS scores ($p < 0.001$), and more frequent MSI-H ($p < 0.001$). See Table 3, below.

Table 3. 2L Cohort Patients.

	Chemo (N=263)	**ICPI (N=99)**	**Total (N=362)**	**p value**
Age				0.11
Median (Q1, Q3)	65.0 (55.0, 71.5)	68.0 (57.5, 74.0)	65.0 (56.0, 72.0)	
gender				0.9
F	70 (26.6%)	27 (27.3%)	97 (26.8%)	
M	193 (73.4%)	72 (72.7%)	265 (73.2%)	
Practice type				0.183
Academic	18 (6.8%)	11 (11.1%)	29 (8.0%)	
Community	245 (93.2%)	88 (88.9%)	333 (92.0%)	
Group stage				0.447

Stage I	5 (1.9%)	1 (1.0%)	6 (1.7%)	
Stage II	14 (5.3%)	10 (10.1%)	24 (6.6%)	
Stage III	32 (12.2%)	14 (14.1%)	46 (12.7%)	
Stage IV	181 (68.8%)	61 (61.6%)	242 (66.9%)	
Unknown/not documented	31 (11.8%)	13 (13.1%)	44 (12.2%)	
Smoking status				0.8
History of smoking	179 (68.1%)	66 (66.7%)	245 (67.7%)	
No history of smoking	84 (31.9%)	33 (33.3%)	117 (32.3%)	
Surgery				0.246
No	204 (77.6%)	71 (71.7%)	275 (76.0%)	
Yes	59 (22.4%)	28 (28.3%)	87 (24.0%)	
ECOG				0.452
0	2 (0.8%)	0 (0.0%)	2 (0.6%)	
1	66 (26.8%)	21 (23.6%)	87 (26.0%)	
2	134 (54.5%)	46 (51.7%)	180 (53.7%)	
3+	44 (17.9%)	22 (24.7%)	66 (19.7%)	
Missing Observations	17	10	27	
HER2 IHC				0.424
Negative	216 (82.1%)	86 (86.9%)	302 (83.4%)	
Positive	16 (6.1%)	6 (6.1%)	22 (6.1%)	
unknown	31 (11.8%)	7 (7.1%)	38 (10.5%)	
TMB				< 0.001
Median (Q1, Q3)	3.5 (1.7, 6.1)	5.0 (2.5, 9.2)	3.8 (1.7, 6.3)	
MSI				< 0.001
MSI unknown	66 (25.1%)	16 (16.2%)	82 (22.7%)	
MSI-H	5 (1.9%)	16 (16.2%)	21 (5.8%)	
MSI-I	1 (0.4%)	1 (1.0%)	2 (0.6%)	
MSS	191 (72.6%)	66 (66.7%)	257 (71.0%)	
PDL1				< 0.001
CPS 0	36 (13.7%)	10 (10.1%)	46 (12.7%)	
CPS 1-4	34 (12.9%)	13 (13.1%)	47 (13.0%)	
CPS 20+	25 (9.5%)	23 (23.2%)	48 (13.3%)	
CPS 5-19	31 (11.8%)	20 (20.2%)	51 (14.1%)	
Unknown	137 (52.1%)	33 (33.3%)	170 (47.0%)	
ALB				0.962
Below LLN	83 (33.1%)	32 (33.3%)	115 (33.1%)	
Normal	168 (66.9%)	64 (66.7%)	232 (66.9%)	
Missing Observations	12	3	15	
Bilirubin				0.791
Above ULN	18 (7.4%)	6 (6.6%)	24 (7.2%)	
Normal	224 (92.6%)	85 (93.4%)	309 (92.8%)	
Missing Observations	21	8	29	

[0389] *Sequential Cohort*: After selection, 65 patients received 1st line platinum chemotherapy followed by 2nd line ICPI. Of these, 17 had TMB ≥ 10 and 48 had TMB < 10. Differences of $p < 0.05$ were not observed by TMB group for age, sex, stage at diagnosis, smoking status, prior surgery, ECOG, albumin, bilirubin, or PD-L1. However, TMB and MSI were highly correlated ($p < 0.001$); of the TMB ≥ 10 group, 14 had MSI-H, 2 had MSS and 1 had unknown MSI status. Among the TMB < 10 group, 0 were MSI-H, 37 were MSS, and 11 were MSI unknown. See Table 4, below.

Table 4. Sequential cohort patients.

	TMB < 10 (N=48)	**TMB10+ (N=17)**	**Total (N=65)**	**p value**
Age				0.442

Median (Q1, Q3)	68.0 (56.8, 73.2)	69.0 (63.0, 75.0)	68.0 (58.0, 74.0)	
gender				0.593
F	11 (22.9%)	5 (29.4%)	16 (24.6%)	
M	37 (77.1%)	12 (70.6%)	49 (75.4%)	
Practice type				0.745
Academic	4 (8.3%)	1 (5.9%)	5 (7.7%)	
Community	44 (91.7%)	16 (94.1%)	60 (92.3%)	
Group stage				0.2
Stage I	0 (0.0%)	1 (5.9%)	1 (1.5%)	
Stage II	4 (8.3%)	0 (0.0%)	4 (6.2%)	
Stage III	7 (14.6%)	2 (11.8%)	9 (13.8%)	
Stage IV	32 (66.7%)	10 (58.8%)	42 (64.6%)	
Unknown/not documented	5 (10.4%)	4 (23.5%)	9 (13.8%)	
Smoking status				0.854
History of smoking	35 (72.9%)	12 (70.6%)	47 (72.3%)	
No history of smoking	13 (27.1%)	5 (29.4%)	18 (27.7%)	
Surgery				0.778
No	38 (79.2%)	14 (82.4%)	52 (80.0%)	
Yes	10 (20.8%)	3 (17.6%)	13 (20.0%)	
ECOG				0.762
1	20 (46.5%)	8 (57.1%)	28 (49.1%)	
2	18 (41.9%)	5 (35.7%)	23 (40.4%)	
3+	5 (11.6%)	1 (7.1%)	6 (10.5%)	
Missing Observations	5	3	8	
HER2 IHC				0.493
Negative	36 (75.0%)	15 (88.2%)	51 (78.5%)	
Positive	1 (2.1%)	0 (0.0%)	1 (1.5%)	
unknown	11 (22.9%)	2 (11.8%)	13 (20.0%)	
TMB				< 0.001
Median (Q1, Q3)	3.8 (1.3, 5.4)	31.3 (13.8, 36.5)	5.0 (2.5, 10.4)	
MSI				< 0.001
MSI unknown	11 (22.9%)	1 (5.9%)	12 (18.5%)	
MSI-H	0 (0.0%)	14 (82.4%)	14 (21.5%)	
MSS	37 (77.1%)	2 (11.8%)	39 (60.0%)	
PDL1				0.495
CPS 0	3 (6.2%)	0 (0.0%)	3 (4.6%)	
CPS 1-4	9 (18.8%)	1 (5.9%)	10 (15.4%)	
CPS 20+	11 (22.9%)	4 (23.5%)	15 (23.1%)	
CPS 5-19	8 (16.7%)	3 (17.6%)	11 (16.9%)	
unknown	17 (35.4%)	9 (52.9%)	26 (40.0%)	
ALB				0.909
Below LLN	6 (13.6%)	2 (12.5%)	8 (13.3%)	
Normal	38 (86.4%)	14 (87.5%)	52 (86.7%)	
Missing Observations	4	1	5	
Bilirubin				< 0.001
Normal	44 (100.0%)	16 (100.0%)	60 (100.0%)	
Missing Observations	4	1	5	

[0390] *1L Comparative Effectiveness Cohort*: After selection, 659 patients received 1st line platinum chemotherapy, and 33 patients received 1st line ICPI. Compared to patients receiving chemotherapy, patients receiving ICPI were older (median 70 vs. 66, $p = 0.038$), less likely to be Stage IC at diagnosis (27.3% vs. 66.5%, $p < 0.001$), more likely to have had prior surgery (69.7% vs. 20%, $p < 0.001$), and more likely to have PD-L1 testing available ($p < 0.001$). See Table 5, below.

Table 5. 1L Cohort Patients.				
	Chemo (N=659)	**ICPI (N=33)**	**Total (N=692)**	**p value**
Age				0.038
Median (Q1, Q3)	66.0 (57.0, 72.0)	70.0 (59.0, 75.0)	66.0 (57.0, 73.0)	
gender				0.766
	1 (0.2%)	0 (0.0%)	1 (0.1%)	
F	164 (24.9%)	10 (30.3%)	174 (25.1%)	
M	494 (75.0%)	23 (69.7%)	517 (74.7%)	
Practice Type				0.306
Academic	52 (7.9%)	1 (3.0%)	53 (7.7%)	
Community	607 (92.1%)	32 (97.0%)	639 (92.3%)	
Stage at Dx				< 0.001
Stage I	11 (1.7%)	1 (3.0%)	12 (1.7%)	
Stage II	59 (9.0%)	5 (15.2%)	64 (9.2%)	
Stage III	100 (15.2%)	11 (33.3%)	111 (16.0%)	
Stage IV	438 (66.5%)	9 (27.3%)	447 (64.6%)	
Unknown/not documented	51 (7.7%)	7 (21.2%)	58 (8.4%)	
History of Smoking				0.192
History of smoking	421 (63.9%)	16 (48.5%)	437 (63.2%)	
No history of smoking	237 (36.0%)	17 (51.5%)	254 (36.7%)	
Unknown/Not documented	1 (0.2%)	0 (0.0%)	1 (0.1%)	
Prior Surgery				< 0.001
No	527 (80.0%)	10 (30.3%)	537 (77.6%)	
Yes	132 (20.0%)	23 (69.7%)	155 (22.4%)	
ECOG				0.005
1	228 (39.0%)	4 (12.5%)	232 (37.7%)	
2	261 (44.7%)	18 (56.2%)	279 (45.3%)	
3+	95 (16.3%)	10 (31.2%)	105 (17.0%)	
Missing Observations	75	1	76	
HER2 IHC				0.793
Negative	504 (76.5%)	25 (75.8%)	529 (76.4%)	
Positive	10 (1.5%)	1 (3.0%)	11 (1.6%)	
unknown	145 (22.0%)	7 (21.2%)	152 (22.0%)	
TMB				0.323
Median (Q1, Q3)	3.8 (1.7, 6.3)	3.8 (2.5, 8.8)	3.8 (1.7, 6.3)	
MSI by NGS				0.549
	46 (7.0%)	0 (0.0%)	46 (6.6%)	
MSI unknown	88 (13.4%)	6 (18.2%)	94 (13.6%)	
MSI-H	30 (4.6%)	2 (6.1%)	32 (4.6%)	
MSI-I	1 (0.2%)	0 (0.0%)	1 (0.1%)	
MSS	494 (75.0%)	25 (75.8%)	519 (75.0%)	
PDL1				< 0.001
CPS 0	72 (10.9%)	2 (6.1%)	74 (10.7%)	
CPS 1-4	96 (14.6%)	7 (21.2%)	103 (14.9%)	
CPS 20+	67 (10.2%)	11 (33.3%)	78 (11.3%)	
CPS 5-19	89 (13.5%)	6 (18.2%)	95 (13.7%)	
unknown	335 (50.8%)	7 (21.2%)	342 (49.4%)	
Albumin				0.032
Below LLN	143 (23.8%)	13 (40.6%)	156 (24.7%)	
Normal	457 (76.2%)	19 (59.4%)	476 (75.3%)	
Missing Observations	59	1	60	
Bilirubin				0.15
Above ULN	36 (6.1%)	0 (0.0%)	36 (5.8%)	
Normal	555 (93.9%)	32 (100.0%)	587 (94.2%)	
Missing Observations	68	1	69	

[0391] Adjusting for known treatment assignment imbalances, patients receiving 2nd line ICPI vs. chemotherapy had more favorable outcomes when TMB ≥ 10 mut/Mb, but not TMB < 10 (FIGs. 8A-8D). In the TMB < 10 subgroup, all features had SMB < 10% after weighting. In the TMB ≥ 10 subgroup, while imbalances were greatly reduced, residual imbalances of SMD ≥ 10% were present, such that patients receiving ICPI were more likely to have had surgery, have PD-L1 CPS ≥ 5, be older, have abnormal labs, and higher TMB. Among patients treated with single-agent ICPI vs. non-platinum chemotherapy in 2nd line (after prior platinum chemotherapy in 1st line), those with TMB < 10 had comparable TTNT (HR: 0.96, 95%CI: 0.69 – 1.34, p = 0.81) and OS (HR: 1.1, 95%CI: 0.73 – 1.64, p = 0.66). However, patients with TMB ≥ 10 had more favorable TTNT (HR: 0.16, 95%CI: 0.07 – 0.35, p < 0.0001) and OS (HR: 0.21, 95%CI: 0.09 – 0.49, p = 0.0004). Sensitivity analyses unadjusted for imbalances show similar results (**FIGs. 9A-9D**).

[0392] Patients receiving 1st line chemotherapy followed by 2nd line ICPI had more favorable outcomes on 2nd line ICPI compared to 1st line chemotherapy when TMB ≥ 10 but not TMB <10. TTNT of 1st line chemotherapy are visualized for those with TMB < 10 (**FIG. 10A**) and those with TMB ≥ 10 (**FIG. 10B**), with the bars colored by MSI status. MSI status was highly correlated with this TMB threshold in this cohort, with no patients with MSI-H having TMB < 10, and 14 of 17 with TMB ≥ 10 having MSI-H (2 were MSS, 1 was MSI unknown). The two with MSS and TMB ≥ 10 do not yet have record of next treatment after ICPI start, with 9.7 and 16.0 months so far. The median TTNT of 2nd line ICPI in the TMB < 10 group is 3.3 months (95%CI: 2.2 – 8.0). Point estimates and confidence intervals from Cox models comparing intra-patient TTNT are shown in **FIG. 10C**. Unadjusted overall survival is shown from the time of 1st line chemotherapy start by TMB in **FIG. 10D**.

[0393] TMB ≥ 10 and MSI-H are stronger predictive biomarkers for ICPI vs. chemotherapy benefit than PD-L1 CPS ≥ 5. MSI-H status is highly correlated with increasing TMB values (**FIGs. 11A-11B**), while PD-L1 CPS ≥ 5 does not have particular enrichment in patients with high TMB (Table 6, below), suggesting a degree of independence in these cohorts. PD-L1 scoring was not available for 47% of the 2nd line comparative effectiveness cohort and for 40% of the sequential cohort. However, the prevalence of patients with PD-L1 CPS ≥ 5 was much higher than TMB ≥ 10 or MSI-H. In the 2L ICPI vs. chemotherapy cohort as well as the sequential analysis, patients identified by TMB ≥ 10 and/or MSI-H had similar prevalence, and enrichment for observed outcomes favoring ICPI vs. chemotherapy (**FIGs. 11A-11C**). There was a modest enrichment for observed outcomes favoring ICPI vs. chemotherapy in patients identified by PD-L1 CPS ≥ 5 in TTNT, but not OS.

Table 6. Biomarker enrichment.

TMB	PD-L1	2L ICPI vs. Chemo Cohort (patients)	1L Chemo then 2L ICPI Cohort (Patients)
TMB < 10	CPS < 5	86	12
TMB < 10	CPS ≥ 5	88	19

TMB < 10	Unknown	146	17
TMB ≥ 10	CPS < 5	7	1
TMB ≥ 10	CPS ≥ 5	11	7
TMB ≥ 10	Unknown	24	9

[0394] KeyNote-061 and real-world cohorts have very different patient populations, similar drug-class specific TMB associations. Using ECOG performance scores as a proxy for overall patient frailty across cohorts, the broad characteristics are shown from the Phase III randomized controlled trial KeyNote-061 comparing 2nd line pembrolizumab to paclitaxel (see Shitara et al., "Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomized, open-label, controlled, phase 3 trial," *Lancet* 392:123-133, 2018), compared to the ECOG scores in the 2nd line comparative effectiveness cohort (**FIG. 12A**). The overall survival subgroup analysis for high TMB in KeyNote-061 was reported as a post-hoc analysis by the FDA (Marcus et al., "FDA Approval Summary: Pembrolizumab for the Treatment of Tumor Mutational Burden-High Solid Tumors," *Clin Cancer Res*, 2021), and is shown alongside the intra-patient OS assessments of the 2nd line comparative effectiveness cohort (**FIG. 12B**). The clinical trial assay used for KeyNote-061 TMB assessment was a whole exome sequencing assay, and "high" TMB was determined by a cut point most concordant to the FDA-approved assay at TMB ≥ 10^6.

[0395] Adjusting for known treatment assignment imbalances, patients receiving 1st line ICPI vs. chemotherapy have more favorable outcomes when TMB ≥ 10 but not TMB < 10 (**FIGs. 13A-13D**). In the TMB < 10 subgroup, residual imbalances remained such that patients receiving ICPI were more likely to not have a PD-L1 score, and were less likely to have had abnormal labs. In the TMB ≥ 10 subgroup, patients receiving ICPI were more likely to have been stage IV at diagnosis. Among patients treated with single-agent ICPI vs. non-platinum chemotherapy in 1st line, those with TMB < 10 had inferior TTNT (HR: 2.42, 95%CI: 1.29 – 4.57, p = 0.0062) and comparable to worse OS (HR: 1.71, 95%CI: 0.82 – 3.57, p = 0.16). However, patients with TMB ≥ 10 had more favorable TTNT (HR: 0.14, 95%CI: 0.04 – 0.52, p = 0.0034) and comparable to favorable OS (HR: 0.45, 95%CI: 0.11 – 1.82, p = 0.26). Sensitivity analyses unadjusted for imbalances show similar results (**FIGs. 14A-14D**).

[0396] In conclusion, the results show the clinical validity of TMB ≥ 10 in a diverse, real-world population of patients less eligible for clinical trials. Using two complementary approaches for comparative effectiveness that partially overcome their respective limitations, consistent strong enrichment for ICPI vs. chemotherapy benefit was observed in both inter-patient and intra-patient assessments for TMB ≥ 10, as well as NGS-assessed MSI. Consistent results of same magnitude were not observed with PD-L1 CPS ≥ 5. TMB ≥ 10 robustly identifies metastatic gastric patients who have favorable outcomes on 2nd line single-agent ICPI compared to chemotherapy in patient populations

and treatment settings more diverse than registrational clinical trials. The effects in 1st line data are consistent with the 2nd line observations. The results suggest that a 1st line trial of ICPI without chemotherapy vs. chemotherapy might be successful if selected by TMB ≥ 10.

EXAMPLE 3: TUMOR MUTATIONAL BURDEN AS A PREDICTIVE BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR VS. TAXANE CHEMOTHERAPY BENEFIT IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER: REAL-WORLD BIOMARKER ANALYSIS.

[0397] This example shows a comparison of the treatment class-specific outcomes of patients with metastatic castration resistant prostate cancer (mCRPC) on ICPI vs. taxane chemotherapy stratified by TMB score.

[0398] Genomic data was associated with clinical variables and outcomes in a cohort of patients with mCRPC. Longitundinal de-identified clinical data from ~280 U.S. academic or community-based cancer clinics were derived from electronic health records, curated via technology-enabled abstraction and linked to genomic testing by a comprehensive genomic assay. 45 patients (14 with TMB of at least 10 and 31 with TMB less than 10) received single-agent anti-PD-1 axis ICPI. 696 (30 with TMB of at least 10 and 666 with TMB less than 10) received single-agent taxanes, at discretion of physician without randomization. For time to next therapy (TTNT) and overall survival (OS) assessments, imbalances between treatment groups were adjusted with propensity weighting.

[0399] 741 patients were identified and included in the analysis. Patients receiving ICPI vs. taxanes had higher TMB (median 3.5, vs. 2.5, $p < 0.001$), higher ECOG scores ($p = 0.057$), and greater prior taxane use (73.3% vs. 53.7%, $p = 0.01$). Baseline patient characteristics overall and within ICPI versus taxane subgroups, as well as comparisons of characteristics between subgroups, is provided in Table 7, below.

Table 7. Patient characteristics.

	ICPI (N=45)	Taxane (N=696)	Total (N=741)	p value
Age: median (Q1, Q3)	72.0 (64.0, 76.0)	70.0 (64.0, 76.0)	70.0 (64.0, 76.0)	0.72
TMB (mut/Mb): median (Q1, Q3)	3.5 (1.7, 15.0)	2.5 (1.3, 3.8)	2.5 (1.3, 3.8)	< 0.001
ECOG: No. (%) with data				0.057
0	5 (13.9)	158 (29.4)	163 (28.4)	
1	20 (55.6)	283 (52.6)	303 (52.8)	
2+	11 (30.6)	97 (18.0)	108 (18.8)	
PSA (ng/mL): median (Q1, Q3)	63.9 (10.8, 183.2)	80.8 (19.4, 256.1)	79.4 (19.0, 254.0)	0.21
Hemoglobin (g/dL): median (Q1, Q3)	10.8 (9.5, 11.9)	11.2 (9.8, 12.6)	11.2 (9.7, 12.6)	0.11
Alkaline phosphatase (IU/L): median (Q1, Q3)	91.0 (77.0, 145.0)	110.0 (77.0, 186.0)	109.0 (77.0, 184.0)	0.2
Treatment setting: No. (%)				0.064
1st line mCRPC	5 (11.1)	92 (13.2)	97 (13.1)	
2nd line mCRPC	5 (11.1)	167 (24.0)	172 (23.2)	
3rd line mCRPC	9 (20.0)	167 (24.0)	176 (23.8)	

4th+ line mCRPC	26 (57.8)	270 (38.8)	296 (39.9)	
Practice type: No. (%)				0.51
Academic	5 (11.1)	102 (14.7)	107 (14.4)	
Community	40 (88.9)	594 (85.3)	634 (85.6)	
Prior NHT (yes vs. no): No. (%)	35 (77.8)	552 (79.3)	587 (79.2)	0.81
Prior Taxane (yes vs. no): No. (%)	33 (73.3)	374 (53.7)	407 (54.9)	0.01
Prior opioid use (recorded vs. no): No. (%)	25 (55.6)	402 (57.8)	427 (57.6)	0.77
Treatment received: No. (%)				<0.001
Atezolizumab	2 (4.4)	0 (0.0)	2 (0.3)	
Cabazitaxel	0 (0.0)	342 (49.1)	342 (46.2)	
Docetaxel	0 (0.0)	354 (50.9)	354 (47.8)	
Nivolumab	9 (20.0)	0 (0.0)	9 (1.2)	
Pembrolizumab	34 (75.6)	0 (0.0)	34 (4.6)	
Biopsy site: No. (%)				0.11
Liver	5 (11)	92 (13.2)	97 (13.1)	
Lymph node	2 (4.4)	120 (17.2)	122 (16.5)	
Other	14 (31.1)	199 (28.6)	213 (28.7)	
Prostate	24 (53.3)	285 (40.9)	309 (41.7)	

[0400] **FIG. 15** shows PSA response for patients evaluable for PSA response receiving single-agent taxane therapy and stratified by TMB less than 10 and TMB of at least 10. **FIG. 16** shows PSA response for patients evaluable for PSA response receiving single-agent anti-PD1 axis therapy. The majority of patients with TMB of at least 10 responded to single-agent anti-PD1 axis therapy, whereas a majority of patients with TMB less than 10 did not. Among patients with evaluable PSA response, no difference was observed on taxanes by TMB level. No patients had PSA decline 50% or more on ICPI if TMB was less than 10. 4 of 9 patients with TMB of at least 10 had PSA decline of 50% or more.

[0401] **FIGs. 17A-17D** show TTNT and OS stratified by TMB of less than 10 or of at least 10. Patients with TMB less than 10 receiving ICPI vs. taxanes had worse TTNT (median 2.4 vs. 4.1 months; HR: 2.7, 95%CI:1.7-4.0, p<0.001) and numerically worse OS (median 4.2 vs. 6.0 months, HR: 1.08; 95%CI:0.68-1.7, p=0.73). In contrast, for TMB of at least 10 ICPI vs. taxane use was associated with more favorable TTNT (median 8.0 vs. 2.4 months; HR: 0.37, 95% CI:0.15-0.87, p = 0.022) and OS (median 19.9 vs. 4.2 months; HR:0.23, 95% CI:0.10-0.57, p=0.0014). Among all 741 patients, 44 had TMB of at least 10, 22 had high microsatellite instability (MSI-H), and 20 had both. Treatment interactions with TMB of at least 10 (TTNT:p<0.001, OS:p=0.021) were stronger than MSI-H (TTNT:p=0.0038, OS:p=0.080).

[0402] In conclusion, these data show that ICPI is a viable alternative to taxane chemotherapy for patients with mCRPC who have a TMB of at least 10.

EXAMPLE 4A: CLINICAL AND GENOMIC CHARACTERISTICS OF PATIENTS WITH DURABLE BENEFIT FROM IMMUNE CHECKPOINT INHIBITORS (ICPI) IN ADVANCED NON-SMALL CELL LUNG CANCER (aNSCLC)

Materials and Methods

Patient Selection

[0403] Patients with confirmed diagnosis of advanced NSCLC who were included in a de-identified clinico-genomic database were selected for evaluation.

[0404] All patients underwent genomic testing using genomic profiling (CGP) assays. Additionally, some patients underwent a PD-L1 DAKO 22C3 or Ventana SP142 IHC Assay. De-identified clinical data originated from approximately 280 US cancer clinics (~800 sites of care). Retrospective longitudinal clinical data were derived from electronic health records (EHR), comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports, which were linked to genomic data derived testing by de-identified, deterministic matching (Singal G, Miller PG, Agarwala V et al. Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database. Jama 2019; 321: 1391-1399). Clinical data included demographics, clinical and laboratory features, timing of treatment exposure, and survival.

[0405] Patient were included in this study if they received a first line single-agent anti-PD1 agent (pembrolizuab) or anti-PD1 (pembrolizumab) + chemotherapy and had tumor mutational burden (TMB) assessed via a tissue specimen collected before start of first-line (1L) therapy. Patients must have additionally tested negative for *EGFR* mutations, and *ALK/ROS1/RET* rearrangements via comprehensive genomic profiling (CGP). Patients who were diagnosed with advanced NSCLC greater than 90 days prior to their first structured activity or who received their CGP report greater than 60 days after their last structured activity date were excluded to (a) ensure all therapies received prior to CGP were captured and (b) exclude patients who left the network prior to CGP. Before the study described in this Example began, the study protocol was approved by an Institutional Review Board.

Comprehensive Genomic Profiling

[0406] Comprehensive genomic profiling (CGP) was performed on hybridization-captured, adaptor ligation-based libraries using DNA and/or RNA extracted from FFPE tumors in a Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory. The samples were sequenced for up to 406 cancer related genes and select gene rearrangements (Frampton GM, Fichtenholtz A, Otto GA et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013; 31: 1023-1031). Tumor mutational burden (TMB) was determined on up to 1.24 Mb of sequenced DNA (Chalmers ZR, Connelly CF, Fabrizio D et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017; 9: 34).

DAKO PD-L1 IHC 22C3 Assay

[0407] DNA and/or RNA samples extracted from FFPE tumors of certain patients were also evaluated via PD-L1 DAKO 22C3 IHC assay or VENTANA SP142 assay, i.e., in parallel with CGP. The results of the DAKO 22C3 PD-L1 IHC and the VENTANA SP142 assays were interpreted according to manufacturer instructions for TPS and TC, respectively. Results from DAKO 22C3 PD-L1 IHC assays were interpreted using the DAKO tumor proportion scoring (TPS) method where tumor cell expression of PD-L1 was quantified. The DAKO TPS scoring method was defined as TPS = # PD-L1 positive tumor cells / (total # of PD-L1 positive + PD-L1 negative tumor cells) (DAKO. PD-L1 IHC 22C3 pharmDx Interpretation Manual – NSCLC). The Ventana SP142 assay also assessed the tumor cell score (TC) where percent of PD-L1 positive tumor cells / (total # of PD-L1 positive + PD-L1 negative tumor cells), similar to the DAKO assay.

Outcomes

[0408] Overall survival (OS) was calculated from start of treatment to death from any cause, and patients with no record of mortality were right censored at the date of last clinic visit or structured activity. Because patients were not entered into the database until a CGP report was delivered, OS risk intervals were left truncated to the date of CGP report to account for immortal time (McGough SF, Incerti D, Lyalina S et al. Penalized regression for left-truncated and right-censored survival data. Stat Med 2021; Brown S, Lavery JA, Shen R et al. Implications of Selection Bias Due to Delayed Study Entry in Clinical Genomic Studies. JAMA Oncology 2021). Database mortality information was a composite derived from 3 sources: documents within the electronic health records (EHR), Social Security Death Index, and a commercial death dataset mining data from obituaries and funeral homes. This mortality information was externally validated in comparison to the National Death Index with > 90% accuracy (Zhang Q, Gossai A, Monroe S et al. Validation analysis of a composite real-world mortality endpoint for patients with cancer in the United States. Health Services Research. 2021 Dec;56(6):1281-1287). Progression free survival (PFS) was calculated from start of treatment to the first progression date >14 days after treatment start or to death. Patients were censored at their last clinic note date if no progression or death was observed. Median PFS and OS values were estimated in months with 95% confidence intervals.

Statistical Analysis and Interpretations

[0409] Differences in time-to-event outcomes were assessed with the log-rank test and Cox proportional hazard (PH) models. Chi-square tests and Wilcoxon rank sum tests were used to assess differences between groups of categorical and continuous variables, respectively. P values were corrected for multiple tests. Multivariable Cox proportional hazard models were fitted on PFS and OS to estimate the adjusted hazard ratio and its significance. Features included in the Cox model were TMB status, PDL1 status, age at treatment start, Eastern Cooperative Oncology Group (ECOG) Performance Status score (0~1, 2+, and unknown), metastasis status, history of smoking, disease

histology, stage at initial diagnosis, and STK11 mutation status. For interaction analysis, the same multivariable Cox models were fitted with an interaction term between therapy class and biomarker.

[0410] Propensity analyses made use of inverse probability of treatment weights targeting the average treatment effect when comparing outcomes between biomarker positive vs negative cohorts., implemented with R package 'MatchIt'. Features used in propensity models were: ECOG (0~1, 2+, and unknown), age at therapy start, PD-L1 (TPS ≥1 vs. not), stage at diagnosis (stage IV vs. not), metastasis status, and history of smoking. Standardized mean difference (SMD) was utilized to assess balance, and within 10% considered acceptable (Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat Med 2015; 34: 3661-3679).

[0411] Predictive biomarker associations (*see* Ballman KV. Biomarker: Predictive or Prognostic? J Clin Oncol 2015; 33: 3968-3971) made use of inverse-propensity weighted multivariable Cox proportional hazards regression models containing at minimum the following variables: drug class (ICPI or ICPI + chemo), TMB (high vs. low) and the interaction term between drug class and biomarker. Models evaluating intra-patient treatment interactions in the Sequential Cohort were additionally clustered on the individual patient, making use of robust variances calculated by generalized estimating equations within a working independence structure. Hazard ratios were then generated from adjusted Cox models stratified by group (i.e. TMB high vs. low). R version 3.6.3 software was used for all statistical analyses.

[0412] Non-proportional hazards over time between treatment groups can limit the interpretability of the hazard ratio as an effect measure. For this reason, pre-specified methods of hazard ratio estimates were augmented with analyses of three-year restricted mean survival times (Liang F, Zhang S, Wang Q, Li W. Treatment effects measured by restricted mean survival time in trials of immune checkpoint inhibitors for cancer. Ann Oncol 2018; 29: 1320-1324; Pak K, Uno H, Kim DH et al. Interpretability of Cancer Clinical Trial Results Using Restricted Mean Survival Time as an Alternative to the Hazard Ratio. JAMA Oncol 2017; 3: 1692-1696).

Results

Patient cohort

[0413]

A total of 15149 unique NSCLC patients were in the clinico-genomic database cohort with 10139 patients with information regarding prior line(s) of therapy. *See* **FIG 18**. Of the 10139 patients, 2344 received immune checkpoint inhibitor (ICPI) monotherapy or immune checkpoint inhibitor therapy in combination with chemotherapy in the first line. Patients without PD-L1 immunohistochemistry results, with specimens collected after start of immune checkpoint inhibitor therapy, and with *EGFR* mutations or *ALK/ROS1/RET* rearrangements were excluded. A total of 1722 patients remained in the analysis. Of these 1722 patients, 630 received ICPI monotherapy and

1092 received ICPI therapy + chemotherapy. When comparing patient characteristics between the ICPI monotherapy vs the ICPI therapy + chemotherapy group, it was observed that patients in the ICPI monotherapy group were predominantly, older, smokers, females, had a higher ECOG scores, were diagnosed at an earlier stage, had higher TMB scores, and had PD-L1 tumor proportion score (TPS)/PD-L1 staining tumor cells (TC) >50% as compared to the patients in the ICPI therapy + chemotherapy group *See* ***Tables 8A*** and ***8B***.

Table 8A. Patient characteristics of the primary cohort - subgroups for TMB

	Total (N=1722)	**ICPI (N=630)**	**Chemo +ICPI (N=1092)**	**p value**
Age at Advanced Dx				< 0.001
Median (Q1, Q3)	70.0 [62.0;76.0]	72.0 [65.0;78.0]	69.0 [62.0;74.0]	
Gender:				<0.001
F	819 (47.6%)	340 (54.0%)	479 (43.9%)	
M	903 (52.4%)	290 (46.0%)	613 (56.1%)	
Race:				0.120
White	1190 (69.1%)	455 (72.2%)	735 (67.3%)	
Black or African American	129 (7.49%)	39 (6.19%)	90 (8.24%)	
Asian	17 (0.99%)	4 (0.63%)	13 (1.19%)	
Others	386 (22.4%)	132 (21.0%)	254 (23.3%)	
Practice Type:				0.274
ACADEMIC	103 (5.98%)	32 (5.08%)	71 (6.50%)	
COMMUNITY	1619 (94.0%)	598 (94.9%)	1021 (93.5%)	
Histology:				0.054
Non-squamous cell carcinoma	1293 (75.1%)	455 (72.2%)	838 (76.7%)	
NSCLC histology NOS	75 (4.36%)	26 (4.13%)	49 (4.49%)	
Squamous cell carcinoma	354 (20.6%)	149 (23.7%)	205 (18.8%)	
Stage At Dx:				<0.001
I	165 (9.58%)	87 (13.8%)	78 (7.14%)	
II	97 (5.63%)	50 (7.94%)	47 (4.30%)	
III	208 (12.1%)	101 (16.0%)	107 (9.80%)	
IV	1227 (71.3%)	380 (60.3%)	847 (77.6%)	
Unkown	25 (1.45%)	12 (1.90%)	13 (1.19%)	
History of Smoking:				0.009
History of smoking	1625 (94.4%)	607 (96.3%)	1018 (93.2%)	
No history of smoking	97 (5.63%)	23 (3.65%)	74 (6.78%)	
ECOG:				0.023
0~1	1175 (68.2%)	406 (64.4%)	769 (70.4%)	
2+	388 (22.5%)	164 (26.0%)	224 (20.5%)	
Unknown	159 (9.23%)	60 (9.52%)	99 (9.07%)	
TMB:				<0.001
<10	1014 (58.9%)	329 (52.2%)	685 (62.7%)	
10~19	505 (29.3%)	203 (32.2%)	302 (27.7%)	
>=20	203(11.8%)	98(15.6%)	105(9.62%)	
PDL1:				<0.001
Negative	508 (29.5%)	63 (10.0%)	445 (40.8%)	
1~49%	493 (28.6%)	98 (15.6%)	395 (36.2%)	
>=50%	721 (41.9%)	469 (74.4%)	252 (23.1%)	
MSI:				0.317
MSI-H	3 (0.17%)	2 (0.32%)	1 (0.09%)	
MSI-I	1 (0.06%)	1 (0.16%)	0 (0.00%)	
MSS	1526 (88.6%)	554 (87.9%)	972 (89.0%)	
Unknown	192 (11.1%)	73 (11.6%)	119 (10.9%)	

Table 8B. Patient characteristics of the primary cohort

	Total (N=1722)	TMB < 10 (N = 1014)	TMB 10~ 19 (N = 505)	TMB >=20 (N=203)	p value
Age at Advanced Dx					< 0.001
Median (Q1, Q3)	70.0 [62.0;76.0]	71.0 [63.0;77.0]	69.0 [62.0;76.0]	67.0 [59.0;73.0]	
Gender:					0.485
F	819 (47.6%)	478 (47.1%)	250 (49.5%)	91 (44.8%)	
M	903 (52.4%)	536 (52.9%)	255 (50.5%)	112 (55.2%)	
Race:					NA
White	1190 (69.1%)	720 (71.0%)	340 (67.3%)	130 (64.0%)	
Black or African American	129 (7.49%)	69 (6.80%)	41 (8.12%)	19 (9.36%)	
Asian	17 (0.99%)	13 (1.28%)	3 (0.59%)	1 (0.49%)	
Others	386 (22.4%)	212 (20.9%)	121 (24.0%)	53 (26.1%)	
Practice Type:					0.412
ACADEMIC	103 (5.98%)	67 (6.61%)	25 (4.95%)	11 (5.42%)	
COMMUNITY	1619 (94.0%)	947 (93.4%)	480 (95.0%)	192 (94.6%)	
Histology:					0.001
Non-squamous cell carcinoma	1293 (75.1%)	766 (75.5%)	362 (71.7%)	165 (81.3%)	
NSCLC histology NOS	75 (4.36%)	41 (4.04%)	19 (3.76%)	15 (7.39%)	
Squamous cell carcinoma	354 (20.6%)	207 (20.4%)	124 (24.6%)	23 (11.3%)	
Stage At Dx:					NA
I	165 (9.58%)	94 (9.27%)	49 (9.70%)	22 (10.8%)	
II	97 (5.63%)	62 (6.11%)	26 (5.15%)	9 (4.43%)	
III	208 (12.1%)	105 (10.4%)	74 (14.7%)	29 (14.3%)	
IV	1227 (71.3%)	740 (73.0%)	347 (68.7%)	140 (69.0%)	
Unkown	25 (1.45%)	13 (1.28%)	9 (1.78%)	3 (1.48%)	
History of Smoking:					<0.001
History of smoking	1625 (94.4%)	927 (91.4%)	498 (98.6%)	200 (98.5%)	
No history of smoking	97 (5.63%)	87 (8.58%)	7 (1.39%)	3 (1.48%)	
ECOG:					0.282
0~1	1175 (68.2%)	707 (69.7%)	329 (65.1%)	139 (68.5%)	
2+	388 (22.5%)	214 (21.1%)	131 (25.9%)	43 (21.2%)	
Unknown	159 (9.23%)	93 (9.17%)	45 (8.91%)	21 (10.3%)	
Therapy:					<0.001
Immune checkpoint inhibitor monotherapy (ICPI)	630 (36.6%)	329 (32.4%)	203 (40.2%)	98 (48.3%)	
ICPI + chemotherapy	1092 (63.4%)	685 (67.6%)	302 (59.8%)	105 (51.7%)	
PDL1:					0.150
Negative	508 (29.5%)	314 (31.0%)	143 (28.3%)	51 (25.1%)	
1~49%	493 (28.6%)	300 (29.6%)	136 (26.9%)	57 (28.1%)	

	Total (N=1722)	TMB < 10 (N = 1014)	TMB 10~ 19 (N = 505)	TMB >=20 (N=203)	p value
>=50%	721 (41.9%)	400 (39.4%)	226 (44.8%)	95 (46.8%)	
MSI:					<0.001
MSI-H	3 (0.17%)	0 (0.00%)	1 (0.20%)	2 (0.99%)	
MSI-I	1 (0.06%)	0 (0.00%)	0 (0.00%)	1 (0.49%)	
MSS	1526 (88.6%)	854 (84.2%)	478 (94.7%)	194 (95.6%)	
Unknown	192 (11.1%)	160 (15.8%)	26 (5.15%)	6 (2.96%)	

[0414] **FIGs 19A** and **19B** provide adjusted Kaplan-Meier plots of real-world progression free survival (rwPFS) and **FIGs 19C** and **19D** provide real-world overall survival (rwOS) for patients who were treated with ICPI monotherapy (**FIGs 19A** and **19C**) or ICPI therapy + chemotherapy (**FIGs 19B** and **19D**). Outcomes were stratified by TMB<10 and TMB>= 10, with a TMB of 10 being the cutoff for high TMB. To adjust for imbalance, full matching was performed between TMB <10 vs TMB >=10 cohorts. Variables included for matching: age at therapy start, ECOG PS (0~1, 2+, and unknown), metastasis, history of smoking, stage at diagnosis. The results shown in **FIGs 19A-D** suggest that tumor mutation burden is prognostic for ICPI-containing regimens. **FIGs 20A** and **20B** provide Kaplan-Meier plots of rwPFS and **FIGs 20C** and **20D** provide rwOS for patients who were treated with ICPI monotherapy (**FIGs 20A** and **20C**) or ICPI therapy + chemotherapy (**FIGs 20B** and **20D**). Outcomes were stratified by TMB<10, TMB 10~19, and TMB>= 20. The results shown in **FIGs 20A-D** suggest that tumor mutation burden is prognostic for ICPI-containing regimens in first line.

[0415] **FIG 21A** provides a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<10 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<10 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=10 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=10 (i.e., PDL1+ / TMB+). **FIG 21B** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwPFS. **FIG 21C** provides a Kaplan-Meier plot of rwOS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<10 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<10 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=10 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=10 (i.e., PDL1+ / TMB+). **FIG 21D** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwOS. **FIG 22A** provides a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<20 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<20 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=20 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=20 (i.e., PDL1+ / TMB+). **FIG 22B** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwPFS. **FIG 22C** provides a Kaplan-Meier plot of rwOS for patients who were treated

with ICPI-containing regimens. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<20 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<20 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=20 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=20 (i.e., PDL1+ / TMB+). **FIG 22D** provides results from multivariate CoxPh model detecting associations between clinical or genomic features and rwOS. The results of FIGS **21A-D** and **22A-D** suggest that TMB and PD-L1 expression are independent markers for ICPI outcomes.

[0416] **FIG 23A** shows the point estimation and 95% confidence interval of HR (hazard ratio) for biomarker and treatment interaction are shown for different TMB or PDL1 cutoffs for rwPFS. **FIG 23B** shows the point estimation and 95% confidence interval of HR (hazard ratio) for biomarker and treatment interaction are shown for different TMB or PDL1 cutoffs for rwOS. **FIG 23C** provides a Kaplan-Meier plot of rwPFS for patients with PD-L1 scores between 1-49% who were treated with ICPI monotherapy or ICPI therapy + chemotherapy. **FIG 23D** provides a Kaplan-Meier plot of rwPFS for patients with PD-L1 scores >=50% who were treated with ICPI monotherapy or ICPI therapy + chemotherapy. The results shown in FIGs **23A-23D** suggest that PD-L1 is a predictive biomarker for ICPI monotherapy vs. ICPI therapy + chemotherapy benefit.

[0417] **FIG 24** shows the top 30 altered genes in the ICPI monotherapy cohort (-) and ICPI therapy + chemotherapy cohort (+). Multi: multiple alterations in the indicated gene; RE: rearrangement; CN: copy number change; SV: short variant mutation (base substitution or insertion/deletion).

[0418] **FIG 25A** shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI monotherapy. **FIG 25B** shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI therapy + chemotherapy. **FIG 25C** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI monotherapy. **FIG 25D** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI therapy + chemotherapy. In **FIGs 25A-25D**, outcomes were stratified by PDL1 TPS < 1% and PDL1 TPS >= 1%. To adjust for imbalance, full matching was performed between PDL1 <1% vs PDL1 >=1% cohorts. Variables included for matching: age at therapy start, ECOG PS (0~1, 2+, and unknown), metastasis, history of smoking, stage at diagnosis. **FIG 26A** shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI monotherapy. **FIG 26B** shows an Adjusted Kaplan-Meier plot of rwPFS for patients who were treated with ICPI therapy + chemotherapy. **FIG 26C** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI monotherapy. **FIG 26D** shows an Adjusted Kaplan-Meier plot of rwOS for patients who were treated with ICPI therapy + chemotherapy. In **FIGs 26A-26D**, outcomes were stratified by PDL1 TC <50% and PDL1 TC >= 50%. To adjust for imbalance, full matching was performed between PDL1 TC <50 % vs PDL1 TC >= 50% cohorts. Variables included for matching: age at therapy start, ECOG PS (0~1, 2+, and unknown), metastasis, history of smoking, stage at diagnosis. The results shown in **FIGs 25A-D** and **26A-D** suggest that PDL1 is prognostic for ICPI-containing treatment regimens.

[0419] **FIG 27** provides box plots of TMB levels in different PDL1 subgroups to show association between PDL1 expression and TMB. Kruskal-Wallis test p = 0.007, effect size: 0.0046. ***Tables 9A*** and ***9B*** show the Likelihood-Ratio tests and significances to compare the nested models.

Table 9A.

Factors in Model (PFS)	Log likelihood	Likelihood ratio x2	DF of X2	P- Value
covariates*	-5728.3			
Covariates + PDL1	-5722.9	10.791	1	0.00102 **
Covariates + PDL1 + TMB	-5716.4	12.948	1	0.0003202 ***
Covariates + PDL1 + TMB + PDL1*TMB	-5716.3	0.2793	1	0.5971
Covariates + PDL1 + TMB + STK11	-5699.9	33.061	1	8.931 e-09 ***
Covariates + PDL1 + TMB + STK11 + TP53	-5699.9	0.0019	1	0.9649

Table 9B.

Factors in Model (OS)	Log likelihood	Likelihood ratio x2	DF of X2	P- Value
covariates*	-5800.0			
Covariates + PDL1	-5797.2	5.6927	1	0.01704 *
Covariates + PDL1 + TMB	-5787.4	19.42	1	1.049e-05 ***
Covariates + PDL1 + TMB + PDL1*TMB	-57185.8	3.3656	1	0.06657
Covariates + PDL1 + TMB + STK11	-5776.5	21.808	1	3.014 e-06 ***
Covariates + PDL1 + TMB + STK11 + TP53	-5773.4	0.0019	1	0.1164

[0420] **FIG 28A** shows a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI monotherapy. **FIG 28B** shows a Kaplan-Meier plot of rwPFS for patients who were treated with ICPI therapy + chemotherapy. **FIG 28C** shows a Kaplan-Meier plot of rwOS for patients who were treated with ICPI monotherapy. **FIG 28D** shows a Kaplan-Meier plot of rwOS for patients who were treated with ICPI therapy + chemotherapy. Outcomes were stratified by TMB and PDL1 levels: PDL1<1% and TMB<10 (i.e., PDL1- / TMB-), PDL1 > 1% and TMB<10 (i.e., PDL1+ / TMB-), PDL1<1% and TMB>=10 (i.e., PDL1- / TBM +), and PDL1>1% and TMB>=10 (i.e., PDL1+ / TMB+).

[0421] The results shown in **FIGs 27** and **28A-28D** and ***Tables 9A*** and ***9B*** suggest that TMB and PD-L1 expression are independent markers for ICPI outcomes.

Discussion

[0422] In this study, it was shown that TMB and PD-L1 immunohistochemistry (IHC) are independent biomarkers for first line NSCLC patients treated with ICPI-containing regimens (e.g., ICPI monotherapy or ICPI therapy + chemotherapy). Specifically, both the single biomarker positive (TMB-High/PD-L1^{neg} and TMB-Low/PD-L1^{pos}) groups had higher rwPFS than the double biomarker negative group (TMB-Low/PD-L1^{neg}). A significantly higher median rwOS (20.1 months) was observed among TMB-High/PD-L1^{pos} when compared to the 10.5-12 months survival in the other three groups (i.e., TMB-Low/PD-L1^{pos}, TMB-Low/PD-L1^{neg}, and TMB-High/PD-L1^{neg}). In sum, in addition to TMB and PD-L1 IHC being independent biomarkers to predict outcomes to ICPI containing regimens, the combined positivity of both biomarkers predicts the strongest response to ICPI containing regimens. The data here suggest that in addition to PD-L1 IHC, TMB testing should also be performed for 1L NSCLC patients.

[0423] As an independent biomarker, TMB-High was highly prognostic for 1L NSCLC in both the ICPI monotherapy and ICPI therapy + chemotherapy groups. This was exemplified by almost doubling of both rwPFS and rwOS in the TMB-High group when compared to the TMB-Low group in the ICPI monotherapy cohort. The same trends were seen in the ICPI therapy + chemotherapy group where a median rwPFS of 10 months was observed in TMB-High group, as opposed to 6.8 months in the TMB-Low group; and a less pronounced but significant difference in rwOS. While TMB was approved as a companion diagnostic in patients that have progressed following previously treatment, emerging real-world evidence has been shown in urothelial carcinoma that TMB has predictive value in the first line setting. In totality, these real-world data suggest that TMB is a highly prognostic biomarker for ICPI-containing regimens in multiple tumor types. PD-L1 is also an independent prognostic biomarker for first line NSCLC at both the TPS $\geq$1 and TPS $\geq$50 cut-off (Pak K, Uno H, Kim DH et al. Interpretability of Cancer Clinical Trial Results Using Restricted Mean Survival Time as an Alternative to the Hazard Ratio. JAMA Oncol 2017; 3: 1692-1696).

[0424] While the most widely used TMB cut-off is currently 10 muts/Mb, a higher cut-off at TMB $\geq$20 muts/Mb can help select patient who are exceptional responders to ICPI. In this Example, it was seen that both in the ICPI monotherapy and ICPI therapy + chemotherapy groups, patients with TMB $\geq$20 responded exceedingly well to therapy. Specially, in the rwOS of the ICPI monotherapy group, the TMB $\geq$20 cohort (median 33.7months) had more than 3 times the survival when compared to the <10 mut/Mb group (median 9.4 months). This same trend was observed when the TMB and PD-L1 defined groups were examined. rwOS in the double positive (PD-L1^{pos}/TMB^{pos}) group had an median rwOS of 33.7 months when compared to the double negative (PD-L1^{neg}/TMB^{neg}) group which had rwOS of 11.2 months. Lastly, patients with durable benefit >2 years after starting ICPI therapy in NSCLC represent a unique population of immune survivors with a median OS of almost 5 years; 41% of patients stopped ICPI before the 2-year mark. Based on these data, further investigation of TMB at

higher cut-offs such as 20 muts/Mb as a biomarker for prolonged benefit to ICPI in NSCLC is warranted.

[0425] The predictive value of TMB and PD-L1 for ICPI monotherapy vs ICPI therapy + chemotherapy was assessed. In this cohort, it was not observed that TMB (at a cut-off of 10 and 20 muts/MB) was able to predict a more favorable rwPFS or rwOS in ICPI monotherapy vs ICPI therapy + chemotherapy. However, patients with PD-L1 IHC at a TPS of 1-49 had a significantly higher rwPFS in the ICPI therapy + chemotherapy when compared to the ICPI monotherapy group (median rwPFS: 7.3months vs 2.9 months) suggesting that PD-L1 IHC could potentially be a biomarker to help guide decision on ICPI monotherapy vs ICPI therapy + chemotherapy. This same trend was seen in the rwOS and is consistent with a previous FDA pooled analysis (Akinboro O, Vallejo JJ, Mishra-Kalyani PS et al. Outcomes of anti-PD-(L1) therapy in combination with chemotherapy versus immunotherapy (IO) alone for first-line (1L) treatment of advanced non-small cell lung cancer (NSCLC) with PD-L1 score 1-49%: FDA pooled analysis. Journal of Clinical Oncology 2021; 39: 9001-9001). Overall, this data suggests that future clinical trials should be performed to better assess the predictive power of PD-L1 in this context.

[0426] ***Conclusions:*** In this Example, a large real-world cohort of patients was used to show that TMB adds additional prognostics value to 1L NSCLC patients in addition to PD-L1 IHC. Such results suggests that these patients should be tested for both TMB and PD-L1 IHC. In addition, real world evidence that PD-L1 could help predict whether to treat 1L NSCLC patients with ICPI monotherapy vs. ICPI therapy + chemotherapy was presented.

EXAMPLE 4B: CLINICAL AND GENOMIC CHARACTERISTICS OF PATIENTS WITH DURABLE BENEFIT FROM IMMUNE CHECKPOINT INHIBITORS (ICPI) IN ADVANCED NON-SMALL CELL LUNG CANCER (aNSCLC)

[0427] ***Background:*** The 2-year mark has become a new milestone in patients with aNSCLC receiving immunotherapy. In patients who are progression free at that point, a subset experience ongoing disease control even after stopping active treatment. Some patients experience such impressive durability beyond 2 years, raising a question about potential cure. A real-world ("rw") clinico-genomic database (CGDB) was queried to better understand these patients with durable benefit and their clinical and genomic features.

[0428] ***Methods:*** Using a nationwide (~280 US cancer clinics) de-identified electronic health record (EHR)-derived clinicogenomic database (CGDB) linked to genomic data, patients treated with immune checkpoint inhibitor therapy (ICPI), either as monotherapy or in combination with chemotherapy, were selected. RW progression (rwP) was obtained via technology-enabled abstraction of EHR data. Durable benefit was classified as absence of rwP, death or treatment failure (indicated by switch to a new line of therapy) within 24 months of beginning ICPI therapy.

[0429] ***Results:*** In a cohort of 4,030 evaluable aNSCLC patients, 184 (4.6%) were free of rwP or treatment failure at 24 mos. Of these 184 patients with durable benefit, 84% received ICPI

monotherapy and 16% received ICPI with chemotherapy; ICPI treatment was more often first line (1L, 43%) or 2L (38%). 59% with durable benefit were still on ICPI at the 2-year mark, whereas 41% had stopped a median of 11.4 months after therapy start. Of 109 patients remaining on ICPI for 2-years, median time on ICPI was 36.3 months from therapy start. Overall, patients with durable benefit had a median real-world progression-free survival (rwPFS) of 37.1 months and median real world overall survival (rwOS) of 58.8 months from start of ICPI therapy. Compared to patients with rwP on ICPI therapy before 24 months, those with durable benefit were more likely to have history of smoking (94% vs 86%) and absence of liver, brain or bone metastases (all $p<0.001$). High tumor mutational burden (TMB $\geq$10) was more common (62% vs 35%, $p<0.001$) and *STK11, CDKN2B, PIK3CA,* and *EGFR* alterations were less common in patients with durable-benefit vs those with rwP on ICPI therapy before 24 mos. In a multivariate cox model of rwPFS beyond 24 months in patients with durable benefit, TMB $\geq$20 was significantly associated with longer rwPFS (HR 0.45 95% CI 0.24-0.83, $p=0.01$) while TMB $\geq$10 was marginally significant (HR 0.65 95% CI 0.40-1.03, $p=0.07$); treatment with ICPI with chemotherapy was significantly associated with worse rwPFS (HR 1.84 95% 1.093.12, $p=0.02$). High PD-L1 >50% was noted in 728 (19%) of those without durable benefit and 38 (21%) of those with durable benefit, though many in the data set had unknown PD-L1 status.

[0430] ***Conclusion:*** Patients with durable benefit >2 years after starting ICPI therapy for aNSCLC represent a unique population of immune survivors with a median OS of almost 5 years; 41% of patients stopped ICPI before the 2-year mark. Elevated TMB was associated with durable benefit on ICPI as well as prolonged rwPFS after the 2-year mark and deserves further investigation as a biomarker for prolonged benefit to ICPI in aNSCLC.

EXAMPLE 5: TUMOR MUTATIONAL BURDEN (TMB) MEASUREMENT FROM CGP ASSAY AND REAL-WORLD OVERALL SURVIVAL ON SINGLE-AGENT IMMUNE CHECKPOINT INHIBITORS (ICPI).

[0431] ***Background:*** The applicability and reliability of TMB across cancer types and assays from different manufacturers. This Example investigated TMB 10 mut/Mb or higher as a pan-solid tumor companion diagnostic for single-agent pembrolizumab in 2nd or further lines of therapy. Using a large real-world dataset with well-validated outcome measures, the clinical validity of the TMB measurement by a CGP in over 8,000 patients across 24 cancer types who received single agent ICPI.

[0432] ***Methods:*** Using a nationwide (~280 US cancer clinics) de-identified electronic health record (EHR)-derived clinicogenomic database (CGDB) linked to genomic data, patients treated with immune checkpoint inhibitor therapy (ICPI), either as monotherapy **(Table 10)** or in combination with chemotherapy, were selected. For patients with advanced or metastatic cancers, lines of therapy were eligible if they received ICPI, had evaluable tissue-assessed TMB, met the 90-day gap rule, and the line of therapy was not entirely immortal time. The pan-tumor cohort included patients from 24 cancer types of interest with advanced/metastatic disease treated with single-agent anti-PD(L)1 therapy in the FH network between November 1, 2021 -September 30, 2022. This Example used the

TMB algorithm from a CGP assay supporting pan-tumor companion diagnostics and survival measure validated against the national death index.

Table 10: Summary of ICPI monotherapy cohort

	TMB < 10 (N=5411)	TMB 10+ (N=3029)	Total (N=8440)	p value
Disease Area				< 0.001
Nsclc	2095 (38.7%)	1483 (49.0%)	3578 (42.4%)	
Anal	60 (1.1%)	16 (0.5%)	76 (0.9%)	
Breast	164 (3.0%)	64 (2.1%)	228 (2.7%)	
Cervical	72 (1.3%)	30 (1.0%)	102 (1.2%)	
Cholangio	56 (1.0%)	14 (0.5%)	70 (0.8%)	
Crc	189 (3.5%)	214 (7.1%)	403 (4.8%)	
Cup	62 (1.1%)	44 (1.5%)	106 (1.3%)	
Endometrial	110 (2.0%)	116 (3.8%)	226 (2.7%)	
Gastric	561 (10.4%)	91 (3.0%)	652 (7.7%)	
Gbm	32 (0.6%)	11 (0.4%)	43 (0.5%)	
Hcc	77 (1.4%)	1 (0.0%)	78 (0.9%)	
Hnc	337 (6.2%)	59 (1.9%)	396 (4.7%)	
Lnec	26 (0.5%)	16 (0.5%)	42 (0.5%)	
Melanoma	147 (2.7%)	381 (12.6%)	528 (6.3%)	
Merkel	33 (0.6%)	18 (0.6%)	51 (0.6%)	
Ovarian	112 (2.1%)	18 (0.6%)	130 (1.5%)	
Pancreatic	76 (1.4%)	14 (0.5%)	90 (1.1%)	
Prostate	50 (0.9%)	27 (0.9%)	77 (0.9%)	
Rcc	296 (5.5%)	7 (0.2%)	303 (3.6%)	
Sarcoma	100 (1.8%)	14 (0.5%)	114 (1.4%)	
Sclc	69 (1.3%)	39 (1.3%)	108 (1.3%)	
Skin	6 (0.1%)	8 (0.3%)	14 (0.2%)	
Smallint	14 (0.3%)	10 (0.3%)	24 (0.3%)	
Urothelial	667 (12.3%)	334 (11.0%)	1001 (11.9%)	

Age at Treatment Initiation				< 0.001
Median (Q1, Q3)	68.0 (60.0, 75.0)	69.0 (61.0, 76.0)	69.0 (61.0, 76.0)	
Practice Type				0.017
Academic	659 (12.2%)	338 (11.2%)	997 (11.8%)	
Academic/Community	68 (1.3%)	21 (0.7%)	89 (1.1%)	
Community	4684 (86.6%)	2670 (88.1%)	7354 (87.1%)	
EHR Abstracted Race				0.099
White	3791 (70.1%)	2129 (70.3%)	5920 (70.1%)	
Asian	123 (2.3%)	44 (1.5%)	167 (2.0%)	
Black or African American	315 (5.8%)	178 (5.9%)	493 (5.8%)	
Other Race	844 (15.6%)	470 (15.5%)	1314 (15.6%)	
Unknown/not documented	338 (6.2%)	208 (6.9%)	546 (6.5%)	
Genetic Ancestry				< 0.001
AFR	397 (7.3%)	232 (7.7%)	629 (7.5%)	
AMR	327 (6.0%)	112 (3.7%)	439 (5.2%)	
EAS	144 (2.7%)	44 (1.5%)	188 (2.2%)	
EUR	4497 (83.1%)	2626 (86.7%)	7123 (84.4%)	
SAS	46 (0.9%)	15 (0.5%)	61 (0.7%)	
Socioeconomic Status				0.164
1 - Lowest SES	642 (11.9%)	383 (12.6%)	1025 (12.1%)	
2	798 (14.7%)	496 (16.4%)	1294 (15.3%)	
3	1005 (18.6%)	561 (18.5%)	1566 (18.6%)	
4	1065 (19.7%)	599 (19.8%)	1664 (19.7%)	
5 - Highest SES	837 (15.5%)	443 (14.6%)	1280 (15.2%)	

Unknown	1064 (19.7%)	547 (18.1%)	1611 (19.1%)	
ECOG				0.162
0	1307 (24.2%)	788 (26.0%)	2095 (24.8%)	
1	2367 (43.7%)	1293 (42.7%)	3660 (43.4%)	
2+	1057 (19.5%)	600 (19.8%)	1657 (19.6%)	
Unknown	680 (12.6%)	348 (11.5%)	1028 (12.2%)	
Line of Therapy				< 0.001
1st	1906 (35.2%)	1521 (50.2%)	3427 (40.6%)	
2nd	1949 (36.0%)	1028 (33.9%)	2977 (35.3%)	
3rd+	1556 (28.8%)	480 (15.8%)	2036 (24.1%)	
Sex				0.588
Female	2466 (45.6%)	1399 (46.2%)	3865 (45.8%)	
Male	2945 (54.4%)	1630 (53.8%)	4575 (54.2%)	
Opioid Rx Pre-Therapy				< 0.001
No	3487 (64.4%)	2098 (69.3%)	5585 (66.2%)	
Yes	1924 (35.6%)	931 (30.7%)	2855 (33.8%)	
MSI by NGS				< 0.001
MSS	5156 (95.3%)	2440 (80.6%)	7596 (90.0%)	
MSI-H	16 (0.3%)	459 (15.2%)	475 (5.6%)	
MSI-L	122 (2.3%)	77 (2.5%)	199 (2.4%)	
Unknown	117 (2.2%)	53 (1.7%)	170 (2.0%)	

[0433] ***Results:*** 8,440 patients from 24 cancer types met inclusion criteria for the pan-tumor cohort. Briefly, patients with at least one line of therapy in the advanced/metastatic setting of one of the diseases of interest were identified. Entries where no advanced/metastatic diagnosis in one of the diseases of interest and/or no lines of therapy in the advanced/metastatic setting were filtered out. Of the remaining entries, those with no TMB score, had a potential EHR gap and/or not treated with ICPI

were also eliminated from analysis. Patients that received ICPI as monotherapy or combination therapy were identified, and those who received anti-PD1 or anti-PDL1 ICPI monotherapy were selected for further analysis. The hazard ratio for time to next treatment {HR, (95% CI)} across the entire cohort, relative to TMB < 5, was 0.90 (0.85 – 0.96) for TMB 5-10, 0.72 (0.67 – 0.77) for TMB 10-20, and 0.51 (0.46 – 0.56) for TMB 20+ (**FIG. 29A**). Relative hazards of death by TMB level were assessed using Cox PH models adjusted for ECOG PS, prior treatment, sex, age, opioid rx pre-therapy, genetic ancestry, and socioeconomic assessment. Pan-tumor models were additionally adjusted for MSI status, with baseline hazards stratified by cancer type. Relative hazards of death {HR, (95% CI)} across the entire cohort, relative to TMB < 5, was 0.95 (0.89 – 1.02) for TMB 5-10, 0.79 (0.73 – 0.85) for TMB 10-20, and 0.52 (0.47 – 0.58) for TMB 20+ (**FIG. 29B**). Adjusted Cox models comparing TMB ≥ 10 vs. TMB < 10 were pre-specified for cancer types with at least 15 death events in each group. Compared to patients with a TMB lower than 10, patients with a TMB ≥ 10 showed improved time to next treatment (TTNT) and overall survival (OS) when treated with single agent ICPI in multiple cancer types (**FIGs. 30A-B**).

[0434] The cohorts were further analyzed based on TMB score for those cancers exhibiting MSS status (**FIGs. 31A-B**). For certain cancer types such as NSCLC, urothelial, gastric, head and neck cancer, melanoma, and cervical cancer, patients with TMB ≥ 10 and designated as MSS demonstrated an improved hazard ratio for TTNT when treated with single agent ICPI (**FIG. 31A**). For certain cancer types such as NSCLC, urothelial, head and neck cancer, and melanoma, patients with TMB ≥ 10 and designated as MSS demonstrated an improved hazard ratio for OS when treated with single agent ICPI (**FIG. 31B**).

[0435] ***Conclusions:*** Across a heterogenous cohort, and within individual cancer types with sufficient power, elevated TMB using the TMB measurement from the FDA-approved test was associated with more favorable survival on ICPI monotherapy than for similar patients with lower TMB levels. Similarly, elevated TMB using the TMB measurement from the FDA-approved test associated with more favorable time to next treatment for patients on ICPI monotherapy than for similar patients with lower TMB levels. For patients with MSS tumors, patients with (a) elevated TMB scores and (b) that were given ICPI monotherapy were also associated with more favorable survival and time to next treatment outcomes compared to the patients with (a) low TMB scores and (b) that were given ICPI monotherapy.

EXAMPLE 6: TUMOR MUTATIONAL BURDEN (TMB) MEASUREMENT FROM AN FDA-APPROVED ASSAY AND REAL-WORLD OVERALL SURVIVAL ON COMBINATION IMMUNE CHECKPOINT INHIBITOR THERAPY (ICPI).

[0436] 8,440 patients from 24 cancer types met inclusion criteria for the pan-tumor cohort, patients with at least one line of therapy in the advanced/metastatic setting of one of the diseases of interest were identified. Entries where no advanced/metastatic diagnosis in one of the diseases of interest

and/or no lines of therapy in the advanced/metastatic setting were filtered out. Of the remaining entries, those with no TMB score, had a potential EHR gap and/or not treated with ICPI were also eliminated from analysis. Patients that received ICPI as monotherapy were filtered out, and patients that received ICPI combination therapy were identified.

[0437] The hazard ratios {HR, (95% CI)} for time to next treatment across the entire cohort, relative to TMB < 5, was 0.92 (0.86 – 0.99) for TMB 5-10, 0.77 (0.71 – 0.84) for TMB 10-20, and 0.59 (0.52 – 0.67) for TMB 20+ (**FIG. 32A**).

[0438] The hazard ratios {HR, (95% CI)} for overall survival across the entire cohort, relative to TMB < 5, was 1.05 (0.97 – 1.13) for TMB 5-10, 0.91 (0.83 – 1.01) for TMB 10-20, and 0.68 (0.60 – 0.78) for TMB 20+ (**FIG. 32B**).

[0439] ***Conclusions:*** Across a heterogenous cohort, elevated TMB using the TMB measurement from the FDA-approved test was associated with more favorable survival for patients on combination ICPI than for similar patients with lower TMB levels. Similarly, elevated TMB using the TMB measurement from the FDA-approved test associated with more favorable time to next treatment for patients on ICPI than for similar patients with lower TMB levels.

CLAIMS

What is claimed is:

1. A method for treating an individual having a cancer, the method comprising:
   (a) determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual; and
   (b) treating the individual with an immune checkpoint inhibitor therapy if the TMB score is at least a threshold TMB score;
   wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

2. The method of claim 1, further comprising assessing microsatellite instability, wherein (b) is further based on the cancer being microsatellite instability-high (MSI-H), wherein microsatellite instability is assessed by next generation sequencing (NGS).

3. The method of claim 1, wherein the threshold TMB score is about 8 mutations/Mb, about 9 mutations/Mb, about 10 mutations/Mb, about 11 mutations/Mb, about 12 mutations/Mb, about 13 mutations/Mb, about 14 mutations/Mb, about 15 mutations/Mb, about 16 mutations/Mb, about 17 mutations/Mb, about 18 mutations/Mb, about 19 mutations/Mb, or about 20 mutations/Mb.

4. The method of claim 1, wherein the TMB score is determined based on between about 100 kb to about 10 Mb of sequenced DNA.

5. The method of claim 1, wherein the TMB score is determined based on between about 0.8 Mb to about 1.1 Mb of sequenced DNA.

6. The method claim 1, further comprising treating the individual with an immune checkpoint inhibitor if the TMB score is at least the threshold TMB score.

7. The method of claim 1, wherein the immune checkpoint inhibitor comprises a small molecule inhibitor, an antibody, a nucleic acid, an antibody-drug conjugate, a recombinant protein, a fusion protein, a natural compound, a peptide, a PROteolysis-TArgeting Chimera (PROTAC), a cellular therapy, a treatment for cancer being tested in a clinical trial, an immunotherapy, or any combination thereof.

8. The method of claim 1, wherein the immune checkpoint inhibitor is a PD-1 inhibitor, and the PD-1 inhibitor comprises one or more of nivolumab, pembrolizumab, cemiplimab, or dostarlimab.

9. The method of claim 1, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor, and the PD-L1 inhibitor comprises one or more of atezolizumab, avelumab, or durvalumab.

10. The method of claim 1, wherein the immune checkpoint inhibitor is a CTLA-4 inhibitor, wherein the CTLA-4 inhibitor comprises ipilimumab.

11. The method of claim 1, wherein the individual previously received treatment with an anti-cancer therapy for the cancer.

12. The method of claim 11, wherein the anti-cancer therapy is one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

13. The method of claim 1, wherein the immune checkpoint inhibitor therapy is a single-active-agent therapy.

14. The method of claim 1, wherein the immune checkpoint inhibitor therapy comprises two or more active agents.

15. The method of claim 1, wherein the immune checkpoint inhibitor therapy comprises a first round of an immune checkpoint inhibitor and a subsequent round of therapy with a different immune checkpoint inhibitor.

16. The method of claim 1, wherein the immune checkpoint inhibitor therapy is the first line therapy for the cancer.

17. The method of claim 1, wherein the immune checkpoint inhibitor therapy is the second line therapy for the cancer.

18. The method of claim 1, further comprising treating the individual with an additional anti-cancer therapy, wherein the additional anti-cancer therapy comprises one or more of a small molecule inhibitor, a chemotherapeutic agent, a cancer immunotherapy, an antibody, a cellular

therapy, a nucleic acid, a surgery, a radiotherapy, an anti-angiogenic therapy, an anti-DNA repair therapy, an anti-inflammatory therapy, an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or any combination thereof.

19. The method of claim 1, wherein the TMB score or microsatellite instability is determined by sequencing, wherein the sequencing comprises use of a massively parallel sequencing (MPS) technique, whole genome sequencing (WGS), whole exome sequencing (WES), targeted sequencing, direct sequencing, next-generation sequencing (NGS), or a Sanger sequencing technique.

20. The method of claim 1, wherein the individual is a human.

21. The method of claim 1, wherein if the TMB score is at least the threshold TMB score, the individual is predicted to have increased time to next treatment (TTNT), improved overall survival (OS), or improved progression free survival (PFS) when treated with an immune checkpoint inhibitor, as compared to a chemotherapy.

22. The method of claim 1, further comprising treating the individual with a chemotherapy if the TMB score is less than the threshold TMB score.

23. The method of claim 1, wherein the threshold TMB score is about 10 mutations/Mb.

24. A method for identifying an individual having a cancer for treatment with an immune checkpoint inhibitor therapy comprising determining a tumor mutational burden (TMB) score for a tumor biopsy sample obtained from the individual, wherein if the TMB score is at least a threshold TMB score the individual is identified for treatment with an immune checkpoint inhibitor therapy, wherein the cancer is a metastatic urothelial carcinoma, metastatic gastric adenocarcinoma, metastatic endometrial cancer, prostate cancer, or non-small cell lung cancer (NSCLC).

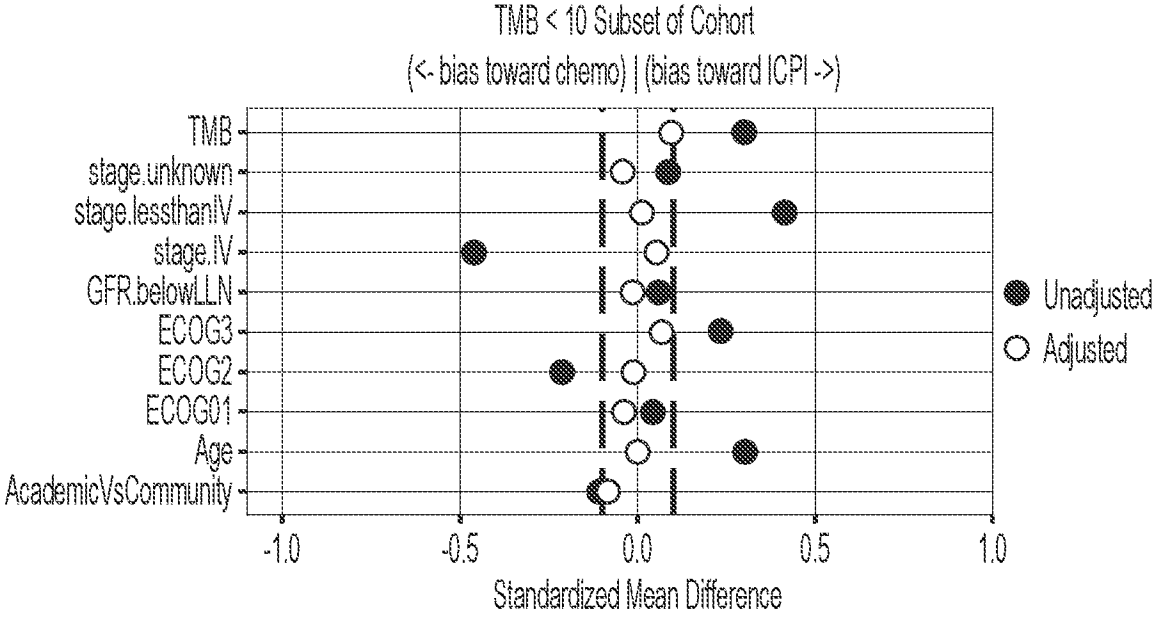

TMB < 10 Subset of Cohort
(<- bias toward chemo) | (bias toward ICPI ->)
TMB
stage.unknown
stage.lessthanIV
stage.IV
GFR.belowLLN
ECOG3
ECOG2
ECOG01
Age
AcademicVsCommunity
Unadjusted
Adjusted
-1.0
-0.5
0.0
0.5
1.0
Standardized Mean Difference

FIG. 1A


TMB ≥ 10 Subset of Cohort
(<- bias toward chemo) | (bias toward ICPI ->)
TMB
stage.unknown
stage.lessthanIV
stage.IV
GFR.belowLLN
ECOG3
ECOG2
ECOG01
Age
AcademicVsCommunity
Unadjusted
Adjusted
-1.0
-0.5
0.0
0.5
1.0
Standardized Mean Difference

FIG. 1B

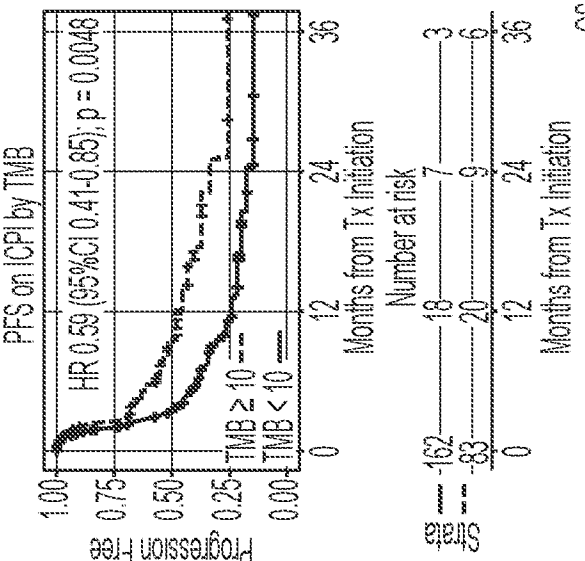


FIG. 2A

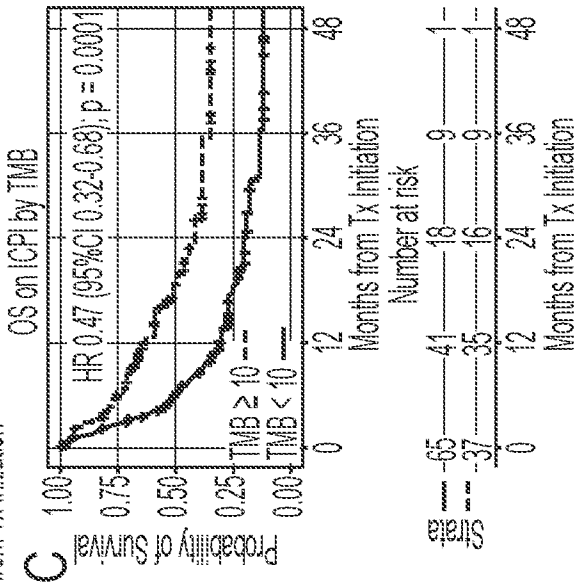


FIG. 2C

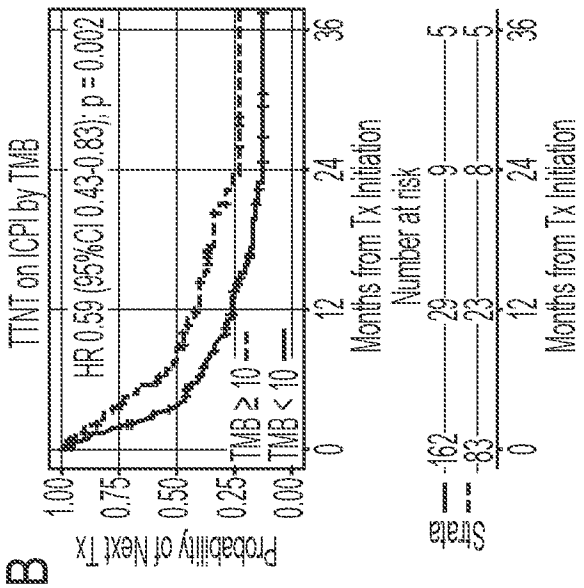


FIG. 2B

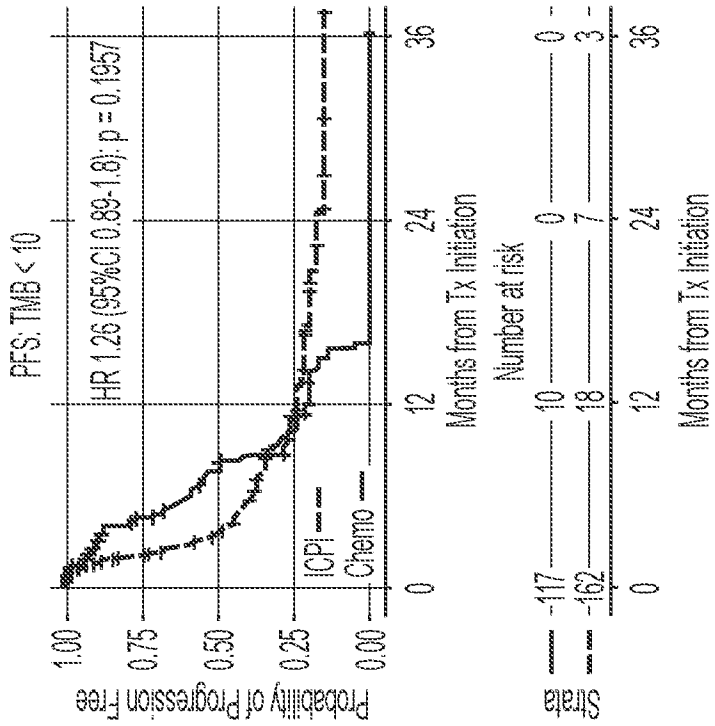

PFS: TMB < 10
HR 1.26 (95%CI 0.89-1.8); p = 0.1957
Probability of Progression Free
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
ICPI 117 10 0 0
Chemo 162 18 7 3
0
12
24
36
Months from Tx Initiation

FIG. 3A

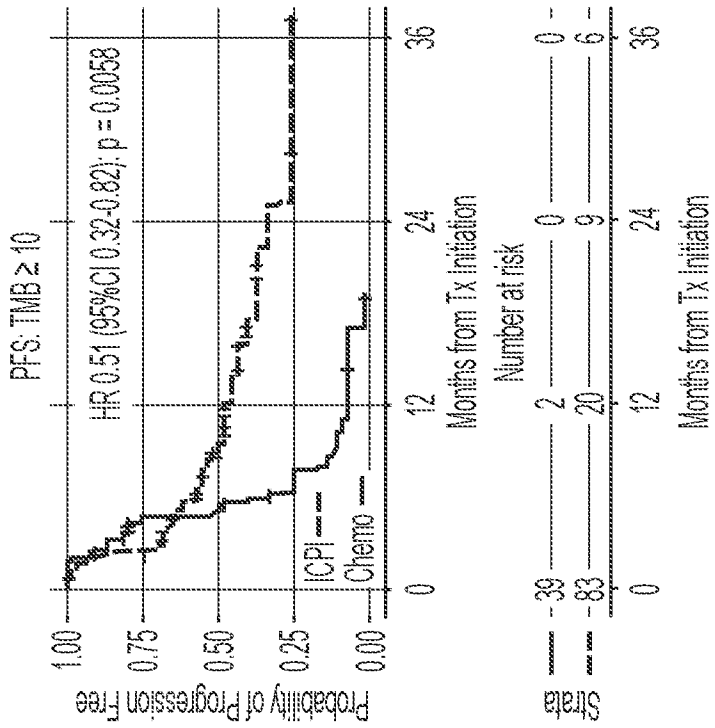

PFS: TMB ≥ 10
HR 0.51 (95%CI 0.32-0.82); p = 0.0058
Probability of Progression Free
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
ICPI 39 2 0 0
Chemo 83 20 9 6
0
12
24
36
Months from Tx Initiation

FIG. 3B

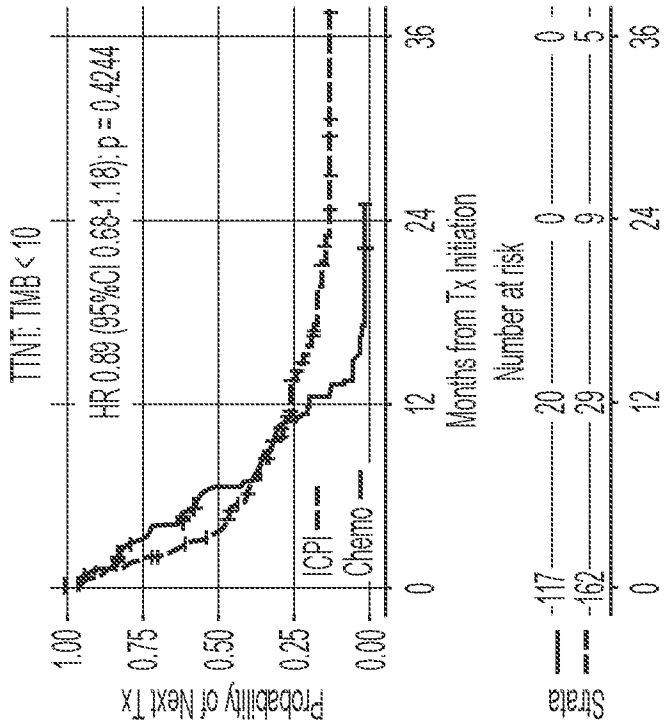

TTNT, TMB < 10
HR 0.89 (95%CI 0.68-1.18); p = 0.4244
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
117
162
20
29
0
9
0
5
0
12
24
36
Months from Tx Initiation

FIG. 3C

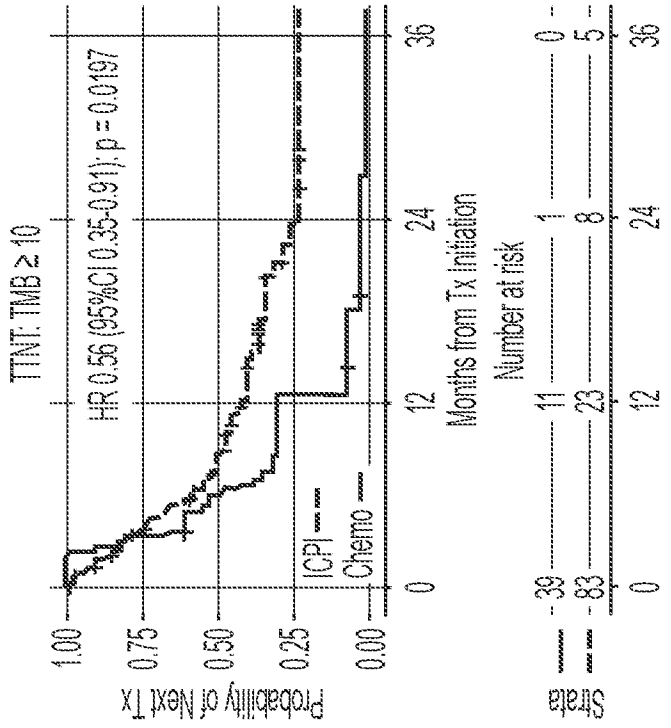

TTNT, TMB ≥ 10
HR 0.56 (95%CI 0.35-0.91); p = 0.0197
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
39
83
11
23
1
8
0
5
0
12
24
36
Months from Tx Initiation

FIG. 3D

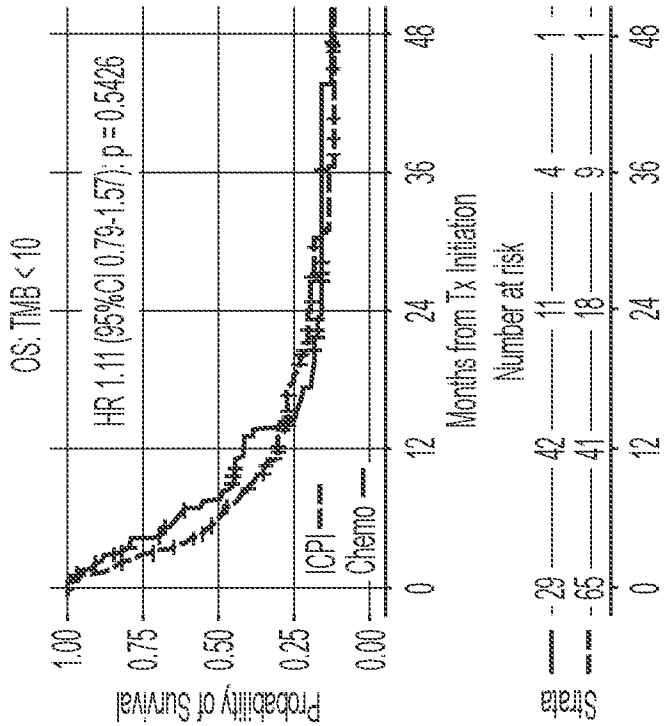

OS: TMB < 10
HR 1.11 (95%CI 0.79-1.57); p = 0.5426
Probability of Survival
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
48
Months from Tx Initiation
Number at risk
Strata
ICPI 29 42 11 4 1
Chemo 65 41 18 9 1
0
12
24
36
48
Months from Tx Initiation

FIG. 3E

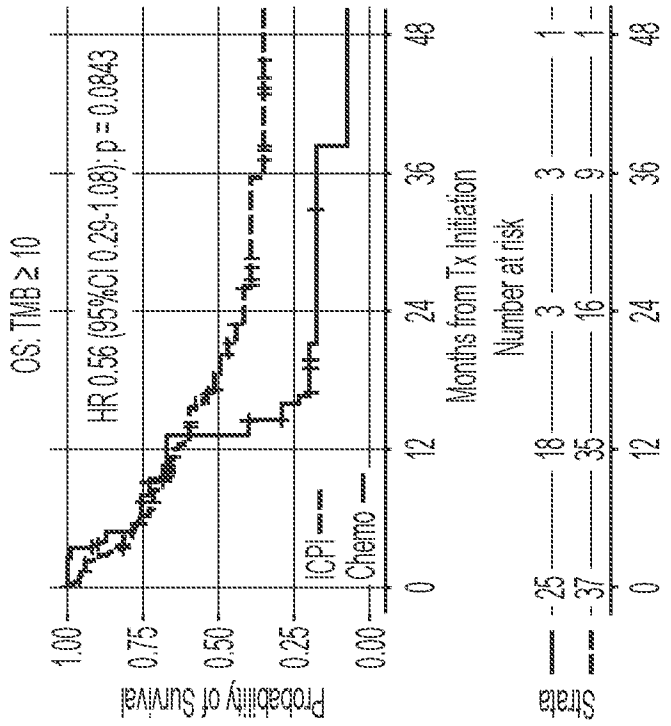

OS: TMB ≥ 10
HR 0.56 (95%CI 0.29-1.08); p = 0.0843
Probability of Survival
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
48
Months from Tx Initiation
Number at risk
Strata
ICPI 25 18 3 3 1
Chemo 37 35 16 9 1
0
12
24
36
48
Months from Tx Initiation

FIG. 3F

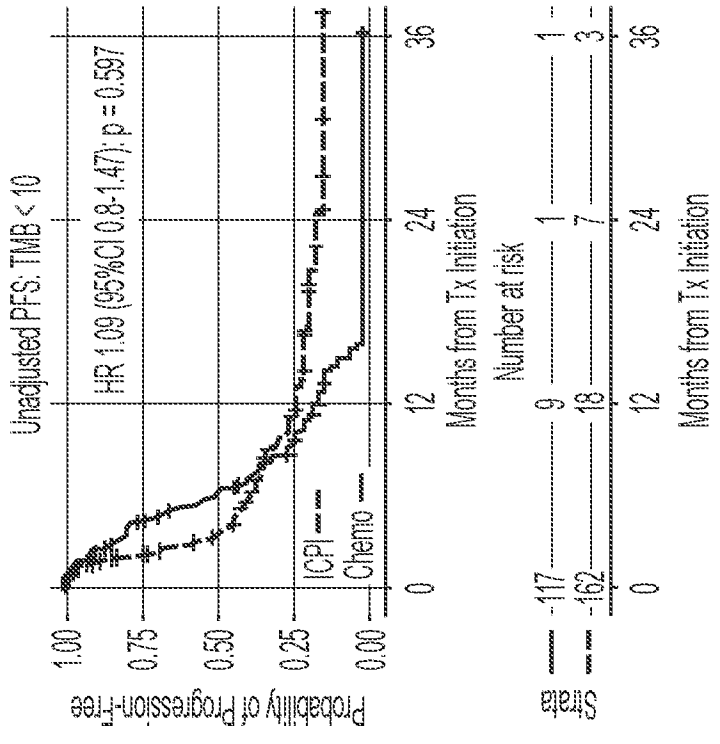

Unadjusted PFS: TMB < 10
HR 1.09 (95%CI 0.8-1.47); p = 0.597
Probability of Progression-Free
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
117
162
9
18
1
7
1
3
0
12
24
36
Months from Tx Initiation

FIG. 4A

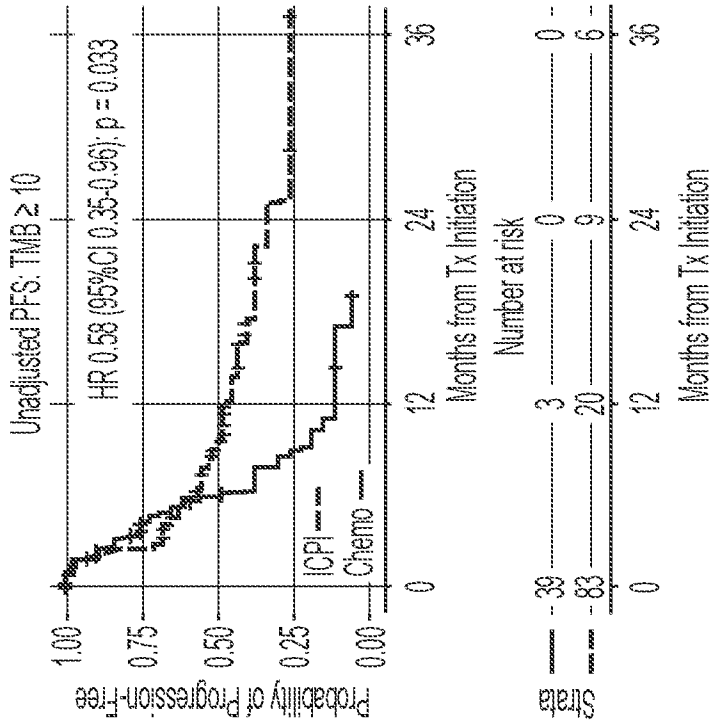

Unadjusted PFS: TMB ≥ 10
HR 0.58 (95%CI 0.35-0.96); p = 0.033
Probability of Progression-Free
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
39
83
3
20
0
9
0
6
0
12
24
36
Months from Tx Initiation

FIG. 4B

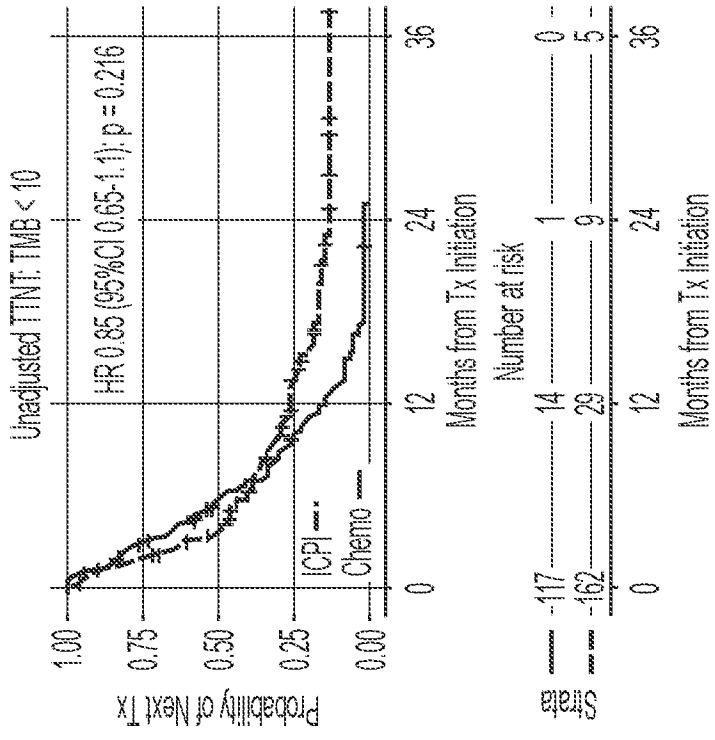

Unadjusted TTNT: TMB < 10
HR 0.85 (95%CI 0.65-1.1); p = 0.216
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
117
162
14
29
1
9
0
5
0
12
24
36
Months from Tx Initiation

FIG. 4C

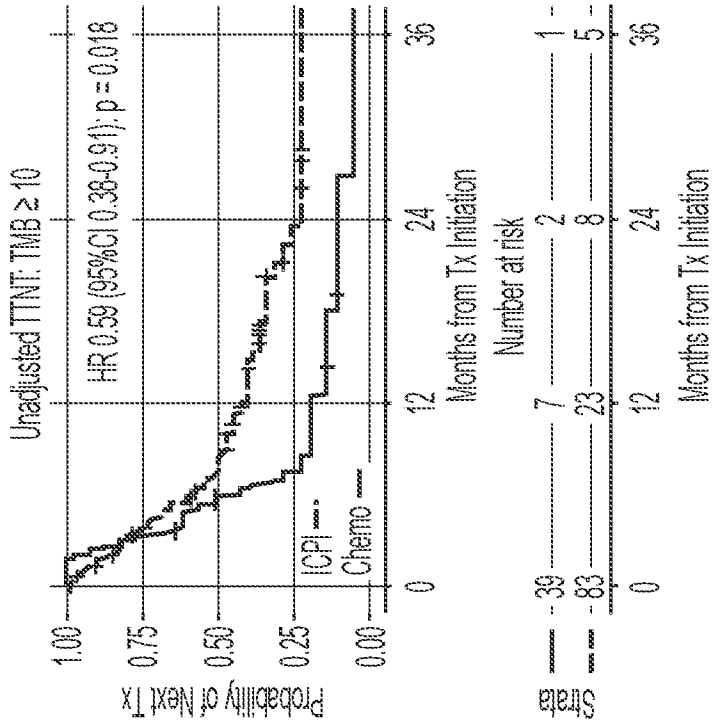

Unadjusted TTNT: TMB ≥ 10
HR 0.59 (95%CI 0.38-0.91); p = 0.018
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
Months from Tx Initiation
Number at risk
Strata
39
83
7
23
2
8
1
5
0
12
24
36
Months from Tx Initiation

FIG. 4D

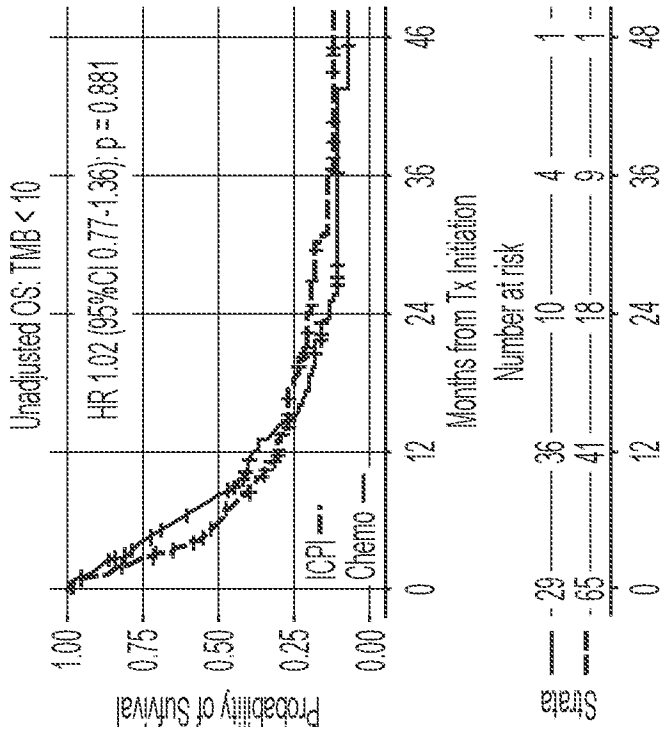

Unadjusted OS: TMB < 10
HR 1.02 (95%CI 0.77-1.36); p = 0.881
Probability of Survival
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
46
Months from Tx Initiation
Number at risk
Strata
ICPI 29 36 10 4 1
Chemo 65 41 18 9 1
0
12
24
36
48
Months from Tx Initiation

FIG. 4E

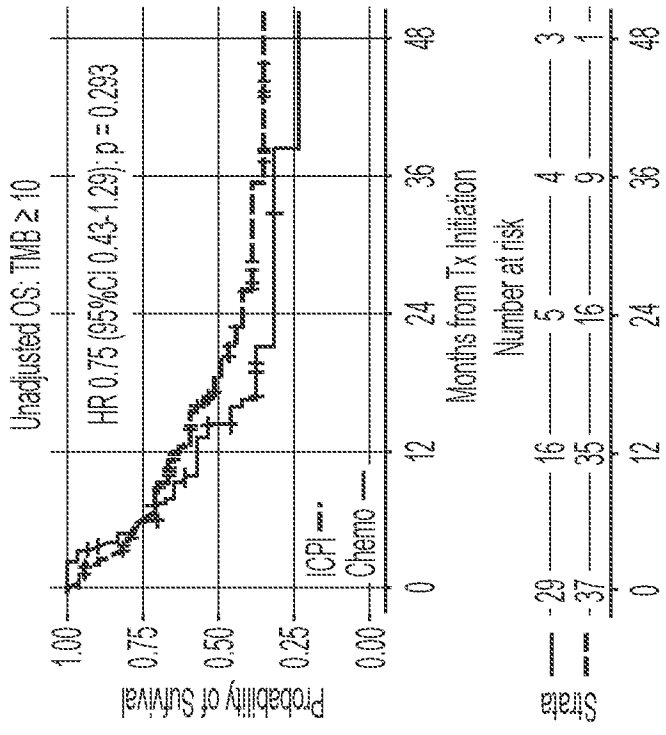

Unadjusted OS: TMB ≥ 10
HR 0.75 (95%CI 0.43-1.29); p = 0.293
Probability of Survival
1.00
0.75
0.50
0.25
0.00
ICPI
Chemo
0
12
24
36
48
Months from Tx Initiation
Number at risk
Strata
ICPI 29 16 5 4 3
Chemo 37 35 16 9 1
0
12
24
36
48
Months from Tx Initiation

FIG. 4F

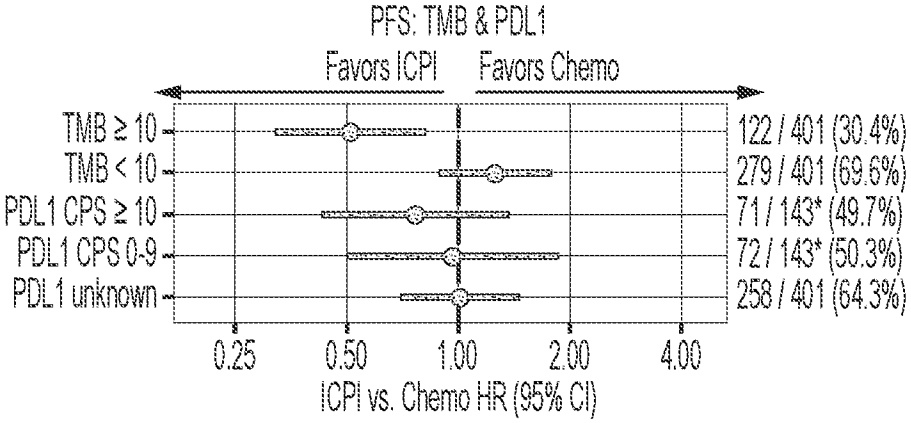
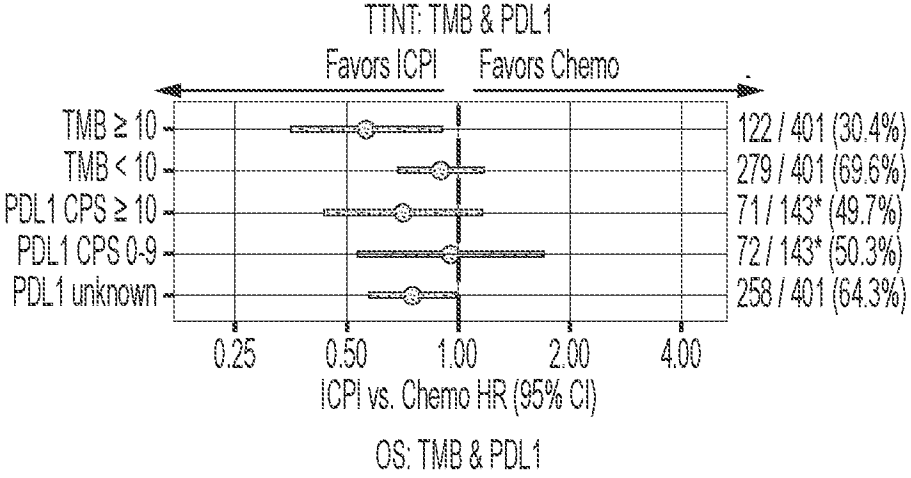


FIG. 5A



OS: TMB & PDL1

FIG. 5B

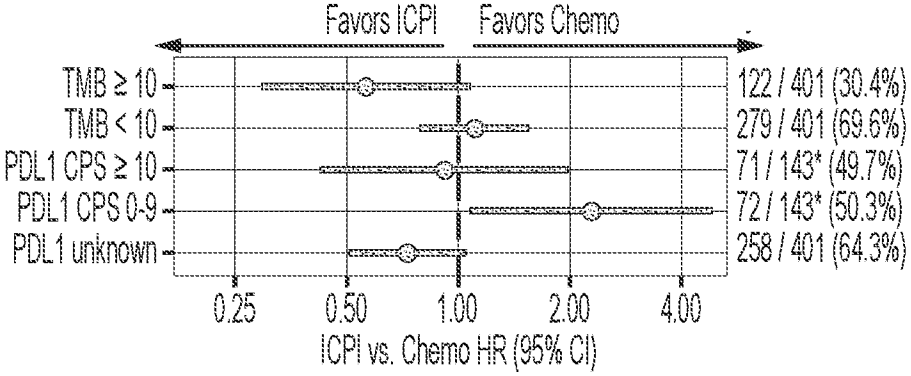


FIG. 5C

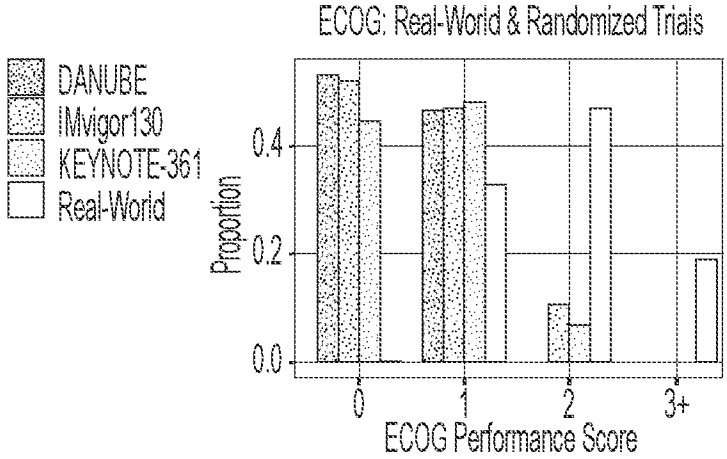


FIG. 6A

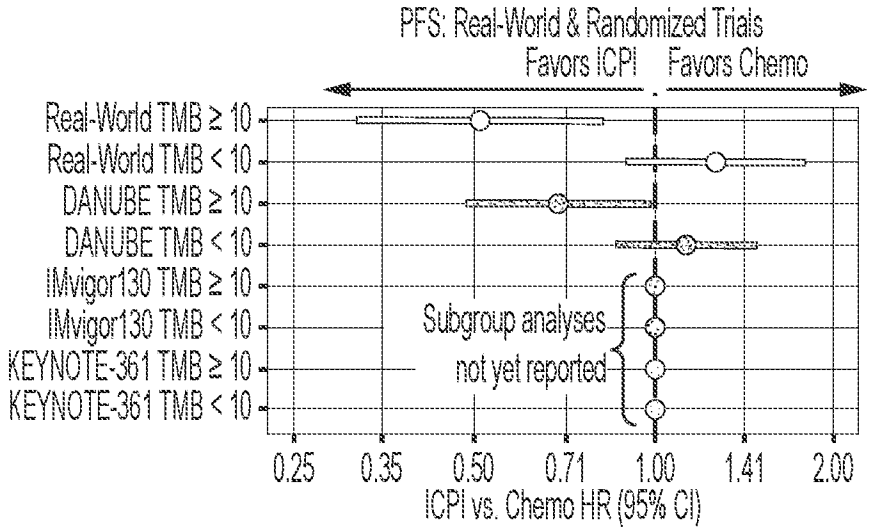


FIG. 6B

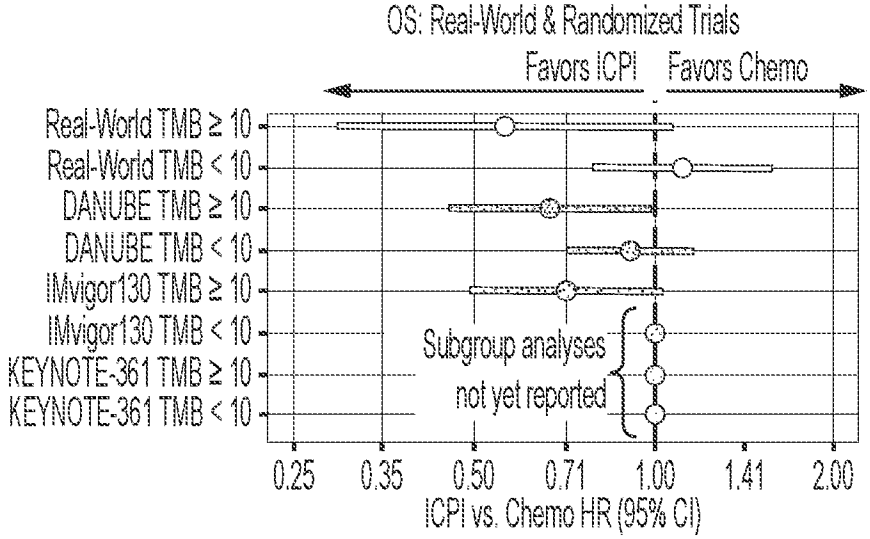


FIG. 6C

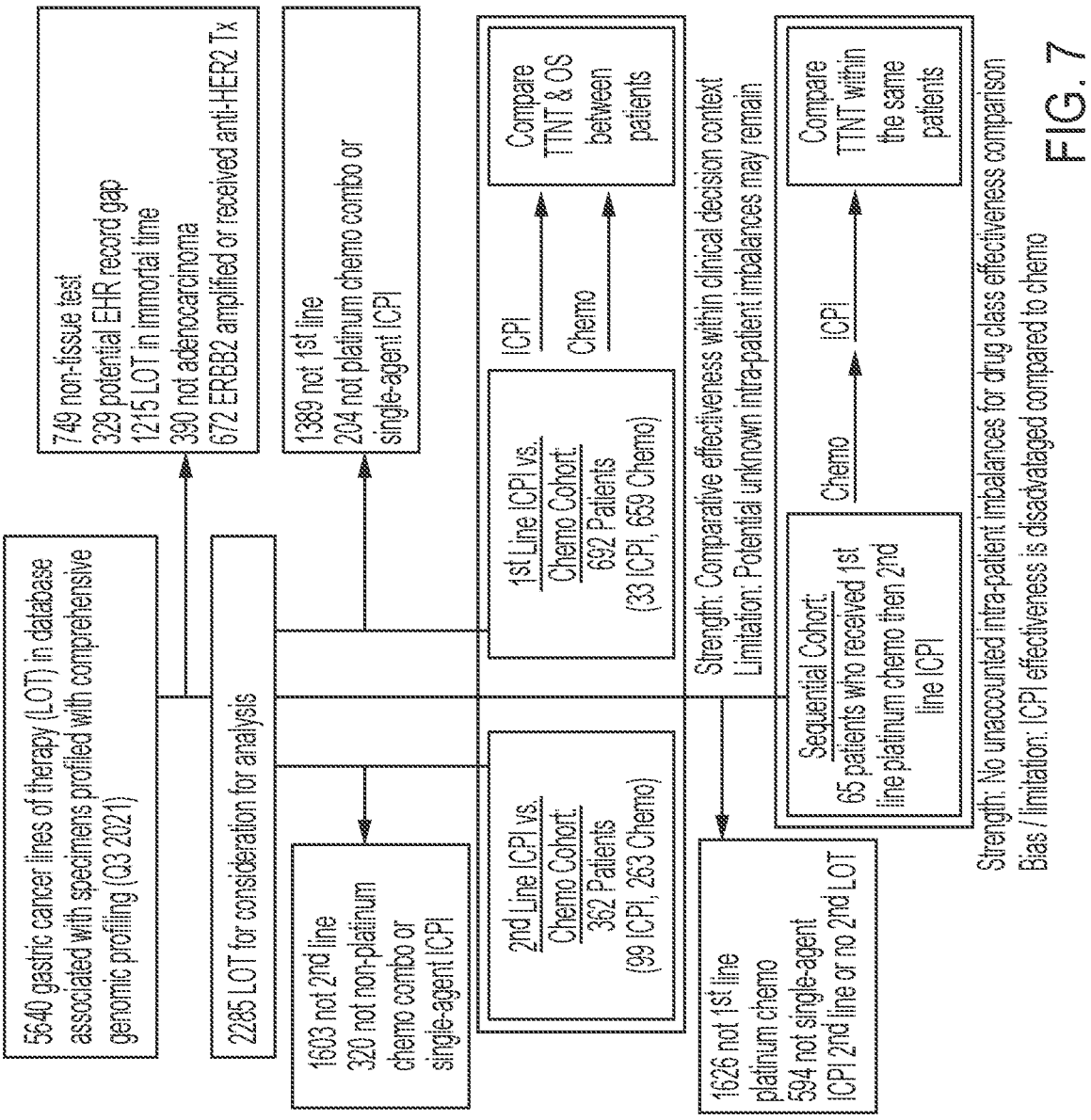

5640 gastric cancer lines of therapy (LOT) in database associated with specimens profiled with comprehensive genomic profiling (Q3 2021)
749 non-tissue test
329 potential EHR record gap
1215 LOT in immortal time
390 not adenocarcinoma
672 ERBB2 amplified or received anti-HER2 Tx
2285 LOT for consideration for analysis
1389 not 1st line
204 not platinum chemo combo or single-agent ICPI
1603 not 2nd line
320 not non-platinum chemo combo or single-agent ICPI
2nd Line ICPI vs. Chemo Cohort:
362 Patients
(99 ICPI, 263 Chemo)
1st Line ICPI vs. Chemo Cohort:
692 Patients
(33 ICPI, 659 Chemo)
ICPI
Chemo
Compare TTNT & OS between patients
Strength: Comparative effectiveness within clinical decision context
Limitation: Potential unknown intra-patient imbalances may remain
1626 not 1st line platinum chemo
594 not single-agent ICPI 2nd line or no 2nd LOT
Sequential Cohort:
65 patients who received 1st line platinum chemo then 2nd line ICPI
Chemo
ICPI
Compare TTNT within the same patients
Strength: No unaccounted intra-patient imbalances for drug class effectiveness comparison
Bias / limitation: ICPI effectiveness is disadvantaged compared to chemo

FIG. 7

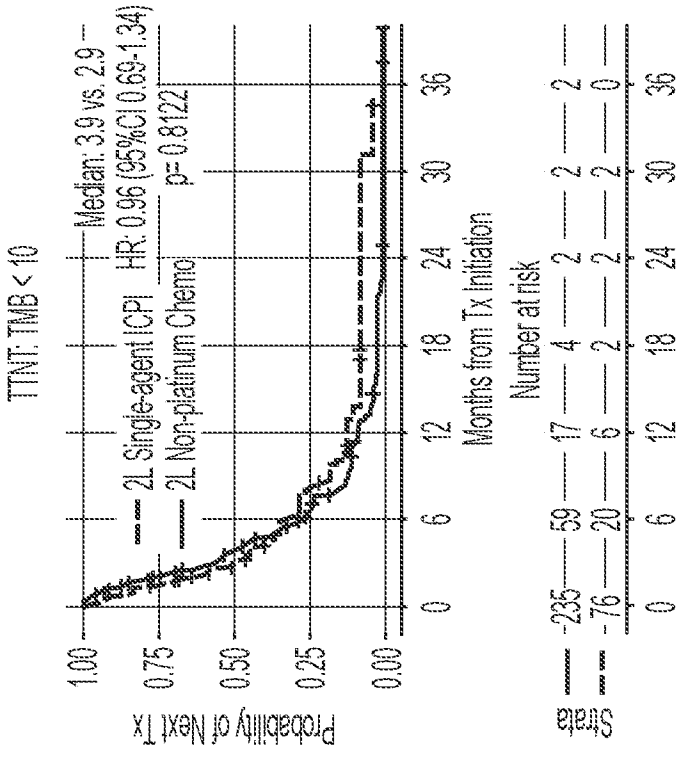

TTNT: TMB < 10
Median: 3.9 vs. 2.9
2L Single-agent ICPI
HR: 0.96 (95%CI 0.69-1.34)
2L Non-platinum Chemo
p= 0.8122
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
235
59
17
4
2
2
2
76
20
6
2
2
2
0
0
6
12
18
24
30
36
Months from Tx Initiation

FIG. 8A

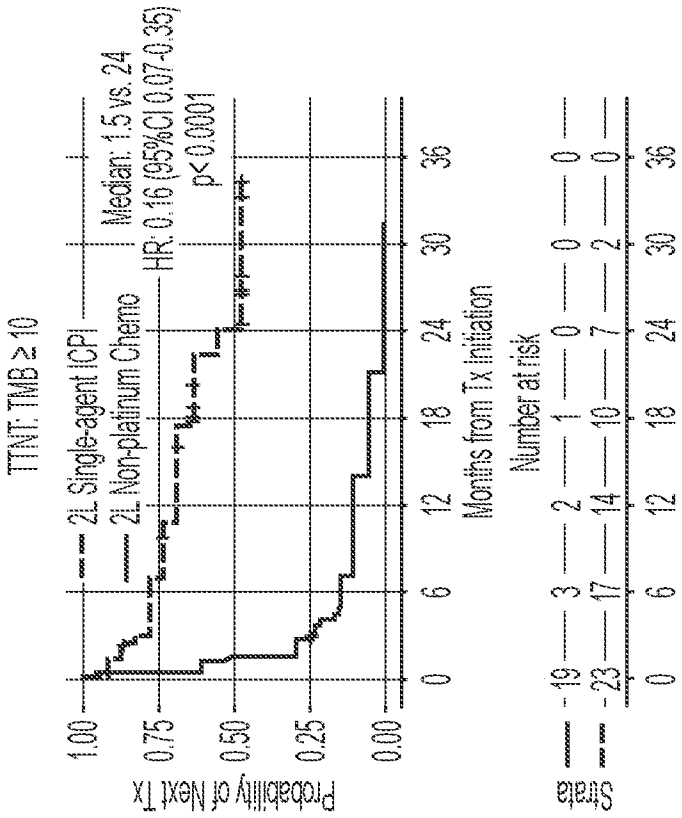

TTNT: TMB ≥ 10
2L Single-agent ICPI
2L Non-platinum Chemo
Median: 1.5 vs. 24
HR: 0.16 (95%CI 0.07-0.35)
p< 0.0001
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
19
3
2
1
0
0
0
23
17
14
10
7
2
0
0
6
12
18
24
30
36
Months from Tx Initiation

FIG. 8B

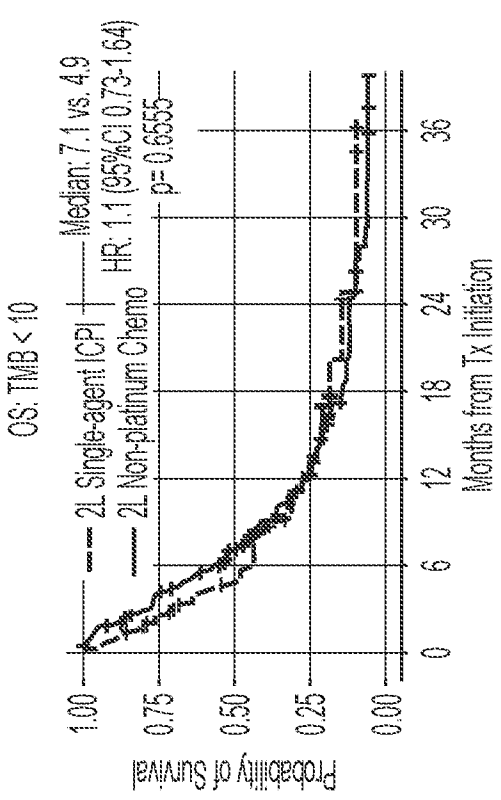

OS: TMB < 10
2L Single-agent ICPI
2L Non-platinum Chemo
Median: 7.1 vs. 4.9
HR: 1.1 (95%CI 0.73-1.64)
p= 0.6555
Probability of Survival
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
150 112 50 19 15 6 2
68 28 13 5 4 2 1
0 6 12 18 24 30 36
Months from Tx Initiation

FIG. 8C

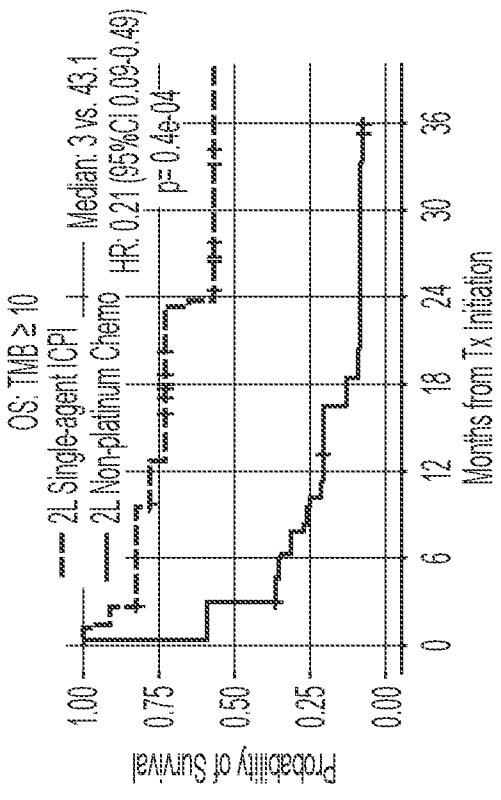

OS: TMB ≥ 10
2L Single-agent ICPI
2L Non-platinum Chemo
Median: 3 vs. 43.1
HR: 0.21 (95%CI 0.09-0.49)
p= 0.4e-04
Probability of Survival
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
16 8 5 3 2 2 0
23 18 16 11 7 3 1
0 6 12 18 24 30 36
Months from Tx Initiation

FIG. 8D

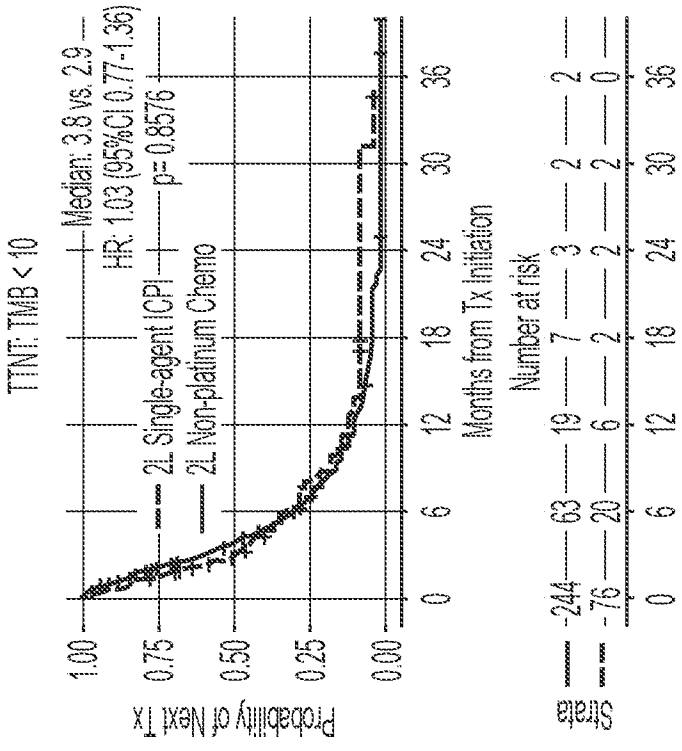

TTNT: TMB < 10
Median: 3.8 vs. 2.9
HR: 1.03 (95%CI 0.77-1.36)
p= 0.8576
2L Single-agent ICPI
2L Non-platinum Chemo
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
244
63
19
7
3
2
2
76
20
6
2
2
2
0
0
6
12
18
24
30
36
Months from Tx Initiation

FIG. 9A

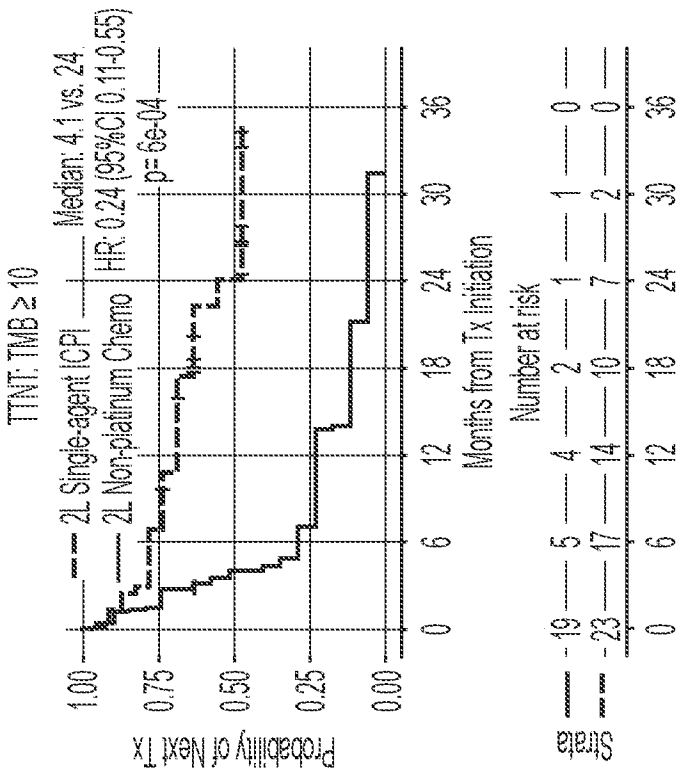

TTNT: TMB ≥ 10
Median: 4.1 vs. 24
HR: 0.24 (95%CI 0.11-0.55)
p= 6e-04
2L Single-agent ICPI
2L Non-platinum Chemo
Probability of Next Tx
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
19
5
4
2
1
1
0
23
17
14
10
7
2
0
0
6
12
18
24
30
36
Months from Tx Initiation

FIG. 9B

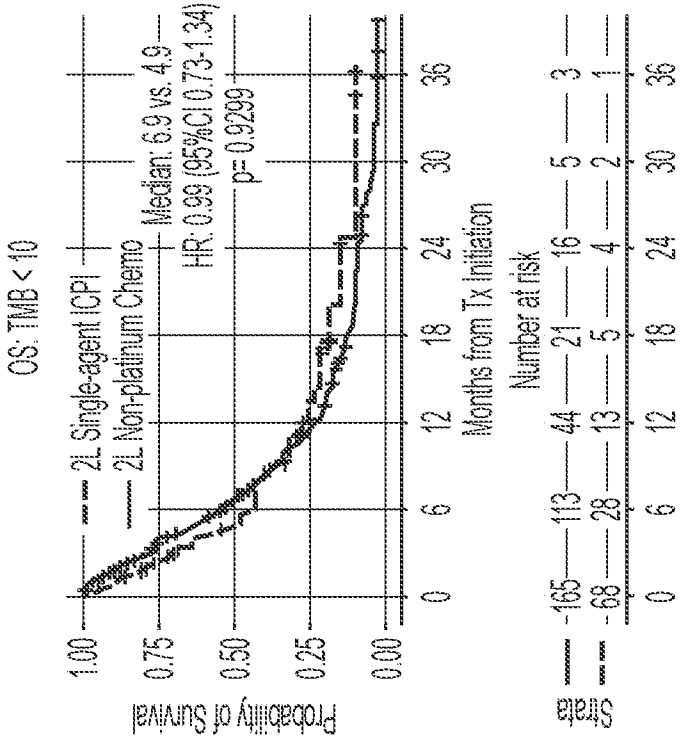

OS: TMB < 10
2L Single-agent ICPI
2L Non-platinum Chemo
Median: 6.9 vs. 4.9
HR: 0.99 (95%CI 0.73-1.34)
p= 0.9299
Probability of Survival
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
165 113 44 21 16 5 3
68 28 13 5 4 2 1
Months from Tx Initiation

FIG. 9C

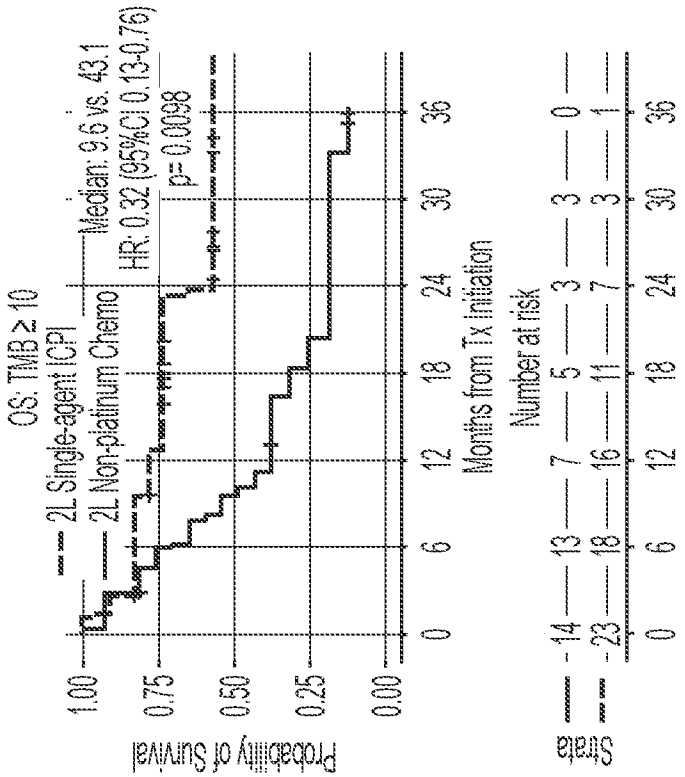

OS: TMB ≥ 10
2L Single-agent ICPI
2L Non-platinum Chemo
Median: 9.6 vs. 43.1
HR: 0.32 (95%CI 0.13-0.76)
p= 0.0098
Probability of Survival
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
Months from Tx Initiation
Number at risk
Strata
14 13 7 5 3 3 0
23 18 16 11 7 3 1
Months from Tx Initiation

FIG. 9D

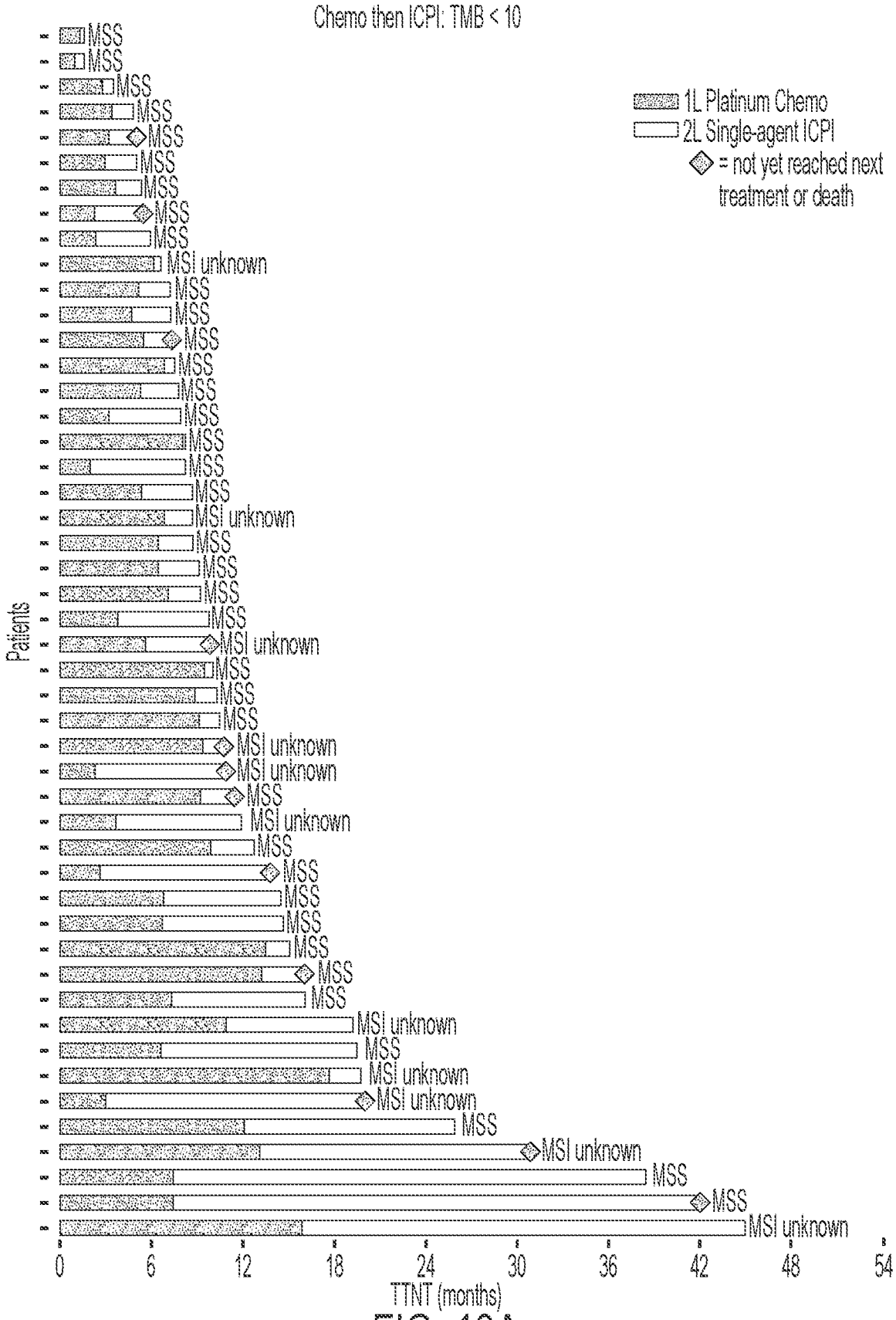

Chemo then ICPI: TMB < 10
1L Platinum Chemo
2L Single-agent ICPI
◇ = not yet reached next treatment or death
Patients
MSS
MSI unknown
0
6
12
18
24
30
36
42
48
54
TTNT (months)

FIG. 10A

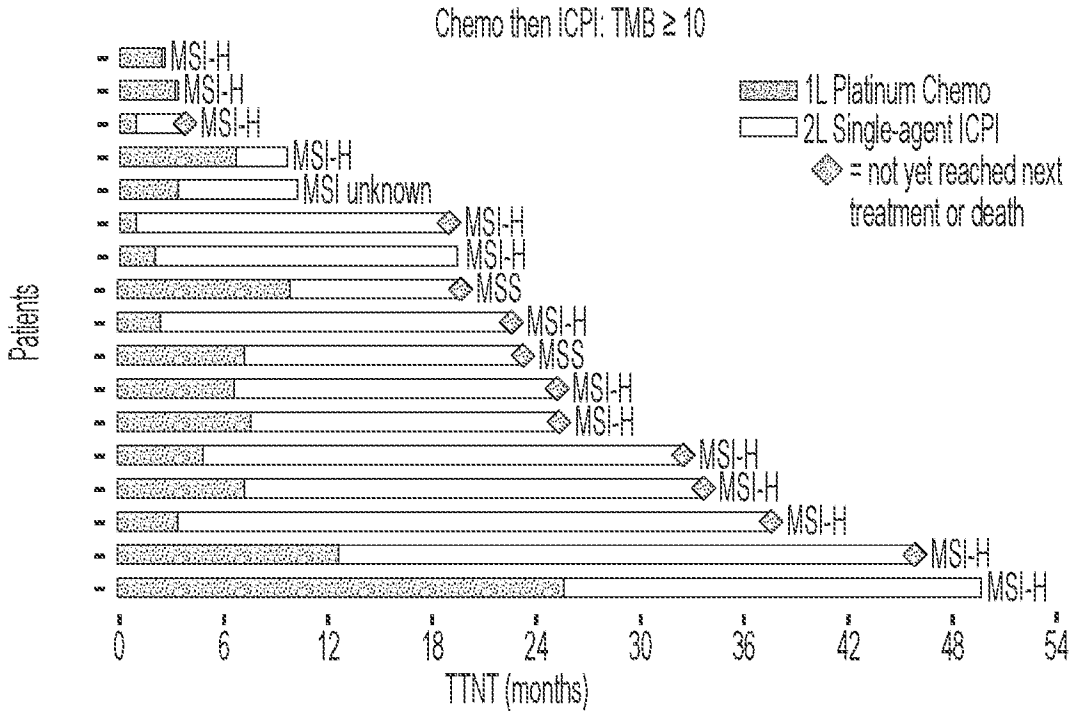

Chemo then ICPI: TMB ≥ 10
1L Platinum Chemo
2L Single-agent ICPI
= not yet reached next treatment or death
MSI-H
MSI-H
MSI-H
MSI-H
MSI unknown
MSI-H
MSI-H
MSS
MSI-H
MSS
MSI-H
MSI-H
MSI-H
MSI-H
MSI-H
MSI-H
MSI-H
Patients
0
6
12
18
24
30
36
42
48
54
TTNT (months)

FIG. 10B

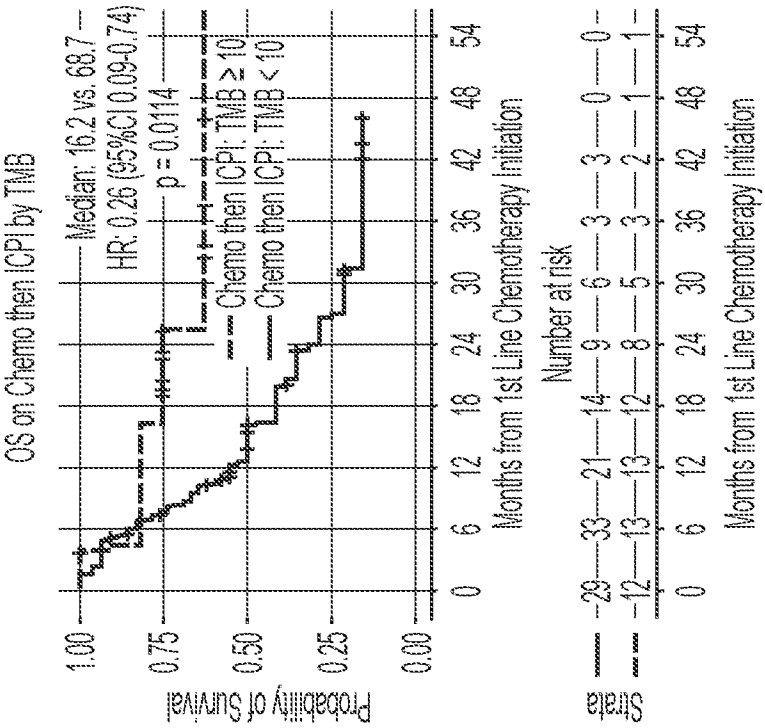


FIG. 10D

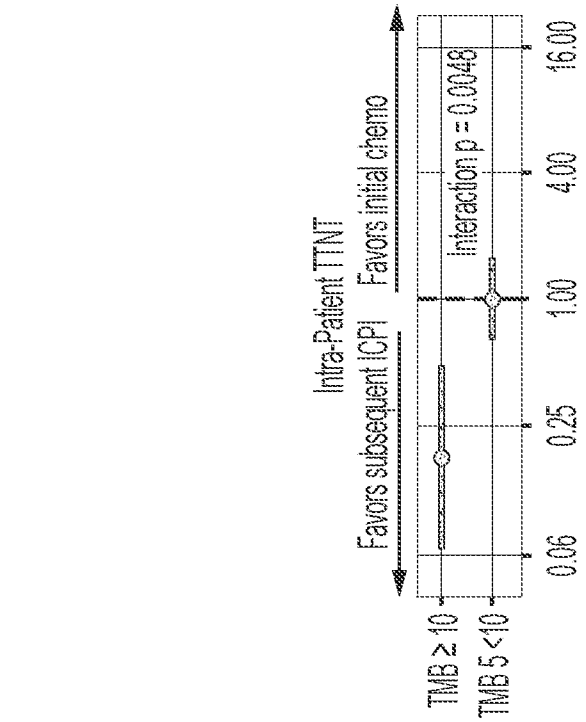


FIG. 10C

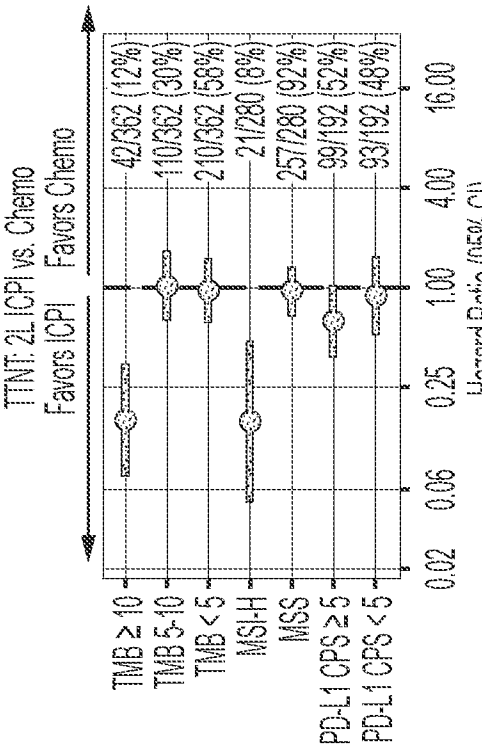


FIG. 11A

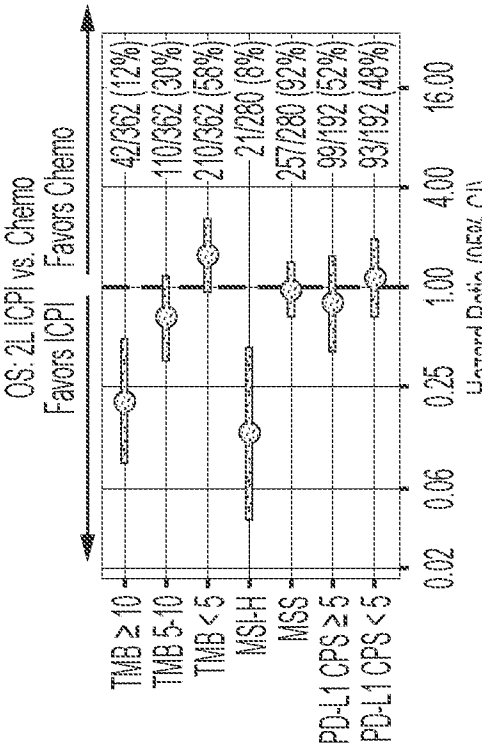


FIG. 11B

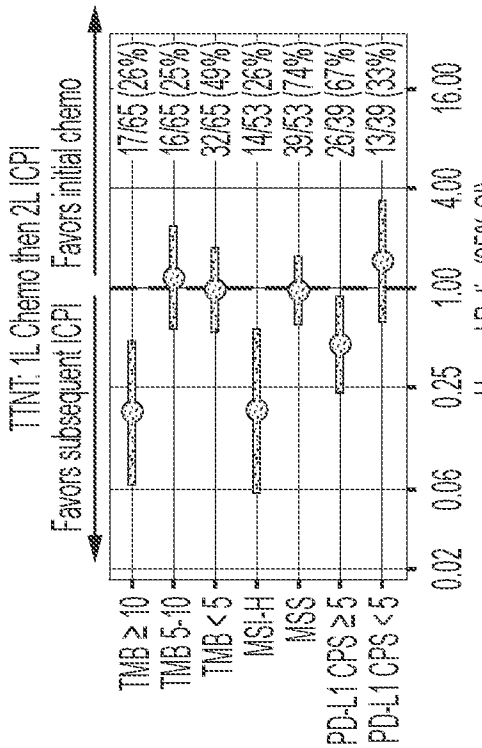


FIG. 11C

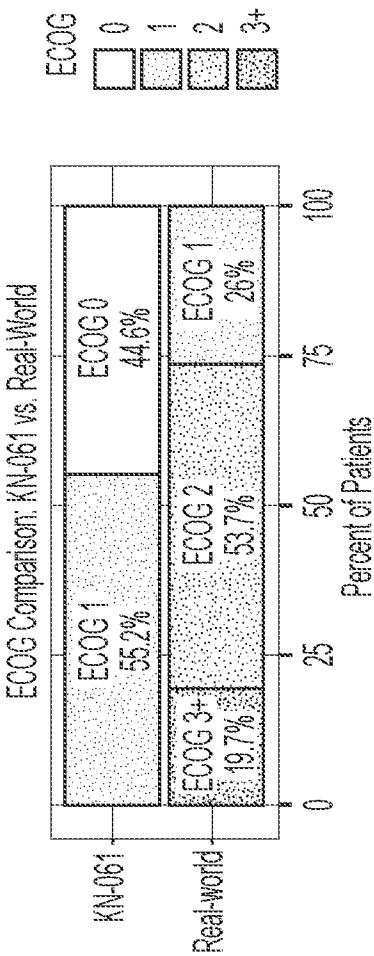


FIG. 12A

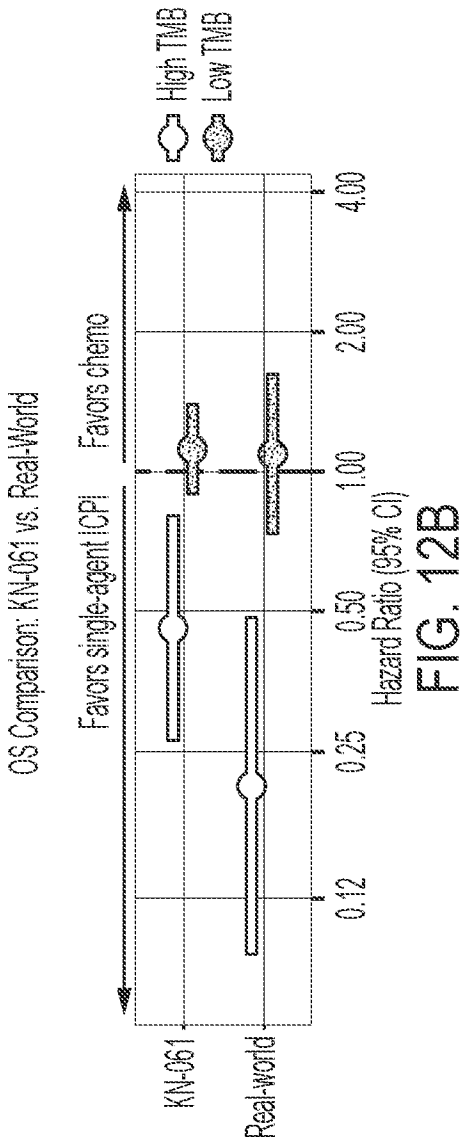


FIG. 12B

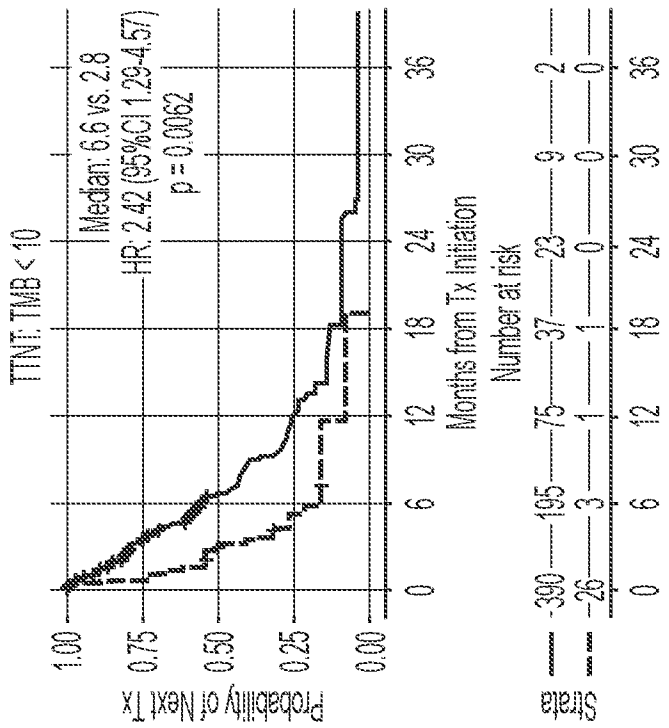


FIG. 13A

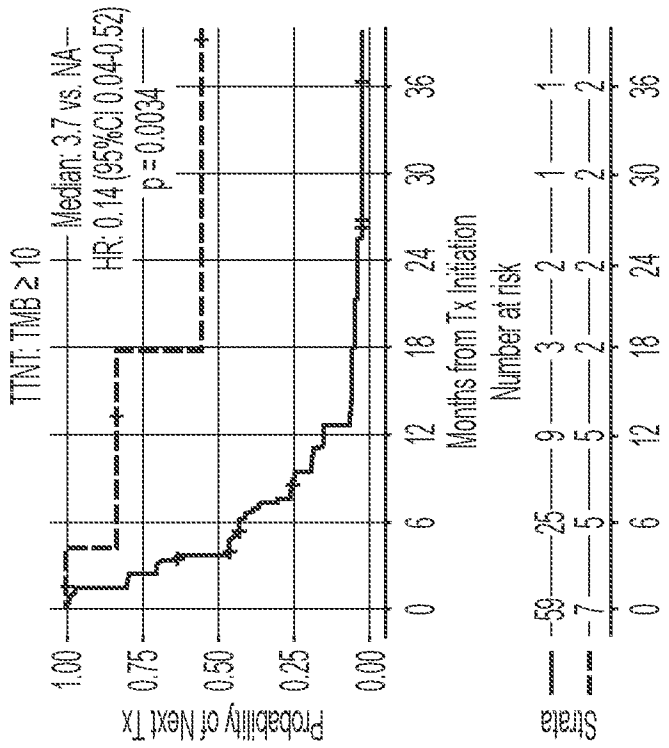


FIG. 13B

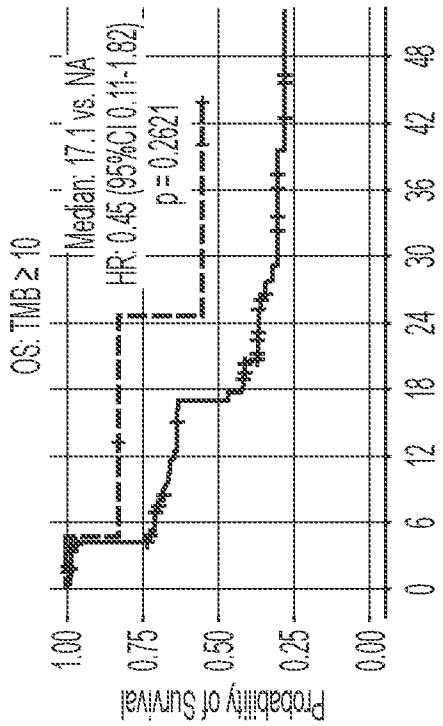


FIG. 13D

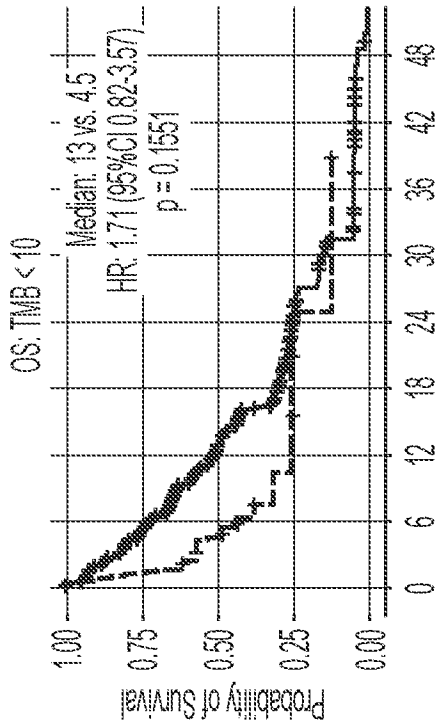


FIG. 13C

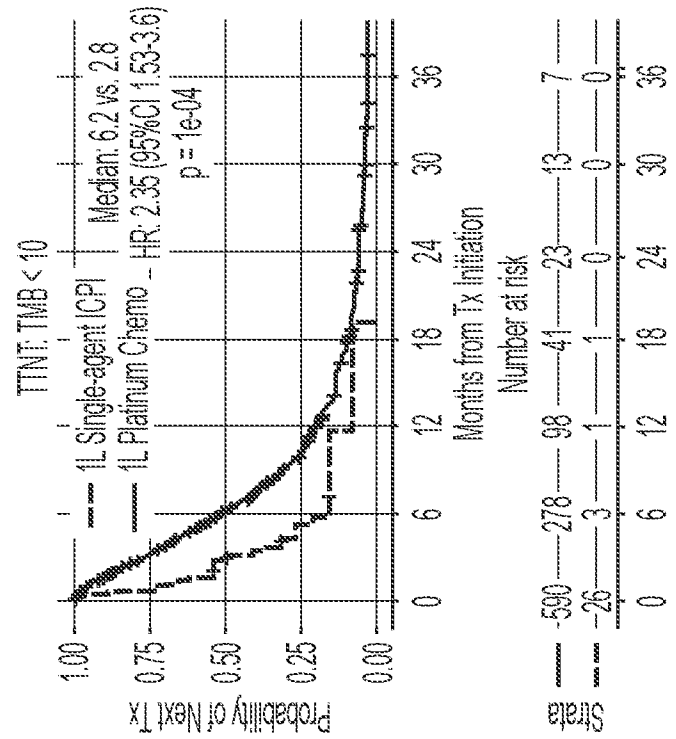


FIG. 14A

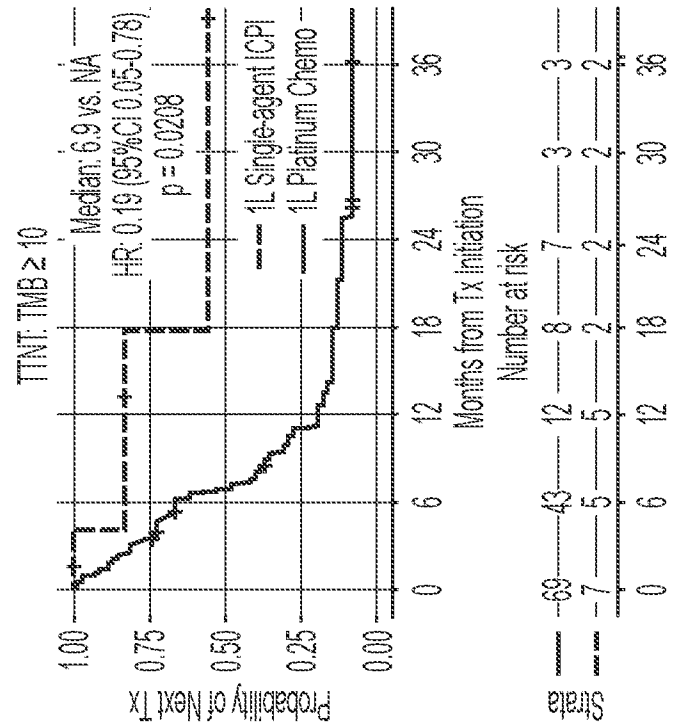


FIG. 14B

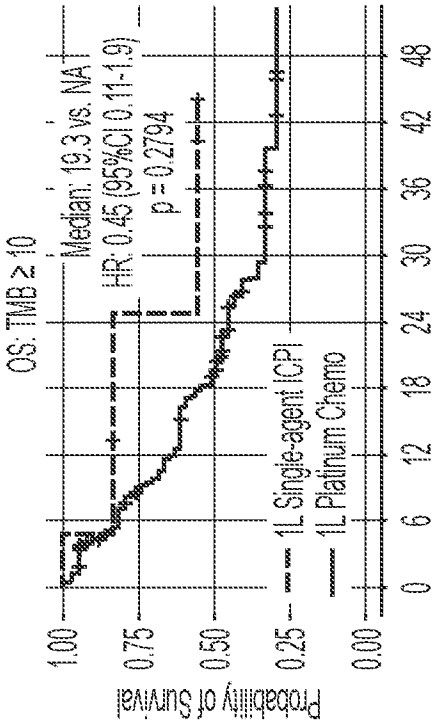


FIG. 14D

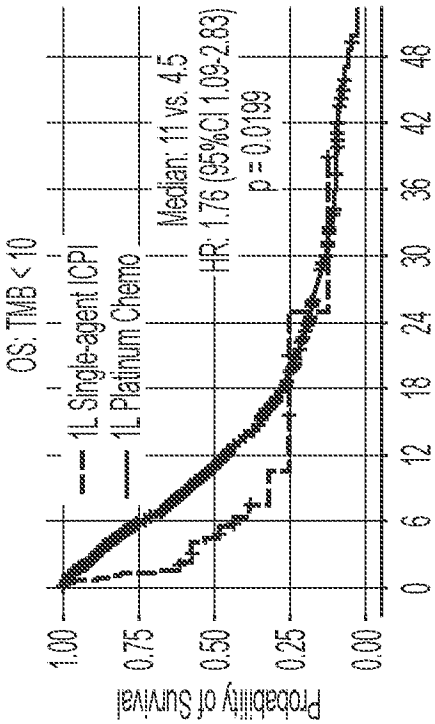


FIG. 14C

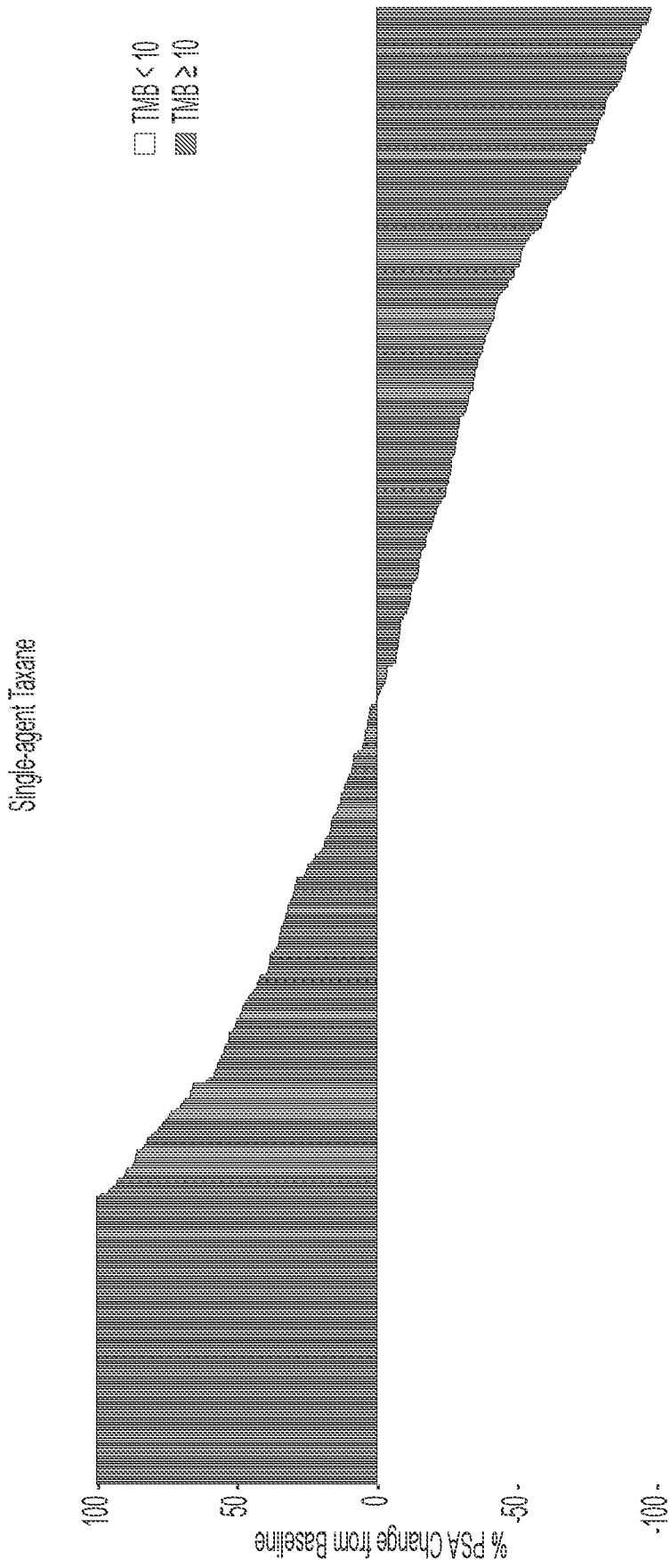

TMB < 10
TMB ≥ 10
Single-agent Taxane
Patients Evaluable for PSA Response
% PSA Change from Baseline
100
50
0
-50
-100

FIG. 15

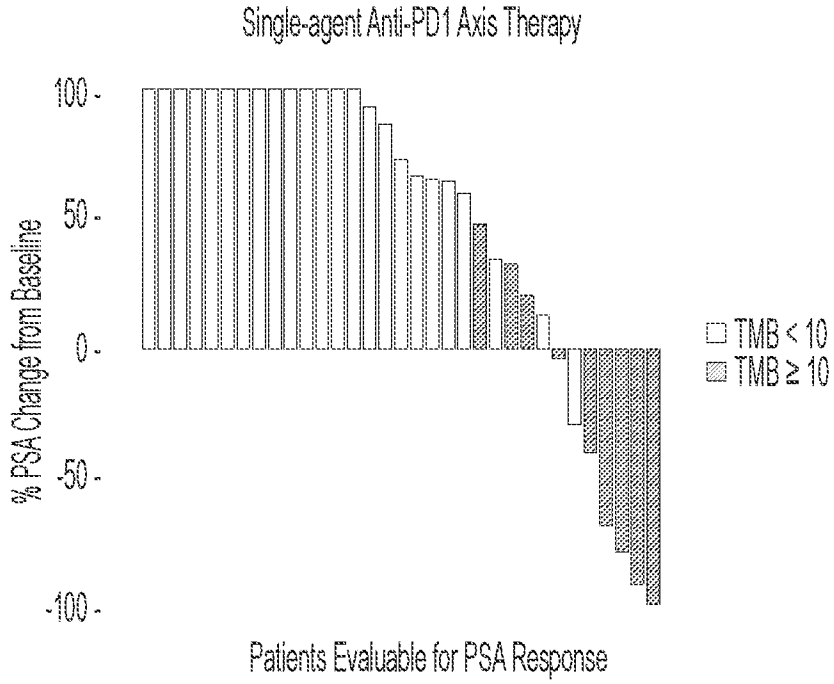

Single-agent Anti-PD1 Axis Therapy
100
50
0
-50
-100
% PSA Change from Baseline
TMB < 10
TMB ≥ 10
Patients Evaluable for PSA Response

FIG. 16

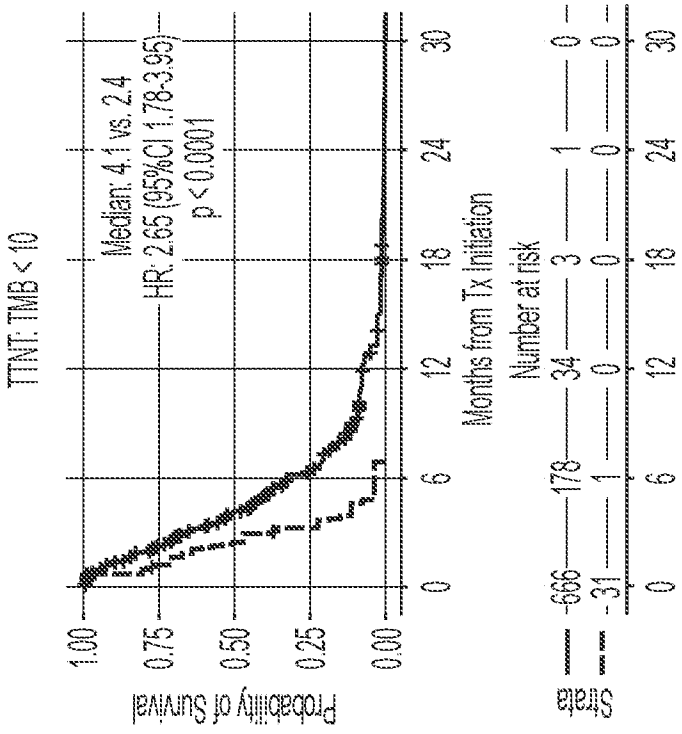


FIG. 17A

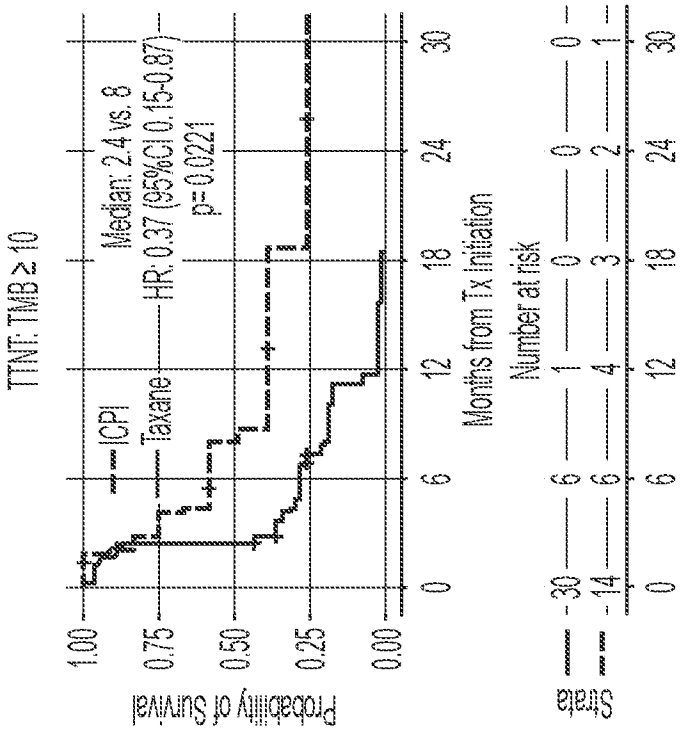


FIG. 17B

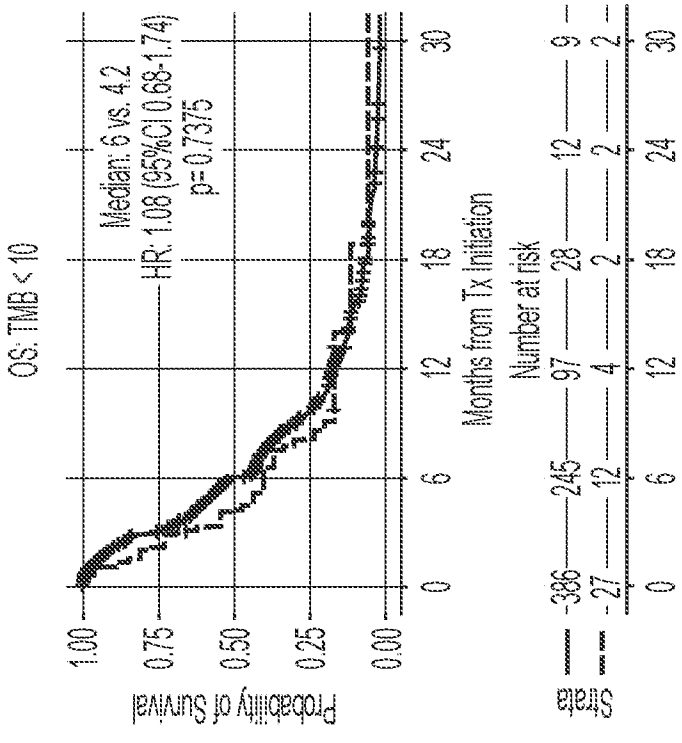


FIG. 17C

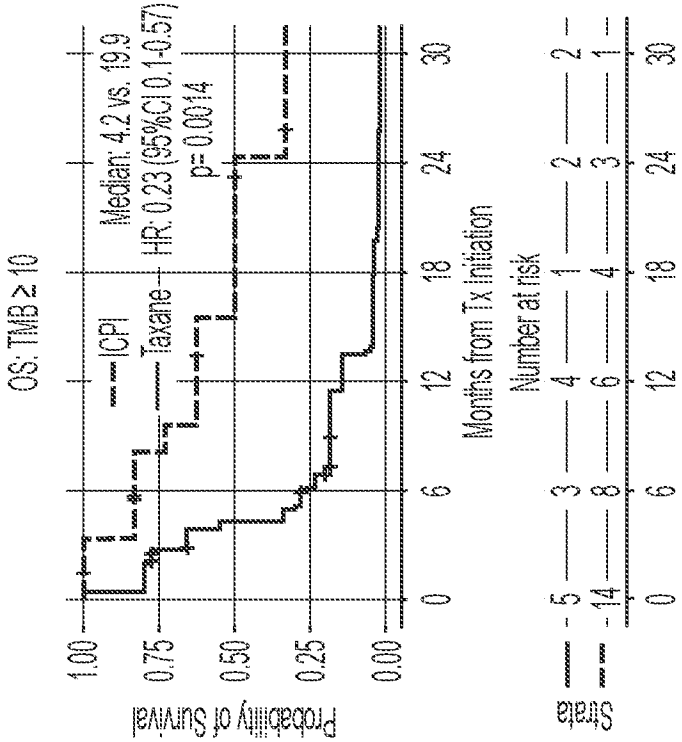


FIG. 17D

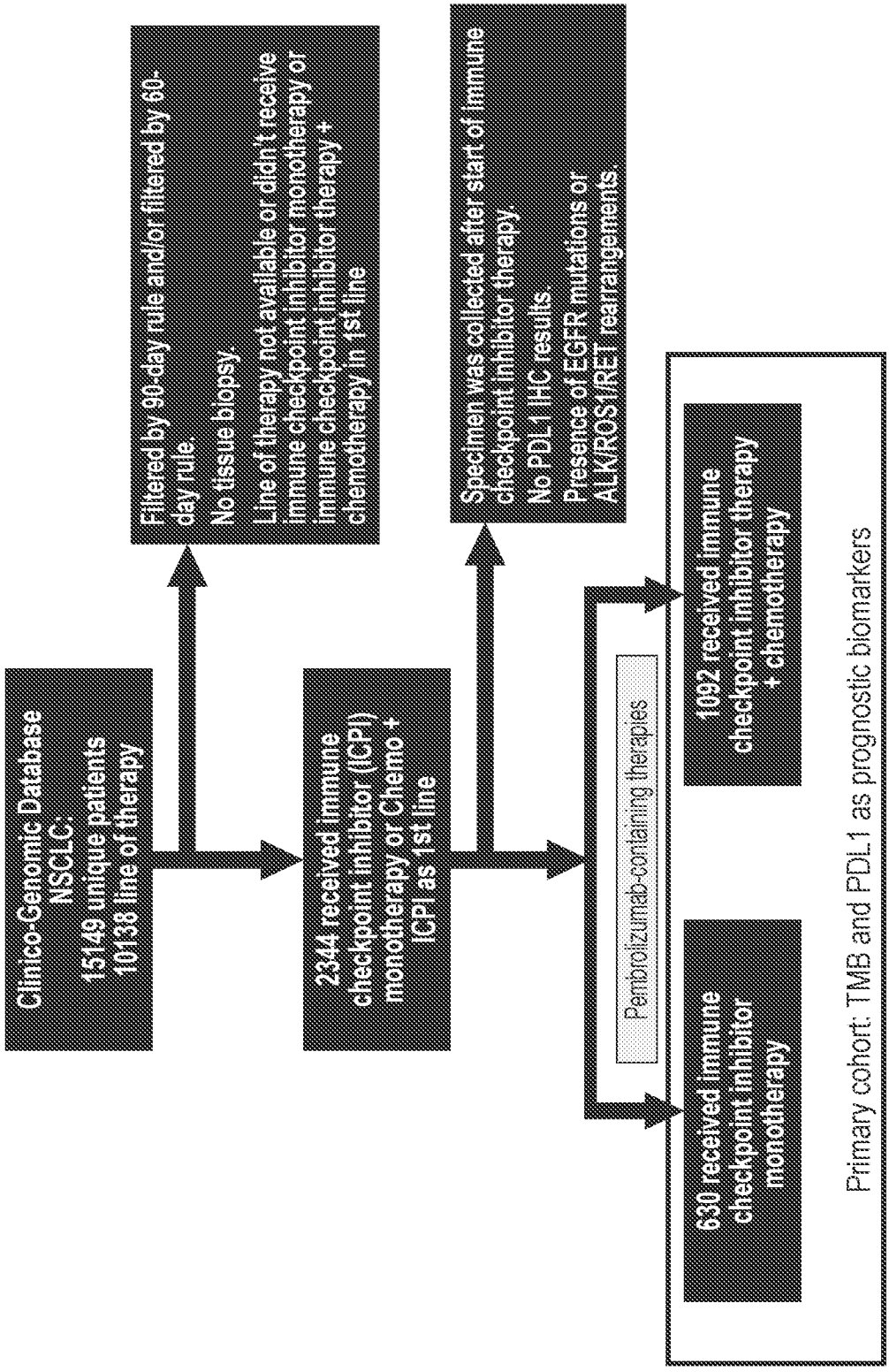

Clinico-Genomic Database
NSCLC:
15149 unique patients
10138 line of therapy
Filtered by 90-day rule and/or filtered by 60-day rule
No tissue biopsy.
Line of therapy not available or didn't receive immune checkpoint inhibitor monotherapy or immune checkpoint inhibitor therapy + chemotherapy in 1st line
2344 received immune checkpoint inhibitor (ICPI) monotherapy or Chemo + ICPI as 1st line
Specimen was collected after start of immune checkpoint inhibitor therapy
No PDL1 IHC results.
Presence of EGFR mutations or ALK/ROS1/RET rearrangements.
Pembrolizumab-containing therapies
630 received immune checkpoint inhibitor monotherapy
1092 received immune checkpoint inhibitor therapy + chemotherapy
Primary cohort: TMB and PDL1 as prognostic biomarkers

FIG. 18

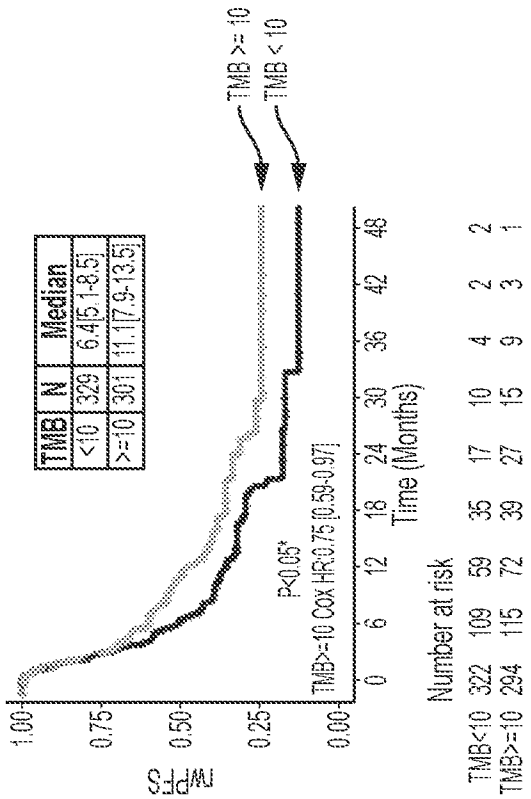


FIG. 19A

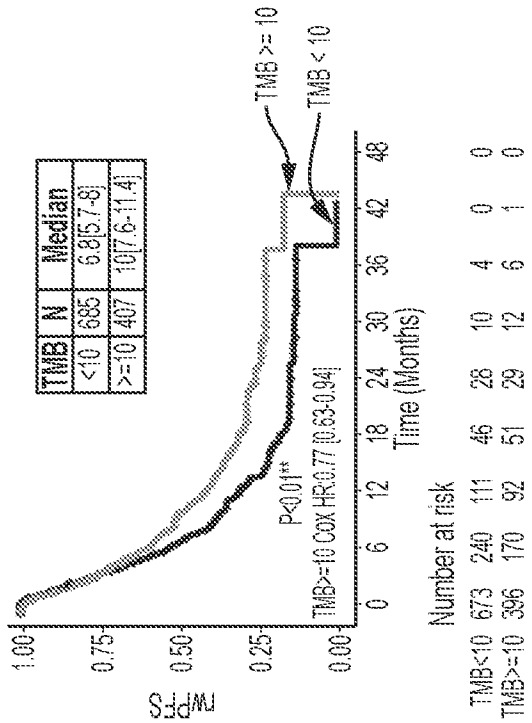


FIG. 19B

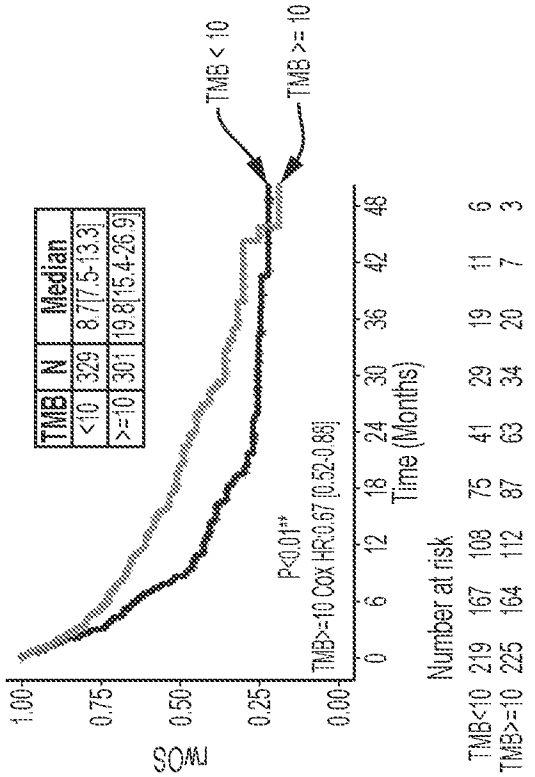


FIG. 19C

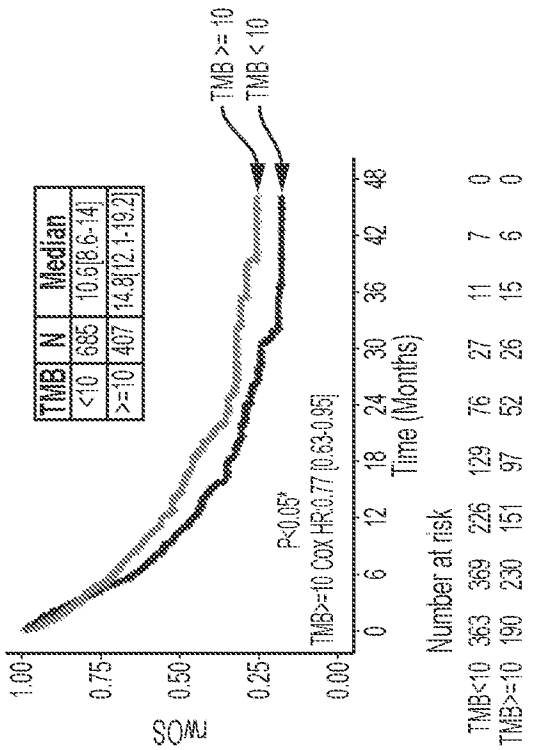
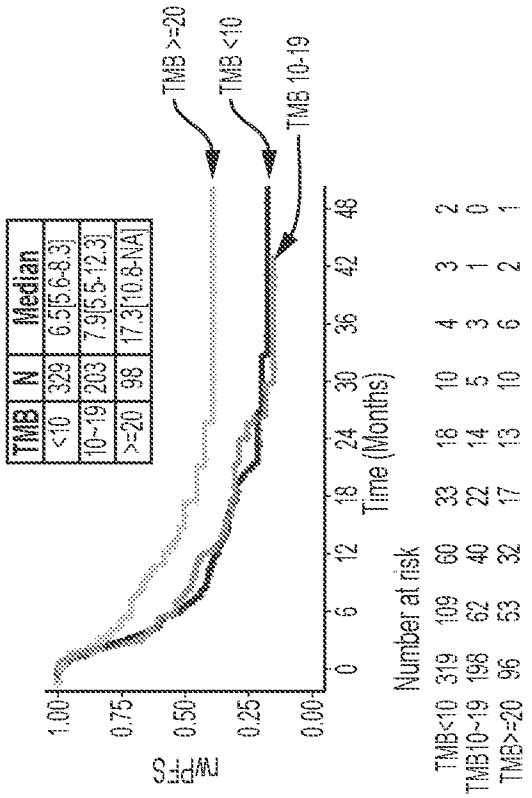
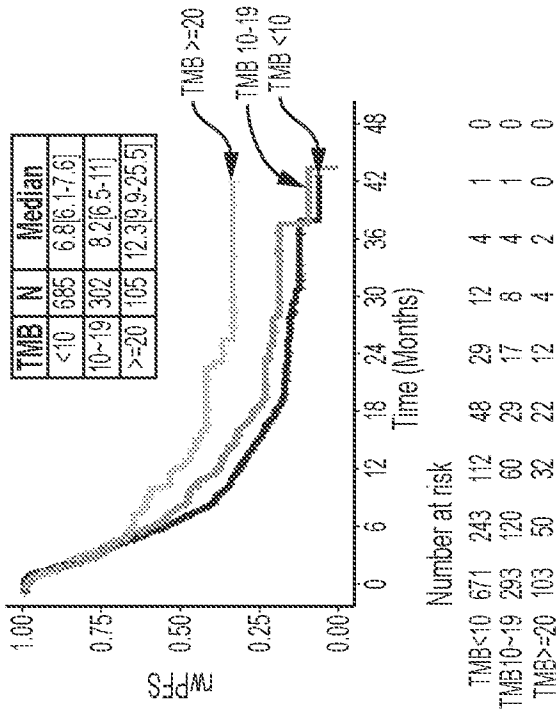


FIG. 19D



TMB	N	Median
<10	329	6.5[5.6-8.3]
10~19	203	7.9[5.5-12.3]
>=20	98	17.3[10.8-NA]

FIG. 20A



TMB	N	Median
<10	685	6.8[6.1-7.6]
10~19	302	8.2[6.5-11]
>=20	105	12.3[9.9-25.5]

FIG. 20B

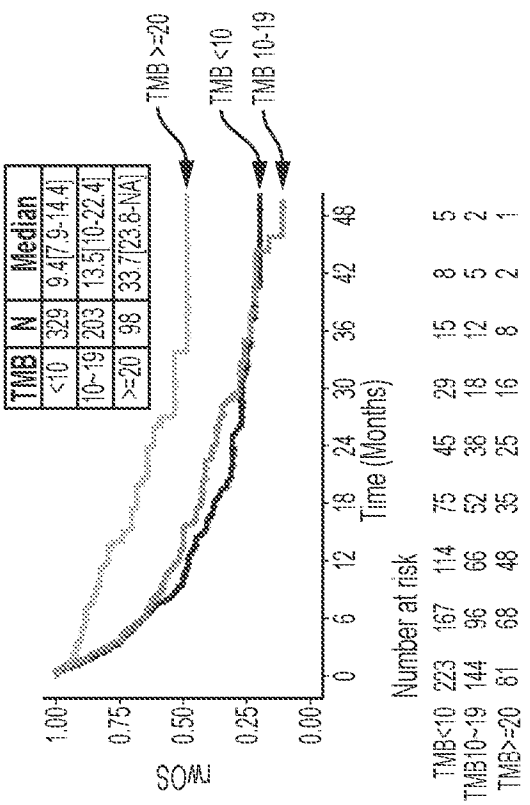


FIG. 20C

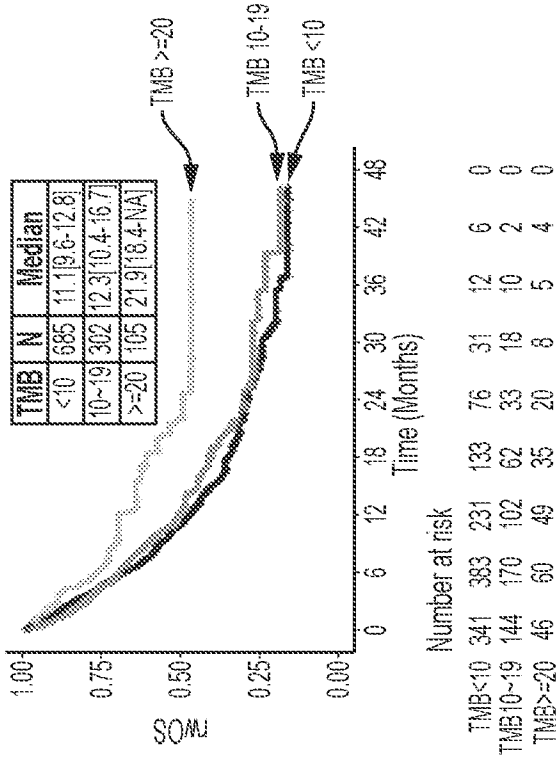


FIG. 20D

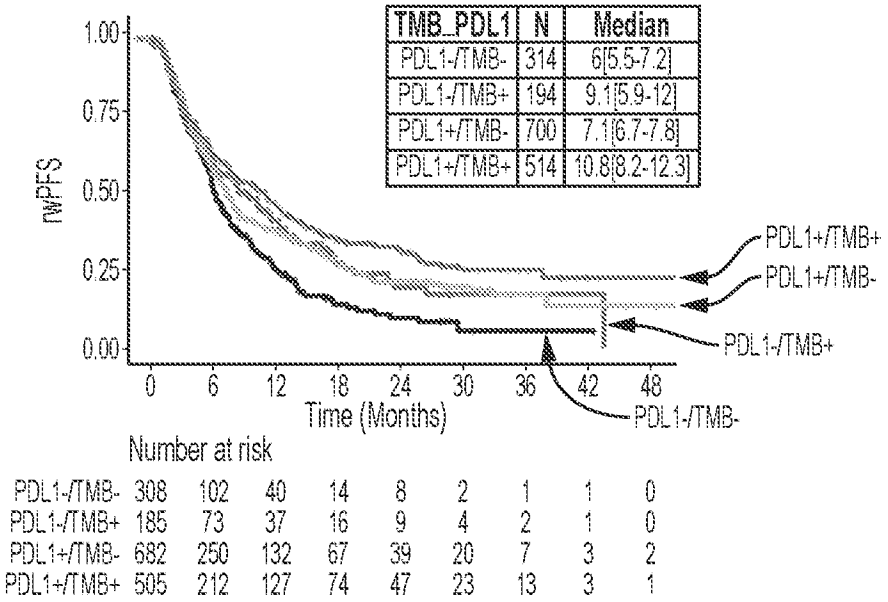


FIG. 21A

Variable		N	Hazard ratio	p
TMB	<10	1014		Reference
	>=10	708		0.79 (0.69, 0.91) 0.001
PDL1	<1%	508		Reference
	>=1%	1214		0.86 (0.74, 0.99) 0.037
STK11		1722		1.74 (1.45, 2.09) <0.001
AgeTherapyStart		1722		1.00 (0.99, 1.00) 0.239
ECOG	0~1	1175		Reference
	2+	388		1.05 (0.88, 1.25) 0.580
	Unknown	159		1.08 (0.84, 1.37) 0.553
Metastasis	no metastasis	89		Reference
	bone only	122		1.24 (0.77, 1.98) 0.378
	brain	386		2.00 (1.34, 2.98) <0.001
	liver	230		2.42 (1.61, 3.62) <0.001
	brain and liver	88		2.94 (1.87, 4.63) <0.001
	other metastases	807		1.45 (0.99, 2.12) 0.059
Smoking	History of smoking	1625		Reference
	No history of smoking	97		1.14 (0.87, 1.59) 0.347
Histology	Non-squamous cell carcinoma	1293		Reference
	NSCLC histology NOS	75		1.22 (0.89, 1.67) 0.210
	Squamous cell carcinoma	354		1.30 (1.09, 1.55) 0.004
StageAtDiagnosis	I	165		Reference
	II	97		1.19 (0.84, 1.68) 0.331
	III	208		1.19 (0.89, 1.59) 0.249
	IV	1227		1.07 (0.85, 1.37) 0.558
	Unknown	25		1.00 (0.53, 1.89) 0.993

FIG. 21B

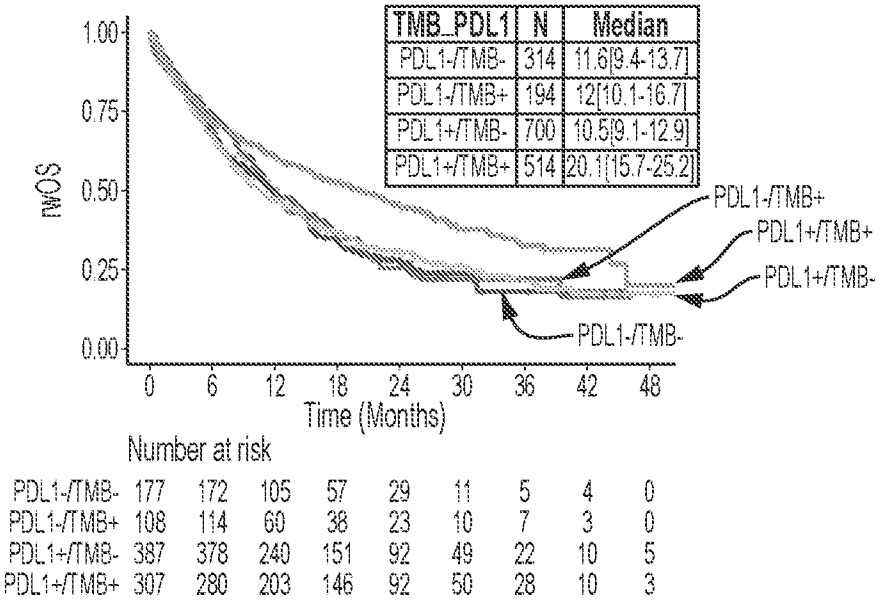


FIG. 21C

Variable		N	Hazard ratio	p
TMB	<10	1014	Reference	
	>=10	708	0.73 (0.64, 0.85)	<0.001
PDL1	<1%	508	Reference	
	>=1%	1214	0.90 (0.78, 1.05)	0.171
STK11		1722	1.56 (1.30, 1.86)	<0.001
AgeTherapyStart		1722	1.01 (1.00, 1.01)	0.098
ECOG	0~1	1175	Reference	
	2+	388	1.83 (1.57, 2.13)	<0.001
	Unknown	159	1.18 (0.93, 1.50)	0.177
Metastasis	no metastasis	89	Reference	
	bone only	122	1.19 (0.75, 1.87)	0.461
	brain	386	1.32 (0.88, 1.97)	0.185
	liver	230	1.63 (1.08, 2.46)	0.020
	brain and liver	88	1.81 (1.15, 2.86)	0.011
	other metastases	807	1.18 (0.80, 1.74)	0.406
Smoking	History of smoking	1625	Reference	
	No history of smoking	97	0.99 (0.75, 1.31)	0.946
Histology	Non-squamous cell carcinoma	1293	Reference	
	NSCLC histology NOS	75	1.09 (0.78, 1.50)	0.620
	Squamous cell carcinoma	354	1.31 (1.10, 1.56)	0.002
StageAtDiagnosis	I	165	Reference	
	II	97	0.68 (0.46, 1.00)	0.050
	III	208	1.05 (0.78, 1.41)	0.753
	IV	1227	1.09 (0.87, 1.37)	0.466
	Unknown	25	1.25 (0.72, 2.17)	0.428

FIG. 21D

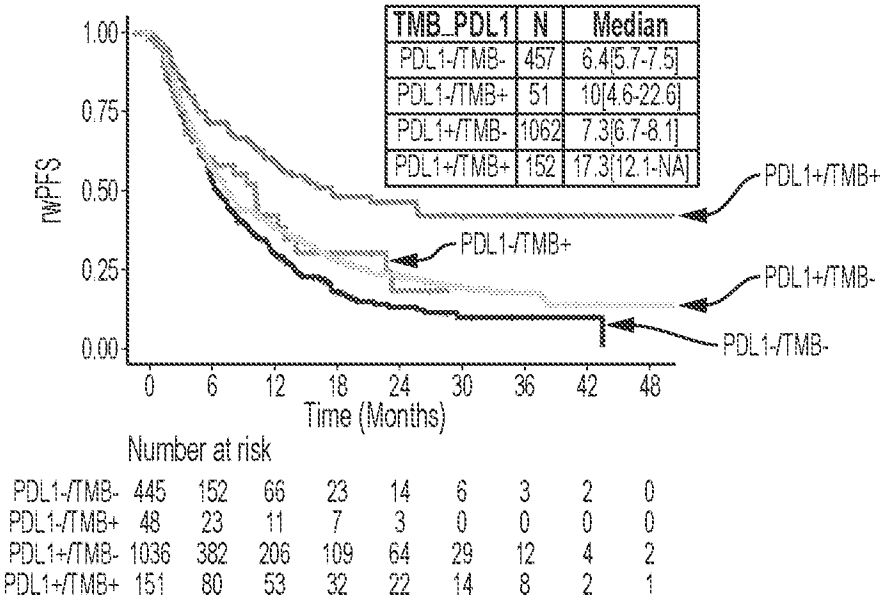


FIG. 22A

Variable		N	Hazard ratio	p
TMB	<20	1519		Reference
	>=20	203		0.62 (0.49, 0.77) <0.001
PDL1	<1%	508		Reference
	>=1%	1214		0.86 (0.75, 1.00) 0.053
STK11		1722		1.73 (1.44, 2.07) <0.001
AgeTherapyStart		1722		0.99 (0.99, 1.00) 0.141
ECOG	0~1	1175		Reference
	2+	388		1.05 (0.88, 1.25) 0.608
	Unknown	159		1.07 (0.84, 1.37) 0.568
Metastasis	no metastasis	89		Reference
	bone only	122		1.29 (0.81, 2.07) 0.284
	brain	386		2.03 (1.36, 3.02) <0.001
	liver	230		2.48 (1.65, 3.72) <0.001
	brain and liver	88		3.03 (1.93, 4.76) <0.001
	other metastases	807		1.50 (1.02, 2.20) 0.038
Smoking	History of smoking	1625		Reference
	No history of smoking	97		1.16 (0.88, 1.52) 0.293
Histology	Non-squamous cell carcinoma	1293		Reference
	NSCLC histology NOS	75		1.25 (0.91, 1.71) 0.162
	Squamous cell carcinoma	354		1.27 (1.06, 1.51) 0.008
StageAtDiagnosis	I	165		Reference
	II	97		1.20 (0.85, 1.70) 0.306
	III	208		1.17 (0.87, 1.56) 0.303
	IV	1227		1.05 (0.83, 1.34) 0.677
	Unknown	25		0.96 (0.51, 1.80) 0.892

FIG. 22B

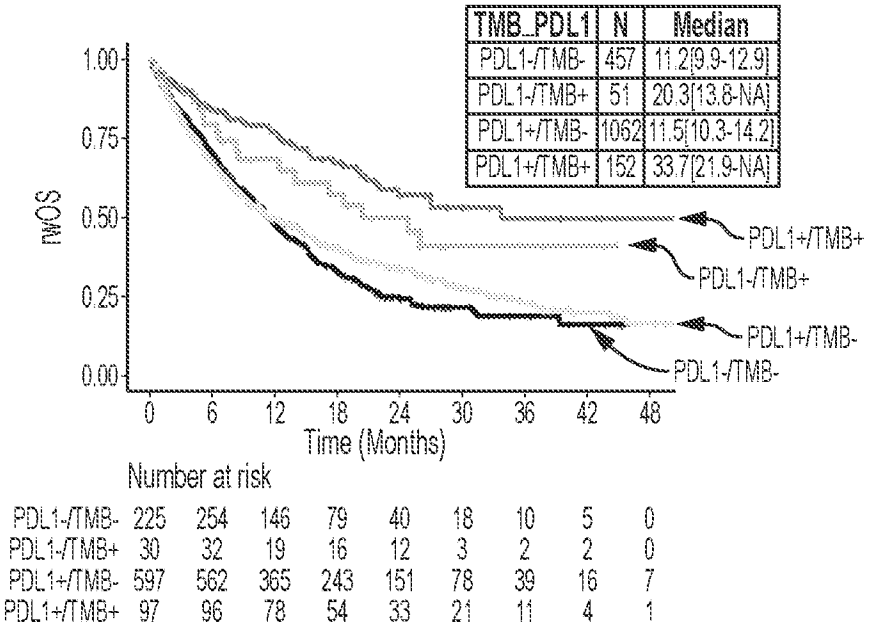


FIG. 22C

Variable		N	Hazard ratio	p
TMB	<20	1519		Reference
	>=20	203		0.49 (0.38, 0.63) <0.001
PDL1	<1%	508		Reference
	>=1%	1214		0.91 (0.78, 1.05) 0.204
STK11		1722		1.54 (1.29, 1.84) <0.001
AgeTherapyStart		1722		1.00 (1.00, 1.01) 0.228
ECOG	0~1	1175		Reference
	2+	388		1.80 (1.55, 2.10) <0.001
	Unknown	159		1.19 (0.94, 1.52) 0.154
Metastasis	no metastasis	89		Reference
	bone only	122		1.24 (0.78, 1.95) 0.364
	brain	386		1.31 (0.88, 1.97) 0.187
	liver	230		1.66 (1.10, 2.50) 0.016
	brain and liver	88		1.84 (1.16, 2.90) 0.009
	other metastases	807		1.22 (0.83, 1.79) 0.319
Smoking	History of smoking	1625		Reference
	No history of smoking	97		1.02 (0.77, 1.34) 0.912
Histology	Non-squamous cell carcinoma	1293		Reference
	NSCLC histology NOS	75		1.13 (0.82, 1.57) 0.464
	Squamous cell carcinoma	354		1.28 (1.07, 1.52) 0.006
StageAtDiagnosis	I	165		Reference
	II	97		0.69 (0.47, 1.01) 0.056
	III	208		1.01 (0.76, 1.36) 0.925
	IV	1227		1.07 (0.85, 1.34) 0.586
	Unknown	25		1.18 (0.68, 2.05) 0.548

FIG. 22D

FIG. 23A

FIG. 23B

FIG. 23C

FIG. 23D

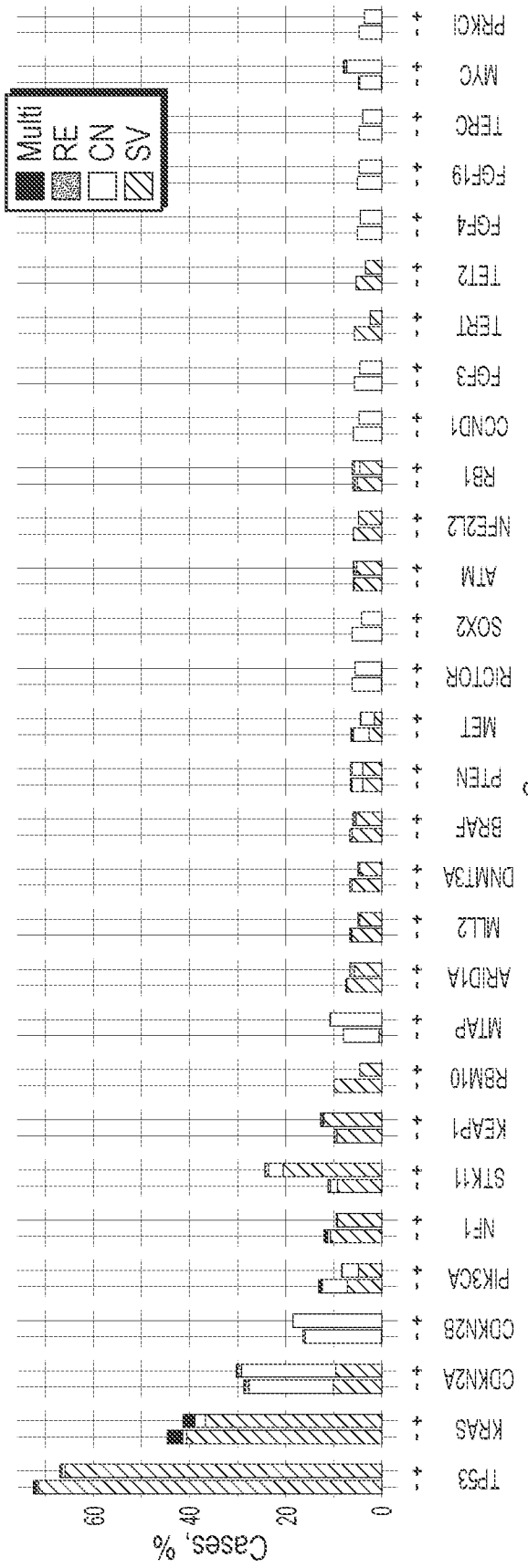

Multi
RE
CN
SV
Cases, %
60
40
20
0
Gene
TP53
KRAS
CDKN2A
CDKN2B
PIK3CA
NF1
STK11
KEAP1
RBM10
MTAP
ARID1A
MLL2
DNMT3A
BRAF
PTEN
MET
RICTOR
SOX2
ATM
NFE2L2
RB1
CCND1
FGF3
TERT
TET2
FGF4
FGF19
TERC
MYC
PRKCI

FIG. 24

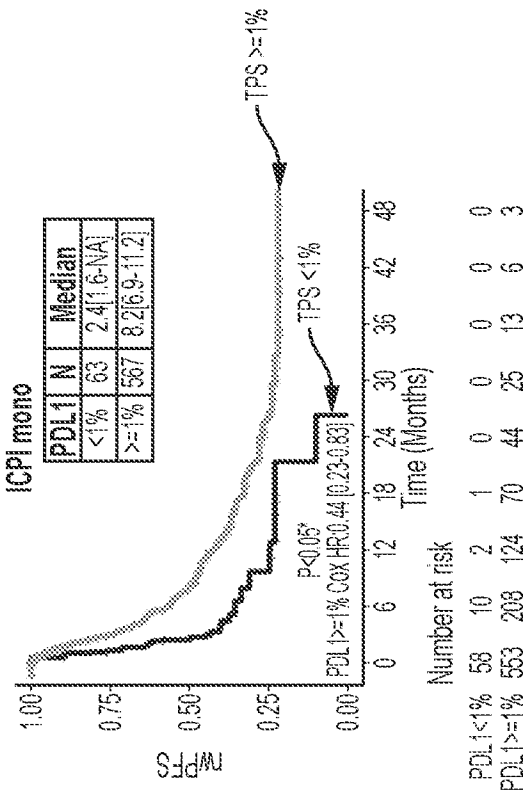


FIG. 25A

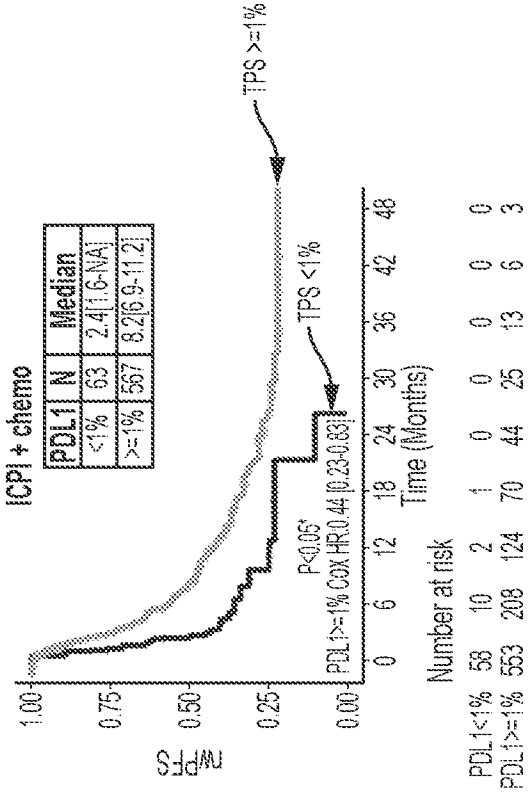


FIG. 25B

FIG. 25C

FIG. 25D

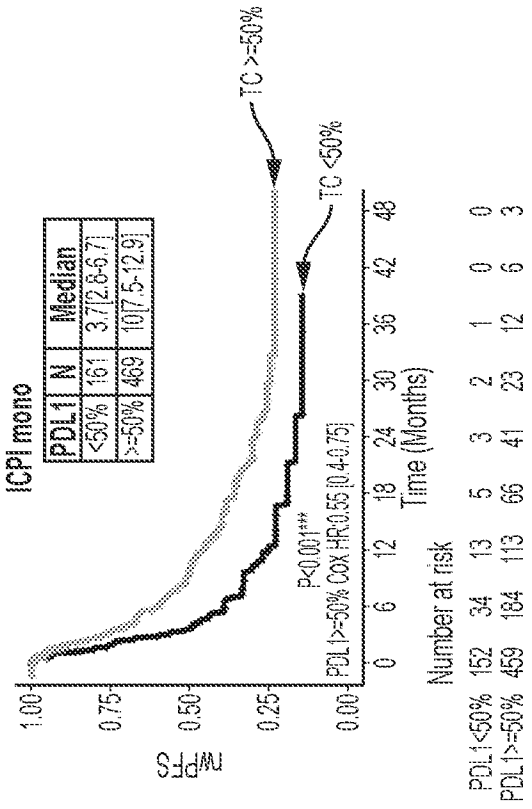


FIG. 26A

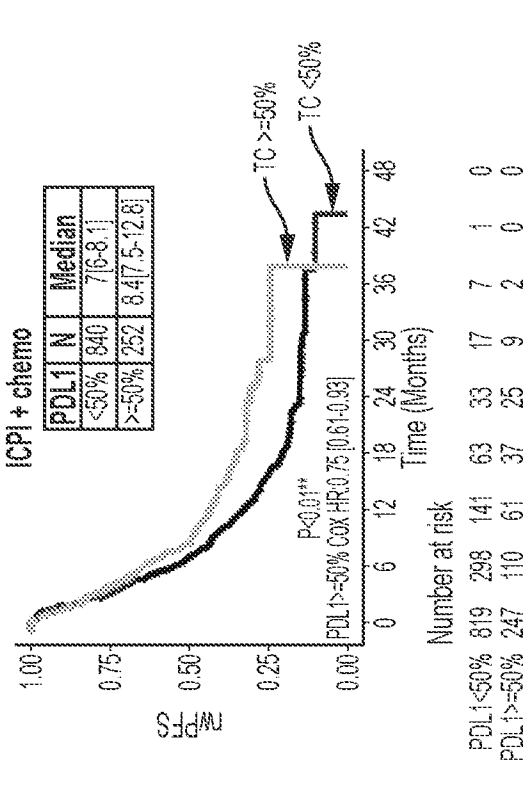


FIG. 26B

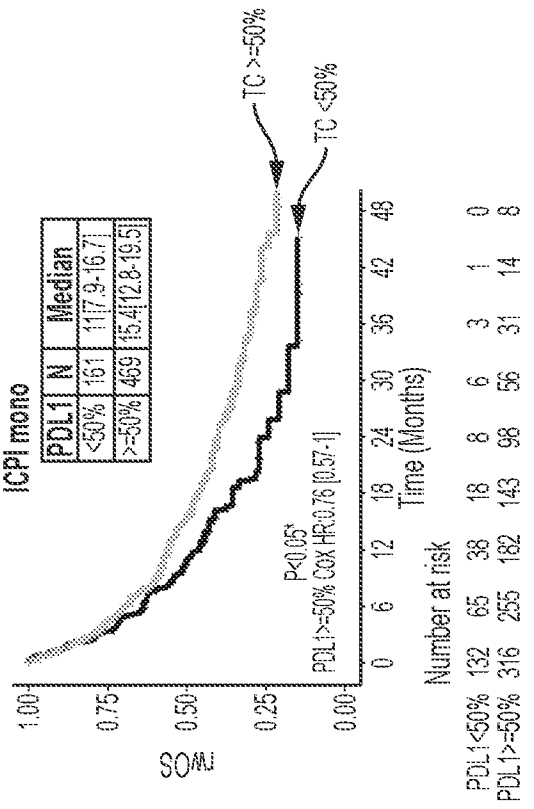


FIG. 26C

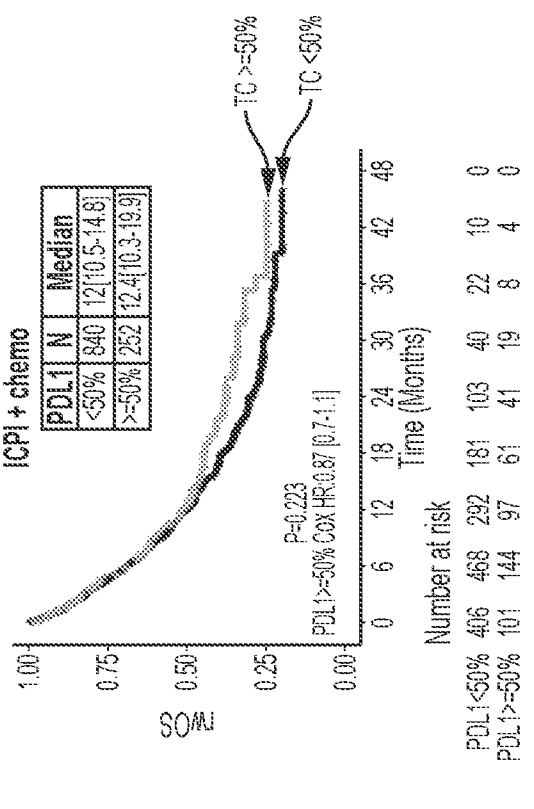


FIG. 26D

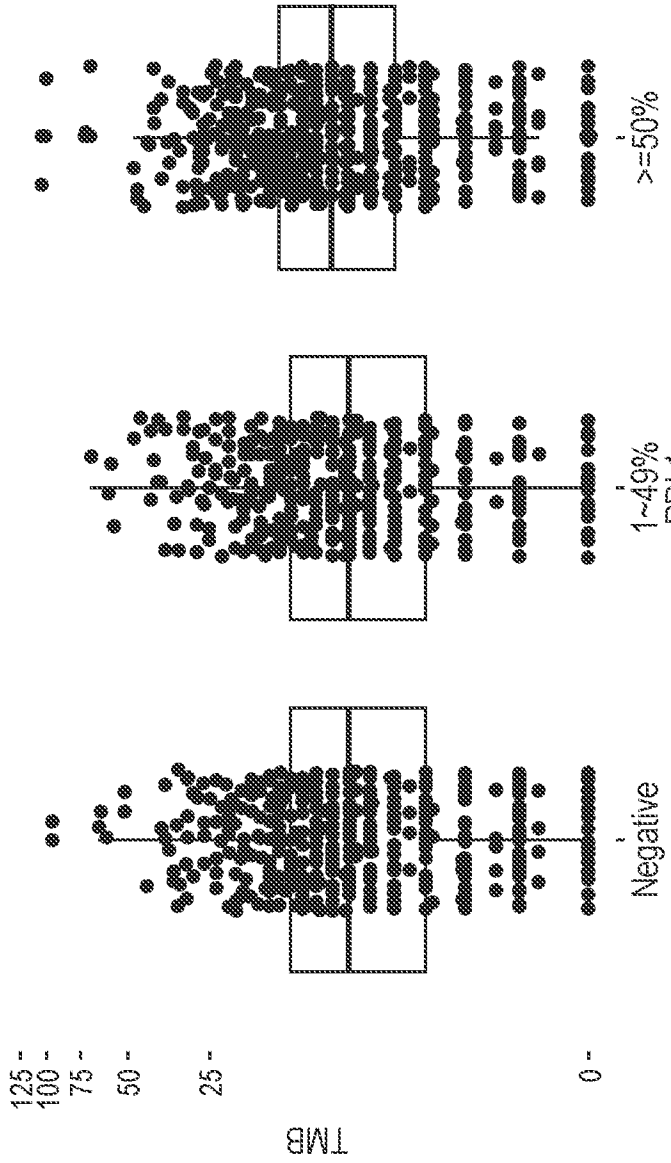


FIG. 27

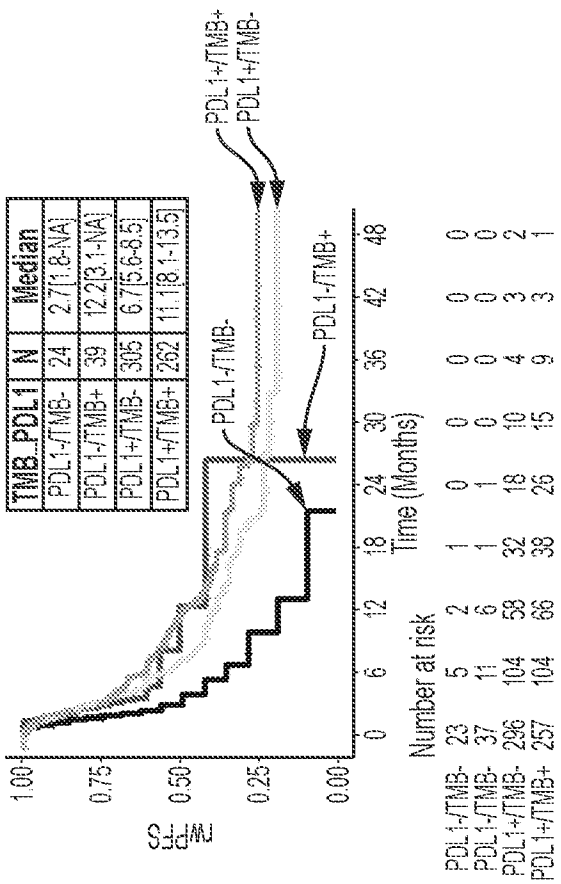


FIG. 28A

FIG. 28B

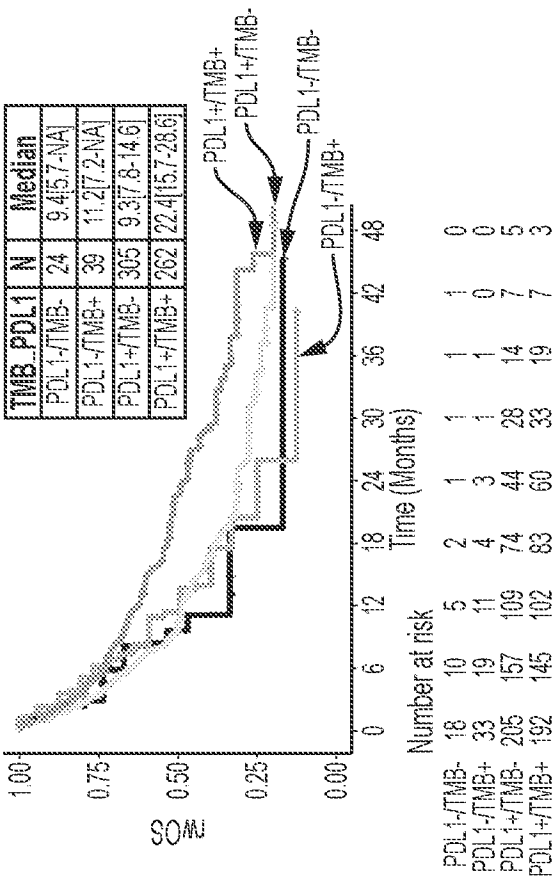


FIG. 28C

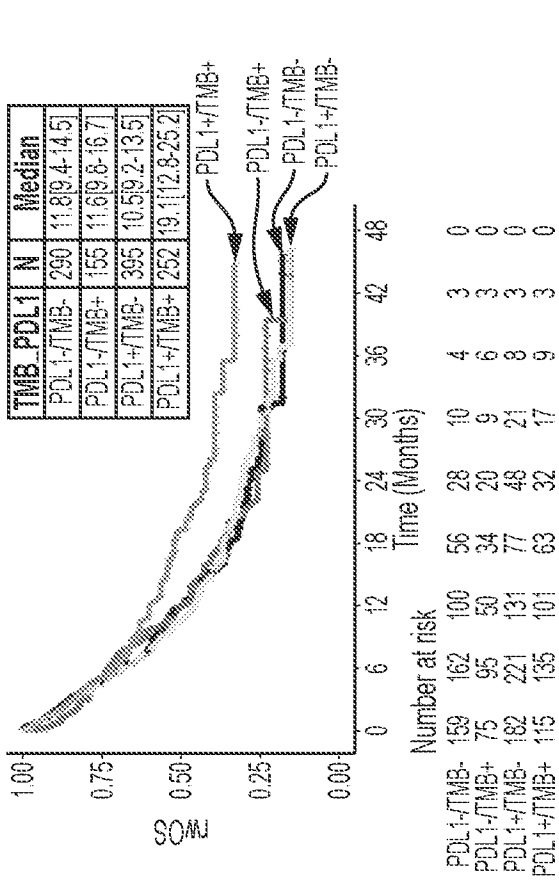


FIG. 28D

Time to Next Treatment

Variable		Pts	Events	Estimate	Estimate (95% CI)	p-value
ECOG	0	2095	1550		Reference	
	1	3660	2984		1.21 (1.14, 1.29)	<0.001
	2+	1657	1365		1.74 (1.62, 1.88)	<0.001
	Unknown	1028	786		1.16 (1.07, 1.27)	<0.001
Line of Therapy	1sl	3427	2483		Reference	
	2nd	2977	2441		1.14 (1.07, 1.20)	<0.001
	3rd+	2036	1761		1.33 (1.24, 1.43)	<0.001
Sex	Female	3865	3031		Reference	
	Male	4575	3654		1.07 (1.02, 1.13)	0.009
Age (per decade increase)		8440			0.98 (0.96, 1.00)	0.114
Genetic Ancestry	AFR	629	504		Reference	
	AMR	439	332		0.89 (0.77, 1.03)	0.113
	EAS	188	147		0.95 (0.79, 1.16)	0.634
	EUR	7123	5655		0.95 (0.86, 1.05)	0.336
	SAS	61	47		1.08 (0.79, 1.46)	0.627
Socioeconomic Status	1 - Lowest SES	1025	819		Reference	
	2	1294	1016		0.98 (0.89, 1.07)	0.652
	3	1566	1253		0.98 (0.90, 1.07)	0.667
	4	1664	1290		0.95 (0.87, 1.04)	0.290
	5 - Highest SES	1280	1005		1.03 (0.93, 1.13)	0.574
	unknown	1611	1302		1.00 (0.91, 1.09)	0.922
Opioid Rx Pre-Therapy	No	5585	4332		Reference	
	Yes	2855	2353		1.26 (1.19, 1.32)	<0.001
MSI	MSS	7596	6135		Reference	
	MSI-H	475	245		0.59 (0.50, 0.69)	<0.001
	MSI-L	199	162		0.89 (0.75, 1.06)	0.199
	unknown	170	143		0.89 (0.75, 1.05)	0.162
TMB Range	TMB < 5	3256	2797		Reference	
	TMB 5-10	2155	1774		0.90 (0.85, 0.96)	0.001
	TMB 10-20	1666	1284		0.72 (0.67, 0.77)	<0.001
	TMB 20+	1363	830		0.51 (0.46, 0.56)	<0.001



FIG. 29A

Overall Survival

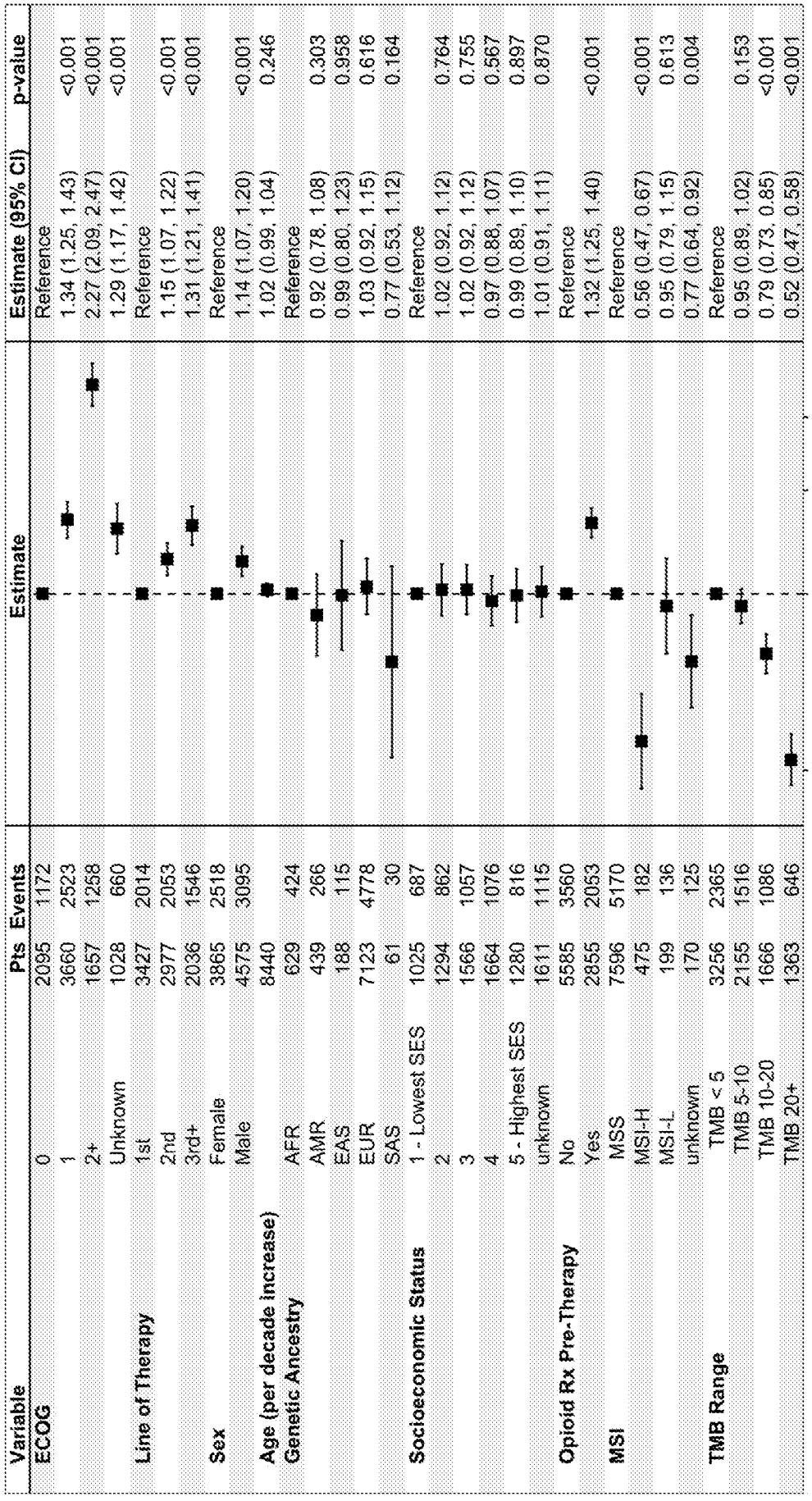


FIG. 29B

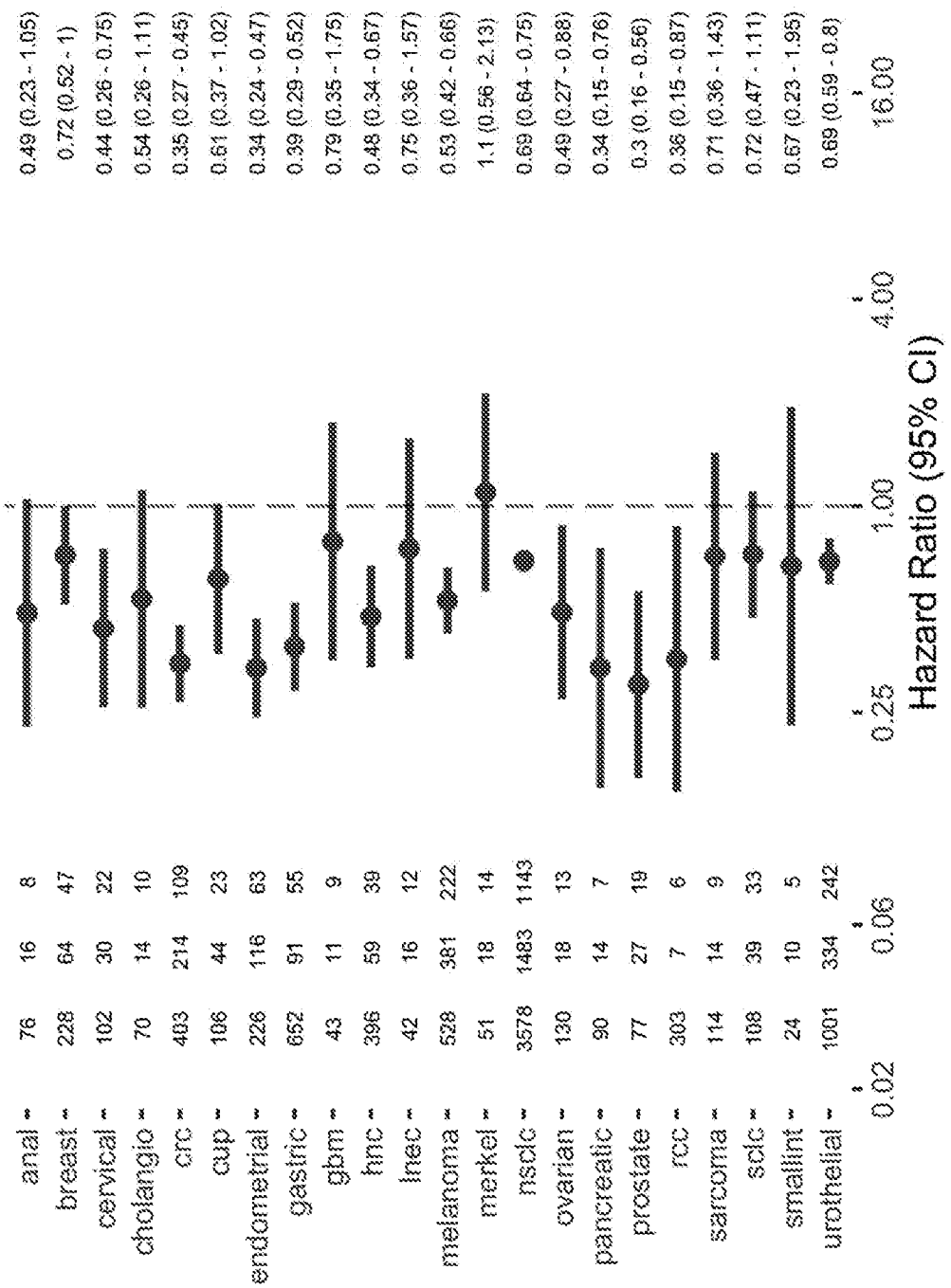

TTNTD: TMB10+ vs. TMB < 10 Per Cancer Type
anal
breast
cervical
cholangio
crc
cup
endometrial
gastric
gbm
hnc
lnec
melanoma
merkel
nsclc
ovarian
pancreatic
prostate
rcc
sarcoma
sclc
smallint
urothelial
76 16 8
228 64 47
102 30 22
70 14 10
483 214 109
106 44 23
226 116 63
652 91 55
43 11 9
396 59 39
42 16 12
528 381 222
51 18 14
3578 1483 1143
130 18 13
90 14 7
77 27 19
303 7 6
114 14 9
108 39 33
24 10 5
1001 334 242
0.49 (0.23 - 1.05)
0.72 (0.52 - 1)
0.44 (0.26 - 0.75)
0.54 (0.26 - 1.11)
0.35 (0.27 - 0.45)
0.61 (0.37 - 1.02)
0.34 (0.24 - 0.47)
0.39 (0.29 - 0.52)
0.79 (0.35 - 1.75)
0.48 (0.34 - 0.67)
0.75 (0.36 - 1.57)
0.53 (0.42 - 0.66)
1.1 (0.56 - 2.13)
0.69 (0.64 - 0.75)
0.49 (0.27 - 0.88)
0.34 (0.15 - 0.76)
0.3 (0.16 - 0.56)
0.36 (0.15 - 0.87)
0.71 (0.36 - 1.43)
0.72 (0.47 - 1.11)
0.67 (0.23 - 1.95)
0.69 (0.59 - 0.8)
0.02
0.06
0.25
1.00
4.00
16.00
Hazard Ratio (95% CI)

FIG. 30A

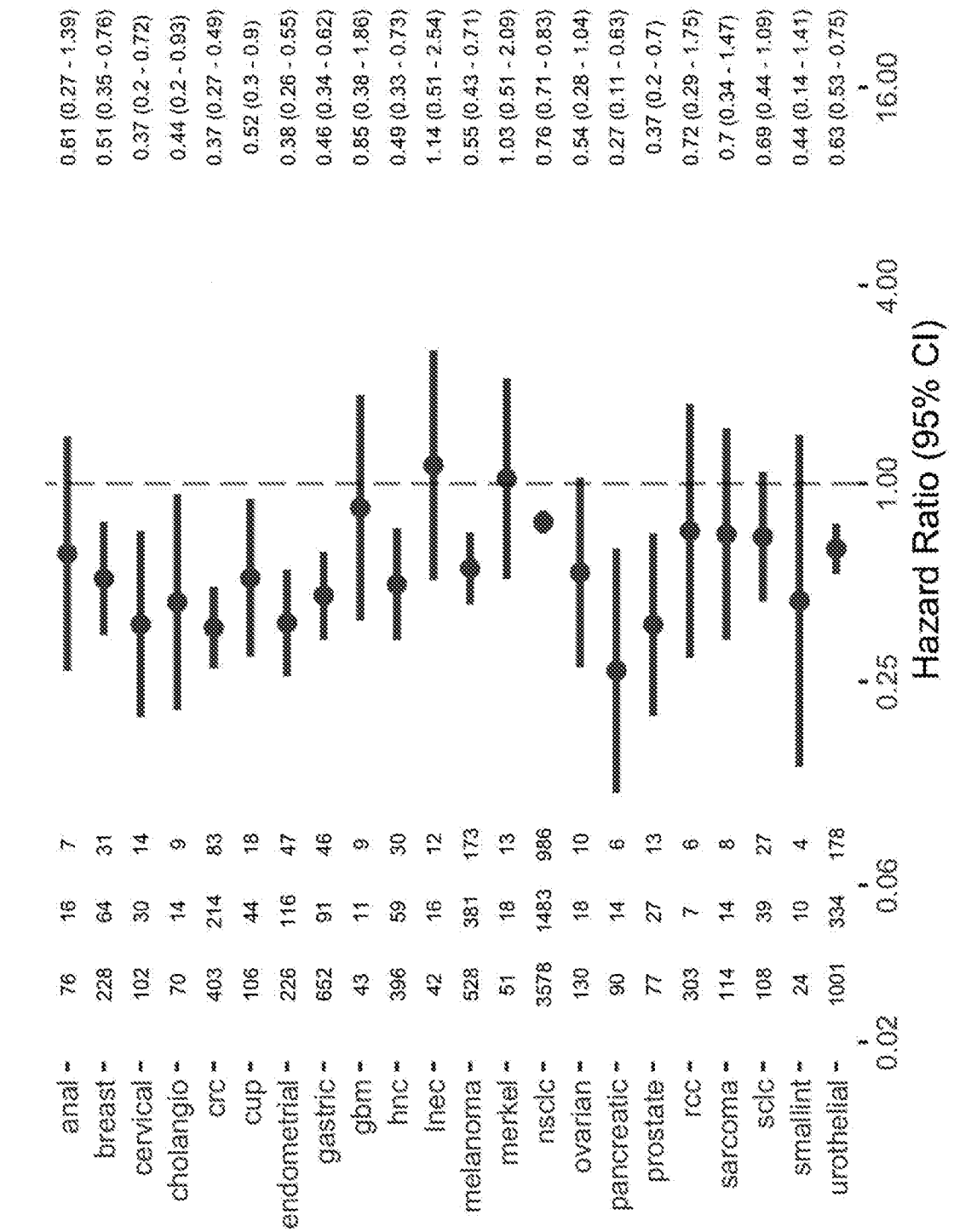

OS: TMB10+ vs. TMB < 10 Per Cancer Type
anal
breast
cervical
cholangio
crc
cup
endometrial
gastric
gbm
hnc
inec
melanoma
merkel
nsclc
ovarian
pancreatic
prostate
rcc
sarcoma
sclc
smallint
urothelial
76 16 7 0.81 (0.27 - 1.39)
228 64 31 0.51 (0.35 - 0.76)
102 30 14 0.37 (0.2 - 0.72)
70 14 9 0.44 (0.2 - 0.93)
403 214 83 0.37 (0.27 - 0.49)
106 44 18 0.52 (0.3 - 0.9)
226 116 47 0.38 (0.26 - 0.55)
652 91 46 0.46 (0.34 - 0.62)
43 11 9 0.85 (0.38 - 1.86)
396 59 30 0.49 (0.33 - 0.73)
42 16 12 1.14 (0.51 - 2.54)
528 381 173 0.55 (0.43 - 0.71)
51 18 13 1.03 (0.51 - 2.09)
3578 1483 986 0.76 (0.71 - 0.83)
130 18 10 0.54 (0.28 - 1.04)
90 14 6 0.27 (0.11 - 0.63)
77 27 13 0.37 (0.2 - 0.7)
303 7 6 0.72 (0.29 - 1.75)
114 14 8 0.7 (0.34 - 1.47)
108 39 27 0.69 (0.44 - 1.09)
24 10 4 0.44 (0.14 - 1.41)
1001 334 178 0.63 (0.53 - 0.75)
0.02
0.06
0.25
1.00
4.00
16.00
Hazard Ratio (95% CI)

FIG. 30B

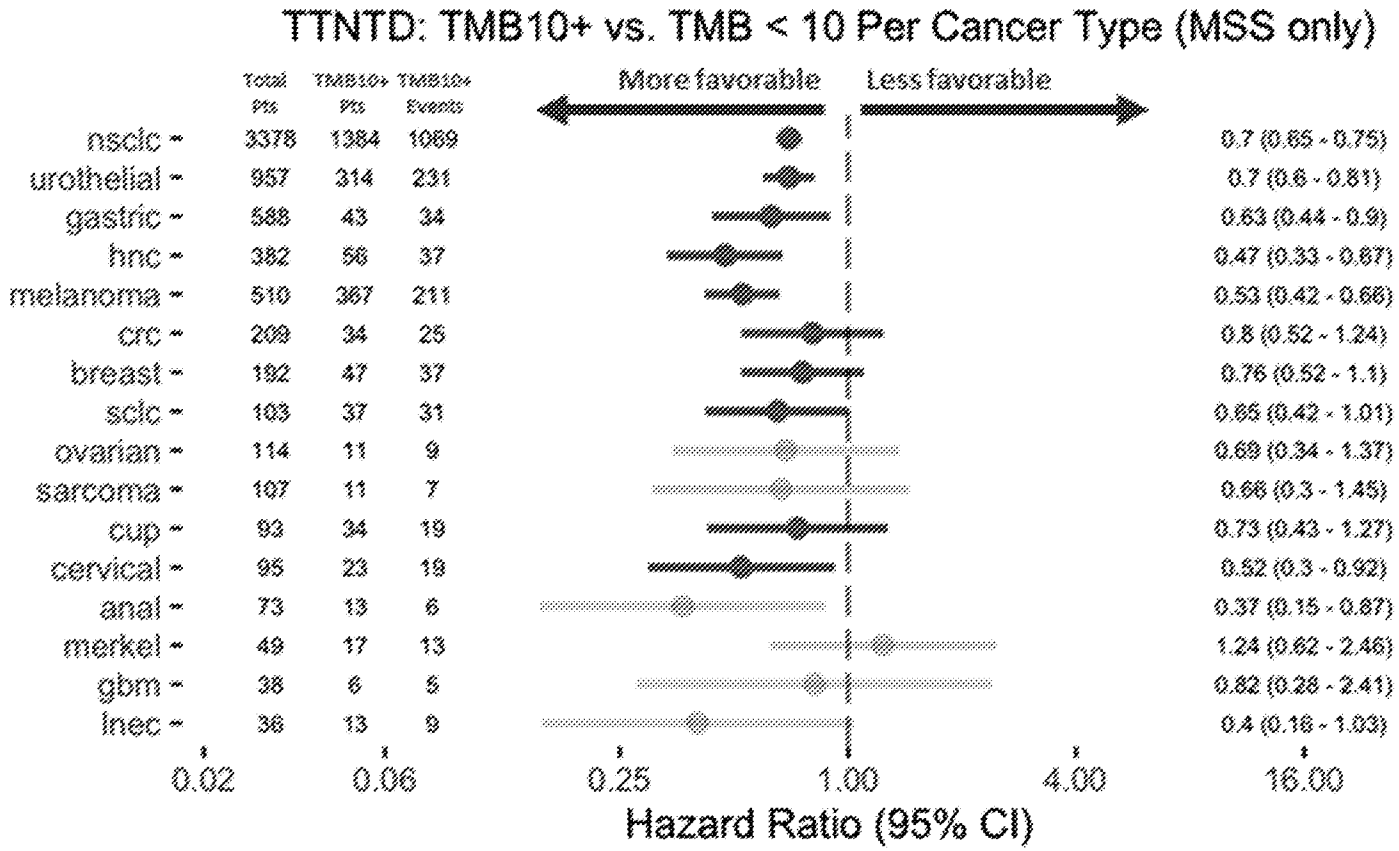

TTNTD: TMB10+ vs. TMB < 10 Per Cancer Type (MSS only)
Total Pts
TMB10+ Pts
TMB10+ Events
More favorable
Less favorable
nsclc ~ 3378 1384 1069 0.7 (0.65 - 0.75)
urothelial ~ 957 314 231 0.7 (0.6 - 0.81)
gastric ~ 588 43 34 0.63 (0.44 - 0.9)
hnc ~ 382 56 37 0.47 (0.33 - 0.67)
melanoma ~ 510 367 211 0.53 (0.42 - 0.66)
crc ~ 209 34 25 0.8 (0.52 - 1.24)
breast ~ 192 47 37 0.76 (0.52 - 1.1)
sclc ~ 103 37 31 0.65 (0.42 - 1.01)
ovarian ~ 114 11 9 0.69 (0.34 - 1.37)
sarcoma ~ 107 11 7 0.66 (0.3 - 1.45)
cup ~ 93 34 19 0.73 (0.43 - 1.27)
cervical ~ 95 23 19 0.52 (0.3 - 0.92)
anal ~ 73 13 6 0.37 (0.15 - 0.87)
merkel ~ 49 17 13 1.24 (0.62 - 2.46)
gbm ~ 38 6 5 0.82 (0.28 - 2.41)
lnec ~ 36 13 9 0.4 (0.16 - 1.03)
0.02
0.06
0.25
1.00
4.00
16.00
Hazard Ratio (95% CI)
Color Key:
Adequate sample size
Intermediate sample size

FIG. 31A

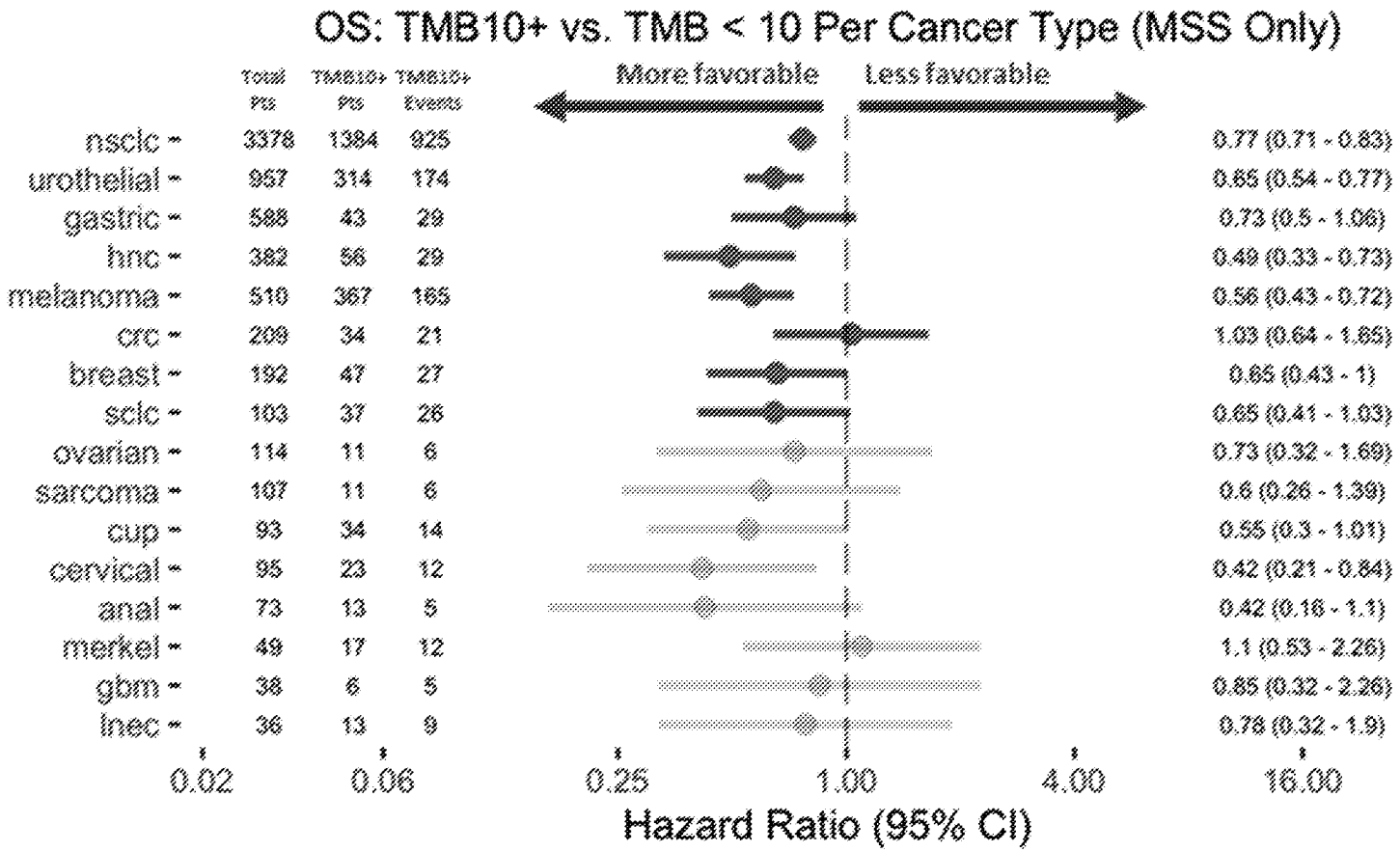

OS: TMB10+ vs. TMB < 10 Per Cancer Type (MSS Only)
Total Pts
TMB10+ Pts
TMB10+ Events
More favorable
Less favorable
nsclc
urothelial
gastric
hnc
melanoma
crc
breast
sclc
ovarian
sarcoma
cup
cervical
anal
merkel
gbm
hnec
3378 1384 925
957 314 174
588 43 29
382 56 29
510 367 165
209 34 21
192 47 27
103 37 26
114 11 6
107 11 6
93 34 14
95 23 12
73 13 5
49 17 12
38 6 5
36 13 9
0.77 (0.71 - 0.83)
0.65 (0.54 - 0.77)
0.73 (0.5 - 1.06)
0.49 (0.33 - 0.73)
0.56 (0.43 - 0.72)
1.03 (0.64 - 1.65)
0.65 (0.43 - 1)
0.65 (0.41 - 1.03)
0.73 (0.32 - 1.69)
0.6 (0.26 - 1.39)
0.55 (0.3 - 1.01)
0.42 (0.21 - 0.84)
0.42 (0.16 - 1.1)
1.1 (0.53 - 2.26)
0.85 (0.32 - 2.26)
0.78 (0.32 - 1.9)
0.02
0.06
0.25
1.00
4.00
16.00
Hazard Ratio (95% CI)
Color Key:
Adequate sample size
Intermediate sample size

FIG. 31B

Time to Next Treatment: ICI combo therapy

Variable		Pts	Events	Estimate	Estimate (95% CI)	p-value
ECOG	0	2084	1471		Reference	
	1	2684	1985		1.11 (1.04, 1.19)	0.003
	2+	943	746		1.59 (1.45, 1.75)	<0.001
	Unknown	843	610		1.23 (1.11, 1.35)	<0.001
Line of Therapy	1st	4259	3029		Reference	
	2nd	1260	939		1.15 (1.06, 1.24)	<0.001
	3rd+	1035	844		1.38 (1.26, 1.51)	<0.001
Sex	Female	3322	2454		Reference	
	Male	3232	2358		1.09 (1.02, 1.16)	0.010
Age (per decade increase)		6554			0.99 (0.96, 1.02)	0.479
Genetic Ancestry	AFR	632	471		Reference	
	AMR	415	299		1.00 (0.86, 1.16)	0.970
	EAS	195	142		1.06 (0.87, 1.29)	0.575
	EUR	5257	3864		0.96 (0.86, 1.06)	0.406
	SAS	55	36		1.19 (0.84, 1.69)	0.318
Socioeconomic Status	1 - Lowest SES	829	577		Reference	
	2	1009	721		1.00 (0.90, 1.12)	0.961
	3	1098	823		1.05 (0.94, 1.17)	0.355
	4	1229	898		1.11 (0.99, 1.23)	0.066
	5 - Highest SES	1146	834		1.11 (1.00, 1.24)	0.059
	unknown	1243	959		1.09 (0.98, 1.21)	0.121
Opioid Rx Pre-Therapy	No	4194	3007		Reference	
	Yes	2360	1805		1.27 (1.19, 1.35)	<0.001
MSI	MSS	6244	4611		Reference	
	MSI-H	127	75		0.85 (0.66, 1.10)	0.218
	MSI-L	145	94		0.75 (0.60, 0.93)	0.010
	unknown	38	32		0.78 (0.54, 1.11)	0.166
TMB Range	TMB < 5	3025	2305		Reference	
	TMB 5-10	1763	1340		0.92 (0.86, 0.99)	0.024
	TMB 10-20	1139	792		0.77 (0.71, 0.84)	<0.001
	TMB 20+	627	375		0.59 (0.52, 0.67)	<0.001

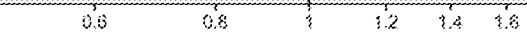


FIG. 32A

Overall Survival: ICI combo therapy

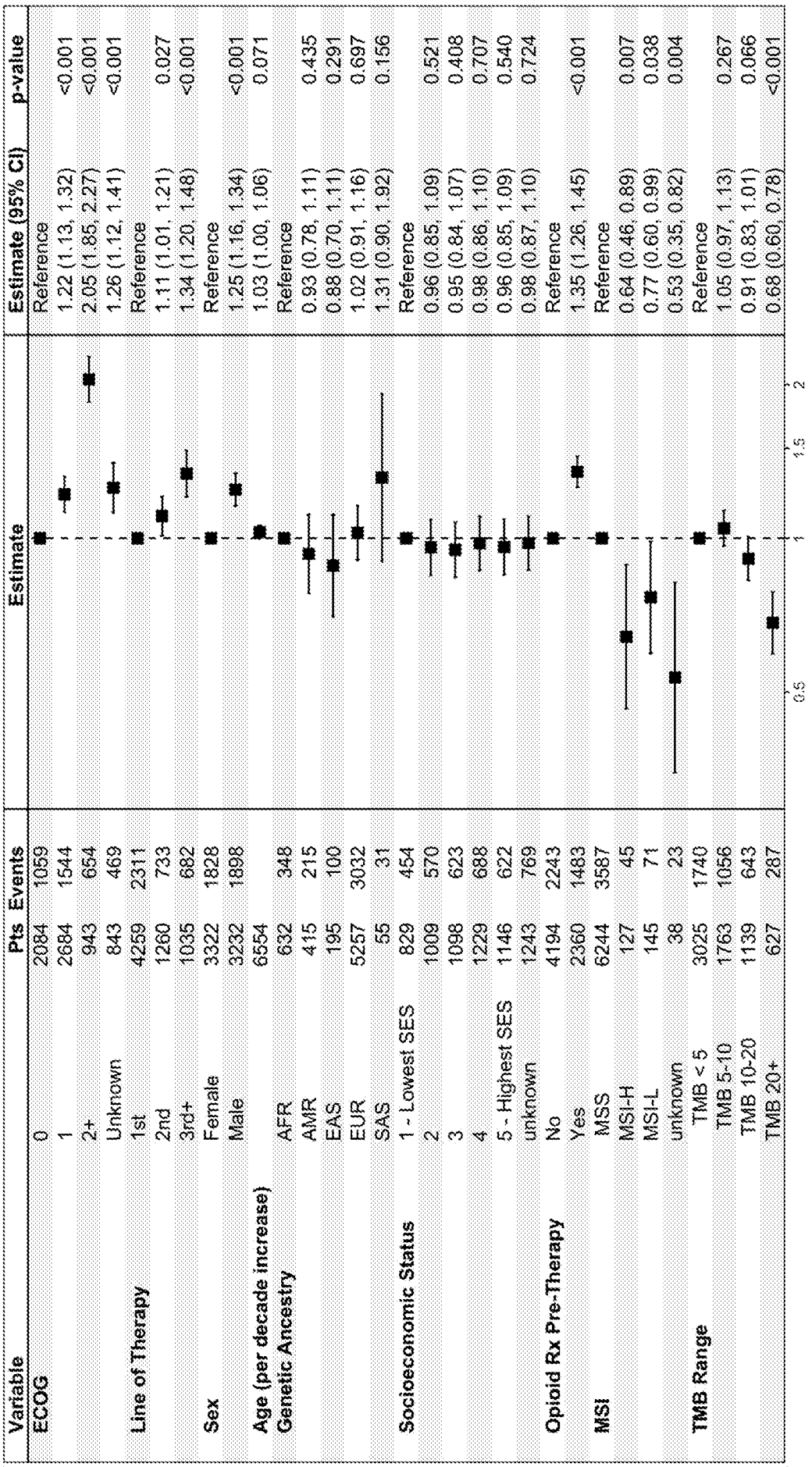


FIG. 32B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/62434

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12Q 1/6886, C12Q 1/6827, C12Q 1/6869 (2023.01)
ADD. A61K 39/395, A61P 35/00 (2023.01)

CPC - INV. C12Q 1/6886, C12Q 1/6827, C12Q 1/6869
ADD. C12Q 2600/106, C12Q 2600/156, A61K 39/395, A61P 35/00

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2019/0219586 A1 (GENENTECH, INC. et al.) 18 July 2019 (18.07.2019); para [0024], [0035], [0066], [0185], [0215], [0217], [0219], [0230], [0304], [0307]-[0309], [0313]; claim 1, 5, 9-10, 28, 39, 59-60, 69, 85, 89	1-24
A	WO 2021/092221 A1 (BRISTOL-MYERS SQUIBB COMPANY) 14 May 2021 (14.05.2021); entire document	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 April 2023	MAY 17 2023
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300